UNIVERSITÉ DE LORRAINE

AVERTISSEMENT

Ce document est le fruit d'un long travail approuvé par le jury de soutenance et mis à disposition de l'ensemble de la communauté universitaire élargie.

Il est soumis à la propriété intellectuelle de l'auteur. Ceci implique une obligation de citation et de référencement lors de l'utilisation de ce document.

D'autre part, toute contrefaçon, plagiat, reproduction illicite encourt une poursuite pénale.

Contact : ddoc-theses-contact@univ-lorraine.fr

LIENS

Code de la Propriété Intellectuelle. articles L 122. 4
Code de la Propriété Intellectuelle. articles L 335.2- L 335.10
http://www.cfcopies.com/V2/leg/leg_droi.php
http://www.culture.gouv.fr/culture/infos-pratiques/droits/protection.htm

Thèse

Présentée et soutenue publiquement pour l'obtention du titre de

DOCTEUR DE L'UNIVERSITE DE LORRAINE

Mention « Biologie et écologie des forêts et des agrosystèmes »

par Lauralie MANGEOT-PETER

Etude des facteurs biotiques et abiotiques influant sur la structuration et la composition du microbiote racinaire du Peuplier

Soutenance le 3 mars 2020

Membres du jury :

Dr Feth-el-Zahar HAICHAR - Rapportrice	Maître de Conférences, Université de Lyon
Pr Daniel WIPF - Rapporteur	Professeur, Université de Bourgogne
Pr Michel CHALOT - Examinateur	Professeur, Université de Lorraine
Dr Stéphane HACQUARD - Examinateur	Chargé de Recherche, Max Planck Institute
Dr Francis MARTIN - Directeur de thèse	Directeur de Recherche, INRAE Nancy
Dr Aurélie DEVEAU - Co-directrice de thèse	Chargée de Recherche, INRAE Nancy

UMR 1136 INRAE / Université de Lorraine, Interactions Arbres – Micro-organismes
INRAE Centre Grand Est - Nancy – 54280 Champenoux
Faculté des Sciences et Technologies – 54500 Vandoeuvre-lès-Nancy

Remerciements

Pour commencer, je souhaite remercier les membres de mon jury de thèse Zahar Haichar, Daniel Wipf, Stéphane Hacquard et Michel Chalot qui ont accepté d'évaluer mes travaux.
Je remercie également Matthieu Barret, Jean-Louis Hilbert et Claire Veneault-Fourrey, les membres de mon comité de suivi qui m'ont aidé tout au long de ces trois années.

Je remercie tout particulièrement mes deux directeurs de thèse Aurélie Deveau et Francis Martin. Un grand merci pour tout ce que vous m'avez appris ainsi que pour votre confiance et vos conseils avisés. Francis, merci pour ton aide et pour toutes les opportunités que tu m'as offertes. Aurélie, un grand merci pour ta patience, le temps que tu as su me consacrer et, surtout, ton soutien.

Un grand merci à l'ensemble de l'équipe Ecogénomique pour votre accueil et votre sympathie :

Merci Seb pour tes encouragements.

Merci Claire, Annegret et Elena pour votre soutien, vos conseils et votre bonne humeur permanente.

Merci Stéphane pour ton aide, ta disponibilité et nos longues discussions qui, certes, parfois m'ont fait douter mais toujours encouragée.

Merci Marc pour ta bonne humeur et pour les bons moments passés à Prague à l'occasion de l'ICOM9.

Merci Claude de m'avoir offert la chance d'animer le stand INRAE-IAM lors du Salon International de l'Agriculture.

Merci Patrice pour ton aide précieuse lors, notamment, des « manips » en serre S2, pour nos discussions, ton côté révolutionnaire et pour ta compagnie dans le bus.

Merci Christine pour les commandes, les pauses-thé réconfortantes, les séances de gym, les conseils bien-être et, surtout, tes séances de Shiatzu reposantes.

Merci à Nicole, Agnès et Marie-Claude pour votre aide administrative, votre gentillesse et votre disponibilité.

Merci Manue et Lucas pour votre indispensable aide informatique et pour votre bonne humeur.

Merci à Béa, Jean-Louis et Cyril.

Merci Kenza, Léa, Mehdi et Yoann pour votre aide précieuse au laboratoire et dans les serres. Je ne pouvais pas espérer meilleurs stagiaires.

Un énorme merci à vous, François et Océane, pour m'avoir soutenu, moi que ne connaissait pas grand chose à R. Merci Cécile pour m'avoir passé le flambeau de « responsable du Journal Club » et pour tous les bons moments partagés. Merci Feng, Cora et Igor. Je suis très heureuse d'avoir pu croiser votre route.

Merci à Milena pour ton sourire permanent. Merci Veronica et Clémence, mes deux acolytes du « Journal Club ». Merci à toi, Félix, pour les nombreux services rendus ainsi que ta présence lors de notre escapade scientifique à Guémené, copilote de choc.

Merci à mes camarades de bureau Océane, François, Maira, Clémence, Chloé et Laura pour nos fous-rires, nos débats parfois enflammés et surtout nos commérages.

Un immense merci Laurette, Fred, Flora et Maira pour votre soutien, vos rires et tous les bons moments que nous avons partagés au et en dehors du labo et pour ceux à venir.

Merci à Pascale et Annick pour leur sourire et leur accueil dans le laboratoire « Tous Chercheurs ». Ce fut une expérience très enrichissante pour moi et, même, un moment de répit dans la dernière ligne droite. J'espère avoir encore l'occasion d'y participer. Merci également à toi, Jonas, pour les formations et les sorties.

Je remercie mille fois mes amies (les docteurs ☺) Emilie et Mélanie, pour les verres en terrasse et les coups de fil réconfortants pendant la phase critique de rédaction.

Merci à la famille du sud, Laura, Teddy et Pascal (mon cher collègue), pour les bons moments passés ensemble et votre soutien sans faille.

Je remercie bien évidemment ma famille et belle-famille pour leur soutien et leurs encouragements tout au long de mes études.

Enfin, je remercie de tout mon cœur Maxime, pour son aide et son soutien durant cette aventure et, avec qui, je suis impatiente d'affronter les prochains défis qui nous attendent.

Résumé

Les écosystèmes forestiers couvrent plus de 30 % du territoire métropolitain français et ont un rôle écologique et socio-économique important. Les forêts sont très vulnérables aux changements globaux, tels que l'augmentation de la température, la diminution des précipitations et l'invasion de pathogènes. Différentes stratégies de gestion sylvicole sont, aujourd'hui, envisagées pour faire face aux changements climatiques actuels et futurs afin d'atténuer les stress biotiques et abiotiques qui affectent les arbres. Ces différentes approches sont i) choisir des essences d'arbres plus résistantes aux contraintes environnementales, ii) exploiter des types de sols adaptés riches en eau et nutriments et iii) amender les sols pour garantir une certaine fertilité. Cependant, ces pratiques sont compliquées à mettre en place pour les gestionnaires forestiers. L'utilisation de micro-organismes bénéfiques à la croissance et à la santé des arbres pourrait alors constituer un levier supplémentaire pour atténuer l'impact des changements globaux. Il est aujourd'hui admis que les plantes interagissent avec une multitude de micro-organismes, en particulier les bactéries et les champignons vivant à leur surface, dans leurs tissus ou dans la rhizosphère. L'ensemble de ces micro-organismes, appelé le microbiote, est donc le siège de diverses interactions allant des symbioses mutualistes aux effets bénéfiques aux interactions pathogènes. Ces interactions influent sur les processus régissant la nutrition, le développement ou la santé des plantes. Mieux comprendre le microbiote des arbres et ses interactions est donc un objectif de recherche prioritaire qui devrait aider au développement de nouveaux modes de gestion forestière durables.

Dans ce contexte, l'objectif de ma thèse est de comprendre l'effet de différents facteurs biotiques et abiotiques influant sur la structure et la composition des communautés de micro-organismes associées aux racines d'arbres, en particulier, du Peuplier choisi comme modèle d'étude. Une approche de barcoding moléculaire (séquençage à haut débit d'amplicons ribosomiques 16S et ITS) est utilisée pour étudier la dynamique de colonisation des racines du peuplier par les communautés bactériennes et fongiques. Cette étude met en évidence une chronologie dans la colonisation des racines par les differentes communautés microbiennes favorisant la mise en place de la symbiose ectomycorhizienne. Ensuite, en utilisant une approche expérimentale originale, couplant écologie microbienne et écophysiologie, nous mettons en évidence l'effet important des facteurs édaphiques et du climat sur la composition et la structure du microbiote racinaire du Peuplier noir, une espèce particulièrement sensible aux effets du changement climatique. Enfin, barcoding moléculaire et métabolomique sont utilisés pour étudier l'impact des variations du microbiote du sol et de l'acide jasmonique, phytohormone impliquée dans les réactions de défense, sur la composition et la structure du microbiote racinaire du peuplier. A l'issue de ces travaux, nous mettons en évidence (1) une corrélation entre les conditions climatiques (ex., sécheresse hivernale) et la composition du microbiote tellurique ; cette dernière affectant la composition du microbiote et du métabolisme racinaire et (2) un rôle potentiel de l'expression ectopique de l'effecteur fongique MiSSP7 sur la composition des communautés microbiennes, en particulier fongiques. L'ensemble de ces résultats suggère que le sol, le climat et l'immunité végétale structurent les communautés microbiennes racinaires et leur fonctionnement.

Abstract

Forests cover more than 30% of France metropolitan territory and have important ecological, economical and societal roles. However, forests are highly vulnerable to global changes, such as increased temperature, reduced precipitation and invasion of pathogens. Different silvicultural management strategies are currently being considered to address current and future climate change in order to mitigate biotic and abiotic stress affecting trees. However, i) choosing tree species that are most resistant to environmental stresses, ii) using suitable soil types that are rich in water and nutrients, and iii) amending soils to ensure fertility are complicated practices to implement. The use of microorganisms beneficial to tree growth and health could then provide additional leverage. It is now clearly recognized that plants and trees interact with a multitude of microorganisms, especially bacteria and fungi living on their surface, in their tissues or around their root systems. All these microorganisms called microbiome are therefore involved in various interactions ranging from symbiosis to beneficial contributing to plant nutrition, development and health or, on the contrary, harmful interactions. A better understanding of tree microbiome is therefore an important research objective that should help in the development of new, more adapted and respectful management methods. In this context, the objective of my thesis is to understand the effects of different biotic and abiotic factors on the structure and composition of communities of microorganisms associated with the roots of the tree, in particular, the Poplar chosen as a model for trees. A metabarcoding approach (high throughput sequencing of 16S amplicons and ITS rRNA) was used to study the dynamics of colonization of poplar roots by bacterial and fungal communities. This study revealed a succession in the colonization of the roots by the different microbial communities, thus favouring the establishment of ectomycorrhizal symbiosis. Then, by using an experimental approach combining microbial ecology and tree ecophysiology, we have highlighted the important effect of soil and climate factors on the composition and structure of the root microbiome of black poplar, a species particularly affected by the effects of climate change. Finally, the combination of metabarcoding and metabolomics approaches was then used to understand the impact of variations in soil microbiome, and jasmonic acid, phytohormones involved in defense, on the composition and structure of the root microbiome in poplar. First, an effect of climatic conditions, particularly that of winter drought, has been correlated with changes in soil microbiome composition, which in turn are responsible for changes in microbiome composition and root metabolism. Secondly, an effect of the host tree, in particular that of the immune system via the expression of the fungal effector MiSSP7, has also been observed on communities of microorganisms, in particular communities of symbiotic fungi. Finally, All the results of my thesis strongly suggest that the soil type, climate and immunity of the host tree are key parameters in the taxonomic and functional structuring of root-associated bacterial and fungal communities.

.

Liste des figures et tableaux 1

Liste des abréviations 5

Glossaire 7

Chapitre I : Synthèse bibliographique 11

I. LA RHIZOSPHERE, L'ENDOSPHERE ET LEURS MICROBIOTES 13
1. DEFINITION ET CARACTERISTIQUES 13
2. DES NICHES SPECIFIQUES 15
3. REGULATION DU MICROBIOTE RACINAIRE : LES FACTEURS BIOTIQUES 15
3. A. Les exsudats racinaires et les métabolites 15
3. B. Le système immunitaire des plantes 17
3. C. La régulation hormonale des défenses chez les plantes 19
4. REGULATION DU MICROBIOTE RACINAIRE : LES FACTEURS ABIOTIQUES 26
4. A. Le sol 26
4. B. Le climat et les variations saisonnières 27
5. IMPACT DU MICROBIOME SUR LA PLANTE & INGENIERIE ECOLOGIQUE 28

II. INTERACTIONS RACINES-CHAMPIGNONS 29
1. LES SYMBIOSES MYCORHIZIENNES 31
1. A. La symbiose ectomycorhizienne 31
1. B. La symbiose endomycorhizienne 33
2. LES CHAMPIGNONS ENDOPHYTES 33
3. LES AUTRES TYPES DE CHAMPIGNONS DE LA RHIZOSPHERE 34

III. LES INTERACTIONS RACINES-BACTERIES 36

III. LES INTERACTIONS CHAMPIGNONS-BACTERIES 37

IV. LES MODELES D'ETUDE *POPULUS* ET *LACCARIA BICOLOR* 39
1. LE PEUPLIER 39
1. A. *Populus tremula x alba* 41
1. B. *Populus nigra* 41
2. LE CHAMPIGNON ECTOMYCORHIZIEN *LACCARIA BICOLOR* 42
3. ROLE DE L'EFFECTEUR MISSP7 43

V. LE MICROBIOTE DU PEUPLIER 47

ARTICLE N°1 (EN PREPARATION) : CURRENT KNOWLEDGE OF THE POPULUS MICROBIOME : IMPROVING THE HOLISTIC PERCEPTION OF TREE-MICROBES INTERACTIONS 48

VI. OBJECTIFS DE LA THESE 61

Chapitre II : Dynamique de colonisation des racines du peuplier par les communautés de micro-organismes 65

I. CONTEXTE GENERAL 67

II. OBJECTIFS 68

III. DEMARCHE EXPERIMENTALE 68

ARTICLE N°2 (EN PREPARATION) : COLONIZATION DYNAMIC OF *POPULUS TREMULA X ALBA* BY SOIL MICROBIAL COMMUNITIES 69

IV. CONCLUSIONS 98

Chapitre III : Effet du type de sol, du génotype et du climat sur le microbiote racinaire du Peuplier noir (*Populus nigra* L.) 101

I. CONTEXTE GENERAL 103

II. OBJECTIFS 109

III. DÉMARCHE EXPÉRIMENTALE 110

ARTICLE N°3 (EN PREPARATION) : A MESOCOSM TRANSPLANT EXPERIMENT TO INVESTIGATE HOW CLIMATE, SOIL PROPERTIES AND PLANT GENETICS DETERMINE THE STRUCTURE OF THE ROOT MICROBIOME OF *POPULUS NIGRA* SEEDLINGS 111

IV. CONCLUSIONS 145

Chapitre IV : Impact des variations de la composition et de la structure du microbiote du sol sur la colonisation racinaire et le métabolome du Peuplier (*Populus tremula x alba*) 149

I. CONTEXTE GENERAL 151

II. OBJECTIFS 152

III. DÉMARCHE EXPÉRIMENTALE 152

ARTICLE N° 4 : IMPACTS OF SOIL MICROBIOME VARIATIONS ON ROOT COLONIZATION BY FUNGI AND BACTERIA AND ON THE METABOLOME OF *POPULUS TREMULA X ALBA* 153

IV. CONCLUSIONS 170

Chapitre V : Etude de l'expression hétérologue de l'effecteur fongique MiSSP7 sur la structuration et la composition du microbiote racinaire et sur le métabolome du Peuplier 173

I. CONTEXTE GENERAL 175

II. OBJECTIFS 175

III. DÉMARCHE EXPÉRIMENTALE 176

IS JASMONIC ACID SIGNALING PATHWAY A HUB FOR CONTROLLING THE *POPULUS TREMULA X ALBA* ROOT MICROBIOME COLONIZATION ? 177

IV. CONCLUSIONS 209

Chapitre VI : Conclusion générale, discussion et perpectives 213

I. RESULTATS MAJEURS DE LA THESE 215
1. MISE EN PLACE DU MICROBIOTE RACINAIRE DE L'ARBRE 215
2. EFFET DU TYPE DE SOL SUR LE MICROBIOTE RACINAIRE DE L'ARBRE 216
2. A. Origine du sol 217
2. B. Variations du microbiote du sol au cours du temps 218
3. EFFET DU CLIMAT SUR LE MICROBIOTE RACINAIRE DE L'ARBRE 219
4. EFFET DE LA PLANTE SUR LE MICROBIOTE RACINAIRE DE L'ARBRE 220
4. A. Effet « genotype » 221
4. B. Rôle des hormones de défense 222
5. LE « CORE MICROBIOME » DU PEUPLIER 225

II. PERSPECTIVES 227
1. EFFET DU STRESS HYDRIQUE SUR LE MICROBIOTE RACINAIRE DU PEUPLIER 227
2. APPROCHE SIP (STABLE ISOTOPE PROBING) : DETERMINER LES COMMUNAUTES MICROBIENNES ACTIVES DU MICROBIOTE RACINAIRE DE L'ARBRE 228
3. EFFET DES EXSUDATS RACINAIRES SUR LE MICROBIOTE DU SOL 228
4. EFFET DE L'ACIDE SALYCILIQUE, DE L'ETHYLENE ET DE L'ACIDE GIBBERILIQUE SUR LE MICROBIOTE RACINAIRE DU PEUPLIER 229
5. APPLICATIONS POSSIBLES DES RESULTATS 230

Références bibliographiques 233

Annexes

Annexe 1 1

Annexe 2 9

Annexe 3 27

Annexe 4 53

Liste des figures

Chapitre I : Synthèse bibliographique

Figure 1 - Les différentes niches à l'interface racine-sol (d'après Mendes et al., 2013) 14

Figure 2 - La rhizosphère et les micro-organismes associés (Phillippot et al., 2013)..... 14

Figure 3 - Représentation schématique de l'immunité déclenchée par les MAMPS (d'après Jones & Dangl, 2006) 18

Figure 4 - Représentation schématique de la cascade de régulation conduisant à l'expression des gènes de défense induits par l'acide jasmonique..... 21

Figure 5 - Représentation schématique de la cascade de régulation conduisant à l'expression des gènes de défense induits par l'acide salicylique 23

Figure 6 - Représentation schématique de la cascade de régulation conduisant à l'expression des gènes de défense induits par l'éthylène..... 24

Figure 7 - Echanges de composés nutritifs entre les deux partenaires durant la symbiose mycorhizienne 30

Figure 8 - Caractéristiques structurales des différents types de symbiose mycorhizienne..... 32

Figure 9 - Structures spécifiques des Dark Septate Endophytes dans les racines de la plante hôte..... 35

Figure 10 - Les différentes espèces de peuplier..... 40

Figure 11 - Le champignon ectomycorhizien *Laccaria bicolor* (d'après Martin et al., 2016) 44

Figure 12 - Représentation schématique du modèle de régulation de la voie de signalisation de l'acide jasmonique du peuplier par l'effecteur fongique MiSSP7 (d'après Martin et al., 2016) 46

Figure 13 - Factors driving reciprocal interactions between trees and their microbiome 49

Figure 14 - Number of available publications dealing with *Populus* microbiome by studied microorganisms and influence factors 59

Chapitre II : Dynamique de colonisation des racines du peuplier par les communautés de micro-organismes

Figure 15 - Experimental design and number of samples used for each experimental approach in this study 73

Figure 16 - Rarefaction curves of bacterial and fungal OTUs..... 78

Figure 17 - Root development and ectomycorrhiza formation along time..... 79

Figure 18 - Confocal microscopy image of *Populus tremula x alba* adventive root at the beginning of the experiment 79

Figure 19 - Structure and composition of bacterial communities colonizing *Populus* roots across time 81

Figure 20 - Structure and composition of fungal communities colonizing *Populus* roots across time 84

Figure 21 - Early stage of the fungal colonization dynamic 87

Figure 22 - Dark Septate Endophyte (DSE) colonizing poplar roots from 4 to 30 days of culture 88

Figure 23 - Intermediate stage of the fungal colonization dynamic 90

Figure 24 - Intermediate stage of the fungal colonisation dynamic 91

Figure 25 - Late stage fungal of the colonization dynamic 92

Chapitre III : Effet du type de sol, du génotype et du climat sur le microbiote racinaire du Peuplier noir (*Populus nigra* L.)

Figure 26 - Experimental design 116

Figure 27 - Monitoring of temperature in Loire and Drôme regions 121

Figure 28 - Monitoring of the growth of the aerial part of *Populus nigra* seedlings 123

Figure 29 - Impact of pre-conditioning stage on the microbiomes of sediments of Loire and Drôme 126

Figure 30 - Composition and structure of sediment microbiome 127

Figure 31 - Composition and structure of *Populus nigra* root microbiome 129

Figure 32 - Rhizosphere and root filtering effects in the native and transplant conditions 130

Figure 33 - Impact of sediment origin on the *Populus nigra* root microbiome 132

Figure 34 - Impact of climate on the *Populus nigra* root microbiome 134

Figure 35 - Rhizosphere and root filtering effects in the non-native conditions of culture 135

Figure 36 - Combined impact of sediment origin and climate on the *Populus nigra* root microbiome 137

Figure 37 - Impact of the transplant conditions of sediment and climate on the relative abundance of fungal guilds detected in the endosphere of *Populus nigra* seedlings 138

Chapitre IV : Impact des variations de la composition et de la structure du microbiote du sol sur la colonisation racinaire et le métabolome du Peuplier (*Populus tremula x alba*)

Figure S1 - Scheme of the experimental design and sampling strategy *156*

Figure S2 - Average monthly temperatures and cumulative precipitations recorded by the meteorological station of Essey located 12 km from the sampling site from January to June 2016 (Soil 1) and 2017 (Soil 2) *158*

Figure 1 - Taxonomic representation of the most abundant fungal and bacterial communities (>0.1% in total relative abundance) detected in bulk soil T0 ..... *159*

Figure 2 - Differences in the composition and diversity of fungal and bacterial communities between T0 bulk soils *160*

Figure S3 - Taxonomic representation of the most abundant fungal and bacterial communities (>0.1% in total relative abundance) detected in Populus roots *161*

Figure 3 - Differences in the structure and the composition of root fungal and bacterial communities of cuttings grown in Soil 1 and Soil 2 ..... *162*

Figure 4 - Relative abundance of fungal functional guilds of OTUs detected in roots of Populus tremula x alba harvested at T1 and at T2 grown in Soil 1 and Soil 2 ..... *163*

Figure 5 - Projection to Latent Structures (PLS) of the dominant microbial communities and root metabolites significantly varying between Soil 1 and Soil 2 conditions at T1 and T2 ..... *165*

Chapitre V : Etude de l'expression hétérologue de l'effecteur fongique MiSSP7 sur la structuration et la composition du microbiote racinaire et sur le métabolome du Peuplier

Figure 38 - The relative expression of MiSSP7 in each cutting used in this study 186

Figure 39 -The phenotypic parameters of *Populus*-expressing MiSSP7 and *Populus* WT monitored before, during and after the hydroponic culture in Year 2 190

Figure 40 - Ectomycorrhization rate measured from the roots of *Populus*-expressing MiSSP7 and *Populus* WT collected at T2 in Year 2 191

Figure 41 - The distribution of the most dominant fungal genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T2 in Year 1 192

Figure 42 - The distribution of the most dominant bacterial genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T2 in Year 1 194

Figure 43 - The distribution of the most dominant fungal genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T2 in Year 2 200

Figure 44 - The distribution of the most dominant bacterial genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T2 in Year 2 201

Figure 45 - Proportional distribution of the most active fungal genera detected in the roots of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T2 in Year 2 203

Chapitre VI : Conclusion générale, discussion et perspectives

Figure 46 - Modèle de synthèse de la régulation du microbiote du peuplier réalisé à partir des résultats obtenus au cours de cette thèse 226

Liste des tableaux

Chapitre I : Synthèse bibliographique

Tableau 1 - Les principaux types de symbioses mycorhiziennes (d'après Smith & Read, 2008) 30

Chapitre II : Dynamique de colonisation des racines du peuplier par les communautés de micro-organismes

Table 2 - Diversity of the bacterial community detected in roots across time 85

Table 3 - Diversity of the fungal community detected in roots across time 85

Chapitre IV : Impact des variations de la composition et de la structure du microbiote du sol sur la colonisation racinaire et le métabolome du Peuplier (*Populus tremula x alba*)

Table 1 - List of metabolites detected in roots of Populus tremula x alba harvested after 10 days (T1) and 6.5 weeks (T2) of growth for which a significant difference of concentration has been measured between Soil 1 and Soil 2 ..... *164*

Chapitre V : Etude de l'expression hétérologue de l'effecteur fongique MiSSP7 sur la structuration et la composition du microbiote racinaire et sur le métabolome du Peuplier

Table 5 - Number of samples used for each experimental approach performed in this study 181

Table 6 - Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation in this study (compartments [BS, AS, Rh and E], sampling times [T1 and T2], treatment [MiSSP7 and WT cuttings] or expression level [WT, MiSSP7_Low exp and MiSSP7_High exp]) for fungal and bacterial communities at the OTU level 188

Table 7 - List of metabolites detected in roots of *Populus tremula x alba* WT and *Populus tremula x alba* expressing MiSSP7 roots harvested in Year 1 after 10 days (T1) and 6.5 weeks (T2) 195

Table 8 - Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation in this study (compartments [BS, AS, Rh and E], sampling times [T1 and T2] and expression [WT, MiSSP7_Low exp and MiSSP7_High exp]) for fungal and bacterial communities at the OTU level 198

Liste des abréviations

AJ : Acide Jasmonique

ARNr 16S : ARN ribosomique constituant la petite sous-unité des ribosomes des procaryotes

AM : (champignon) Mycorhizien à Arbuscules

AS : Acide Salicylique

BS : Bulk soil (en français, sol nu)

CLSM : Confocal Laser Scanning Microscopy (en français, microscopie confocale à balayage laser)

CO_2 : dioxyde de carbone

DSE : Dark Septate Endophyte (en français, les endophytes bruns septés)

ECM : (champignon) Ectomycorhizien

ET : Ethylène

ETI : Effector Triggered Immunity (en français, immunité déclenchée par les effecteurs)

ETS : Effector Triggered Susceptibility

GC-MS : Gas Chromatography-Mass Spectrometry (en français, chromatographie en phase gazeuse couplée à la spectrométrie de masse)

ITS : Internal Transcribed Spacer (en français, espaceur interne transcrit)

JAZ : Jasmonate Zim domain

JGI : Joint Genome Institute

MAMP : Microbe Associated Molecular Pattern (en français, patron moléculaire associé aux micro-organismes)

MiSSP : Mycorrhiza Small Secreted Protein (en français, petite protéine sécrétée induite par la mycorhize)

MTI : MAMP Trigered Immunity (en français, immunité déclenchée par les MAMPs)

NMDS : Non-metric Multidimensional Scaling (en français, positionnement multidimensionnel)

PGPR : Plant Growth Promoting Rhizobacteria (en français, rhizobactérie favorisant la croissance des plantes)

PRR : Pattern Recognition Receptor (en français, récepteur de reconnaissance de motifs moléculaires)

SIP : Stable Isotope Probing (en français, marquage à l'aide d'isotope stable)

OTU : Operational Taxonomic Unit (en français, unite taxonomique opérationnelle)

WT : Wild Type (en français, sauvage)

Glossaire

Communauté : ensemble de micro-organismes de différentes espèces vivant dans un même habitat. Chaque espèce contribue pour sa part aux fonctions de la communauté.

Composition : identité des membres de la communauté de micro-organismes détectés dans un échantillon.

Endophyte : (du grec *endo*, « dans », et *phyte*, « végétal ») micro-organisme qui accomplit tout ou partie de son cycle de vie à l'intérieur d'une plante hôte.

Endosphère : ensemble des tissus internes d'une plante.

Habitat : endroit où toutes les conditions sont réunies pour qu'une population d'individus puisse vivre et se développer normalement.

Holobionte : (du grec *holo*, « tout », et *bios*, « vie ») ensemble composé de l'organisme végétal hôte et des communautés de micro-organismes avec lesquelles il interagit.

Indice de Shannon : indice donnant une idée de la diversité spécifique d'un milieu c'est-à-dire du nombre de taxons microbiens détectés dans ce milieu (diversité spécifique) et de la répartition des individus au sein de ces taxons (équitabilité spécifique).

Microbiome : somme des génomes des micro-organismes vivant dans ou sur un hôte.

Microbiote : ensemble des micro-organismes vivant dans un écosystème donné.

Niche : notion permettant d'expliquer la co-existence d'espèces différentes dans un même habitat.

Progénie : ensemble des arbres issus de graines spécifiquement récoltées sur des arbres mères caractérisés génétiquement sur la base d'analyses de polymorphisme de nucléotide unique (SNP en anglais, Single Nucleotide Polymorphism) ou de microsatellites.

Rhizosphere : zone du sol directement sous l'influence des racines, très riche en micro-organismes et en substances biologiques.

Rhizoplan : surface des racines d'une plante.

Richesse : nombre de taxons microbiens détecté dans un échantillon.

Sol nu : zone du sol exempt de racines.

Structure : identité et abondance relative individuelle des membres de la communauté de micro-organismes détectés dans un échantillon

Chapitre I

Synthèse bibliographique

Les forêts tempérées couvrent 31 % du territoire métropolitain français et ont un rôle écologique, économique et sociétal important. Il est maintenant clairement démontré que les racines des arbres sont massivement colonisées par des communautés complexes et très dynamiques de micro-organismes provenant du sol environnant. Certains micro-organismes peuvent avoir un rôle bénéfique pour l'arbre en contribuant fortement à la nutrition et à la santé de leur hôte tandis que d'autres peuvent être des pathogènes et donc être néfastes pour l'hôte. L'ensemble de ces micro-organismes constitue le microbiote racinaire de l'arbre.

L'arbre hôte et son microbiote forment une seule entité appelée « holobionte » (du grec *holos*, « tout », et *bios*, « vie »). Ce méta-organisme est en interaction permanente avec les autres arbres, les plantes des sous-bois, les champignons, les bactéries et d'autres micro-organimes au sein de l'écosystème complexe que forme la forêt.

I. La rhizosphère, l'endosphère et leurs microbiotes

1. Définition et caractéristiques

La rhizosphère a été définie pour la première fois par Hiltner en 1904 comme étant le volume de sol directement sous l'influence des racines d'une plante (Hiltner, 1904 ; Hartmann et al., 2008). La racine peut être divisée en deux sous-compartiments distincts : le rhizoplan qui correspond à la surface externe et l'endosphère définit comme l'ensemble des tissus internes de la racine (Figure 1). Ce sont des zones très favorables à la prolifération et à l'activité de nombreux micro-organismes tels que les bactéries, champignons, archées, oomycètes, virus, protistes, algues et nématodes (Mendes et al., 2013).

L'« effet rhizosphère » est dû à la libération d'exsudats racinaires dans le sol, sous forme d'une multitude de composés tels que des acides organiques, des sucres, des acides aminés, des protéines, des acides gras, des composés de défense tels que les flavonoïdes et des facteurs de croissance telles que les hormones (Badri & Vivanco, 2009 ; Turner et al., 2013). On trouve également dans cette zone une accumulation de cellules végétales mortes et de mucilage participant à la nutrition des micro-organismes (Phillipot et al., 2013 ; Figure 2). La présence de ces molécules en fait donc une zone riche en composés carbonés et donc potentiellement de nutriments, augmentant ainsi la densité microbienne de l'ordre de 10 à 1000 fois par rapport au sol non en contact avec les racines (Ricon-florez et al., 2015). La colonisation de la rhizosphère par diverses communautés de micro-organismes (Figure 1) permet la mise en place d'importantes fonctions de défense contre des pathogènes et d'apports de nutriments bénéfiques à la plante-hôte (Adam et al., 2016).

L'émergence de racines latérales peut être à l'origine de l'apparition de disjonctions cellulaires à la surface de la racine principale formant alors des points d'entrée pour les micro-organismes de la rhizosphère vers l'intérieur de la racine. Les études de la composition microbienne de la rhizosphère, du rhizoplan et de l'endosphère de plusieurs espèces végétales indiquent que ces différents habitats écologiques contiennent des micro-organismes du sol avec des structures phylogénétiques distinctes (Bulgarelli et al., 2012 ; Lundberg et al., 2012).

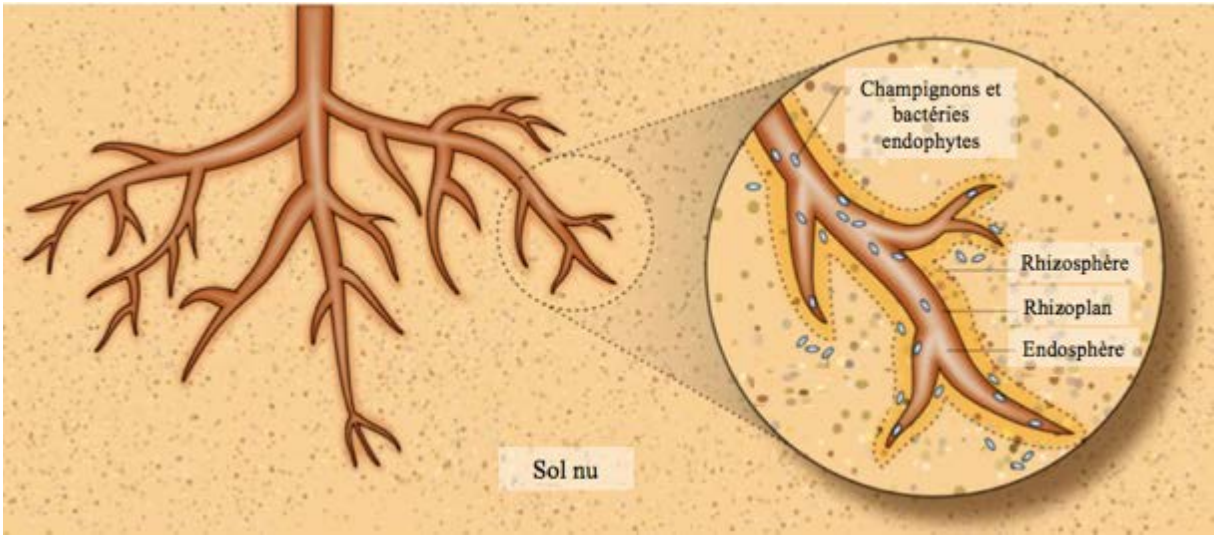


Figure 1 – Les différentes niches à l'interface racine-sol (d'après Mendes et al., 2013).
Différentes parties existent au sein d'une racine : l'endosphère correspond à la partie interne des racines des plantes et abritent champignons et bactéries endophytes tandis que le rhizoplan correspond à la surface externe des racines des plantes.

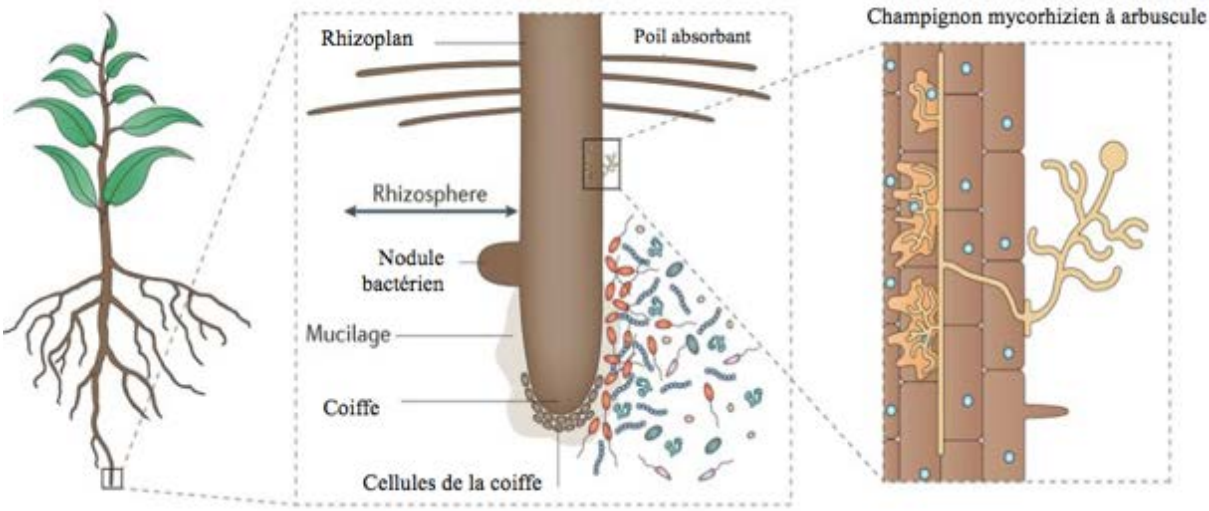


Figure 2 - La rhizosphère et les micro-organismes associés (Phillippot et al., 2013).

2. Des niches spécifiques

L'enrichissement des communautés de micro-organismes spécifiques de la rhizosphère et de l'endosphère a été décrit pour diverses plantes dont la plante herbacée modèle, *Arabidopsis thaliana* (Sperber & Rovira, 1959 ; Newman, 1985 ; Sørensen, 1997 ; Selosse et Le Tacon, 1998 ; Germida et al., 1998 ; Grayston et al., 1998 ; Bulgarelli et al., 2013 ; Lundberg et al., 2012).
L'ensemble des communautés rhizosphériques correspond à un assemblage de micro-organismes issus du sol adaptés aux conditions physico-chimiques associées à la rhizosphère et capables de consommer des métabolites produits par la plante via l'exsudation racinaire (Bulgarelli et al., 2013 ; Lundberg et al., 2012). Concernant les communautés endosphériques, il s'agit de l'ensemble des micro-organismes sous le contrôle de la plante hôte (Bulgarelli et al., 2012).

La composition et la structure du microbiote des plantes sont régulées par différents facteurs biotiques et abiotiques. Par exemble, le type de sol est un facteur déterminant dans le contrôle de la biomasse et de l'activité microbienne (Cao et al., 2016 ; Li et al., 2018). L'affinité de la plante ainsi que les interactions entre les micro-organismes ont également un impact majeur dans l'établissement du microbiote racinaire (Lou et al., 2014 ; Hassani et al., 2018).
Les récentes avancées technologiques en matière de séquençage nous permettent désormais d'étudier en profondeur la diversité microbienne et fongique associée aux plantes et au sol ainsi que de connaître l'influence de l'espèce hôte et de l'origine du sol sur les associations fongiques et bactériennes en interaction avec les racines (Bonito et al., 2014).

3. Régulation du microbiote racinaire : les facteurs biotiques

En écologie, les facteurs biotiques correspondent à l'ensemble des interactions du vivant sur le vivant dans un écosystème. Le génotype ou l'espèce de la plante hôte (appelé « effet hôte ») ont un rôle important dans la structuration et la composition taxonomique et fonctionnelle des communautés de micro-organismes de la rhizosphère et de l'endosphère. Plus précisément, il a été mis en évidence que les traits phénotypiques, la quantité et la qualité des exsudats racinaires ainsi que les systèmes de défense et de protection des plantes sont des facteurs clés de régulation du microbiote racinaire (Hu et al., 2018 ; Haichar et al., 2008 ; Doornbos et al., 2012 ; Turner et al., 2013).

3. A. Les exsudats racinaires et les métabolites

Les plantes sont des organismes autotrophes c'est-à-dire des organismes vivants qui n'ont pas besoin d'une source de carbone organique pour se développer. Elles ont la capacité de fabriquer leur propre carbone grâce à la photosynthèse. La photosynthèse est l'ensemble des réactions permettant aux végétaux (algues et plantes) mais également à certaines bactéries de convertir l'énergie lumineuse en énergie chimique utilisable pour la

synthèse de la matière organique. Durant ce processus, les glucides ($C_6H_{12}O_6$) sont obtenus suite à l'assimilation du dioxyde de carbone (CO_2) grâce à l'ensemble des réactions produisant le pouvoir réducteur (NADPH) et une source d'énergie (ATP) issue de la photolyse de l'eau couplée à un dégagement d'oxygène (Ziska & Brunce, 2006). Ces éléments (lumière, CO_2, eau et nutriments) sont indispensables à la croissance des plantes. Les échanges gazeux sont assurés par des organes particuliers situés sur la face supérieure des feuilles : les stomates. Ces derniers régulent leur ouverture et fermeture en fonction de la température, de la concentration en CO_2, du taux d'humidité et de la lumière qui joue également un rôle déterminant dans la croissance végétale et la réalisation de la photosynthèse.

La production primaire de sucre issu de la photosynthèse est le fructose-6-phosphate transformé par la suite en saccharose pour le transport et en amidon pour le stockage (Heller et al., 1993). Une partie de ces réserves sera utilisée pour la production de molécules plus complexes du métabolisme primaire (acides aminés, lipides, acides organiques…) et de composés associés à la formation des parois cellulaires (cellulose, lignines…) permettant à la plante de constituer sa biomasse aérienne et racinaire. Une autre fraction de ces sucres de réserve sera stockée dans les parties racinaires (Heller et al., 1993). On estime que 5 à 21 % du carbone assimilé par la photosynthèse n'est pas directement utilisé par la plante mais est achéminé dans les racines pour être directement libéré dans le sol, au niveau de la rhizosphère et, donc, transféré aux micro-organismes associés sous forme de sucres solubles, acides aminés ou de métabolites secondaires (Badri & Vivanco, 2009 ; Badri et al., 2013 ; Chaparro et al., 2012).

Les exsudats racinaires des plantes peuvent être divisés en deux classes : les composés de faible poids moléculaire comme les acides aminés, les acides organiques, les sucres, les composés phénoliques et les métabolites secondaires et les composés de haut poids moléculaire tels que les polysaccharides et les protéines (Bais et al., 2006 ; Badri & Vivanco, 2009). Les plantes sont capables de modifier la composition et la structure du microbiote du sol en contact avec les racines en sécrétant des molécules actives dans la rhizosphère. Le stade de croissance et de développement de la plante a un impact sur la quantité et la nature des composés carbonés libérés par la plante (Badri & Vivanco, 2009). La quantité et la qualité en termes de composition des exsudats racinaires dépend également de l'espèce végétale (Uren, 2000 ; Badri & Vivanco, 2009) qui influence la composition et la structure des communautés de micro-organismes du sol suggérant un certain degré de spécifité pour chaque espèce végétale. De plus, certains exsudats racinaires (e.g. acides organiques) sont également susceptibles de modifier le pH et les gradients redox influençant ainsi la composition et la structure des communautés microbiennes (Schmidt et al., 2011).

Les élements sécrétés par les racines de la plante sont à l'origine de diverses interactions plantes-micro-organismes. Ces interactions peuvent être neutres, bénéfiques ou délétères pour les partenaires. L'ensemble de ces micro-organismes consomme différents types de composés sécrétés par les racines. Ils consomment très rapidement les métabolites primaires ce qui rend pratiquement impossible la collecte d'exsudats racinaires purs dans le sol (Oburger & Jones, 2018). Ces micro-organismes peuvent également affecter le processus

d'exsudation. Dans la rhizosphère des plantes herbacées comme la luzerne ou le blé, certains micro-organismes libèrent des composés secondaires qui stimulent l'exsudation d'acides aminés par les racines (Phillipps et al., 2004) alors que d'autres sont capables de modifier le métabolisme de la plante entière (Fernandez et al., 2012). Bien que beaucoup de recherches concernant l'impact des exsudats racinaires sur les communautés de micro-organismes du sol ont été menées sur les plantes de grandes cultures, relativement peu de travaux ont été menés dans la rhizosphère des arbres (Uroz et al 2016). L'allocation du carbone étant très différente entre les plantes annuelles et les plantes à long cycle de vie comme les arbres, les processus de régulation des composés exsudés via les racines devraient être différents. Chez le tremble, la concentration en composés carbonés non structuraux c'est-à-dire le carbone stocké dans les parties pérennes de l'arbre influence les taux d'exsudation du carbone organique et ainsi les communautés de micro-organismes de la rhizosphère (Karst et al., 2017). Les plantes utilisent les composés sécrétés dans le sol pour attirer les communautés de micro-organismes bénéfiques mais également pour se défendre contre les micro-organismes pathogènes grâce à la production des métabolites secondaires comme les phytohormones de défense.

3. B. Le système immunitaire des plantes

Les plantes possèdent des barrières naturelles physiques et chimiques préformées dont l'une des fonctions principales est d'empêcher la pénétration de tissus par des micro-organismes pathogènes. Il s'agit de la cuticule et l'épiderme. Le pathogène doit adhérer sur ces barrières et les rompre pour pouvoir pénétrer à l'intérieur des tissus de la plante. En plus de ces barrières, la plante est capable de produire des molécules toxiques pour les micro-organismes pathogènes tels que des métabolites secondaires (acides phénoliques, flavonoïdes, hormones de défense). Les phytohormones comme l'éthylène, l'acide salicylique et l'acide jasmonique sont généralement impliquées dans la régulation de la résistance des plantes faces aux pathogènes et aux insectes ravageurs (Pusztahelyi et al., 2015). Enfin, le microbiote commensal des plantes défini comme l'ensemble des micro-organismes résidant à la fois dans la rhizosphère et sur la plante et ayant une relation mutualiste avec cette dernière constitue également une barrière. Le mécanisme le plus important de ces micro-organismes qui ne nuisent pas à la plante, lorsqu'elle est en bonne santé, est l'effet de compétition qui empêche l'établissement de micro-organismes pathogènes (Berendsen et al., 2012).

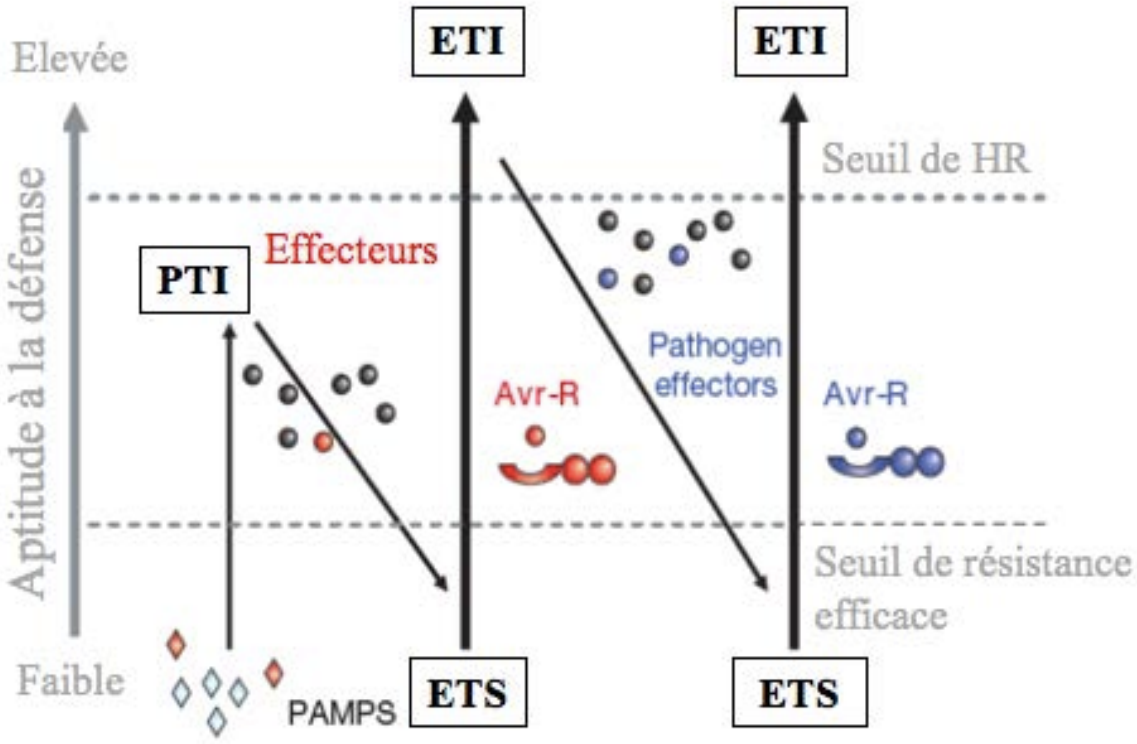


Figure 3 – Représentation schématique de l'immunité déclenchée par les Patrons Moléculaires Associés aux Micro-organismes (MAMPS, MTI, d'après Jones & Dangl, 2006). Durant la première phase, le système immunitaire des plantes détecte les PAMP microbiens grâce aux récepteurs PRR qui mettent en place la résistance basale (PTI, PAMP-Triggered Immunity). Durant la seconde phase, les agents pathogènes libèrent des effecteurs pour contrer la résistance basale et permettre l'installation du pathogène (ETS, Effector-Triggered Susceptibility). Durant la troisième phase, un des effecteurs (en rouge) est reconnu par la protéine R qui déclenche la résistance spécifique (ETI, Effector-Triggered Immunity). Enfin, durant la dernière phase, les agents pathogènes ayant perdu l'effecteur rouge et éventuellement gagné d'autres effecteurs (en bleu) sont sélectionnés. Chez la plante, la sélection va favoriser de nouveaux allèles de gènes R, capables de reconnaître ces nouveaux effecteurs, résultant en une résistance spécifique (d'après Jones & Dangl, 2006).

L'ensemble de ces barrières est efficace contre l'invasion de micro-organismes pathogènes mais une partie de ces derniers est capable de contrer cette première ligne de défense et d'initier la colonisation de la plante hôte. Les plantes peuvent alors répondre via des mécanismes de défense induits. Elles possèdent des systèmes de surveillance et de détections leur permettant de reconnaitre des molécules de surface des micro-organismes qu'ils soient bénéfiques ou, au contraire, pathogènes. Ces molécules de défense constituent les « Patrons Moléculaires Associés aux Micro-organismes » (Microbes-Associated Molecular Patterns, acronyme de MAMPs en anglais, Newman et al., 2013). Ces MAMPs correspondent majoritairement à la flagelline, aux liposaccharides ou encore à la chitine des champignons ou aux glucanes des oomycètes et sont reconnus par la plante via des récepteurs de reconnaissances des MAMPs (Pattern Recognition Receptor ou PRR en anglais). Cette première étape intervenant dans les stades précoces de l'infection est appelée « immunité induite par les MAMPS » (MAMP Triggered Immunity, MTI). En 2006, les chercheurs Jones et Dangl ont proposé à la communauté scientifique un modèle général des mécanismes moléculaires impliqués dans l'immunité chez les plantes (Jones et Dangl, 2006, Figure 3).

Ce modèle appelé « zig-zag » est constitué de plusieurs étapes consécutives et intègre un aspect de coévolution entre la plante hôte et les micro-organismes. Il se divise en deux niveaux de reconnaissance. Le premier niveau correspond à la reconnaissance des MAMPs qui permet le déclenchement de la MTI. Le deuxième niveau correspond à la reconnaissance de protéines microbiennes appelées « effecteurs » qui jouent le rôle de suppresseurs de la MTI. Ce niveau d'immunité est appelé « Immunité médiée par la détection d'effecteur (Effector-triggered immunity ou ETI en anglais, Jones et Dangl, 2006, Figure 3). Enfin, le modèle intègre la notion de coévolution entre les deux protagonistes de l'interaction avec la phase ETS. A cette étape, un nouvel effecteur est sécrété par le micro-organisme et s'en suit une nouvelle phase ETI dans le cas où ce nouvel effecteur est reconnu par une protéine de résistance de la plante hôte.
Les micro-organismes commensaux sont également pourvus de flagelline ou de chitine pouvant être reconnues par la plante hôte. Plusieurs études illustrent comment ce type de micro-organismes a évolué pour échapper directement à la reconnaisaance des récepteurs PRR de la plante hôte par la modification ou l'inhibition des MAMPs ou encore, par l'altération de leur paroi cellulaire (Félix et al., 1999 ; Wawra et al., 2016 ; Hacquard et al., 2017). Ces découvertes suggèrent alors un rôle de gestion plus complexe de la colonisation microbienne par le système immunitaire de la plante capable de contrôler l'accomodation des micro-organismes commensaux ou bénéfiques et l'élimination des pathogènes.

3. C. La régulation hormonale des défenses chez les plantes

Les phytohormones correspondent à des molécules endogènes qui régulent des processus essentiels tels que la croissance, le développement et la défense des plantes face aux stress biotiques et abiotiques. Certaines sont également synthétisées lors de la réponse immunitaire de la plante (Pieterse et al., 2009) lors d'interactions de la

plante avec des agents pathogènes. Il s'agit principalement de l'éthylène (ET), l'acide salicylique (AS) et l'acide jasmonique (AJ).
Bien que les voies de biosynthèse et de signalisation ainsi que le rôle de chacune de ces trois phytohormones dans la mise en place des réactions de défense de la plante soient très étudiées, les connections existant entre ces différentes voies de signalisation forment un réseau très complexe dont la compréhension reste à améliorer.

La voie de signalisation de l'acide jasmonique

L'acide jasmonique est un composé hormonal dérivant de l'acide α-linolénique. Il existe de nombreux dérivés actifs de l'AJ nommés jasmonates. Parmi ces derniers, l'AJ-Isoleucine (AJ-Ile) joue le rôle de régulateur central chez les plantes. Sa synthèse et son accumulation interviennent en réponse à des signaux développementaux et environnementaux. L'AJ-Ile joue un rôle important durant leur réponse immunitaire face aux attaques de pathogènes nécrotrophes et d'insectes herbivores (Turner et al., 2002).
La voie de signalisation de l'AJ est activée grâce à sa conjugaison avec l'isoleucine qui permet la production de la forme active de l'hormone, l'AJ-Ile. Chez *Arabidopsis thaliana*, l'AJ-Ile est perçu par COI1 (CORONATINE INSENSITIVE 1) qui forme un complexe avec des protéines de la famille JAZ (JASMONATE ZIM-DOMAIN-CONTAINING). Ces dernières sont des régulateurs négatifs de la voie de signalisation de l'AJ dont la dégradation entraîne l'activation transcriptionnelle des gènes impliqués dans la défense de la plante (Chini et al., 2007 ; Thines et al., 2007 ; Pauwels & Goossens, 2011). En l'absence de l'AJ, les répresseurs JAZ interagissent avec les protéines MYC. L'activation de la branche MYC lors de l'élimination de JAZ entraine l'expression d'un grand nombre de gènes sensible à l'AJ (Li et al., 2019 ; Figure 4).

Des travaux réalisés sur la plante modèle *Arabidopsis thaliana* ont permis de démontrer que l'activation de la voie de signalisation de l'AJ influence la composition des communautés bactériennes de la rhizosphère (Carvalhais et al., 2015 ; Doornbos et al., 2011). Il a été démontré que la mutation du facteur de transcription myc2 impliqué dans la voie de signalisation de l'hormone est responsable de l'altération de la composition des exsudats racinaires notamment en termes de production d'acides aminés entraînant alors des changements de la composition des communautés bactériennes associées aux racines. Plus précisement, la mutation a entraîné un changement de l'abondance relative de certains taxas bactériens connus pour être abondants dans la rhizosphère comme *Streptomyces* et *Bacillus* (Carvalhais et al., 2015). De plus, la mutation du gène jar1 impliqué dans la voie de biosynthèse de l'AJ est responsable de la diminution de la densité des communautés bactériennes telles que *Pseudomonas* dans la rhizosphère (Doornbos et al., 2011). Chez le blé (*Triticum aestivum*), il a été démontré que l'activation de la voie de signalisation de l'AJ entraîne une diminution de la diversité des communautés bactériennes de l'endosphère mais également des changements dans leur composition (Liu et al., 2017).

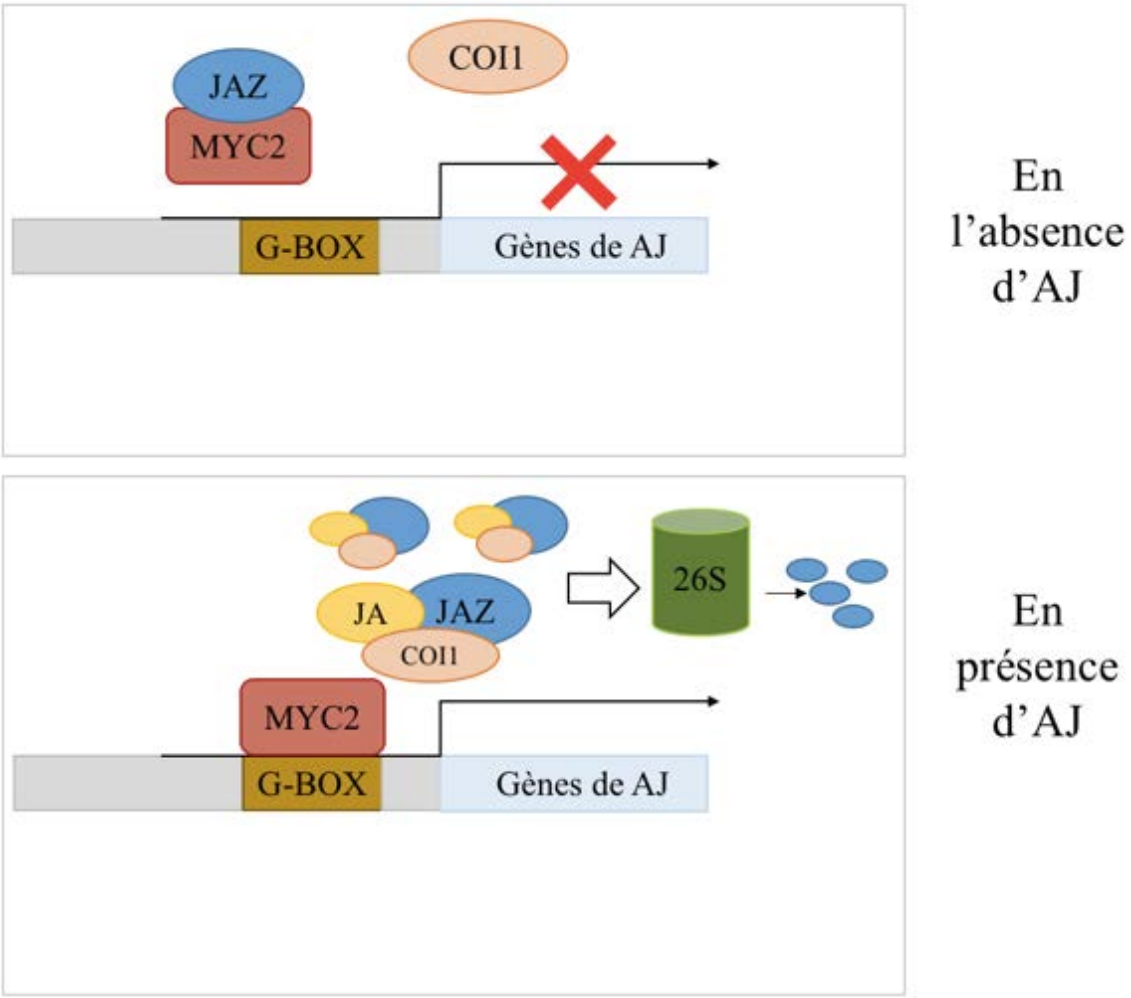


Figure 4 – Représentation schématique de la cascade de régulation conduisant à l'expression des gènes de défense induits par l'acide jasmonique.

Par contre, l'AJ ne semble avoir aucun impact sur la structuration des communautés de bactéries et d'archées au niveau de l'interface sol-racines (le rhizoplan) chez le blé (Liu et al., 2017). Cependant, certaines communautés endophytes connues pour supprimer les phytopathogènes bactériens, fongiques et viraux deviendraient plus abondantes en réponse à l'AJ (Liu et al., 2017).

La voie de signalisation de l'acide salicylique

L'acide salicylique (AS) est un petit composé phénolique intervenant durant les réponses immunitaires de la plante (Seyfferth, Tsuda, 2014). L'induction de l'expression des gènes de défense liée à la production d'AS est complexe mais il semble évident que le régulateur protéique NPR1 (Nonexpressor of Phatogenesis-Related Genes 1) y joue un rôle clé. Les plants mutants *npr1* d'*Arabidopsis thaliana*, ne sont plus capables de percevoir l'AS et sont donc plus sensibles à l'attaque de pathogènes biotrophes que les plants non transformés (Cao et al., 1997).

Un modèle existe à l'heure actuelle concernant l'activation de la voie de signalisation de l'AS dans les cellules végetales (Li et al., 2019). En cas d'attaque de pathogènes, la biosynthèse de l'AS augmente considérablement dans les plastes via la voie de ICS/PAL (IsoChorismate Synthase/Phenylalanine Ammonia Lyase). L'enzyme SA methyl transferase (ou SAMT) catalise la conversion de l'AS en methyl-AS (MeSA) qui se diffuse dans le cytoplasme où il est converti à nouveau en AS grâce à l'activité de SABP2. L'augmentation de la concentration en AS dans le cytoplasme est alors responsable du passage de NPR1 de la forme oligomère à monomère et de sa migration vers le noyau. Dans ce dernier, le monomère de NPR1 active la transcription des gènes de défense induit par l'AS tels que les gènes PR (pour Pathogenesis Related, Durant & Dong, 2004 ; Pieterse & Van Loon, 2004) en interagissant avec les facteurs de transcription de la famille TGA (TGACG motif-binding factor) (Figure 5). Ces facteurs TGA activés se lient au promoteur de certains gènes codant les protéines PR et induisent leur transcription (Durrant & Dong, 2004). Durant son interaction avec les facteurs de transcription, NPR1 est phosphorylé puis dégradé par le protéasome.

Cette phytohormone peut avoir un effet positif ou négatif sur les micro-organismes du sol en interaction avec les racines. Il peut promouvoir la croissance de certains groupes de bactéries ou au contraire l'inhiber chez *A. thaliana* (Lebeis et al., 2015). En effet, le microbiote racinaire des plantes altérées dans la voie de signalisation de l'AS présente des différences dans l'abondance relative de groupes bactériens spécifiques par rapport au microbiote des plantes non transformées génétiquement. L'altération de la voie de signalisation de l'AS inhiberait la colonisation racinaire par les Firmicutes et, au contraire, améliorerait la colonisation par les Protéobactéries. Ces deux phyla appartiennent notamment au microbiome central bactérien (« core microbiome » en anglais). Chez *A. thaliana*, la mutation du gène cpr1 impliqué dans la voie de signalisation de l'AS se traduit par un niveau élevé d'AS entraînant ainsi l'expression constitutive des voies de défense dépendantes de l'AS. Une diminution du nombre total de bactéries et particulièrement des bactéries *Pseudomonas* a été observée sur les racines de ces mutants (Doornbos et al., 2011). Ces résultats suggèrent que l'AS est un facteur clé de la régulation du microbiote racinaire (Lebeis et al., 2015).

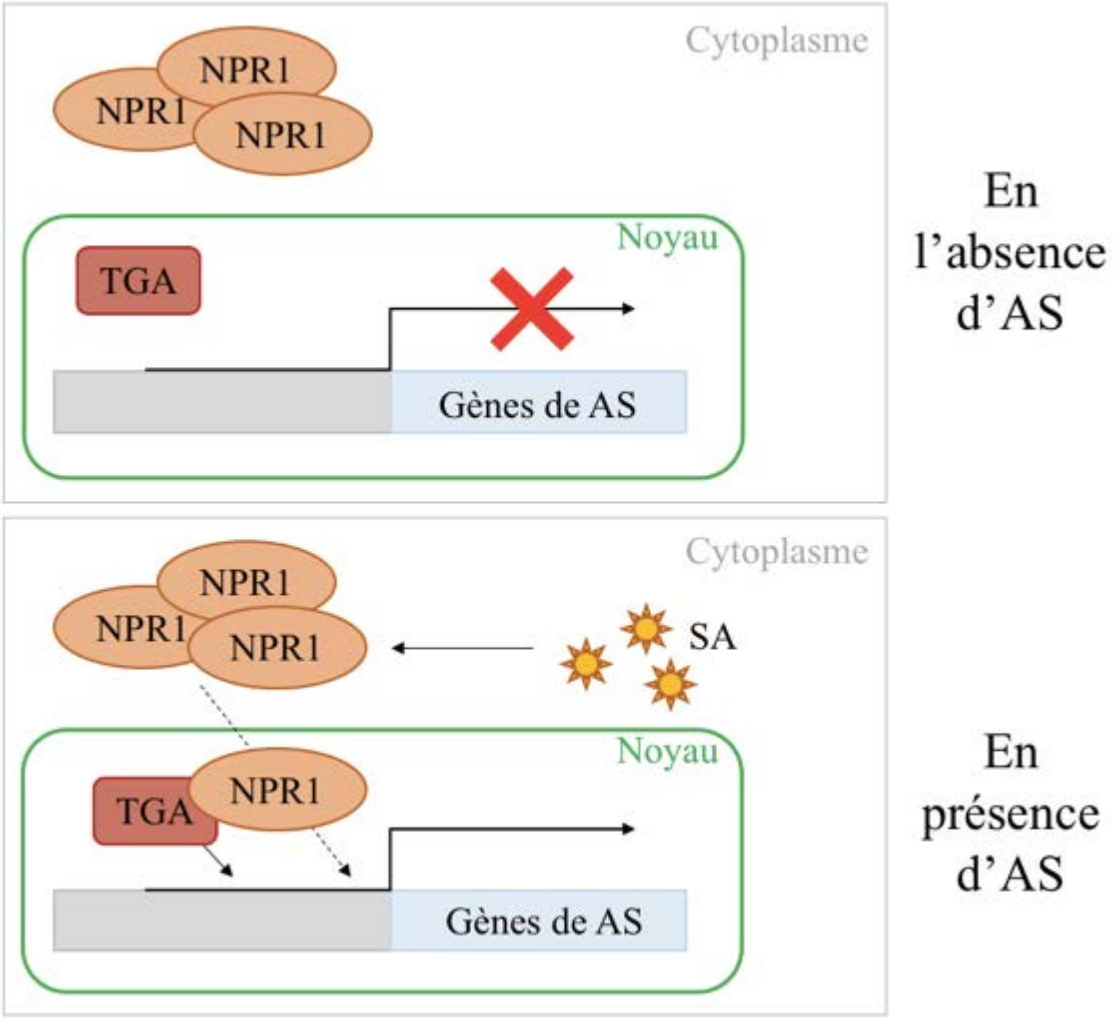


Figure 5 - Représentation schématique de la cascade de régulation conduisant à l'expression des gènes de défense induits par l'acide salicylique.

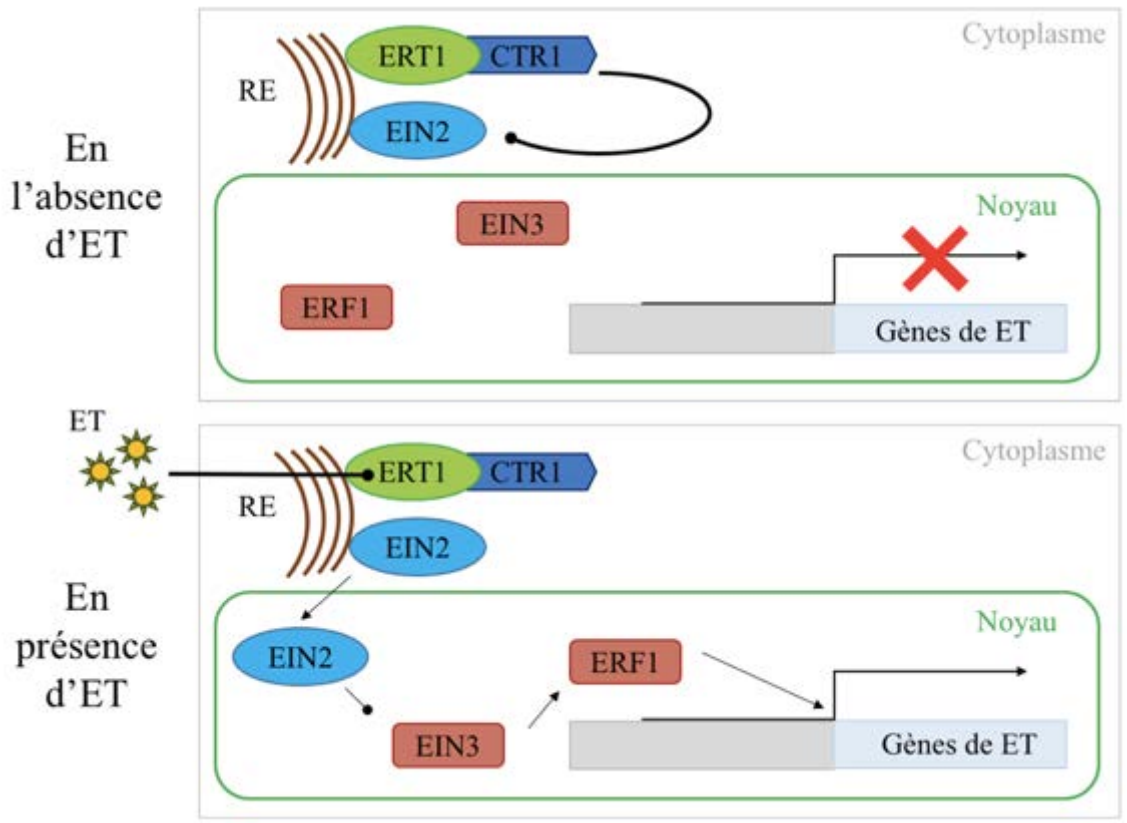


Figure 6 - Représentation schématique de la cascade de régulation conduisant à l'expression des gènes de défense induits par l'éthylène.

La voie de signalisation de l'éthylène

L'ET est un métabolite volatile essentiel à la croissance et au développement des plantes. Il est synthétisé de façon endogène par la quasi-totalité des plantes mais également lors de stress biotiques et abiotiques. L'ET est perçu par des récepteurs membranaires tels que ETR1 (ETHYLENE RESPONSE 1), ERS1 (ETHYLENE RESPONSE SENSOR 1), ETR2 (ETHYLENE RESPONSE 2), ERS2 (ETHYLENE RESPONSE SENSOR 2) et EIN4 (ETHYLENE INSENSITIVE 4 ; Hua et al., 1995 ; Ecker, 1995 ; Bleecker et al., 1988). Ces récepteurs correspondent à des régulateurs négatifs de la voie de signalisation de l'ET (Figure 6). Lorsque le niveau d'ET est faible, ils maintiennent la régulation négative d'une sérine à kinase CTR1 (Constitutive Triple Response 1) sur un régulateur positif localisé dans le réticulum endoplamique EIN2 (ETHYLENE INTENSIVE 2) (Ju & Chang, 2015 ; Lee & Yoon, 2017 ; Bisson et al., 2009).

En absence d'ET, CTR1 intéragit avec EIN2 (Clark et al., 1998). Suite à la perception d'ET, la répression de CTR1 sur EIN2 est levée entraînant alors l'activation des facteurs de transcription comme EIN3 (Chao et al., 1997 ; Ju & Chang, 2015). La présence d'ET et plus précisément EIN2 est à l'origine de l'inhibition de la dégradation des facteurs de transcription EIN3 par le protéasome. Ces facteurs de transcription activent ensuite d'autres facteurs de transcription tels que ERF1 conduisant ainsi à l'expression de gènes inductibles par l'ET (Figure 6).

En condition de salinité élevée, sécheresse, pollution aux métaux lourds ou encore contact avec des phytopathogènes, le niveau endogène d'ET augmente significativement chez les plantes et a un effet négatif sur la croissance (Ahemad, Kibret, 2014).

Les conséquences de l'augmentation de l'ET sur les plantes pourraient être évitées grâce à l'utilisation de rhizobactéries à activité 1-aminocyclopropane-1-carboxylate (ACC) désaminase qui dégrade l'ET. En effet, ces bactéries seraient à l'origine de la réduction des stress abiotiques en contrebalançant l'ET produit par la plante responsable de l'inhibition de la division cellulaire, de la synthèse d'ADN et de la croissance (Gaiero et al., 2013 ; Ahemad, Kibret, 2014).

L'ET et l'ACC jouent un rôle important dans les interactions entre la plante hôte et les communautés bactériennes ayant colonisé l'intérieur des racines (Nascimento et al., 2018). Par exemple, il a été démontré par Iniguez et al., (2005) que l'ET inhibe la colonisation racinaire des bactéries endophytes fixatrices d'azote du genre *Klebsiella*. Dans le cas contraire c'est-à-dire en ajoutant au milieu de culture un inhibiteur de la voie de perception de l'ET (1-methylcyclopropane), la colonisation racinaire de *Klebsiella* augmente. Une autre étude portant sur l'effet de l'hormone sur la bactérie *Agrobacterium tumefaciens* a mis en évidence que l'expression des gènes de virulence de la bactérie est négativement affectée par l'ajout d'ET exogène entraînant ainsi la diminution de l'efficacité de prolifération de la bactérie et donc une réduction de sa pathogénicité (Nonaka et al., 2008).

A l'échelle du microbiote, il a été démontré que la mutation du gène ein2, au centre de la régulation de la voie de signalisation de l'ET, chez *A. thaliana* entraînait une diminution de la densité des populations bactériennes de la

rhizosphère telles que *Pseudomonas* par rapport aux plantes non modifiées génétiquement (Doornbos et al., 2011).

Aucune étude n'est aujourd'hui publiée concernant l'impact de l'ensemble de ces trois voies de signalisation sur le microbiote racinaire des plantes. L'ensemble des travaux de recherche évoqués dans cette partie de l'introduction ont été réalisés à partir de mutations génétiques affectant les voies de signalisation hormonale. Les hormones de défense étant toutes impliquées également dans la croissance et le développement de la plante, il semble être complexe de créer une plante mutante déficiente dans les trois voies de signalisation ou de synthèse de l'AJ, l'AS et l'ET.

4. Régulation du microbiote racinaire : les facteurs abiotiques

En écologie, les facteurs abiotiques correspondent à l'ensemble des actions du non-vivant sur le vivant d'un écosystème. Les paramètres physico-chimiques de la matrice sol ainsi que les variations environnementales et météorologiques constituent les facteurs abiotiques majeurs de régulation du microbiote racinaire des plantes.

4. A. Le sol

Le sol forme un écosystème dynamique et complexe. Du fait de ses multiples fonctions, il n'en existe pas de définition universelle. Pour les agronomes, le sol est un milieu de croissance pour les plantes. Pour les géologues, il s'agit d'une phase courte dans le cycle géologique alors que pour les pédologues, le sol est le résultat de l'interaction entre le temps, les organismes vivants et morts, le climat et la topographie (Certini & Ugolini, 2013 ; Bockheim et al., 2014).

De grandes différences existent entre les caractéristiques des sols forestiers et celles des sols agricoles. Contrairement aux sols agricoles, les sols forestiers sont généralement peu ou pas amendés et ne sont que très faiblement travaillés. Les sols forestiers sont en moyenne bien plus acides, plus pauvres en éléments nutritifs mais plus riches en matière organique que les sols agricoles (Badeau et al., 1999).
Le sol offre de nombreux habitats pour les micro-organismes grâce à la diversité de sa structure et sa contenance en éléments nutritifs essentiels à la vie (Baldrian et al., 2017). Leur activité est notamment alimentée par la décomposition de la matière organique et par le carbone assimilé par la photosynthèse des plantes qui pénètre dans le sol sous forme d'exsudats racinaires et grâce au mycelium des champignons mycorhiziens (Clemmensen et al., 2013). La présence d'une grande quantité de mycéliums de champignons ectomycorhiziens est d'ailleurs une des caractéristiques des forêts tempérées et boréales. Cette quantité peut atteindre jusqu'à un tiers de la biomasse microbienne totale du sol (Högberg, 2002). Le sol apparaît donc comme un réservoir important de micro-organismes (Fierer, 2017) potentiellement capables de coloniser les racines des plantes. En effet, dans le sol forestier, on dénombre de 10^7 à 10^9 cellules bactériennes par gramme de sol, de 0,1 à 0,6

tonnes par hectare de biomasse fongique, de 0,2 à 0,7 milligrammes de mycélium fongique par gramme de sol et, enfin, la production de 0,2 tonne de carpophores par hectare et par année (Baldrian et al., 2017).

Chez les plantes de cultures et chez les arbres, le type de sol est un facteur important de structuration et de composition des communautés de micro-organismes du sol et de la rhizophère (Berg & Smalla, 2009 ; Schreiter et al., 2014 ; Edwards et al., 2015 ; Bonito et al., 2014 ; Bonito et al., 2019). Chaque type de sol possède son propre cortège microbien. Cette spécificité est notamment due au pH qui peut varier considérablement d'un sol à un autre (Fierer & Jackson, 2006). Des études menées sur les arbres ont démontré que la composition et la diversité des communautés bactériennes semblent être largement influencées par le pH (Hartmann et al., 2008 ; Gottel et al., 2011 ; Shakya et al., 2013).

Si le pH est un facteur clé de la structuration et de la composition du microbiote du sol mais selon le type sol, d'autres paramètres sont également à prendre en considération car ils peuvent également agir sur la structure des communautés de micro-organismes. C'est le cas, par exemple, de (i) la disponiblité en nutriments et le contenu en carbone et en azote qui favorisent la prolifération de bactéries copiotrophes appartenant aux phyla des Bacteroidetes et Beta-Protéobactéries et des champignons de l'ordre des *Agaricales* (Fierer et al., 2007 ; Lauber et al., 2008), (ii) l'humidité des sols profitant aux bactéries des phyla Cyanobactéries, Verrucomicrobia et Chloroflexi ainsi qu'aux Ascomycètes (Meisner et al., 2018) et (iii) la salinité, responsables de l'augmentation de l'abondance relative des bactéries halophyles ou halotolérantes telles que les Protéobactéries, Actinobactéries et Bacteroidetes (Zhang et al., 2019).

4. B. Le climat et les variations saisonnières

Les micro-organismes du sol jouent un rôle essentiel dans le cycle du carbone et des nutriments dans les écosystèmes. Leurs activités sont régulées par différents facteurs abiotiques comme la quantité de litière apportée, la température et l'humidité.

Les changements atmosphériques comme la concentration en CO_2 et les variations météorologiques et saisonnières comme la température, le taux d'humidité et les niveaux de précipitations ont un impact à la fois sur les propriétés physico-chimiques du sol et sur la physiologie des plantes. De plus, ils peuvent modifier de façon directe ou indirecte les communautés de micro-organismes du sol (Compant et al., 2010).

L'augmentation de la concentration atmosphérique en CO_2 semble stimuler la physiologie des arbres et modifier la quantité et la qualité des exsudats racinaires (Pritchard, 2011). Il n'est donc pas étonnant que la composition et la structure des communautés de micro-organismes associées aux racines des plantes soient affectées par ces changements. Il a notamment été rapporté que des quantités élevées de CO_2 (jusqu'à trois fois supérieures aux quantités de CO_2 ambient moyennes actuelles) modifient la structure et l'abondance des communautés de micro-organismes rhizosphériques des prairies et des sols agricoles (Hayden et al., 2012 ; Schortemeyer et al., 1996 ; Compant et al., 2010). L'altération des précipitations a un effet significatif sur les communautés actives des écosystèmes forestiers en favorisant certaines espèces microbiennes mieux adaptées aux environnements secs

ou humides telles que les champignons de la famille des *Cortinariaceae* et les bactéries de l'ordre des *Bacillales* (Richard et al., 2011 ; Felsmann et al., 2015). Enfin la hausse des températures, responsable de l'assèchement des sols et des périodes de sécheresse, affecte significativement les communautés bactériennes du sol directement en sélectionnant les communautés résistantes à la dessication ou indirectement en modifiant le métabolisme de la plante et/ou en altérant les propriétés du sol (Naylor & Coleman-Derr, 2018). Les réseaux bactériens et fongiques du sol ont des propriétés différentes et réagissent différemment à la sécheresse, cette dernière ayant un impact beaucoup plus prononcé sur les réseaux bactériens que sur les réseaux fongiques (De Vries et al., 2018).

Dans les écosystèmes forestiers, les variations saisonnières sont à l'origine de la variation de la disponiblité en nutriments ainsi que de la variation des températures et de l'humidité du sol. Ces fluctuations sont responsables de l'altération des processus microbiens du sol impliqués dans la dégradation de la matière organique. En effet, les changements temporels de l'activité enzymatique des communautés microbiennes du sol suggèrent un changement du substrat principal à décomposer entre l'été et l'automne (Kaiser et al., 2010, 2011). L'abattage des arbres empêchant ainsi l'augmentation de l'allocation du carbone dans le sol, comme c'est naturellement le cas en automne, affecte fortement ces processus microbiens (Ekberg et al., 2007). Toutefois, il a également été démontré que les altérations saisonnières et expérimentales de la disponibilité des ressources et des facteurs abiotiques induisent des changements dans la composition des communautés microbiennes du sol (Kaiser et al., 2010 ; Fierer et al., 2012) ainsi que des différences fonctionelles entre les communautés microbiennes (Koranda et al., 2013).

5. Impact du microbiote sur la plante & ingénierie écologique

La plante modifie son environnement grâce à l'apport de la litière et de ses racines, au prélèvement de nutriments de la rhizosphère et grâce à la production des exsudats racinaires. La plante, par ce biais, est donc capable de réguler directement et indirectement les communautés de son microbiote racinaire et de sélectionner celles qui sont bénéfiques à sa croissance et sa santé (Mendes et al., 2013). Il est aujourd'hui établi que les micro-organismes jouent un rôle essentiel dans les cycles biogéochimiques terrestres et le fonctionnement des écosystèmes. Le microbiote est connu pour promouvoir la croissance et la résistance des plantes hôtes face aux facteurs biotiques et abiotiques. Plusieurs études ont fait le parallèle entre le microbiote intestinal des Hommes et des animaux et le microbiote racinaire des plantes (Berendson et al., 2012 ; Hacquard, 2015). Ces deux types de microbiotes possèdent les mêmes fonctions tels que l'acquisition de nutriments, la protection de l'hôte contre les micro-organismes pathogènes et la modulation de l'immunité de l'hôte (Berendson et al., 2012). Le microbiote constitue un véritable réservoir génétique pour la plante hôte. La théorie d'un « super-organisme » formé par l'hôte et son microbiote encourage les travaux de recherche actuels à adapter une vision plus holistique où l'hôte et son microbiote ne forment plus qu'une seule entité (Vandenkoornhuyse et al., 2015 ; Hacquard & Schadt, 2015).

Ainsi, le microbiote contribue au phénotype étendu de la plante. Du fait de sa très grande plasticité, de sa dynamique très rapide et de la présence d'un ensemble de gènes bien supérieur au génome des plantes, on pourrait se demander si le microbiome pourrait aider les plantes hôtes à s'adapter à des changements environnementaux et à répondre aux stress biotiques et abiotiques. Accroître nos connaissances sur le microbiote des arbres nous permettrait donc potentiellement de l'exploiter pour améliorer la croissance et la survie des plantes. Cette idée a notamment été vérifiée en démontrant que des manipulations sur le microbiote de l'arabette et du chou entraînent la modification de la date de floraison de ces végétaux (Panke-Buisse et al., 2015).

Enfin, des manipulations réalisées sur le microbiote de plusieurs plantes de grandes cultures ont pu réduire l'incidence de maladies, augmenter la production agricole et réduire l'utilisation d'intrants chimiques (Turner et al., 2013).

Concernant les arbres forestiers, de telles manipulations seraient difficiles à entreprendre à l'échelle d'une forêt entière. Cependant, des manipulations du microbiote racinaire ont été réalisées sur des arbres cultivés pour la production de biomasse tels que le saule, le peuplier, le frêne, le hêtre ou encore l'eucalyptus (Mercado-Blanco et al., 2018). Ces travaux ont montré que l'établissement d'intéractions bénéfiques entre l'arbre et les communautés fongiques favorise l'adaptation des arbres dans des conditions pédologiques peu favorables. De plus, des effets positifs ont été également observés sur la croissance des arbres fruitiers quelques années après leur plantation (Mercado-Blanco et al., 2018).

II. Interactions racines-champignons

Les deux types de mycorhizes les plus fréquents dans les écosystèmes tempérés sont les ectomycorhizes (EcM) et les endomycorhizes (AM). Ils se distinguent par le partenaire fongique mais également par les relations physio-anatomiques entre les deux partenaires (Smith & Read, 2008 ; Tableau 1). Quel que soit le type de symbiose mycorhizienne, cellules végétales et fongiques sont étroitement liées au cours de la formation de l'organe symbiotique. Selon le type de mycorhize, les hyphes fongiques peuvent traverser la paroi végétale et coloniser les cellules racinaires de la plante (AM) ou non (ECM).

	Ectomycorhizes		Endomycorhizes				
	EcM	Ectendomycorrhize	AM	ERM	Mycorhize arbutoïdes	Mycorhize monotropoïdes	Mycorhize à orchidée
Anotomie des hyphes	Septés	Septés	Aseptés	Septés	Septés	Septés	Septés
Colonisation fongique intracellulaire	-	+	+	+	+	+	+
Manteau	+	+ / -	-	-	+ / -	+	-
Réseau de Hartig	+	-	-	-	+	+	-
Arbuscules ou vésicules	-	-	+	-	-	-	-
Pelotons	-	-	-	+	-	-	+
Taxon fongique impliqué	Basidiomycètes Ascomycètes (Glomeromycètes)	Basidiomycètes Ascomycètes	Glomeromycetes	Ascomycètes	Basidiomycètes	Basidiomycètes	Basidiomycètes
Taxon végétal impliqué	Gymnospermes Angiospermes	Gymnospermes Angiospermes	Bryophytes Pteridophytes Gymnospermes Angiospermes	Ericales Bryophytes	Ericales	Monotropacées	Orchidées

Tableau 1 - Les principaux types de symbioses mycorhiziennes (d'après Smith & Read, 2008)

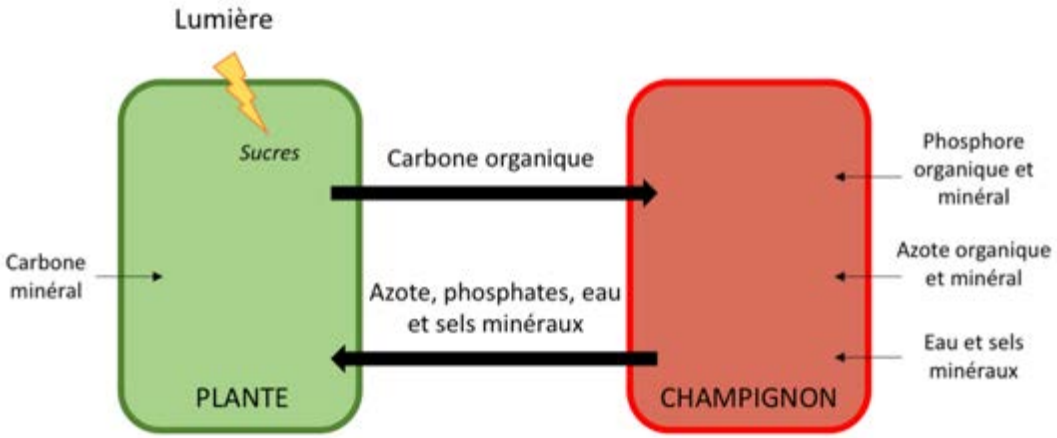


Figure 7 - Echanges de composés nutritifs entre les deux partenaires durant la symbiose ectomycorhizienne.

1. Les symbioses mycorhiziennes

Décrites pour la première fois en 1885 puis en 1887 par les botanistes Albert Bernhard Frank et Anton de Bary, les symbioses mycorhiziennes sont des associations mutualistes complexes entre les champignons du sol et les racines des plantes. Il s'agit de l'association symbiotique la plus commune et la plus répandue décrite à ce jour. Elle permet un échange mutualiste de nutriments entre la plante et le champignon favorisant leurs santés et leurs croissances. La plante transfère des composés carbonés issus de la photosynthèse à son partenaire fongique tandis que ce dernier améliore de manière très significative les prélèvements en eau et en nutriments du sol de la plante mais également l'acquisition de phosphore et d'azote (Harley & Smith, 1983 ; Figure 7).

1. A. La symbiose ectomycorhizienne

La symbiose ectomycorhizienne (EcM) est caractérisée par la formation de mycorhizes externes. Elle concerne en majorité les arbres des forêts tempérées et boréales et, dans une moindre mesure, tropicales appartenant à la famille des Fagacées, des Pinacées, des Salicacées et des Bétulacées et des champignons appartenant majoritairement aux Basidiomycètes mais aussi aux Ascomycètes (Tableau 1). Les hyphes du champignon ectomycorhizien forment un manteau autour des racines et pénètrent entre les cellules du cortex racinaire pour former le réseau de Hartig, siège des échanges entre les deux partenaires symbiotiques. Un réseau d'hyphes extramatriciels se développe également en parallèle assurant ainsi le lien entre le sol et le manteau de l'ectomycorhize (Figure 8).

A noter également, l'existence des ectendomycorhizes caractérisées par leur capacité à former un manteau autour de la racine et une colonisation intracellulaire comme celle des endomycorhizes (Yu et al., 2001, Tableau 1, Figure 8).

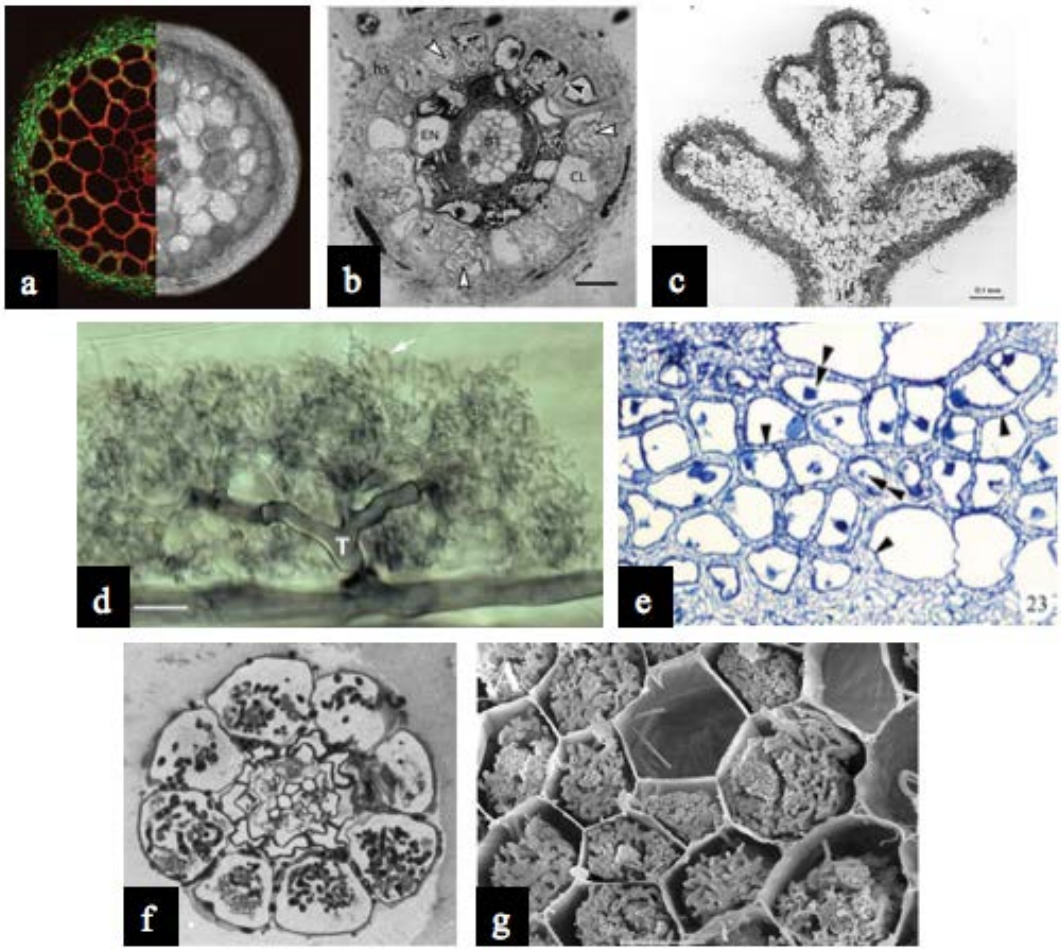


Figure 8 - Caractéristiques structurales des différents types de symbiose mycorhizienne

a – Ectomycorhize : Coupe transversale d'une racine de Populus trichocarpa colonisée par Laccaria bicolor au microscope confocal. Les hyphes du champignon (en vert) se développent entre les cellules épidermiques et corticales (en rouge) pour former le réseau de Hartig (Martin et al., 2016).
b – Ectendomycorhize : Coupe transversale d'une mycorhize de Cavendishia nobilis var. capitata avec gaine hyphale (hs), hyphes intercellulaires (flèche noire) et hyphes intracellulaires (flèche blanche). La racine présente une stèle (ST), un endoderme (EN) et une couche cellulaire corticale (CL). Barre, 20 µm (Setaro et al., 2005).
c – Mycorhize arbutoïde : Observation au microscope électronique à balayage d'une mycorhize d'Arbutus menziesii-Pisolithus tinctorius (Massicotte et al., 1993).
d – Mycorrhizes à arbuscule : Observation au microscope optique d'arbuscules matures de Glomus mosseae dans une cellule corticale racinaire de Allium porrum L. (Brundrett et al., 1984).
e – Mycorhize monotropoïde : Observation au microscope optique d'une section paradermique de mycorhize de Monotropa uniflora. La pénétration du réseau de Hartig est exclusivement épidermique (Massicotte et al., 2005)
f – Mycorhize ericoïde : Observation au microscope optique de la coupe transversale d'une racine de Calluna vulgaris colonisée par un champignon mycorhizien éricoïde (Perotto & Bonfante, 2012).
g – Mycorhize à orchidée : Observation des pelotons de Vanilla planifolia au microscope électronique à balayage (Gonzalez-Chavez et al., 2017).

1. B. La symbiose endomycorhizienne

Les endomycorhizes sont la forme la plus répandue. Contrairement aux champignons ectomycorrhiziens, les champignons endomycorrhiziens pénètrent à l'intérieur des cellules racinaires et forment différents types d'endomycorhizes Tableau 1, Figure 8) :

- les endomycorhizes à arbuscules (AM) sont formées par des champignons appartenant aux Gloméromycètes et une importante variété de végétaux incluant les Bryophytes, les Angiospermes et les Gymnospermes. Ce type d'interaction concerne essentiellement les plantes herbacées, les arbres tropicaux et quelques dizaines d'essences des forêts tempérées et boréales appartenant par exemple aux genres *Fraxinus*, *Fagus, Populus* ou *Acer* (Hiesalu et al., 2014 ; Liu et al., 2015 ; Lang et al., 2015). Il s'agit de l'association mycorhizienne la plus ancienne puisqu'elle semble être apparue il y environ 450 millions d'années juste après la colonisation de la surface terrestre par les plantes, suggérant un rôle important de la mycorhization dans la survie et l'adaptation des plantes (Corradi et Bonfante, 2012 ; Strullu-Derrien et al., 2018).

- les mycorhizes éricoïdes (ERM) sont formées par des Ascomycètes des genres *Hymenocyphus* et *Oidiodendron*. Les hyphes de ces derniers se développent uniquement au niveau des cellules corticales des Ericacées dépourvus d'épiderme et forment des pelotons à l'intérieur des cellules racinaires.

- les mycorhizes arbutoïdes et les mycorhizes monotropoïdes sont formées par des Basidiomycètes. Les plantes impliquées dans ce genre de symbioses appartiennent aussi bien aux Ptéridophytes qu'aux Angiospermes.

- les mycorhizes à orchidées sont formés par des champignons appartenant au genre *Rhizoctonia*. Ce dernier type d'endomycorhize apparaît durant les premiers stades de développement des orchidées.

2. Les champignons endophytes

Le terme « endophyte » a été introduit pour la première fois en 1866 par Anton De Bary. L'endophytisme des champignons est une interaction biologique caractérisée par le fait d'effectuer, pour un individu, tout ou partie de son cycle de vie à l'intérieur d'une plante, sans provoquer aucun symptôme. Omniprésents dans le règne végétal, ces champignons endophytes sont majoritairement affiliés au phylum des Ascomycètes (Rodriguez et al., 2008). Ils prélèvent des nutriments à la plante hôte sans lui être délétère et peuvent croître de façon intracellulaire ou extracellulaire. Deux modes de transmission ont été observés chez les champignons endophytes : la transmission verticale et horizontale. Ces deux mécanismes sont très importants pour le champignon car ils lui permettent de coloniser une autre plante à partir de la plante hôte initiale. La transmission verticale correspond à la colonisation d'un nouvel hôte végétal descendant de l'hôte initial. Elle consiste à la pénétration de l'hyphe fongique dans une graine, un grain de pollen (Hodgson et al., 2014 ; Gibert et al., 2015) ou à un explant de l'hôte initial. Par conséquent, le champignon endophyte reste génétiquement identique mais peut coloniser un hôte génétiquement différent issu d'une ou plusieurs nouvelles générations ou coloniser un clone. La transmission horizontale correspond au mode de transmission le plus répandue chez les endophytes.

Elle se caractérise par la colonisation d'une nouvelle plante hôte grâce à la dissémination des spores fongiques ou par l'intermédiaire d'hyphes provenant du sol ou d'une autre plante. Généralement, après germination de ces spores, les hyphes pénètrent le nouvel hôte au travers de l'épiderme. Certains endophytes ne colonisent spécifiquement qu'une partie de la plante hôte. C'est le cas des Dark Septate Endophytes (DSE) qui colonisent uniquement les racines de la plante hôte. Ces endophytes se distinguent par l'anatomie de leurs hyphes : septés et mélanisés. Principalement présents dans le cortex racinaire, ils forment des micro-sclérotes (Jumpponen et al., 1998 ; Ban et al., 2012 ; Figure 9).

3. Les autres types de champignons de la rhizosphère

Outre les champignons mycorhiziens et endophytes, d'autres champignons sont fortement présents dans la rhizosphère. C'est le cas des champignons saprotrophes qui, comme les champignons ectomycorhiziens, sont responsables de la décomposition de la matière organique contenue dans la litière et les premiers horizons du sol (Lindahl & Tunlid, 2015). Les communautés fongiques saprotrophes minéralisent les produits de fractionnement issus de la décomposition assurée dans les premiers stades par le macrofaune et la mésofaune grâce à la production d'enzymes extracellulaires (Hättenschwiler et al., 2005 ; Pant et al., 2017). Les composés ainsi libérés sont disponibles pour les micro-organismes et les plantes.

On distingue également la présence de champignons pathogènes (Rey & Schornack, 2013). Bien que les plus fréquents colonisent les parties aériennes des arbres, certains champignons présents dans les sols infectent les racines ou bien s'introduisent dans les parties souterraines des plantes dans les systèmes vasculaires et colonisent petit à petit toute la plante en remontant les vaisseaux conducteurs provoquant des maladies telles que la pourriture vasculaire ou la pourriture des racines.

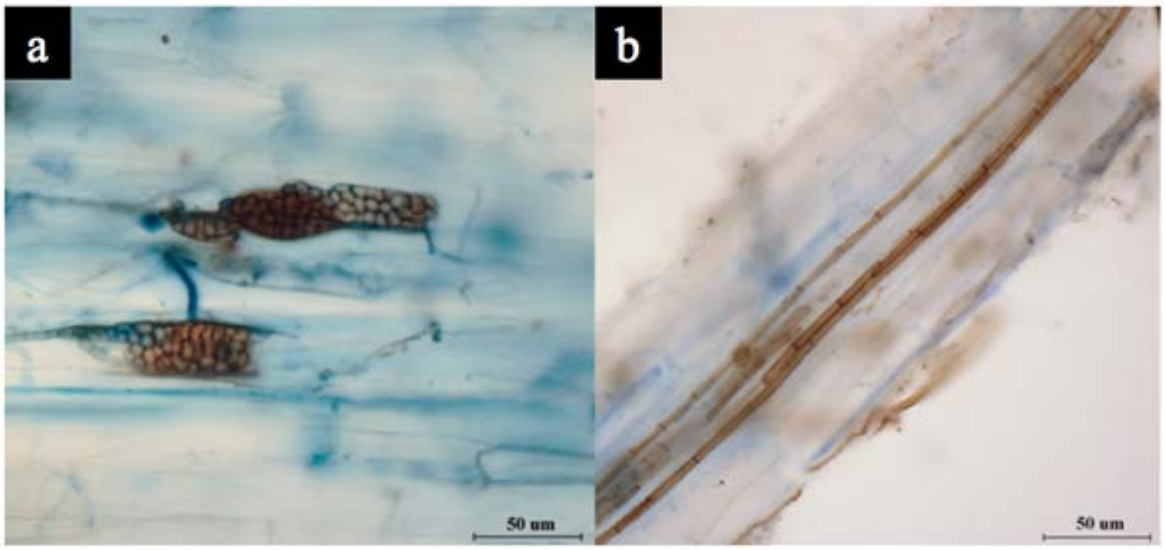


Figure 9 - Structures spécifiques des DSE dans les racines de la plante hôte. Micro-sclérotes formées par P. chrysanthemicola (a). Colonisation intracellulaire par un hyphe septé mélanisé formé par G. cylindrosporum (b) (Ban et al., 2012).

III. Les interactions racines-bactéries

Les bactéries jouent un rôle majeur dans les cycles biogéochimiques tels que les cycles du carbone et de l'azote. La décomposition de la matière organique est une des activités microbiennes les plus importantes dans les sols forestiers. La capacité de dégradation de composés organiques carbonés chez les bactéries (et les champignons) est bien connue. Elles sont capables de dégrader des molécules complexes telles que la cellulose, la pectine, la lignine, la lignocellulose ou encore la chitine (De Boer et al, 2005 ; Khatoon et al., 2017). Ces molécules peuvent être décomposées par des enzymes aspécifiques telles que les peroxydases (Tian et al., 2014). L'ensemble de ces activités de décomposition de la matière organique participe de façon intense à l'apport de carbone du sol et des plantes. Les bactéries libres fixatrices d'azote atmosphérique telles que les bactéries du genre *Azotobacter* et *Nostoc* contribuent à compenser une partie des pertes en azote, élément indispensable à la croissance des plantes, des sols résultant du lessivage et de la dénitrification. Cette fixation n'est possible que par les organismes procaryotes et constitue une des formes d'acquisition de l'azote les plus importantes chez les plantes terrestres bien qu'elle soit rare chez les arbres (Cleveland et al., 1999). Toutefois, de nombreuses plantes et en particulier les légumineuses, réalisent cette fixation d'azote, de manière indirecte, en symbiose avec des bactéries du genre *Frankia* et *Rhizobium* qui se localisent généralement dans des nodosités situées sur les racines (Brockwell et al., 1995).

Les bactéries jouent également un rôle indirect dans la nutrition des plantes grâce à leur capacité d'altération minérale permettant d'augmenter la concentration des éléments nutritifs essentiels au développement et à la croissance des plantes. Cette dissolution microbienne des minéraux est réalisée principalement via la production de protons, d'acides organiques ou de molécules chélatantes comme les sidérophores. Les genres bactériens *Collimonas*, *Bulkholderia* et *Bacillus* sont été décrits comme étant particulièrement efficaces pour altérer les minéraux du sol. Outre leur capacité à altérer les minéraux, les bactéries participent aussi activement à la dégradation de la matière très récalcitrante (Pérez et al. 2002 ; Bugg et al. 2011 ; Tian et al. 2016).

Enfin, certaines bactéries du sol vont également avoir une influence directe sur la production végétale de manière positive ou négative. Celles ayant un effet négatif sur la croissance végétale sont des bactéries pathogènes et parasites des plantes (Mendes et al., 2013). Certaines bactéries pathogènes sont capables de transformer génétiquement leur hôte végétal pour créer un habitat favorable. C'est le cas, par exemple, d'*Agrobacterium tumefaciens* provoquant la galle du collet (Escobar et Dandekar, 2003 ; Abarca-Grau et al., 2011). A l'inverse, d'autres bactéries sont connues pour leur capacité à stimuler la croissance des plantes. Il s'agit des bactéries promotrices de la croissance des plantes (ou PGPR, acronyme de Plant Growth Promoting Rhizobacteria) qui ont également la capacité d'interférer avec les voies de signalisation de la plante via différents mécanismes comme la phytostimulation qui correspond à l'amélioration directe de la croissance végétale grâce à la production de phytohormones (Gaeiro et al., 2013). Les PGPR produisent également des substances antibiotiques, des enzymes bactériolytiques et diverses toxines capables d'éliminer certaines bactéries compétitrices (Beneduzi et al., 2012 ; Backer et al., 2018). C'est notamment grâce à la production de ces

composés qu'elles sont utilisées comme agents de biocontrôle et de lutte biologique contre la colonisation des racines par des micro-organismes pathogènes.

Les micro-organismes endophytes bactériens correspondent aux individus résidant au moins une partie de leur vie à l'intérieur des tissus racinaires de la plante, l'endosphère. Ils présentent des caractéristiques métaboliques différentes par rapport aux micro-organismes de la rhizosphère suggérant que tous ces derniers ne peuvent pas entrer dans la plante (Turner et al., 2013). Les travaux de Timm et al., (2015) ont notamment démontré que les souches bactériennes *Pseudomonas fluorecens* isolées à partir de l'endosphère du peuplier étaient davantage pourvues d'éléments génomiques dédiés au métabolisme des composés d'origine végétale comme la synthèse ou la modification des hormones végétales ou le catabolisme des nucléosides et des acides sucrés, molécules complexes riches en carbone et très abondantes dans les racines. Comme les autres bactéries rhizosphèriques, les endophytes utilisent les ressources de la plante hôte comme source principale de nutriments mais elles ont, en retour, un effet neutre sur la plante hôte grâce à différents mécanismes. La distribution des endophytes dépend de leur capacité de colonisation mais également des ressources de la plante qui diffèrent d'un organe à l'autre.

Concernant les archées, peu d'informations sont disponibles par rapport aux communautés bactériennes. Bien que ces micro-organismes soient probablement très abondants dans la rhizosphère et l'endopshère de certaines plantes, leurs activités fonctionnelles et les liens qu'ils entretiennent avec leurs hôtes sont méconnus (Buée et al., 2009). Les archées jouent également un rôle clé dans les cycles globaux du carbone et de l'azote, par exemple dans la méthanogénèse, l'oxydation anaérobie du méthane et l'oxydation de l'ammoniac (Deveau et al., 2018). Une étude récente réalisée sur la végétation naturelle des tourbières alpines a révélé que ce type de micro-organismes interagit avec la plante. Cette interaction basée sur des fonctions telles que la promotion de la croissance de la plante par la biosynthèse d'auxines, l'apport de nutriments et la protection de la plante contre le stress abiotique ont été détectés par des approches de métagénomique (Taffner et al., 2018).

IV. Les interactions champignons-bactéries

Champignons et bactéries du sol et de la rhizosphère partagent le même habitat mais également souvent les mêmes ressources. Ces micro-organismes interagissent donc en permanence par l'intermédiaire de multiples types d'interactions bénéfiques ou, au contraire, délétères. Ces interactions peuvent être indirectes ; un micro-organisme peut avoir un effet sur un autre grâce à la production de composés modifiant les propriétés physico-chimiques du milieu (Raaijmakers & Mazzola, 2012 ; Hassani et al., 2018). En parallèle, ces interactions entre micro-organismes peuvent être directes. Champignons et bactéries peuvent interagir de façon mutualiste en collaborant pour acquérir une même ressource qu'ils ne pourraient pas acquérir individuellement. Une telle coopération a déjà été décrite dans les sols forestiers concernant la dissolution des minéraux (Uroz et al., 2007). Des interactions directes et délétères existent également entre bactéries et champignons du sol. Des

phénomènes de compétitions ont déjà été décrits dans les sols. Ce type d'interactions est très répandu entre les bactéries et les champignons et se produit quand les micro-organismes exploitant une même ressource présente dans le milieu en quantité limitée comme, par exemple, le carbone et l'azote (Frey-Klett, et al., 2011). Certains micro-organismes sont également capables de produire des composés ayant un effet délétère sur d'autres micro-organismes. Ce type d'interactions appelé antibiose est courant dans les sols et contribue fortement à la sélection des micro-organismes des différents compartiments. Par exemple, le champignon EcM *Amanita muscaria* entraîne la supression de la production d'antibiotiques par la souche bactérienne *Streptomyces* AcH505 par production d'acides organiques (Riedlinger et al., 2006).

Les hyphes des champignons fournissent des microhabitats pouvant être colonisés par des communautés bactériennes spécifiques (Frey-Klett et al., 2011). La diversité de ces communautés peut varier de quelques centaines à plusieurs centaines d'espèces bactériennes selon le champignon (Shulz-Bohm et al., 2016 ; Ghodsalavi et al., 2017). Alors que certaines bactéries, comme *Burkholderia* sont capables de coloniser un grand nombre d'espèces fongiques en raison de leur capacité à utiliser des métabolites dérivés de champignons et à surmonter les systèmes de défense fongiques, d'autres peuvent avoir une relation plus spécifique avec leurs hôtes fongiques (Stopnisek et al., 2016 ; Warmink et al., 2009).

Les bactéries qui vivent à l'intérieur des cellules fongiques sont appelées bactéries endofongiques ou endobactéries. Ce type de bactéries, dont les mieux étudiées appartiennent à la famille des *Burkholderiaceae*, a été signalé dans des champignons aux modes de vie très variés et d'origine taxonomique très diverse comme les champignons saprotrophes ou symbiotiques. Les endobactéries peuvent avoir des effets sur la biologie de l'hôte fongique, y compris sur certains aspects de sa reproduction, sa croissance et le métabolisme primaire et secondaire (Deveau et al., 2018).

Ainsi, de nombreux types d'interactions existent entre les bactéries et les champignons du sol et de la rhizosphère et ont probablement un rôle important dans la structuration du microbiote. Elles sont dynamiques et très dépendantes des conditions environnementales mais également de la physiologie des micro-organismes. Elles mettent en avant la complexité des études à réaliser pour comprendre comment le microbiote racinaire d'une plante se met en place et est régulé.

IV. Les modèles d'étude *Populus* et *Laccaria bicolor*

1. Le peuplier

Le peuplier est une appellation générique qui désigne un nombre important d'espèces d'arbres à feuilles caduques du genre *Populus* de la famille des Salicacées. Répartis dans l'hémisphère nord, des écosystèmes subtropicaux aux écosystèmes boréaux, les peupliers dominent les bords de rivières et les peuplements forestiers inondés tandis que les trembles correspondent aux feuillus boréaux les plus importants (Peterson & Peterson, 1992). D'après la classification de Eckenwalder (1966), les différentes espèces de peupliers se répartissent en six sections selon leurs caractéristiques phénotypiques et biologiques. Les sections *Aigeiros*, *Tacamahaca* et *Populus* regroupent le plus d'espèces (Figure 10).

En plus des peuplements naturels, les arbres du genre *Populus* sont cultivés dans des plantations en Amérique, en Europe et en Asie. En Europe, les 940 000 ha de plantations produisent actuellement environ 8 millions de m^3 de bois par an. Bien que le bois de peuplier soit aujourd'hui exploité par l'industrie pour produire des objets à faible valeur ajoutée (contreplaqué, pâte à bois, bois scié…), l'utilisation du peuplier comme source de combustible et de biomasse pour la production énergétique a suscité un intérêt croissant durant ces dernières années. C'est un arbre à croissance rapide avec une floraison possible au bout de quatre à cinq ans, permettant ainsi les croisements génétiques. Les espèces de peupliers *Populus deltoides*, *Populus nigra*, *Populus alba* et *Populus trichocarpa* ainsi que leurs hybrides sont les plus couramment utilisés en populiculture (Figure 10). L'intérêt du peuplier en phytoremédiation est également très étudié (Rugh et al., 1998 ; Pillipovic et al., 2006 ; Castro-Rodriguez et al., 2016 ; Foulon et al., 2016). Sur la base des travaux de recherche disponibles, l'utilisation de cultivars est déjà couramment appliquée à la phytoremédiation. L'utilisation du peuplier en phytoremédiation présente l'avantage de stocker du carbone et de produire du bois.

Le peuplier est le premier arbre dont le génome a été entièrement séquencé et mis à disposition de la communauté scientifique (Tuskan et al., 2006). En effet, c'est le génome de *Populus trichocarpa* qui a été séquencé en 2001 par le Joint Genome Institute (JGI, Département de l'énergie américain, Etats-Unis, http://www.jgi.doe.gov/). Il possède un génome diploïde de 485 Mb réparti sur 19 chromosomes (Tuskan et al., 2006). Certains clones de peupliers sont également facilement manipulables par génie génétique. Il peut être transformé génétiquement par agrotransformation et micro-propagé en conditions stériles (Fillatti et al., 1986 ; Parsons et al., 1986). En plus de ces qualités, le peuplier possède la particularité de développer des associations mutualistes avec des champignons mycorhiziens (EcM et AM), des champignons endophytes et des bactéries (Gehring et al., 2006 ; Karlinski et al., 2010, Gottel et al., 2011 ; Shakya et al., 2013, Bonito et al., 2014 ; Bonito et al., 2016 ; Cregger et al., 2018 ; Bonito et al., 2019).

Dans le cadre de cette thèse, deux génotypes de peupliers ont été étudiés : le peuplier gris (*Populus tremula x alba* clone 717-1B4) et le peuplier noir (*Populus nigra* L.).

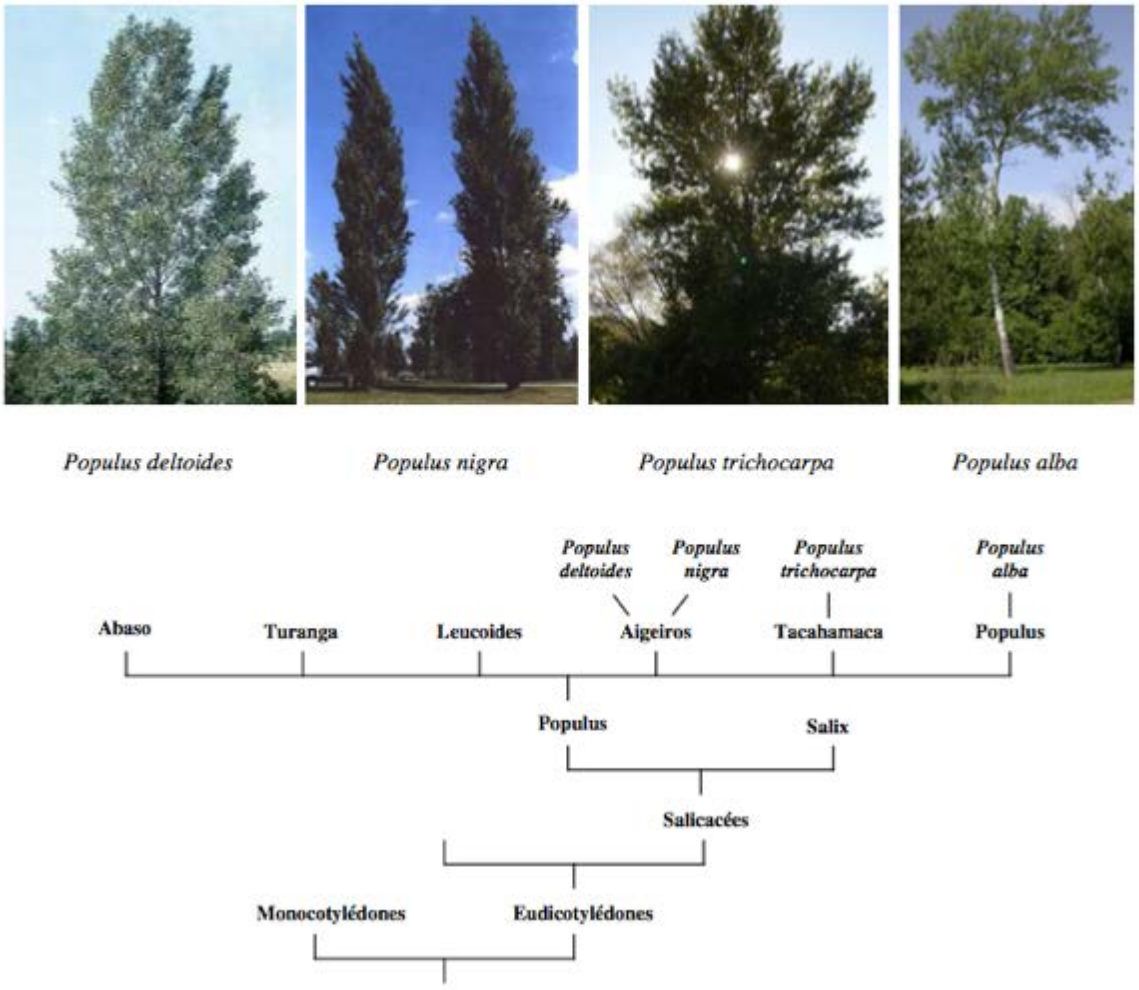


Figure 10 – Les différentes espèces de peuplier. Elles se répartissent en six sections selon leurs caractéristiques phénotypiques et biologiques. Les quatre espèces les plus connues sont indiquées au sein des populations auxquelles elles appartiennent (D'après Eckenwalder, 1996).

1. A. *Populus tremula x alba*

Populus tremula x alba est un hybride interspécifique femelle initialement crée par l'INRA en 1959 dans le but d'obtenir des lignées d'arbres à forte croissance, avec un bois de qualité et résistantes aux pathogènes. Le clone « INRA 717-1B4 » a été remarqué du fait de sa très forte infection par la bactérie *Agrobacterium tumefaciens*. Il s'agit d'un clone femelle, issu d'un croisement entre un individu femelle *Populus tremula* récolté en 1959 dans la forêt de Palroy (Meurthe-et-Moselle, France) et un individu mâle *Populus alba* récolté en 1960 près du Pont du Gard (Gard, France). Cet hybride faisait partie d'un programme de sélection initié par le docteur Michel Lemoine dans les années 1960 (Lemoine, 1973 ; Mader et al., 2016).

Le clone 717-1B4 possède l'avantage d'être facilement manipulable en génie génétique lors de transformation par la bactérie *Agrobacterium tumefaciens*, c'est pourquoi il est largement utilisé en laboratoire dans des expériences de transformations génétiques pour analyser l'expression et la fonction de gènes d'intérêt. La première transformation génétique de *Populus tremula x alba* a été réalisée en 1992 per Leple et al. De nombreuses lignées de peupliers transgéniques basées sur ce clone ont ensuite été testées notamment pour une application commerciale (Walter et al., 2010 ; Pilate et al., 2012). Dans le cadre des études physiologiques et génomiques, le nombre de publications scientifiques qui ont utilisé le clone INRA 717-1B4 comme modèle était de 245 publications en 2016 (Mader et al., 2016). Une ébauche du génome de *Populus tremula x alba* est aujourd'hui disponible grâce à la combinaison d'assemblages *de novo* et d'assemblage de référence (Mader et al., 2016).

1. B. *Populus nigra*

Le peuplier noir (*Populus nigra* L.) est une espèce suscitant de nouveaux intérêts. En effet, il s'agit d'une espèce colonisant un habitat particulier : la forêt alluviale à bois tendre qui représente une zone tampon entre l'espace terrestre largement occupé par l'agriculture et l'urbanisation et le milieu aquatique. Le peuplier noir couvre une aire naturelle très vaste, allant de l'Europe de l'Ouest à l'Ouest de la Chine en passant par le Nord de l'Afrique (Villar & Forestier, 2009).

Cette espèce pionnière, exigeante en lumière et en eau, est une espèce dioïque (sexes séparés) dont les graines peuvent être transportées par le vent et l'eau. Ces dernières possèdent une viabilité très courte de l'ordre de quelques jours. La régénération du peuplier noir dépend donc étroitement du fonctionnement du cours d'eau auprès duquel il est installé. En effet, les graines produites ne peuvent germer que sur des sédiments frais et sont soumises à la dynamique fluviale et aux phénomènes de crues et de décrues. L'espèce possède également un mode de reproduction végétative, par l'intermédiare de boutures qui peuvent être transportées par l'eau ou, et un mode de reproduction par clonage naturel par l'intermédiaire de drageons (Villar & Forestier, 2009).

Le peuplier noir présente de nombreux atouts écologiques ; il est notamment pourvu d'un important système racinaire, piégeur de sédiment, fixateur de berges et épurateur naturel de nutriments (Ruffinoni et al., 2003). Enfin, le peuplier noir apparait comme un élément clé dans l'attractivité et la qualité des paysages.

Cependant, les populations de peupliers noirs ont beaucoup souffert au cours des cinquante dernières années en raison de l'augmentation de l'impact de l'Homme sur les bassins et les corridors fluviaux qui ont fortement affecté la phase critique de recrutement des semis (Corenblit et al., 2014). Le peuplier noir est également menacé par le risque d'abâtardissement des peuplements naturels par des clones de peupliers cultivés dont le pollen peut féconder les fleurs des peupliers noirs femelles (Bastien et al., 2009). Deux types de cultivars présentent des menaces pour les populations sauvages : le peuplier d'Italie (*Populus nigra* var. *italica*) de sexe mâle et les hybrides de peupliers développés pour la production de bois tels que les clones *Populus deltoides* x *Populus nigra* ou *Populus deltoides* x *Populus trichocarpa* de sexe mâle et femelle.

Ces menaces ne portent pas sur la survie de l'espèce qui n'est globalement pas menacée de disparition en France. Néanmoins, la diminution de la qualité et de la diversité de ses ressources génétiques peut présenter un risque important dans ses capacités d'adaptation et de maintien à long terme (Villar & Forestier, 2009).

Dans ce contexte et au cours des deux dernières décennies, plusieurs projets scientifiques européens ont vu le jour afin de mieux comprendre la dynamique écologique et génétique actuelle du peuplier noir et de proposer des stratégies ciblées pour sa conservation et son maintien. C'est le cas, par exemple, du projet POPMICROCLIM (métaprogramme INRA) qui vise à étudier le rôle du microbiote racinaire dans la réponse adaptative du peuplier noir au changement climatique et auquel j'ai participé (http://www.accaf.inra.fr/Actions-et-Projets/Adaptation-et-biodiversite/POPMICROCLIM).

2. Le champignon ectomycorhizien *Laccaria bicolor*

Laccaria bicolor (ou Laccaire bicolore) est une espèce de champignon basiodiomycète de la famille des *Hydnangiacées* et de l'ordre des *Agaricales*. Il s'agit d'un champignon ectomycorhizien vivant en symbiose avec plusieurs essences d'arbres comme le pin, le boulot, le hêtre, et dans une moindre mesure, le peuplier dans les lisières, clairières et bords de chemins en forêts boréales et tempérées (Figure 11).
La souche utilisée couramment dans le laboratoire « Interactions Arbres-Micro-organismes » est la souche S238N. Cette souche a été isolée à partir d'un carpophore recueilli sous un pin Douglas *Tsuga mentenziana* par Molina et Trappe (Oregon, Etats-Unis) puis transféré au laboratoire de Microbiologie de Nancy en 1980. Plus tard, la souche S238N dicaryotique s'est révélée particulièrement efficace pour augmenter la croissance des semis de Douglas *Pseudotsuga menziesii* en pépinière forestière (Le Tacon & Bouchard, 1986). En effet, la croissance des arbres a été doublée par rapport aux plants spontanément mycorhizés par les champignons naturellement présents (Villeneuve et al., 1991).

En 1988, plusieurs monocaryons ont été obtenus à partir de la germination de spores isolées d'un carpophore issu d'une expérience de mycorhization controlée de *L. bicolor* S238N avec *Pseudotsuga menziesii* (Selosse et al., 2000). C'est l'une de ces souches monocaryotiques, S238N-H82 qui a été sélectionnée pour être séquencée. Le génome de *Laccaria bicolor* a été séquencé et publié par le JGI en 2006 (http://genome.jgi-psf.org/Lacbi2/Lacbi2.home.html). L'analyse à l'échelle génomique a été réalisée par le Consortium du Génome de *L. bicolor* (Martin et al., 2008). *L. bicolor* possède un génome de 65 Mb réparti en 12 chromosomes, riche en transposons et en éléments répétés (environ 20% du génome). Comme le peuplier pour l'ensemble des arbres, *L. bicolor* est le premier champignon symbiotique à avoir été séquencé. De plus, sa croissance plus rapide en culture pure en comparaison avec d'autres EcM, sa forte capacité à former des mycorhizes en conditions stériles et contrôlées (Figure 11), et sa transformabilité génétique font de cette espèce de champignon ectomycorhizien un excellent modèle d'étude.

3. Rôle de l'effecteur MiSSP7 dans la formation de la symbiose ectomycorhizienne

Les effecteurs correspondent à des petites protéines sécrétées par les micro-organismes pour modifier la structure et/ou la fonction de la cellule hôte (Kamoun, 2006). Ces effecteurs sont capables de contourner les réactions de défense de la PTI et permettent au micro-organisme pathogène qui les a produits d'infecter la plante hôte malgré les premières lignes de défense. Ils sont très étudiés dans le cadre des interactions plante-pathogènes mais sont également produits par les champignons mycorhiziens (Jones & Dangl, 2006 ; Dodds & Rathjen, 2010 ; Plett et al., 2011 ; Kloppholz et al., 2011).

Les effecteurs sont sécrétés par les micro-organismes pour aider ces derniers à coloniser une plante hôte de différentes manières. Ils permettent aux micro-organismes de pénétrer dans les tissus ou bien dans les cellules de la plante mais également de faciliter l'acquisition de nutriments provenant de la plante. De plus, les effecteurs peuvent contourner le système immunitaire de la plante hôte en empêchant la reconnaissance des MAMPs, en inactivant les phytoenzymes de défense ou encore en inactivant les voies de signalisation impliquées dans la mise en place des mécanismes de défense et l'expression des gènes de défense de la plante hôte.

Lors de la mise place de la symbiose ectomycorrhizienne entre le champignon *L. bicolor* S238N et le peuplier *P. trichocarpa*, l'effecteur fongique MiSSP7 (acronyme de Mycorrhizal Induced Small Secreted Protein 7 kDa) est sécrété par *L. bicolor* et pénètre dans le noyau des cellules racinaires où il se lie avec la protéine régulatrice JAZ6, bloquant ainsi l'induction de la voie de signalisation de l'AJ (Plett et al., 2011).

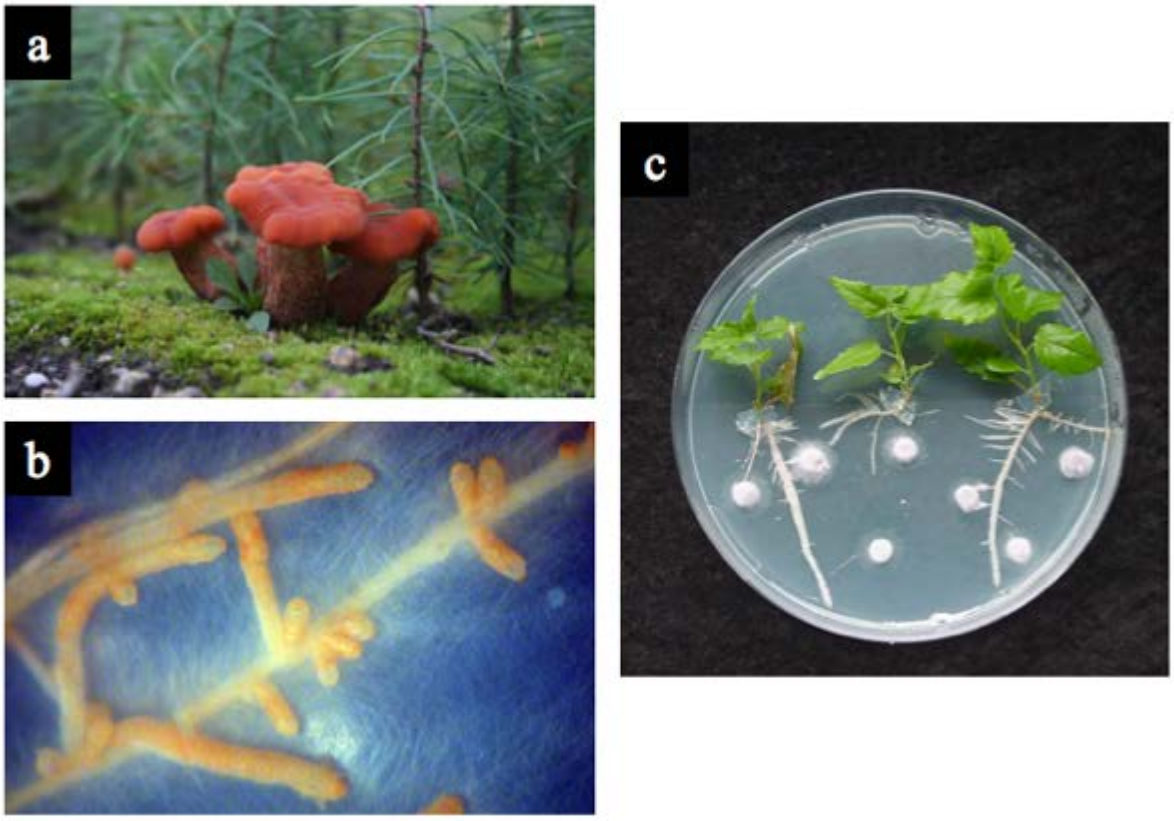


Figure 11 - Le champignon ectomycorhizien Laccaria bicolor (d'après Martin et al., 2016). Carpophores de L. bicolor (a). Ectomycorhizes de L. bicolor obtenues avec Populus tremula x alba (b). Développement in vitro de la symbiose ectomycorhizienne entre L. bicolor et P. tremula x alba par la méthode sandwich (c).

Selon le modèle actuel, MiSSP7 serait ainsi capable de moduler les mécanismes de défense dans les racines du peuplier, et notamment la voie de signalisation de l'AJ, ce qui permettrait la colonisation fongique de l'hôte et ainsi la mise en place de l'interaction. Bien que les gènes impliqués dans l'induction de la voie de signalisation de l'AJ ne soient pas encore totalement connus chez le peuplier, l'action de MiSSP7 dans le noyau des cellules racinaires de l'hôte est aujourd'hui établie.

L'effecteur MiSSP7 sécrété par le champignon *Laccaria bicolor* est capable de moduler la voie de signalisation de l'AJ dans les racines du peuplier. La protéine JAZ6 est une protéine végétale qui bloque l'expression des gènes défensifs induits par l'AJ permettant ainsi aux voies de défense de rester inactives dans les cellules des racines non colonisées. JAZ6 interagit avec des facteurs de transcription comme MYC2. Leur liaison avec JAZ6 empêche ces facteurs de transcription de se lier aux sites de reconnaissance de la boîte G située en amont des gènes répondant à l'AJ. Après la pénétration des hyphes fongiques, l'AJ est libérée et détectée par la protéine COI1 qui entraîne la formation du complexe JAZ6-COI1. COI1 est un composant du complexe d'ubiquitine ligase SCFCOI1. Le complexe est alors dégradé par le protéasome 26S. Par conséquent, MYC2 n'est plus inhibé par JAZ6 et peut jouer son rôle de facteur de transcription en activant la transcription des gènes de défense induits par l'AJ. Après la pénétration des hyphes fongiques dans l'espace apoplastique des racines du peuplier, *Laccaria bicolor* sécrète MiSSP7 qui supprime les mécanismes de défense liés à l'AJ en se liant à JAZ6. Cette liaison empêche sa reconnaissance avec AJ-COI1 et donc sa dégradation maintenant ainsi l'inhibition sur MYC2 et la répression des gènes sensibles à l'AJ (Plett et al., 2014 ; Martin et al., 2016, Figure 12).

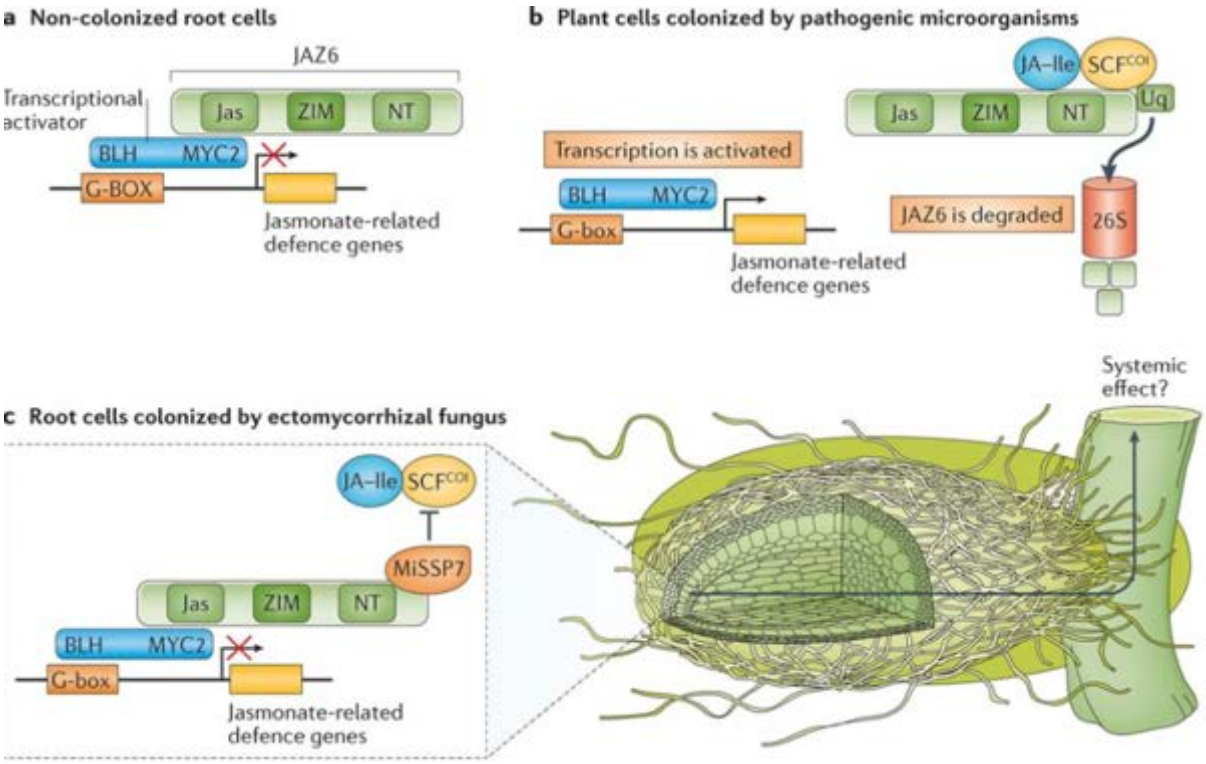


Figure 12 – Représentation schématique du modèle de régulation de la voie de signalisation de l'acide jasmonique du Peuplier par l'effecteur fongique MiSSP7 (d'après Martin et al., 2016).
a - Liaison des facteurs de transcription avec JAZ6 qui empêche les facteurs de transcription associés de se lier aux sites de reconnaissance de la boîte G située en amont des gènes répondant à l'AJ.
b –Formation du complexe JAZ6-COI1 et dégradation par le protéasome 26S suite à la présence d'AJ. MYC2 n'est plus inhibé par JAZ6 et peut jouer son rôle de facteur de transcription en activant la transcription des gènes sensibles à l'AJ.
c – Liaison entre MiSSP7 et JAZ6 qui supprime les mécanismes de défense liés à l'AJ. Cette liaison empêche sa reconnaissance avec AJ-COI1 et donc sa dégradation.

V. Le microbiote du peuplier

Cette partie correspond à un article de synthèse en préparation (et non finalisé dans sa version présentée dans ce manuscrit de thèse) rédigé en anglais.

Dans cet article de synthèse intitulé « Current knowledge of the *Populus* microbiome : improving the holistic perception of tree-microbes interactions », je présente les connaissances scientifiques acquises jusqu'à aujourd'hui concernant la composition et les mécanismes de régulation du microbiote du peuplier. Bien que cette thèse se concentre exclusivement sur les facteurs de régulation du microbiote racinaire, cet article présente également les facteurs de régulation des communautés de micro-organismes associées aux parties aériennes de l'arbre tels que les feuilles et le tronc.

Après un travail exhaustif de recherche bibliographique sur les microbiotes du peuplier et également ceux d'autres arbres, j'ai personnellement pris en charge la rédaction de l'article qui constitue une partie importante de l'introduction générale à ma thèse.

Current knowledge of the *Populus* microbiome: improving the holistic perception of tree-microbes interactions

Lauralie Mangeot-Peter[1], Aurélie Deveau[1]

[1] INRAE, Université de Lorraine, UMR IAM Interactions Arbres/Micro-organismes (IAM) 1136, Laboratoire d'excellence Recherches Avancés sur la Biologie de l'Arbre et les Ecosystèmes Forestiers (LabEx ARBRE), Centre INRAE Grand-Est, 54280 Champenoux, France.

∗Corresponding author: Mailing address: UMR1136 INRAE Université de Lorraine, Interactions Arbres Micro-organismes, 54280 Champenoux, France. Phone: +33 3 83 39 40 88, Fax: +33 3 83 39 40 69. E-mail: aurelie.deveau@inrae.fr

Abstract

Populus have played an increasing role in environmental, commercial and research projects in the past few decades. Thanks to its multiple specificity, *Populus* became an excellent tree model to improve our knowledge on tree microbiome. Interactions between trees and microorgansims are multiple and extremely diverse according to the different tissues of the tree. These associations play important role for tree nutrition, growth and health but they could also help trees to improve their adaptation to environmental conditions, opening exciting opportunities for forestry. Although most of the factors influencing the composition and structure of the microbiome are currently known, there is a real lack of knowledge regarding the specific function of each member of the microbiome and the molecular dialogue occurring between microorganisms and tree host. Filling these knowledge gaps would allow us to improve our holistic perception of tree microbiome.

We review recent studies in *Populus* to highlight the importance of this holistic vision across the rhizosphere, phyllosphere, stem and wood, and discuss potential improvement for future research in *Populus* microbiome and forest ecosystem.

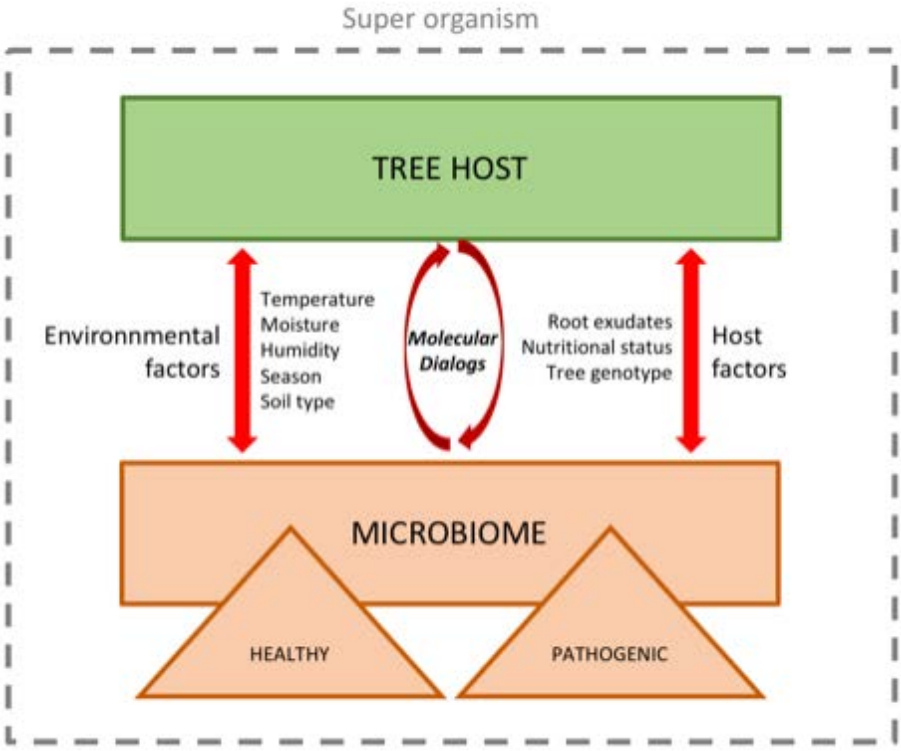


Figure 13 - Factors driving reciprocal interactions between trees and their microbiome

Introduction

Like any other plants, nearly all tissues of trees are colonized by complex and diverse communities of microorganisms, so-called the tree microbiome. The most studied microbial associates of trees are the mycorrhizal fungi that establish mutualistic interactions with the roots of most trees (Lang et al., 2011). Yet tree tissues are home for other fungal types (e.g. endophytic fungi), bacteria, protists, algae and some invertebrates that tightly interact together and with their hosts (Sieber, 2007; Beck et al., 2014; Tedersoo et al., 2016; Proença et al., 2017;). These microorganisms colonize both the surfaces and the inner tissues of trees and fulfil a broad range of functions that overall enhance the health and development of trees (Taghavi et al., 2009; Mercado-Blanco, 2018). If some microorganisms have beneficial effect on their host, some others are deleterious to tree growth and health. Pathogenic agents integrated in the microbiome is called pathobiome (Vayssier-Taussat et al., 2014). Some members of the tree microbiome also contribute to biogeochemical cycles in soils (Akroume et al., 2019) and in the atmosphere (Yip *et al.*, 2018). Understanding the contribution of these different groups of microorganisms and the balance regulating both is therefore crucial. Interactions between trees and their associated microbial communities are extremely complex and the network occurring between these multiple organisms have key role in tree health and disease. Trees and their microbiome should be considering as superorganisms. This observation gave birth to the concept of holobiont which consist of a collective view of the function and interactions between host organism and its associated microorganisms (Vandenkoornhuyse et al., 2015; Figure 13).

Recently, interest for plant microbiome increase and more and more important highlights appears. Most of the researches carried out so far on the interaction between plants and their microbiome have been done on model annual herbaceous plant such as *Aradidopsis thaliana*, which present a very different life cycle compared to the one of tree and which is not able to form association with mycorrhizal partners. In perennial woody plant, nutrient allocations which correspond to a major factor shaping the microbiome are different from those in non-perennial plant. In addition, trees are colonized by other types of microorganisms such as ectomycorrhizal fungi which could modify formation mechanisms of microbiome and response of tree host. They could help tree to have a higher capacity to adapt to many kinds of environmental conditions and climate change (Bulgarelli *et al*., 2013; Timm *et al*., 2016) thanks to a higher degree of plasticity, a shorter-term dynamic and a larger pool of genes of tree-associated microorganisms which form an extension of tree host genome (Panke-Buisse et al., 2015).

Populus has emerged as a model tree to study interactions between trees and their microbiomes (Shakya *et al.,* 2013; Hacquard *et al.,* 2015; Beckers *et al.*, 2017; Cregger *et al.,* 2018). This is likely due to the fact that poplar has become one of the main and powerful model systems in tree research in the past 15 years. Poplars have the advantages to grow rapidly, to have prolific and early sexual reproduction, and to show tight coupling between physiological traits and biomass productivity (Rae *et al*., 2004). In addition, some hybrids can be genetically modified and a number of species can be clonally propagated. Altogether with the numerous molecular tools and

databases that were generated following the sequencing of its genome (Tuskan et al., 2006), all these elements fostered the establishment of Poplar as the main model system for research in biology, genetics, molecular biology of trees and more recently tree-microbiome interactions. *Populus* was notably used to dissect the molecular mechanisms of the establishment of the ectomycorrhizal symbiosis, highlighting the prominent roles of fungal and Poplar small secreted proteins in the molecular dialog between the symbionts (Martin *et al.,* 2016).

The scientific interest for *Populus* not only lies in its usefulness for basic science but also in its environmental and commercial importance. According to the classification established by Eckenwalder in 1996, the genus *Populus* regroups 29 species (plus an important number of hydrids) of deciduous trees in the family of Salicaceae. Distributed across the Northern hemisphere from subtropical to boreal ecosystems, poplars and cottonwoods dominate riparian forests and flooded forest stands (Rood & Mahoney, 1990; Braatne et al. 1996) while aspens are the most important boreal broadleaf trees (Peterson & Peterson, 1992). *Populus* species are cultivated in plantations across America, Europe and Asia. The 940.200 ha of *Populus* plantations in Europe produce about 8 millions m^3 per year. Although most of *Populus* products are nowadays exploited by the wood industry to produce plywood, sawn timber and pulpwood, there has been an increasing interest in the past years for using *Populus* as a source of fuel source and biomass for energy production. Thanks to their high tolerance to pollution and their ability to purify organically polluted soils, fast-growing poplar hybrids are being more and more utilized for phytoremediation (Yadav et al., 2010). New usages of *Populus* have recently stimulated the development of large scale breeding and biotechnology programs across the world to produce new varieties of fast growing Poplars that could be cultivated as crops for large scale production (Bredemeier et al., 2015; Hart et al., 2018).

We reviewed recent advances in *Populus* microbiome study to show the importance of improving holistic view across the different tissues of the tree and to highlight future key elements to be discovered. We will focus only on bacteria, archaea and fungi, as they are considered to be the main contributors to the tree phenotype and they are also the most studied.

The root microbiome

Tree roots are an oasis of nutrients for microorganisms. Trees transfer 20 to 40 % of their photosynthetates to their roots and 10 to 30 % of this carbon is allocated to the root microbiome. Part of it is directly transferred to mycorrhizal fungi, which themselves offer a habitat to specific microbial community, while the other part is secreted as exudates in the zone of soil directly under the influence of roots, the rhizosphere (Lakshmanan et al., 2014). The main compounds of root exudates are the product of photosynthesis and secondary metabolites with important roles as chemical attractants and repellents in the rhizosphere. The composition of root exudates has been studied mainly to understand the potential of some species of *Populus* trees for phytoremediation allowing for the identification of secreted organic acids that are induced upon treatment with heavy metals such as malic acid, citric acid or oxalate (Qin et al., 2007; Naik et al., 2009) but not the one's that are constitutively produced. Thus, a complete description of the compounds secreted by *Populus* roots and their respective roles in selecting for the microbiome is still lacking.

Root microbiome composition

In root, we could distinguish two major habitats: the rhizoplane which corresponds to the external surface of the root directly under the influence of the rhizosphere, and the endosphere which corresponds to the interior of the root. Composition and structure of microbial communities associated with these two different tissues but also the factors that contribute to this assembling were examined in many trees such as *Pinus* (Gallart et al., 2018), *Quercus* (Maeden et al., 2016) and *Fagus* (Goldmann et al., 2016; Colin et al., 2017). A recent study performed by Toju and colleagues in 2018 explored hubs within metacommunity-scale networks of tree-fungi interactions across 8 forest ecosystems.

Dominant bacterial phyla in *Populus deltoides* roots (rhizosphere and endosphere) were Proteobacteria, Actinobacteria, Acidobacteria and Verrucomicrobia and dominant fungal phyla were Ascomycota, Basidiomycota and Chytridiomycota. Observation of rhizosphere and endophyte communities of both bacteria and fungi associated with roots in *Populus deltoides* showed that dominant phyla were present in rhizosphere but in difference abundance, suggesting that endosphere constitute a unique niche for microbial communities (Gottel et al., 2011; Shakya et al., 2013).

Ratio of EcM and AM colonization are different according to different factors such as type of soil and *Populus* genotype (Karlinski *et al.,* 2010). EcM fungi associated with *Populus* included species of *Laccaria*, *Hebeloma*, *Tuber* and *Cortinarius* (Gottel et al., 2011; Guevara et al., 2013). Recent works insighted the key role of Mycorrhizal-Induced Small Secreted Protein (MiSSP) in the formation of EcM-tree symbiosis formation. By studying the interaction model between *Populus* and *Laccaria*, Plett et al. (2014) showed that the production of a MiSSP of 7kDa (MiSSP7) by the EcM fungus and its interactions with the *Populus* transcriptional repressor JASMONATE ZIM DOMAIN protein 6 (JAZ6) altered the hormonal balance of the tree defence system allowing the formation of symbiosis. Future investigations are needed to understand the signalling interactions of *Populus* species with other EcM fungi to improve our knowledge related to model pattern of interactions. For example, other MiSSP are currently be taken in account such as role of MiSSP8 in the ectomycorrhizal symbiosis establishment (Pellegrin *et al*., 2017).

AM fungi associated with *Populus* roots mostly belonged to the genus *Glomus* (Beauchamp et al., 2006; Bonito et al., 2014). AM fungi colonization dominated in early stages of *Populus* life then the ratio of EcM and AM fungi changed during development (Takacs et al., 2005).

Endophytes were defined as microorganisms such as bacteria and fungi inhabiting the plant endosphere during all part of their life cycle regardless of the outcome of association. In roots, both bacterial and fungal endophytes were isolated from endosphere. Bacterial endophytes attached to the rhizoplane and entered in the endosphere by openings where root hairs and lateral roots emerged (Hardoim et al., 2015). In *Populus*, most abundant bacterial endophytes have been characterized to belong to Gammaproteobacteria with *Serratia spp*, *Pseudomonas spp* and *Enterobacter spp*., and Actinobacteria with *Rhodococcus spp* (Taghavi et al., 2009). Dark-septate fungi and other fungal endophytes have been discovered in most *Populus* roots (Helm et al., 1996).

Fungal endophytes associated with *Populus* roots belonged to *Cadophora, Exophiala and Leptodontidium* (Bonito et al., 2016).

Some studies have examined the fungal community composition and structure (Bonito *et al*., 2016) whereas some others have studied the bacterial community composition and structure (Beckers *et al*., 2017). But recently, some root microbiome studies have included bacterial and fungal community composition observation in the same experimental set-up (Shakya *et al.*, 2013; Bonito et al., 2014; Cregger *et al*., 2018; Table 1). In these works, different compartments of the soil and the roots have been defined. The shift in the microbial community composition across these different compartments are today well known (Gottel *et al.*, 2011; Beckers *et al*., 2017) but these works give important insight concerning the effect of different drivers of the root microbiome composition.

The main drivers of the root microbiome composition

The root microbiome composition is influenced by environmental factors, host factors but also by complex molecular dialogs occurring between host plant and microbial communities, particularly for both EcM and AM symbiosis establishment. The factors impacting the structure of bacterial and fungal communities in trees were soil type, temperature variation (Baum and Makeschin, 2000; Bonito et al., 2014), host genotype or host species and tree age (Shakya *et al*., 2013; Cregger *et al*., 2018).

Both bacterial and fungal community composition varied more so across root compartment (rhizosphere vs. endosphere) than between host genotype. This observation suggests an environmental filtering and a selective force for microbial communities of tree roots. Soil type and nutrient availability played an important role on microbial community composition. Soil type had a stronger effect on fungal community composition of *Populus* (Bonito et al., 2014). For example, nitrogen and phosphorus fertilization significantly reduced EcM colonization rate in *P. trichocarpa* and *P. tremula x tremuloides* (Baum & Makeschin, 2000) whereas moisture increased mycorrhizal fungi colonization (Lodge, 1989). In *P. deltoides*, bacterial communities in the roots are more structured by plant host species than by soil type (Bonito et al., 2014). In a lower extent, seasonal variation has been shown to also impact microbial community composition in *Populus* root endosphere (Shakya et al., 2013).

One of *Populus* hybrid, *Populus tremula x alba* also called 717-1B4 clone, and also *P. deltoides* are particularly appreciated by researchers due to its ability to be easy genetically transformed. Comparison between microbial communities associated with wild-type *Populus* and transgenic lines is particularly helpful to study the main drivers of the root microbiome composition. Some studies have been performed with this strategy with trees modified in their cellulose biosynthesis and cell wall composition (Veach et al., 2018) and with trees modified in their lignin biosynthesis (Beckers et al., 2015). Taken together, these works from both greenhouse and field conditions showed that potential modifications in microbial community structure and diversity in roots of *Populus* are the results of some alteration in the host tree primary and secondary metabolism. In ectomycorrhizal (EcM) symbiosis, the fungi exchange soil-derivate nutrients for carbohydrates from the host tree. In 2014, Tschaplinski *et al*., compared metabolomes of EcM root tips in compatible (*P. trichocarpa* with *L. bicolor*) and incompatible (*P. deltoides* with *L. bicolor*) interactions. The result of this study showed that incompatible relationship was

characterized by a reduced number of regulated metabolites in roots in contact with fungus hyphae and a higher accumulation of metabolites related to tree defence.

Main functions of *Populus* root microbiome

The basic functions of mycorrhizal fungi in terms of nutrients and water exchange with tree are well characterized (Bücking et al., 2012). In the tree root microbiome, archaea community composition has been less examined than bacterial community composition probably because their ecological functions for tree host remain unclear. Novel insights obtained by metagenomics analyses shows archaea is an important functional component of the plant microbiome thanks to its ability to promote plant growth through auxin biosynthesis and nutrient supply (Taffner et al., 2018).

Populus is also able to interact with a variety of bacterial and fungal root endophytes. Bacterial root endophytes have been well characterized to have different functions for host *Populus*. They promote *Populus* growth with an important increase of total biomass through production of auxin (IAA), phytohormone involved in plant growth processes (Khan *et al*., 2016). If many studies on interactions between *Populus* and mycorrhizal fungi are available, few works have been performed on fungal root endophytes. The presence of fungal endophytes in plants (Caroll, 1988) and in *Populus* in natural conditions is well established but the diversity and ecological function for host tree are still not well known. Some studies revealed that fungal root endophytes have an important role for plant physiology and health (Varma *et al*., 1999; Lugtenberg *et al*., 2016; Patle *et al*., 2018). One species of this order, *Atractiella rhizophila*, was characterized and *Populus* inoculation assays revealed a faster plant growth and elevated photosynthesis rates (Bonito *et al.,* 2017).

Currently, one of the most important wide-scale perturbation is drought, which has recently been shown to alter the composition and structure of forest. In temperate forest but also in boreal and tropical forest, drought and heat related physiological stress promote tree mortabilty (Law, 2015). A study observed how the bacterial communities associated with *P. deltoides* roots changed in response to water (drought) and light (shade) limitation. It has been shown that these treatments resulted in significant shifts in bacterial community at the phylum level and that a cluster of OTUs either increase or decrease in abundance (Timm et al., 2018). Inoculation of 10 endophytes (9 bacteria and 1 yeast) in *P. deltoides x nigra* resulted in substantial growth promotion with improved leaf physiology after periods of drought (Khan et al., 2016). *Populus* form also associations with EcM fungi communities that are known to help tree contend to drought. *P. euphratica*, a drought-sensitive *Populus* displayed an improved water status in roots thanks to the presence of the EcM fungi *Paxillus involutus* although no mycorrhizae were formed (Luo et al., 2009).

As one of the most often used tree species in phytoremediation, *Populus* play significant role in remediation of contaminated sites thanks to its to high biomass production and rapid growth. Study of bacterial communities associated to the roots of *P. trichocarpa x P. maximowiczii* planted in an industrial phytomanaged site contaminated with mercury revealed contrasting bacterial communities in different tree habitats (soil, roots, leaves and stem; Durand et al., 2018). Copper stress was compared to water and light limitation stress in *Populus deltoides*. Even if metal stress was likely the least severe of the three, as indicated by growth measurements and

transcriptional responses of tree, changes in the microbiome response was observed (Timm et al., 2018). A variety of studies have examined the response of AM fungi to different soil pollutant or to different abiotic stress in *Populus* clones selected for phytoremediation of contaminated soil (Takacs *et al*., 2005; Lingua *et al*., 2008; Liu *et al*., 2015). It is clear AM fungi enhanced tree growth and reduced biomass losses during stress but the mechanism and functioning of AM and EcM fungi dual symbiosis in *Populus* roots are currently not known.

The phyllosphere, stem and wood microbiome

The phyllosphere corresponds to the leaves of plants. It is considered relatively nutrient poor but a much more dynamic habitat in comparison with the rhizosphere because of strong environmental constraints with high fluctuation along the day such as temperature, humidity and solar radiation. Phyllosphere and stem microbiota play a critical role in protecting plants from diseases and promoting their growth by different mechanisms (Turner et al., 2013).

Phyllosphere and stem microbiome composition

Associated microbial communities structure and diversity have been observed in several tree species such as European beech (Cordier *et al*., 2012), pines (Beule *et al*., 2017) or olive tree (Pascazio *et al*., 2015). Some other studies have compared the bacterial (Laforest-Lapointe *et al.,* 2016; Lambais *et al*., 2006) and fungal (Izuno *et al*., 2016) community structure of phyllosphere of tree inhabiting the same type of forest.

Concerning, microbial communities associated with *Populus* leaves and stem, recent works begin to appear. In field-grown *P. tremula x alba* trees, bacterial colonization of the stem and leaves was highly variable compared to rhizosphere colonization in terms of diversity and community composition. This observation confirmed that additional skills are required to colonize the different tree niches (Beckers *et al*., 2017). In *Populus* leaves (surface and endosphere), dominant bacteria phyla were Alphaproteobacteria (43% in terms of relative abundance), Gammaproteobacteria (42%), Actinobacteria (5%), Betaproteobacteria (3%) and Bacteroidetes (3%) and dominant fungal phyla were Ascomycota (85%) and Basidiomycota (9%). In *Populus* stem (surface and endosphere), dominant bacterial phyla were Alphaproteobacteria (58%), Actinobacteria (20%), Gammaproteobacteria (9,5%) and Bacteroidetes (7,5%) and dominant fungal phyla were Ascomycota (70%) and Basidiomycota (25 %) but also Chytridiomycota and Glomeromycota (3% each; Cregger et al, 2018).

About 1000 fungal endophytic isolates belonging to *Dothideomycetes*, *Sordariomycetes* and *Helotiales* were obtained from 175 *P. tremula* leaves (Albrectsen et al., 2010). A total of 513 bacterial endophytic isolates were found in several poplar clones leaves and the majority of these isolates were related to common endophytic and soil bacteria. They belonged to Curtobacteriaum, Plantibacter, Pseudomonas and Xanthomonas (Ulrich et al., 2008).

Endophytic microbes colonized plant roots are able to enter in the plant vascular system to be transferred and thus colonize the aerial tissues of the plant (Whipps *et al.*, 2008). In their *Populus* holobiont study of 2017, Beckers *et al*. demonstrated that *P. tremula x alba* stem endosphere present a decreased richness comparable

with those of the phyllosphere compared to the roots. These results are in concordance with the general view of endophytic colonization. Bacterial and fungal endophytes can be horizontally acquired from the environment in each generation. Endophytic lifestyle is dependent to the ability of bacteria from the soil to actively or passively pass the endodermis and the pericycle, reach the xylem vessels and finally lead to systemic colonization of the plant (Hardoim *et al*., 2008). Microbial endophytes can be also vertically transmitted from generation to generation via seed (Zilber-Rosenberg et al., 2008).

In spite of several studies performed on phyllosphere microbial communities in leaves and stem of *Populus* (Balint et al., 2014; Busby et al., 2015; Doty et al., 2016), our knowledge related to the structure and composition of both bacterial and fungal communities associated with *Populus* leaves and stem remains partial in comparison with *Populus* root microbiome.

Recently, new studies carried out on the whole tree microbiome (soil, root, leaves and stem) allow us to better understand the specific interactions, mechanisms and functions associated with microbial colonization of the aerial tissues of *Populus* (Beckers et al., 2015, 2017; Cregger et al., 2018; Durand et al., 2017, 2018; Table 1).

The main drivers of the phyllosphere and stem microbiome composition

While the microbiome of *Populus* roots in influenced by soil type and host genotype, less is known about the main drivers that impact microbial communities of the phyllosphere and stem. The phyllosphere corresponds to the plant-air interface which is characterized by permanently changing abiotic conditions.

Microorganisms associated with leaves or stem provide and vary in composition and diversity according to seasonal variations as well as in response to environmental disturbance such as rainfall or wind. Both bacterial and fungal community composition shifted more so across habitat (soil, roots, stem and leaves) than between *Populus* tree genotype (Cregger et al., 2018). In *P. balsamifera*, warming strongly influenced the diversity and structure of the foliar fungal microbiome especially fungal endophytic communities (Balint et al., 2014). These observations agree with the hypothesis that microbial communities vary across the different tree compartment due to different environmental stress associated with the different geographical range of *Populus* genotype.

In *P. deltoides* and in *P. trichocarpa x deltoides*, stem had similar bacterial alpha diversity than roots but fungal alpha diversity was higher is stems than in leaf or root habitat (Cregger *et al*., 2018). In *P. balsamifera*, fungal communities associated with leaves of trees growing in a common garden were differently structured according to host genotype (Balint et al., 2013). These results, in comparison with those of Beckers *et al*., suggest that host tree genotype play also a key role in the composition of microbial communities in phyllosphere and stem. Fungal microbiome within leaf habitat varied more so between tree genotypes compared to habitat, likely due to the greater abundance of two dominant fungal pathogens (*Massonina* and *Septoria* cause leaf spots and stem cankers) in the hybrid *P. trichocarpa x deltoides* (Cregger *et al*., 2018). Bacterial communities associated with *Populus* phyllosphere are also influenced by volatile organic compound (VOC) produced by trees. Of the same age as the root exudates, these VOC are emitted by the tree and condition the installation of specific microbes. Isoprene is a climate-active gas produced in huge amount by tree. This compound promoted the *Populus* leaves

colonization by active isoprene-degrading bacterial populations belonging to the genus *Rhodococcus* (Crombie et al., 2018).

Main functions of *Populus* phyllosphere and stem microbiome

As mentioned previously, microbiome is important for plant health and nutrient acquisition. Microbial interactions in the phyllosphere could affect the fitness of tree but in comparison to the roots system and the soil, this tree niche is less studied. In 2016, Doty *et al*. showed that nitrogen (N_2) fixation is possible in wild *Populus trichocarpa* branch by using both $^{15}N_2$ incorporation and acetylene reduction assay. Nitrogen fixation could be performed by endophytic or closely associated nitrogen-fixing microorganisms.

Fungal pathogen presence in leaves raises some questions about the climate change reactions known to increase pathogen abundances in tree host (Balint *et al*., 2014). But some recent works have shown that common foliar fungal antagonist could be used to bolster resistance to rust disease in *Populus* plantations, suggesting that naturally occurring fungal foliar endophytes partially determine disease severity (Busby *et al*., 2015; 2016). In pedunculated oak, another important tree model, a non-pathogenic bacteria strain (*Streptomyces sp*. AcH505) protects tree host from the pathogen responsible of oak powdery mildew (Kurth *et al*., 2014).

Characterization of fungal communities from different *Populus trichocarpa* × *maximowiczii* compartment at a Hg-contaminated phytomanagement site had led to isolate some Hg-resistant yeast strains from the leaf habitat. *Aureobasidium pullulans*, *Cladosporium spp*., and *Alternaria alternate* were highly represented in *Populus* leaves and were known to be an active phyllosphere colonizer with specific capacity to bind heavy metals to the cell surfaces (Durand et al., 2017). The bacterial communities from the phyllosphere were altogether less resistant than the communities of the soil and roots, potentially due to the direct contact between soil bacterial communities and higher amounts of Hg in the soil. In the leaf and stem, OTUs from the genus *Deinococcus* the most abundant OTU and were not detected in soil and roots. This genus of bacteria corresponds to extremophile bacteria resistant to very high doses of radiation and long periods of desiccation (Durand et al., 2018).

The wood microbiome

Microbiome of wood have been studied in several woody plant such as *Pinus* (Proença et al., 2017) and *Quercus* (Denman et al., 2017) with the goal to provide new insight into microbial interactions and tree disease.

The number of publications on *Populus* wood microbiome is very low as compared to root and phyllosphere microbiome (Figure 14), but only few studies recently provided some interesting results concerning the composition of microbial communities associated with wood and factors shaping wood microbiome. Culturable fungal endophytes have been examined within the woody tissues of branches of *Populus angustifolia* (Lamit et al., 2014). In *Populus deltoides*, wood environments are dominated by anaerobic microbes with a prominence

of methanogens in heartwood compared to sapwood. Contrary to root and phyllosphere microbiome, wood associated microbial communities are only shaped by environmental conditions (Yip *et al*., 2018).

Understanding complex tree host – microbes interactions

The *Populus* core microbiota

The principle of a core microbiota has been proposed to describe the microbial community that is systematically associated with a given plant genotype. Based on its taxonomic composition, the core microbiota could provide some key basis related to component species colonizing *Populus* tree and gain further insight into the complex host-microbe interactions.

In addition to this taxonomical approach, functional approach is also possible by using metagenomics and metatranscriptomic analyses to predict potential functions of tree microbiome (Vandenkoornhuyse *et al*., 2015).

In *Populus tremula x alba*, core bacterial microbiome has been defined as the 10 most abundant OTUs of each of the studied tree compartments (rhizosphere soil, roots, stem and leaves). Beckers et al. (2017) observed significant tree compartment effects across core bacterial OTUs with only 16,4 % of total OTUs shared by all tree compartments. In *Populus deltoides* tree, core microbiome of both fungal and bacterial OTUs was identified in root rhizosphere and endosphere of the set of sampled trees. The core bacterial microbiome in the rhizosphere was comprised of 35 OTUs belonging to the order of *Bulkholderiales* and *Rhizobiales* and only one OTU in the root endosphere. The core fungal microbiome was comprised of four rhizosphere OTUs and one endosphere OTU represented by genus *Exophiala*, *Metarhizum*, *Neoseptia* sp. and *Mortierella* known to promote plant growth (Shakya *et al*., 2013). During abiotic stress (drought, shading and copper pollution), the relative abundance of some communities of the core bacterial microbiome in *Populus deltoides* roots changed. This shift highlighted the presence of a “core stress microbiome”, opening some interesting ways in tree-microbial community manipulation to promote and improve tree growth and productivity (Timm *et al*., 2018).

As these two studies on poplar microbiome show, the number of fungal and bacterial OTUs shared by the different compartments of the tree is rather low. A small set of OTUs forming the core microbiome should encourage genome sequencing of isolates and controlled inoculations experiments for understanding the detailed mechanisms of interactions between microbial communities and host *Populus*.

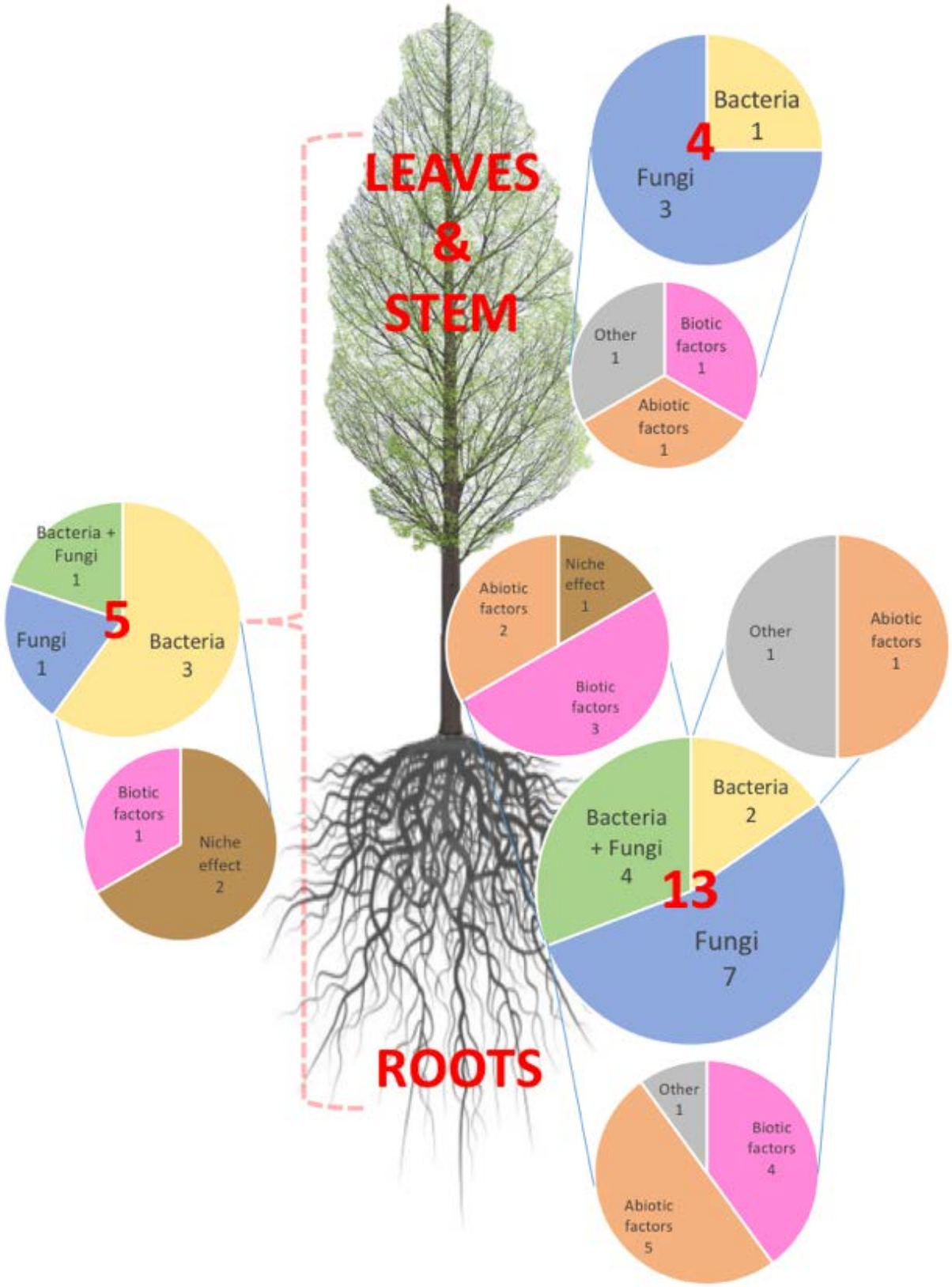


Figure 14 - Number of available publications dealing with Populus microbiome by studied microorganisms and influence factors

The Populus holobiont

Understanding microbial community composition and structure variations in the different plant-habitat from leaves to roots and soil is crucial to improve our knowledge of the impact of microbiome on plant. To understand the role of microbiome, it is very important to pay attention to the different factors which contribute to the microbiome shaping in order to understand the effect of *Populus* holobiont on tree health. A new field of research is currently appearing in which the *Populus* holobiont is taking account (Beckers *et al*., 2017; Cregger *et al*., 2018) but some key informations are still lacking and should be a priority (Figure 14). They concern (1) the function of the microbiome, (2) the molecular dialog occurring between microorganism and tree host, and (3) the assemblage mechanism of microbial community shaping by tree host. Microorganisms live in complex interactions with other members of the microbiome, host plant but also environment. Despite the different omics techniques and data available (Zuniga *et a*l., 2017), deciphering the nature and function of these multitude of interactions is a great challenge for scientists. Investigating the biosynthetic potential of the *Populus* microbiome is now possible by analysing metagenomic data and pool of sequences bacterial isolates taken from the *Populus* roots. The development of this strategy based on bacterial natural products isolation is a great opportunity to discover new bacterial molecules involved in important functions for the tree host or involved in communication and control in the microbiome (Blair et al., 2018).

Understanding the complex host-microorganisms interaction could provide some important basis and help to realize the goal of engineering host phenotype through microbiome manipulation for example the use of these interactions in phytoremediation and sustainable tree culture management (Timm *et al*., 2016). We described recent studies performed on *Populus* microbiome but comparison with other tree model such as *Pinus*, *Quercus* or *Eucalyptus* should provide additional key information to understand the role of tree-associated microorganisms of forest ecosystem.

VI. Objectifs de la thèse

Les associations entre les plantes, les champignons et les bactéries sont ubiquistes dans le règne végétal. Elles peuvent être de différents types, allant de la symbiose mutualiste à des interactions neutres (endophytes) ou néfastes avec les micro-organismes pathogènes. Les communautés microbiennes (microbiote) associées aux plantes jouent donc un rôle majeur dans leur croissance et leur développement.
La plupart des études menées sur le microbiote racinaire concernent la plante modèle herbacée *Arabidopsis thaliana* et les plantes de grandes cultures telles que le blé ou le riz (Lundberg et al., 2012 ; Rascovan et al., 2016 ; Edwards et al., 2015). Les communautés fongiques et bactériennes associées aux racines sont modulées par de nombreux facteurs abiotiques, tels que le type de sol (Bonito et al., 2014 ; Veach et al., 2019) et le climat (variations saisonnières, température, humidité et sécheresse extrême) (Shakya et al., 2013 ; Felsmann et al., 2015 ; Mercado-Blanco et al., 2018), mais également par des facteurs biotiques, tels que la composition des exsudats racinaires, les métabolites secondaires accumulés dans les tissus racinaires et le génotype de l'hôte (Compant et al., 2019).

Sur la base des connaissances acquises et évoquées précédemment, il est clairement établi que la constitution du microbiote racinaire des arbres dépend fortement du réservoir de micro-organismes disponibles dans le sol environnant et de la capacité des arbres à sélectionner activement certains de ces micro-organismes. Toutefois, l'importance relative de ces deux grands facteurs ainsi que les mécanismes par lesquels les arbres sélectionnent leur microbiote ne sont pas encore complétement connus.

Dans ce contexte, nous avons émis trois hypothèses :

1. Les variations naturelles au sein des communautés microbiennes (champignons et bactéries) du sol ont un effet majeur sur la formation du microbiote racinaire de l'arbre,
2. Les modifications du microbiote racinaire altèrent la physiologie de l'holobionte et sa capacité d'adaptation aux potentielles modifications climatiques,
3. La manipulation de la voie de signalisation de l'acide jasmonique via l'effecteur fongique MiSSP7 influence la composition et la structuration du microbiote racinaire et la physiologie de l'holobionte.

Pour tester ces différentes hypothèses, nous avons choisi le peuplier comme système modèle. De plus, différentes approches expérimentales et analytiques ont été employées : couplage du séquençage haut débit de l'ADN microbien (en anglais, DNA metabarcoding), de la microscopie confocale pour caractériser les micro-organismes de la rhizosphère et de l'endosphère de l'arbre, des analyses métabolomiques pour étudier l'impact du microbiote sur le métabolisme de l'holobionte et/ou des analyses métatranscriptomiques pour connaître les communautés de micro-organismes les plus actifs du microbiote racinaire de l'arbre.

Ma thèse est divisée en quatre chapitres avec pour objectifs de répondre aux questions suivantes :

- ✓ Chapitre II : Comment se fait la colonisation des racines vierges de l'arbre par les communautés de champignons et de bactéries du sol ? Cette colonisation de la racine par les micro-organismes rhizosphériques est-elle simultanée ou obéit-elle à une chronoséquence ?
- ✓ Chapitre III : Quelle est la contribution relative du sol, du génotype et du climat sur la composition et la structuration du microbiote racinaire du peuplier noir ?
- ✓ Chapitre IV : Quel est l'impact des variations naturelles au sein des communautés microbiennes du sol sur la composition et la structure du microbiote racinaire de l'arbre ?
- ✓ Chapitre V : En modulant la voie de signalisation de l'acide jasmonique, les champignons ectomycorhiziens influencent-ils la composition et la structure du microbiote racinaire ?

Chapitre II

Dynamique de colonisation des racines du peuplier par les communautés de micro-organismes

I. Contexte général

La composition et la structure du microbiote racinaire d'une plante sont très dynamiques et peuvent être influencées par différents facteurs abiotiques tels que les propriétés du sol et le climat, ainsi que par des facteurs abiotiques tels que le génotype de l'hôte et l'exsudation racinaire (Berg & Smalla, 2009 ; Mendes et al., 2013).
Le microbiote racinaire se compose de communautés de micro-organismes originaires du sol environnant qui représente le réservoir principal de micro-organismes pour la formation du microbiote racinaire (Lareen et al., 2016). Ces micro-organismes colonisent alors différentes parties de la racine (rhizoplan et endosphère) en fonction de leur capacité de pénétration et de leur fonction (Gottel et al., 2011). La production d'exsudats racinaires riches en sucres, acides aminés, protéines et métabolites secondaires joue un rôle important dans le recrutement des membres du microbiote racinaire. La qualité et la quantité de ces exsudats racinaires sont fortement influencées par l'espèce, la physiologie mais également le stade de développement de la plante hôte (Badri & Vivanco, 2009). Les arbres sont des plantes pérennes et ligneuses à longue durée de vie avec un mode de gestion des éléments nutritifs différent par rapport aux plantes herbacées et annuelles comme *Arabidopsis thaliana* ou les plantes de grandes cultures (Grayston et al., 1996 ; Nehls et al., 2008). De plus, les arbres sont capables de s'associer à des champignons ectomycorrhiziens, très abondants dans les sols des forêts boréales et tempérées (Baldrian, 2017) et à divers champignons saprotrophes et endophytes dont le rôle reste encore à élucider ainsi qu'à diverses communautés bactériennes (Liao et al., 2019 ; Terhoven et al., 2019). La présence de champignons mycorhiziens affecte également la composition des communautés bactériennes (Garbaye et al., 1994 ; Frey-Klett et al., 2007).

L'établissement du microbiote est un processus dynamique dans lequel les communautés microbiennes issues essentiellement du sol colonisent progressivement le système racinaire de l'hôte. Des travaux ont été réalisés sur la compartimentation des communautés microbiennes du sol, de la rhizosphère et des racines du peuplier et d'autres arbres afin de comprendre les mécanismes de sélection et de mise en place des communautés de micro-organismes (Gottel et al., 2011 ; Shakya et al., 2013 ; Cregger et al., 2018 ; Uroz et al., 2010 ; Uroz et al., 2016). D'autres études se sont uniquement concentrées sur une seule espèce de bactérie ou de champignon et ont uniquement été fondées sur l'inoculation microbienne en laboratoire (Lilleskov et al., 2003 ; Noirot-Gros et al., 2018 ; Bueno de Mesquita et al., 2018; Mesanza et al., 2019). Or, l'étude de la dynamique de colonisation directement liée au développement racinaire des arbres dans le sol, principal réservoir de micro-organismes, est nécessaire pour aider à comprendre les interactions complexes existantes entre le microbiote et la plante hôte.

II. Objectifs

Dans ce contexte, l'objectif de cette étude est donc de mettre en évidence les différentes étapes de la colonisation des racines du peuplier par les micro-organismes du sol. Plus précisément, deux questions principales se posent :

- Quelle est la dynamique de colonisation des racines de peuplier par les communautés bactériennes et fongiques du sol ?
- Cette colonisation est-elle simultanée ou successive ?

III. Démarche expérimentale

Afin de répondre à notre questionnement, nous avons transplanté des vitroplants de peupliers cultivés en conditions axéniques dans du sol naturel de peupleraie. Huit prélèvements racinaires ont été effectués après 2, 4, 7, 15, 21, 30 et 50 jours de croissance en conditions contrôlées. Les échantillons de racines ainsi prélevés nous ont permis d'étudier la colonisation fongique et microbienne, d'une part, par séquençage MiSeq haut débit des amplicons 16S et ITS, et, d'autre part, par marquage puis visualisation des hyphes fongiques par microscopie confocale.

Les résultats de cette étude sont décrits sous la forme d'un article scientifique actuellement en préparation.
Les tableaux supplémentaires sont disponibles en Annexe (Annexe 1 de la page 1 à la page 8).

Colonization dynamic of *Populus tremula x alba* roots by soil microbial communities

F. Fracchia [a1], L. Mangeot-Peter [a1], C. Veneault-Fourrey [a], F. Martin [a,b], A. Deveau [a*]

[a] Université de Lorraine, INRAE, UMR IAM, 54280 Champenoux, France

[b] Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Haisian District, Beijing, China

[1] F.F and L.M-P contributed equally to this work.

∗ Corresponding author: Mailing address: UMR1136 INRAE Université de Lorraine, Interactions Arbres Micro-organismes, 54280 Champenoux, France. Phone: +33 3 83 39 40 88, Fax: +33 3 83 39 40 69. E-mail: aurelie.deveau@inrae.fr

Abstract

A wide diversity of microorganisms belonging to various trophic guilds (i.e mutualists, endophytes, saprophytes) colonize roots of trees in natural conditions. The soil offers the main reservoir of microorganisms from which roots are colonized and select for specific microbial communities that contribute to the tree nutrition, development and protection against stresses. If it is well known that the formation of the root microbiome is a dynamic process, little is known on how different types of microorganisms colonize the root system and how the selection occurs. We hypothesized that the final composition of the microbiome is the product of several waves of colonization by different guilds of microorganisms To test this hypothesis, we planted sterile rooted cuttings of Grey Poplar obtained from plantlets propageted in axenic conditions in natural soil taken from a poplar stand. We analyzed the root microbiome at different time points between 2 and 50 days of culture by combining high throughput Illumina MiSeq sequencing of fungal rDNA ITS and bacterial 16S rRNA amplicons with Confocal Laser Scanning Microscope observations.

We observed that the colonization dynamic of *Populus* roots was different between bacterial and fungal communities. The rhizosphere effect was visible as early as two days after plantation for bacteria; roots were colonized very quickly and massively by members of the *Burkholderiaceae* family. By contrast, if fungi were also already colonizing the roots after 2 days, the initial communities were very close to the one of the soil and were dominated by saprotrophs and endophytes. Those were slowly replaced by both arbuscular and ectomycorrizal fungi . The replacement of the most abundant fungal guilds and bacterial community members observed over the time of our monitoring of the tree root colonization could suggest potential competition effect between microbial communities and/or a selection by the host tree.

Keywords : microbial communities, root colonization, metabarcoding, microscopy, *Populus*

Introduction

Plants have been recognized as metaorganisms possessing specific microbiomes. Plant microbiomes can be compartimentalised regarding the different plant organs (e.g. leaves, root microbiomes) and is a key determinant of plant health and productivity (Turner et al., 2013; Llado et al., 2017). Associated microorganisms, especially bacteria and fungi undergo different types of relationships with the host plant (Mendes et al., 2013; Hacquard & Schadt, 2015). The root-microbial interactions can be beneficial such as with mycorrhizal symbionts, by promoting plant nutrition and resistance against biotic and abiotic stresses, or detrimental such as with pathogens (Raaijmakers et al., 2009; Meena *et al.*, 2017; Naylor & Coleman-Derr, 2018).

The root microbiome is characterized by its composition and its structure. The composition corresponds to the identity of microbial members of the community while the structure is the product of the combination of the composition of the microbial community and the abundance of individual members. Composition and structure of root microbiomes are highly dynamic and are shaped by abiotic factors such as soil properties and climate, and by biotic factors such as the host genotype, root exudates and plant secondary metabolites (Berg & Smalla, 2009; Mendes et al., 2013).

The root microbiome is mainly recruited from the microbial communities present in the bulk soil which is considered as the main reservoir of microorganisms for plant root microbiome composition (Lareen et al., 2016). Roots provide different habitats for microorganisms that can be divided in three main compartements: the rhizosphere, the soil area directly under the influence of plant roots and the rhizoplane (i.e. the surface of the roots) are mainly colonized by subsets of microorganisms originating from the bulk soil, while the endosphere, i.e. the inner tissues of the roots, is colonized by both a subset of these rhizospheric communities and endophytic microorganisms coming from other plant tissues. The rhizosphere is enriched in compounds that are naturally produced by plant roots and attract microorganisms. Root exudates include sugars, amino acids, proteins and secondary metabolites and form a substantial source of nutrients for the microorganisms (Badri & Vivanco, 2009 ; Huang et al., 2014, Sasse et al. 2018). The quality and quantity of root exudates is strongly influenced by the species and the physiology of the host plant but also by plant developmental stages (Badri & Vivanco, 2009).

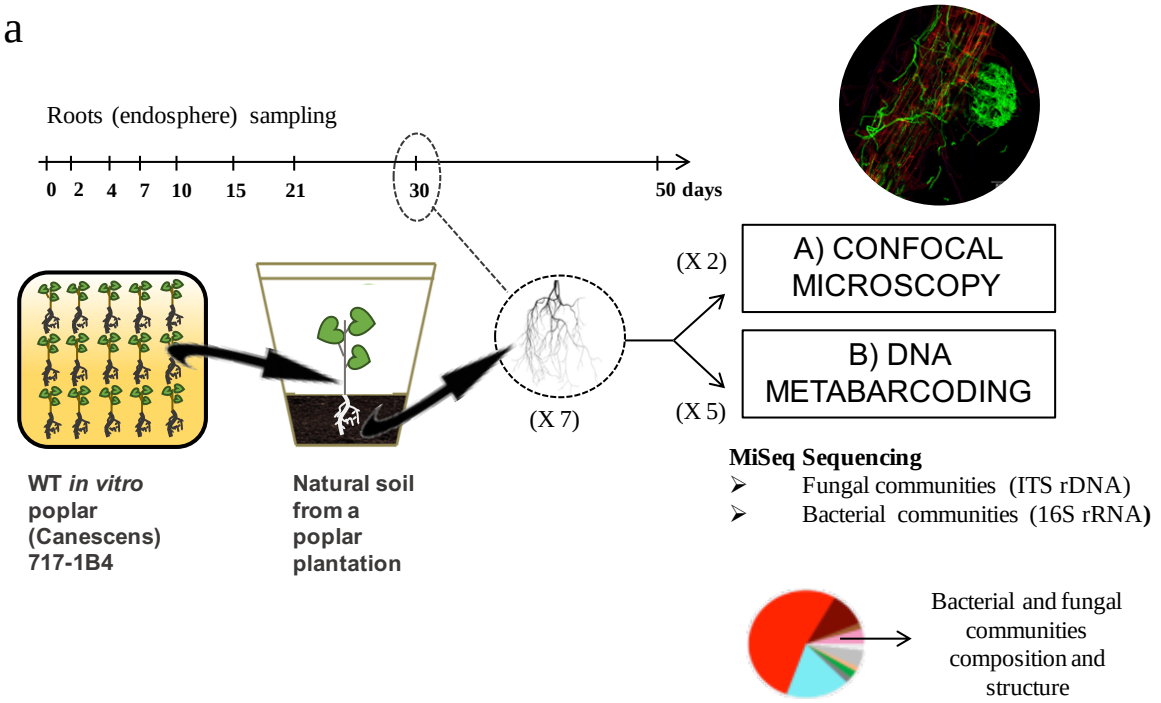
Trees are long-lived woody perennial plants with a different way of management of the nutrient allocation compared to herbaceous and annual plant species such *Arabidopsis thaliana* or crop plants (Grayston et al., 1996; Nehls et al., 2008). Tree root microbiome is also composed of a plethora of microorganisms with potential different functional capacity compared to herbaceous plants. Indeed, tree root are colonized by ectomycorrhizal (EcM) fungi in temperate and boreal forest ecosystem (Baldrian et al., 2017) but also by endophytes and saprotrophic fungi even though their role still remains elusive (Terhoven et al., 2019; Liao et al., 2019). The presence of mycorrhizal fungi also affect the composition of the bacterial part of the microbiome by offering a different habitat than nacked roots to bacteria named the mycorhizosphere (Frey-Klett et al., 2007, Marupakula et

al. 2017). Some rhizobacteria found in tree rhizosphere have been shown to improve mycorrhizal formation (mycorrhizal helper bacteria; Garbaye et al., 1994).

The establishment of the root microbiome is a dynamic process where specific microbial communities originating from the surrounding soil progressively colonise root systems under both the selection by the plant and the microorganisms communities. Previous works on root colonization have been carried out on trees to understand the mechanisms of the establishment of the tree root microbiome and of tree root selection (Lapeyrie et al., 1985; Chilvers et al., 1987; Lodge et al., 1989; Lilleskov et al., 2003; Noirot-Gros et al., 2018). For instance, aspen root colonization by the plant growth promoting bacteria *Pseudomonas* indicated that the spatial and temporal patterns of colonization of roots was different between the four strains of bacteria and was correlated with the ability of bacteria to form biofilm (Noirot-Gros et al., 2018). In pine roots, comparison of the dynamic of root colonization of two EcM fungi revealed different strategies. The ability of *Rhizopogon* to colonize roots rapidly from spores and its important early abundance constrasted with later root colonization and the slow increase in abundance of *Tomentella* (Lilleskov et al., 2003). The work of Lapeyrie et al. (1985) and Chilvers et al. (1987) on EcM and AM colonisation dynamic in eucalyptus roots showed a successionnal replacement of AM by EcM. Similarly, Lodge et al. (1989) showed negative associations among EcM and AM fungi leading to a depletion of AM and an increase in EcM in lateral roots in poplar. Nevertheless, these studies were focused on one or few bacterial or fungal species using microbial inoculation and did not look at the overall dynamic of the microbiome, including endophytes and saprophytes. Yet, pioneering studies on ectomycorrhizal and bacterial communities of the roots of Pines indicate that the full microbiome is likely subject to a complex dynamic during the colonization process (Marupakula et al. 2016). We hypothesized that fungal and bacterial communities originating from the natural soil colonized host roots with a successional turnover.

Investigating the succession of microbial communities colonizing the root system of young trees directly linked to the root development in natural soil, is needed to help the understanding of the complex interactions occurring between microbiota and host plant. To test this hypothesis, we assessed the dynamic of tree roots colonization by fungal and bacterial communities during the first 50 days of contact between naive tree roots and soil microbial communities. We used the grey poplar, *Populus tremula x alba* as a woody and perennial model organism. *Populus* root microbiome interactions and regulation have been studied in several genotypes (Shakya et al., 2013; Beckers et al., 2017; Cregger et al., 2018, Liao et al., 2019; Veach et al., 2019). Poplar trees have the specificity to host different types of mycorhizal fungi (ectomycorrhizal (EcM) and arbuscular mycorhizal (AM) fungi), fungal endophytes (Karlinski et al., 2010) and bacterial communities (Harquard & Schadt, 2015; Timm et al., 2018). Last but not least, poplar is an important species in the Northern hemisphere forestry with 80 million hectares of trees in the world (FAO, 2004). In France, poplar culture represent 23 % of the annual broad leaves trees yields and french industries should have difficulties in supply in 2023 (source CODIFAB).

a



b

	T0 Soil	T0 Roots		T2 Roots		T4 Roots		T7 Roots		T10 Roots		T15 Roots		T21 Roots		T30 Roots		T50 Roots	
Technical approach	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Number of samples collected	3	2	5	2	5	2	5	2	5	2	5	2	5	2	5	2	5	2	5
Completion of rarefaction (ITS)	3		0		5		4		5		4		4		5		5		5
Completion of rarefaction (16S)	3		0		4		5		5		5		5		5		5		4

Figure 15 - Experimental design (a) and number of samples used for each experimental approach in this study (b).

We characterized root colonization by fungal and bacterial communities of *Populus tremula x alba* cuttings cultivated in axenic conditions and transferred in natural soil taken from a *Populus* plantation using 16S and ITS rRNA gene-targeted Illumina MiSeq sequencing and Confocal Laser Scanning Microscopy (CLSM).

Material & Methods

Biological material and sample preparation

Populus tremula x alba (INRAE clone 717-1B4) vitroplants were cultivated on Musharige & Skood (MS) supplemented with IBA (2ml.L^{-1}) during one week before transfering them on MS for two weeks at 24 °C in growth chamber (photoperiodicity of 16h, light intensity of 150 umol.m^{-2}.s^{-1}) until root systems were developed as described in Felten et al (2009). Soil was collected from an 18-year-old poplar stand planted with *Populus trichocarpa* x *deltoides* and located in Champenoux, France (48° 51' 46'' N/2° 17' 15'' E). The first soil horizon (0-15 cm) was collected after pruning of brambles and adventitious plants and litter removal with a ratle. Then, soil was maintained at room temperature and homogenised through sifting at 2mm and fixed at 75% of humidity. Bulk soil was sampled in triplicate and stored at -20°C until DNA extraction.

Rooted vitroplants were selected to be homogeneous in terms of th size of the aerial part and the root system. Selected vitroplants were transplanted in natural soil in transparent plastic pots with a filtered cover allowing gas exchange and a dark area at the ground level to prevent algae development. Plants were cultivated in growth chamber (photoperiodicity of 16h, light intensity of 150 umol.m^{-2}.s^{-1}). Humidity in pots was maintained at 75% during all the experiment by regular wattering. Vitroplants were harvested after 0, 2, 4, 7, 10, 15, 21, 30 and 50 days of growth (Figure 15). At the beginning of the experiment (time-point T0) and at each time point, the root system (corresponding to the endosphere) of five plants were harvested, rinsed in sterile water, scanned to check root growth (WinRHIZO software v. 2009c, Regent Instrumentals, Québec, Canada), freezed in liquid nitrogen and stored at -20°C until DNA extraction. Two additional plants were harvested and roots fixed in a solution containing 3 volumes of 1X phosphate-buffered saline (PBS : 0,13 M NaCl, 7 mM Na2HPO4, 3 mM NaH_2PO_4, pH 7,2) for 1 volume of 3% para-formaldehyde (PFA) overnight at 4°C. At T30 and T50 time point, the root system was sufficiently developed to be splited in two equal part to performed these two technical approach on all plants.

Monitoring of vitroplant growth and EcM root colonization monitoring

Total area of root systems were measured for each vitroplant collected at the different time points on scan images using ImageJ (Schneider et al., 2012) before freezing in liquid nitrogen or PFA fixation. Mycorhization rate of each vitroplants was quantified as previously described (Labbé et al., 2011). Briefly, each root system was rinsed with tap water and analyzed under a dissecting microscope. For each root system, 100 short roots were randomly examined and assessed as mycorrhizal or non-mycorrhiza.

Confocal laser scanning microscopy

Staining of root systems and fungi were adapted from Vierheilig et al., (2005) protocol. In brief, fixed root systems were washed 3 times in one volume of 1X PBS and a last wash in 1 volume PBS / 1 volume of 90% ethanol before clearing them during 2h at 90°C in 20% KOH. After 3 washes in distilled water, samples were incubated overnight in 1X PBS containing 10 µg.ml-1 WGA-Alexa fluor 488, a specific marker of the chitine based fungi cell wall. Then, root systems were washed in 1X PBS and incubated for 15 min in 1X PBS containing 10 µg.ml-1 of propidium iodide, a marker of the root cell wall before 3 wash in 1X PBS. Samples were mounted between slide and cover slip with a drop of SlowFade solution (Life Technologies) containing the DNA staining DAPI. All root samples were observed with a ZEISS LSM 780 (ZEISS International) confocal laser scanning microscope (CLSM). WGA-AF488 was excited using 488 nm excitation wavelenght and detected at 500-540 nm whereas 561 nm excitation wavelenght and detection at 580-660 nm were used regarding propidium iodide. Maximum intensity projections were performed using the ZEN software with z-stack of 30 to 50 µm.

Optic Microscopy

Blue staining of fungal stuctures was adapted from Vierheilig et al., (2005) and Walker (2005). Cleared roots were incubated at 90°C in KOH 10% during 20 min. After few washes in distilled water, root systems were incubated for 10 min in 0,1 N HCL at room temperature. We removed HCL without washing and we incubated the root systems during 30 min at 90°C in acidified ink (5% Waterman ink, 20% lactic acid, 75% water). Finally, roots were washed in distilled water before being mounted between slide and cover slip with a drop of glycerol 20 % for observation under the OLYMPUS BX41 optic microscope.

DNA extraction, Illumina Miseq amplicon sequencing and quantification of microorganisms on roots

Approximatively 250 mg of bulk soil samples was used for each individual soil DNA extraction. Soil DNA was extracted using the DNeasyPowerSoil Kit following the protocol provided by the manufacturer (Quiagen, Venlo, the Netherlands). For root systems, fifty mg of root tissue were crushed in liquid nitrogen with mortar and pestle. DNA was extracted using the DNAeasy Powerplant Kit (Quiagen, Venlo, the Netherlands). All extractions were quantified on a Nanodrop 1000 spectrophotometer (Nanodrop Products, Wilmington, DE, USA).

A two-step PCR approach was performed in this study to barcode tag templates with frameshifting nucleotide primers. Forward and reverse primer mixtures were used to maximize phylogenetic coverage of bacteria and fungi. Primer mixtures for tagging bacterial amplicons were composed of 4 forwards and 2 reverses 515F and 806R primers screening the 16S rRNA V4 gene region in equal concentration (0,1µM; Mangeot-Peter et al., 2020). Primer mixtures for tagging fungal amplicons were composed of 6 forward and 1 reverse for ITS1 – ITS4 rRNA region at equal concentration (0,1 µM; Mangeot-Peter et al., 2020). To inhibit plant material amplification, a mixture of peptide nucleotide acid (PNA) blockers targeted plant mitochondrial and chloroplast 16S rRNA genes and plant 5.8S nuclear rRNA gene were added in PCR reaction mixes (Mangeot-Peter et al., 2020). Polymerase chain reaction (PCR) were performed for three replicates of eac sample (2µl isolated DNA at about 10 ng/µl) using 2.5x Phusion flash high fidelity master mix (ThermoScientific) with 1.5 µl of forward and reverse primer mix,

0.75 µl of PNA probe and 8.5 µl of 0.2µm filtered UV treated DNA free water (Carl Roth, France) in a total reaction volume of 30 µl per sample. Thermal cycler conditions for the primary PCRs for bacterial amplification in soil and root samples were 30 cycles of 98°C for 5s, 78°C for 10s, 52°C for 20s and 72°C for 15s. Primary PCR condition for fungal amplification in soil and root samples were 30 cycles of 98°C for 5s, 78°C for 10s, 55°C for 20s and 72°C for 15s. PCR products without addition of microbial DNA (negative control), mock communities of known fungal or bacterial compositions were added as quality controls. Samples of 50 µl (30 ng DNA per µl) were sent for tagging and MiSeq Illumina Next Generation Sequencing (GeT PlaGe INRAE sequencing platform, Toulouse, France).

Sequence processing

Bacterial sequences were further processed with FROGS (Find Rapidly OTU with Galaxy Solution; Escudié et al., 2018) based on the Galaxy analysis platform (Afgan et al., 2016). Sequences were demultiplexed, dereplicated, sequence quality was checked, oligonucleotides, linker, pads and barcodes were removed from sequences. Sequences were removed from data set, if they are non-barcoded, exhibited ambiguous bases or did not match expectations in amplicon size. Remaining sequences were clustered into operational taxonomic units (OTUs) based on the iterative Swarm algorithm, then chimeras and OTU containing only one, two, three or four one sequences were removed. Bacterial double affiliation was performed by blasting OTUs against SILVA database (Quast et al., 2012) and the ribosomal database project (RDP) classifier (Wang et al., 2007). OTUs with affiliation <100% at the phylum level (indicated by a RDP bootstrap value <1) and corresponding to chloroplasts or mitochondria were removed from the data set. OTUs at lower taxonomic ranks than the phylum level were considered as “unidentified” below when the RDP bootstrap value was < 0.70. OTUs with high abundances in negative controls were excluded from further analysis. Sequencing, and affiliation quality was also evaluated based on the results obtained for the bacterial mock community.

After demultiplexing and quality checking (QC quality score = 30, minimal size = 200 bp), bioinformatics analyses were performed using standard procedures as described in Pérez-Izquierdo et al. (2017).

For both fungal and bacterial data, per-sample rarefaction curves were produced to assess sampling completeness, using function *rarecurve()* in package Vegan v3.5-1 (Oksanen et al., 2015) in R (version 3.4.3 ; R Core Team, 2016). Reads assigned to AM fungi were extracted from the data set before the rarefaction due to the very small number of reads. Samples with insufficient number of sequences according to rarefaction curves were removed. Based on these, subsequent analyses of diversity and community structure were performed on datasets where samples had been rarefied with the Phyloseq (McMurdie and Holmes, 2013) package to achieve equal read numbers according to the minimum number of total reads in any sample (4,964 reads for fungi and 21,142 reads for bacteria). Microbial community composition and structure in bulk soil and roots data were further analysed by using Phyloseq package (McMurdie and Holmes, 2013).

FUNGuild (Nguyen et al., 2016) was used to classify each fungal OTU into an ecological guild. OTUs identified to a guild with a confidence ranking to “highly probable” or “probable” were conserved in our analysis, whereas

those ranking to “probable” or with multiple assignation were called “unclassified”.

Statistical analysis

Statistical analyses and data representations were performed using R software (R Core Team, 2016). Fungal and bacterial community structure was determined using permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrices and compositional differences were visualized using a non-metric dimensional scaling (NMDS) ordination. Analysis of similarities (PERMANOVA) was performed to test statistically whether there is a significant difference between fungal and bacterial communities detected in each sampling time. After checking normality of the data distribution with Shapiro-Wilk test, one-way ANOVA test followed by a Tukey post-hoc test were used to detect significant difference in the relative abundance of dominant fungal and bacterial phyla, orders and genera of the soil and across root systems collected at the different time points. This procedure was also used to compare relative abundance of fungal guild and mycorrhization rate between root samples.

Results

Microbial sequencing

MiSeq sequencing of ITS and 16S rDNA amplicons were performed on soil and roots DNA samples between T0 and T50. After quality filtering and chimera and singleton removal, a total of 450,000 fungal reads (10,714 ± 775 reads per sample) and 1,740,000 bacterial reads (40,510 ± 3,962 reads per sample) were kept for further analyses. After taxonomic assignment, elimination of contaminants and completion of rarefactions, 227 fungal OTUs (59 ± 5 OTUs per sample) and 8,572 bacterial OTUs (1,945 ± 121 OTUs per sample) were detected (Figure 16).

Plantlet development and composition of soil microbiome

In order to investigate the temporal colonisation dynamic of *Populus* roots by fungal and bacterial communities, axenic cuttings of poplar were propagated in vitro to obtain rooted plantlets. Two weeks-old plantlets with a single main root of about 2 cm was then planted in pots containing natural soil. Monitoring of the growth of the root system indicated a slow development of the roots during the first 15 days followed by an acceleration of the growth in the next weeks (Figure 17 a). First short roots and ectomycorrhizae (ECM) were observed at 10 and 15 days, respectively. The rate of ectomycorrhization regularly increased to reach 37 % at 50 days post plantation and nearly doubled between T15 and T50 (Figure 17 b).

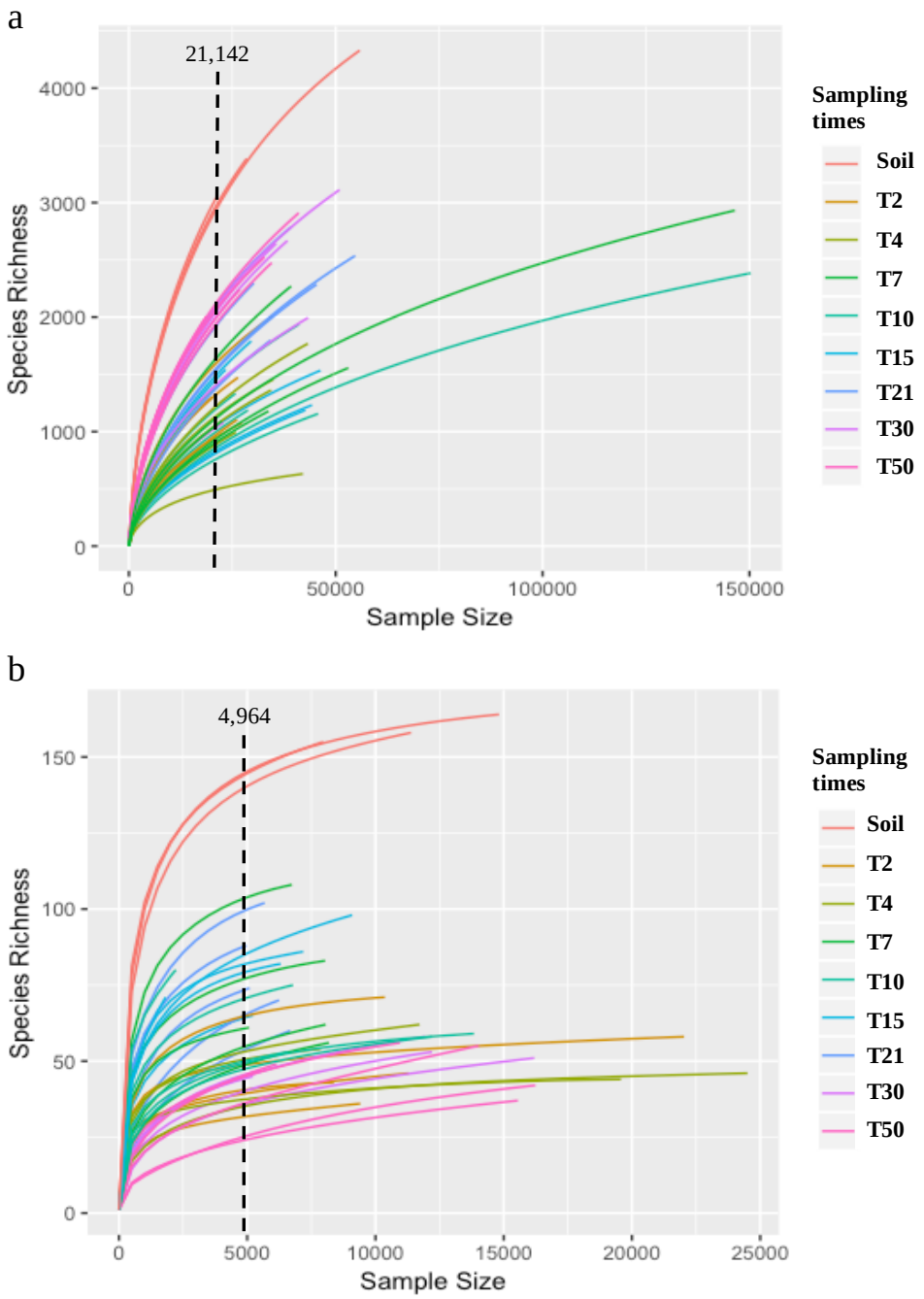


Figure 16 - Rarefaction curves of bacterial (a) and fungal (b) OTUs. Each curve represents one sample and sampling times are color-coded.

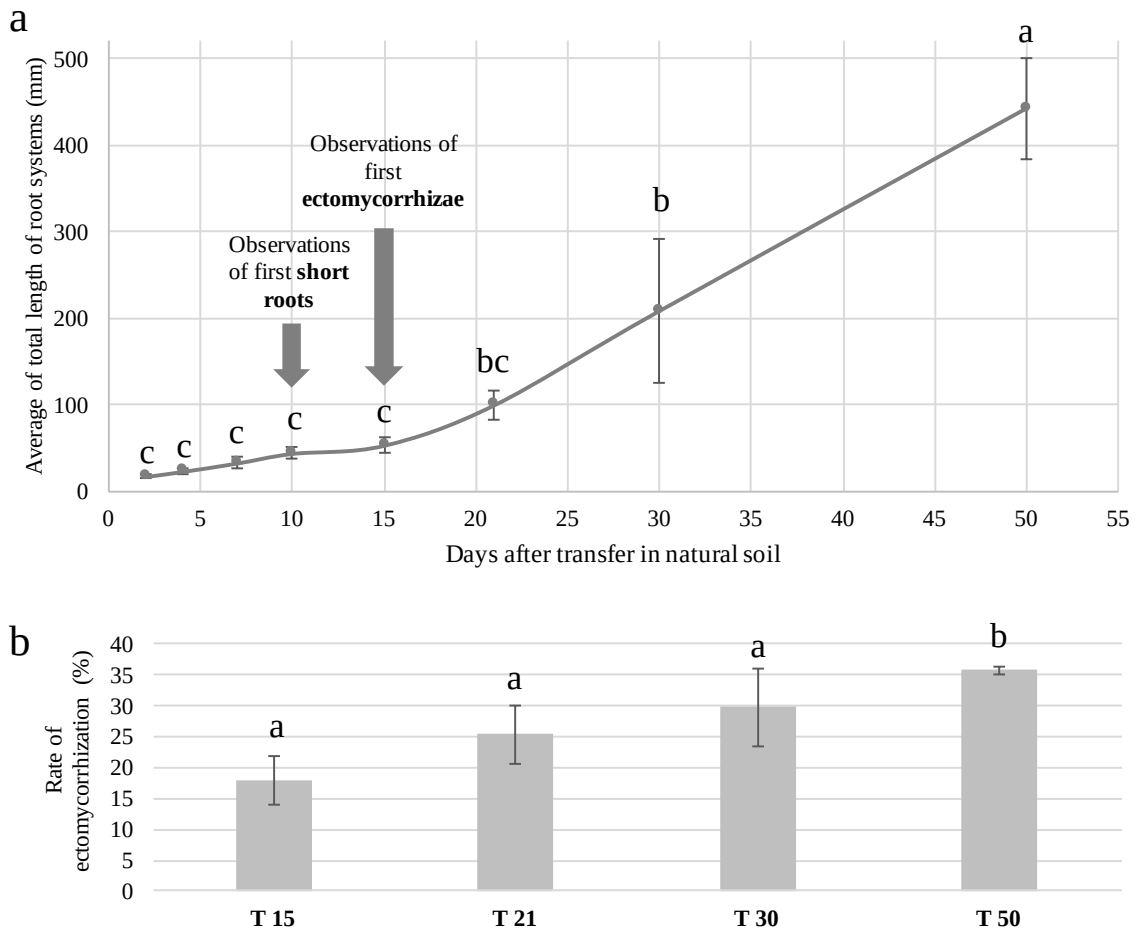


Figure 17 – Root development and ectomycorrhizae formation along time. Total length of the root system measured at each sampling time from T2 to T50 (a). Ectomycorrhization rate of Populus roots from T15 to T50 calculated as the number of fungal colonized lateral roots/ total number of lateral roots (b). Each given value is the average value of 7 replicates +/- SE. Different letters denote significant difference between each sampling time (One-way ANOVA, factor=sampling time, P<0.05).

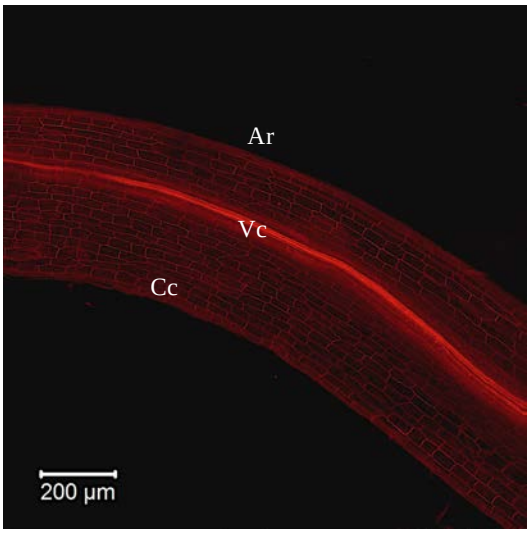


Figure 18 - Confocal microscopy image of Populus tremula x alba adventive root at the beginning of the experiment (T0). Plant cell walls were stained with propidium iodide and appear in red. Ar, Adventive Root; Vc, Vascular cylinder

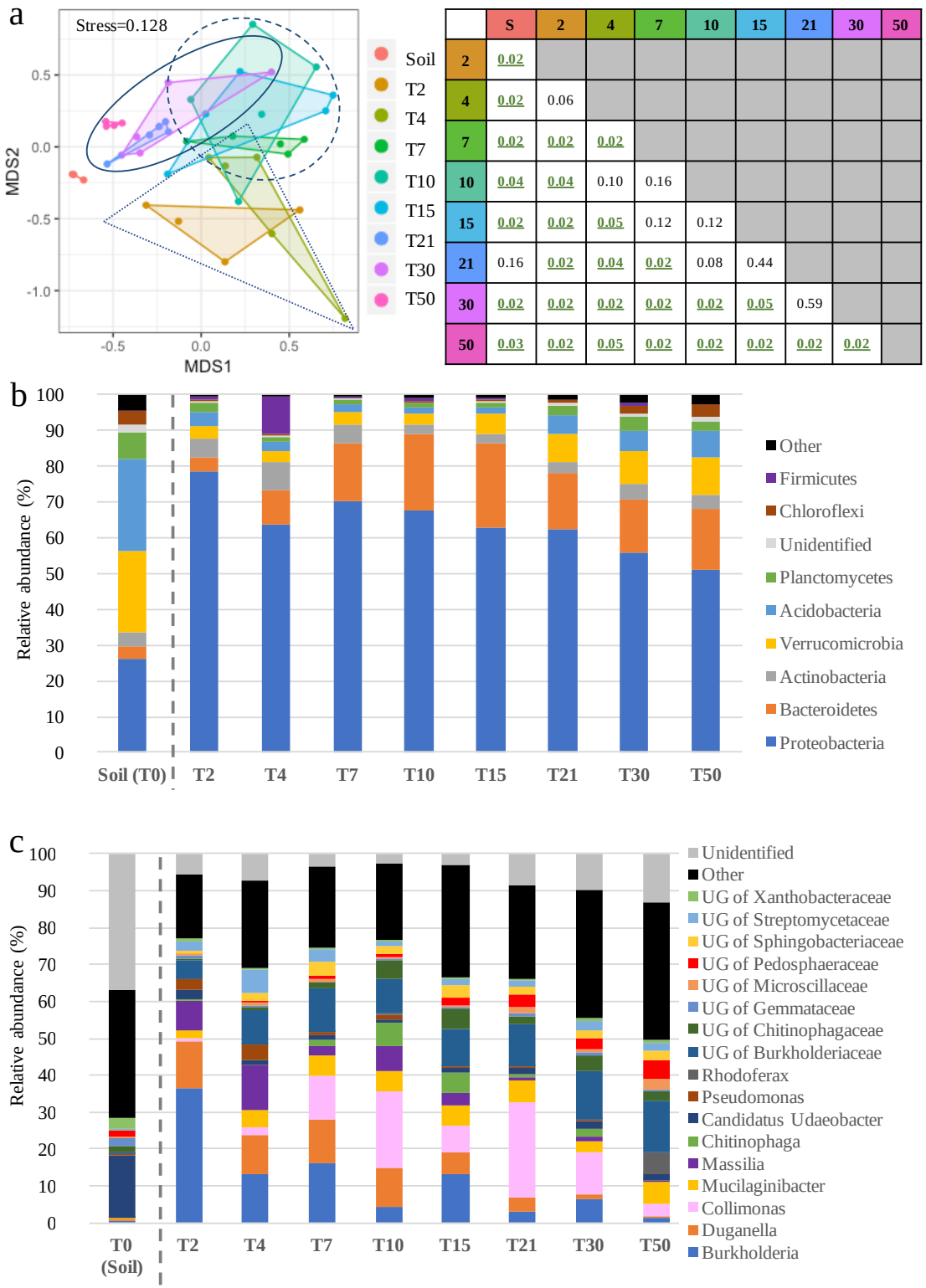
Fungal and bacterial colonization of the roots were tracked using two complementary methods: 16S and ITS rRNA gene-targeted Illumina MiSeq sequencing and confocal microscopy. We first validated the axenic status of the invitro plants: no amplification of ITS and 16S rDNA genes were obtained from samples of roots collected before vitroplants were transfered in natural soil. These results are in accordance with CLSM observations concerning fungal colonisation as no fungal structure could be visualised at T0 (Figure 18). By contrast, soil was heavily colonized by complex bacterial and fungal communities at T0, as expected from a previous study on soil taken from the same poplar plantation (Mangeot-Peter et al., 2020): a total of 4,028 bacterial and 165 fungal OTUs were detected in the soil. The soil inoculum was dominated by nine bacterial (> 1 % in relative abundance) and six fungal phyla. Proteobacteria (26.1 ± 0.4 %), Acidobacteria (25.9 ± 0.1 %) and Verrucomicrobia (22.6 ± 1.2 %) dominated the bacterial community while Basidiomycota (51.6 ± 2.9 %), Zygomycota (24.4 ± 1.3 %) and Ascomycota (20.1 ± 2.3 %) dominated the fungal soil community (Table S1, Table S2). Eight bacterial genera (>1 % in relative abundance) and 11 fungal genera were detected in bulk soil collected at T0. OTUs from *Candidatus Udaeobacter* largely dominated the soil bacterial community as they represented 17 % of the reads in average (Table S1). Members of a few genera also dominated the soil fungal community: *Sebacina* (EcM, 19.1 ± 0.8 %), *Umbelopsis* (saprophyte, 12.3 ± 0.8 %), *Mortierella* (saprophyte, 12.0 ± 1.1 %) and *Cryptococcus* (saprophyte, 8.3 ± 0.8 %) were the most abundant fungal genera detected in soil (Table S2). This initial soil fungal community was made of a mix of EcM fungi (34.9 ± 3.1 %), saprotrophic fungi (16.7 ± 1.8 %), fungal endophytes (13.0 ± 1.2 %) and AM fungi (0,3 %; 14 OTUs) (Table S3).

Structure and composition of bacterial communities associated to *Populus* roots along sampling time

Bacterial colonization of *Populus* roots was observed after two days of growth (T2). At the OTU level, the structure of bacterial communities significantly shifted between roots samples collected from T2 to T50 (except for root samples collected at T21) and bulk soil samples collected at T0 (PERMANOVA, P<0.05, Figure 19 a). In addition, significant shifts in the structure of bacterial communities were also observed between roots samples collected from T2 to T50 although close time points not statistically different (e.g T2-T4, T15-T21…, PERMANOVA test, P<0.05; Figure 19 a).

The number of bacterial OTUs detected in roots tended to increase after 21 days of growth in soil and almost doubled between T2 and T50 (Table 2). Similarly, diversity slowly increased over time as indicated by Shannon index values (Table 2).

A detailed analysis of bacterial community compositions across the different sampling times revealed an early selection of bacterial communities by the *Populus* roots. Community composition of the roots differed greatly from that of the soil as early as two days after planting. Proteobacteria, and particularly *Burkholderiaceae* (*Burkholderia, Duganella, Massilia*, *Cupriavidus*) massively colonized the roots, reaching 36 % of the reads at that time point (Figure 19 b, c).



	S	2	4	7	10	15	21	30	50
2	0.02								
4	0.02	0.06							
7	0.02	0.02	0.02						
10	0.04	0.04	0.10	0.16					
15	0.02	0.02	0.05	0.12	0.12				
21	0.16	0.02	0.04	0.02	0.08	0.44			
30	0.02	0.02	0.02	0.02	0.02	0.05	0.59		
50	0.03	0.02	0.05	0.02	0.02	0.02	0.02	0.02	

Figure 19 - Structure and composition of bacterial communities colonizing Populus roots across time. Non-metric multidimensional scaling (NMDS) ordinations of bacterial OTU across compartments (soil and roots) and sampling times (from T2 to T50). P-values of variances explanation based on permutational multivariate analysis using Euclidean dissimilarity matrix for bacterial communities. P-values < 0.05 appear in green) (a). Distribution of most abundant bacterial phyla (>2 % in relative abundance in at least one sampling time) detected in bulk soil and in Populus roots at each sampling (b). Distribution of most abundant bacterial genera (>1 % in relative abundance) detected in bulk soil and in Populus roots at each sampling time (c).

By contrast, Acidobacteria and Verrucomicrobia were strongly counter-selected compared to the bulk soil at T2. Then, the composition of the root bacterial communities clearly evolved over time from T2 to T50. While still dominant, the proportion of Proteobacteria significantly decreased from 78 % at T2 to reach 55 % at T30 (Figure 19 b, Table S1, ANOVA, $p<0.05$). Proteobacteria were slowly replaced by members of the Verrucomicrobia, Acidobacteria, Bacteroidetes and Chloroflexi. By contrast, proportion of Actinobacteria and Planctomycetes in the roots remained stable all along the time of the analysis. Different types of behaviours were detected at the genus level: some bacterial genera, mainly belonging to the *Burkholderiaceae* family strongly decreased in proportion along time. For instance, OTUs of the *Burkholderia-Caballeronia-Paraburkholderia* genus decreased from 36 % at T2 to 1.2 % at T50. By contrast, OTUs belonging to the *Chitinophaga* or *Sphingomonas* tended to pick between T10 and T15 and then decreased until T50. A third type of OTUs such as *Rhodoferax*, *Rhizobacter* or *Bradyrhizobium* reached their maximum relative abundance at T50. In addition, OTUs belonging to bacterial genera *Clostridium sensu stricto 9*, *Dyella*, and an unidentified genus of *Methylophilaceae* were not detected in the bulk soil but were found at different colonisation time of the experiment. *Clostridium sensu stricto 9* and *Dyella* relative abundance increased at T4 (Table S1) and fluctuated until the end of the expermiment wherease the unidentified genus of *Methylophilaceae* was the most abundant between T15 and T21 (Table S1). Finally, some genera strongly varied in abundance between root systems collected at the same time. For instance, *Collimonas* massively colonized some root systems between T7 and T30, reaching up to 25 % of the reads in some roots while being almost absent from other roots (Table S1).

Structure and composition of fungal communities associated to *Populus* roots

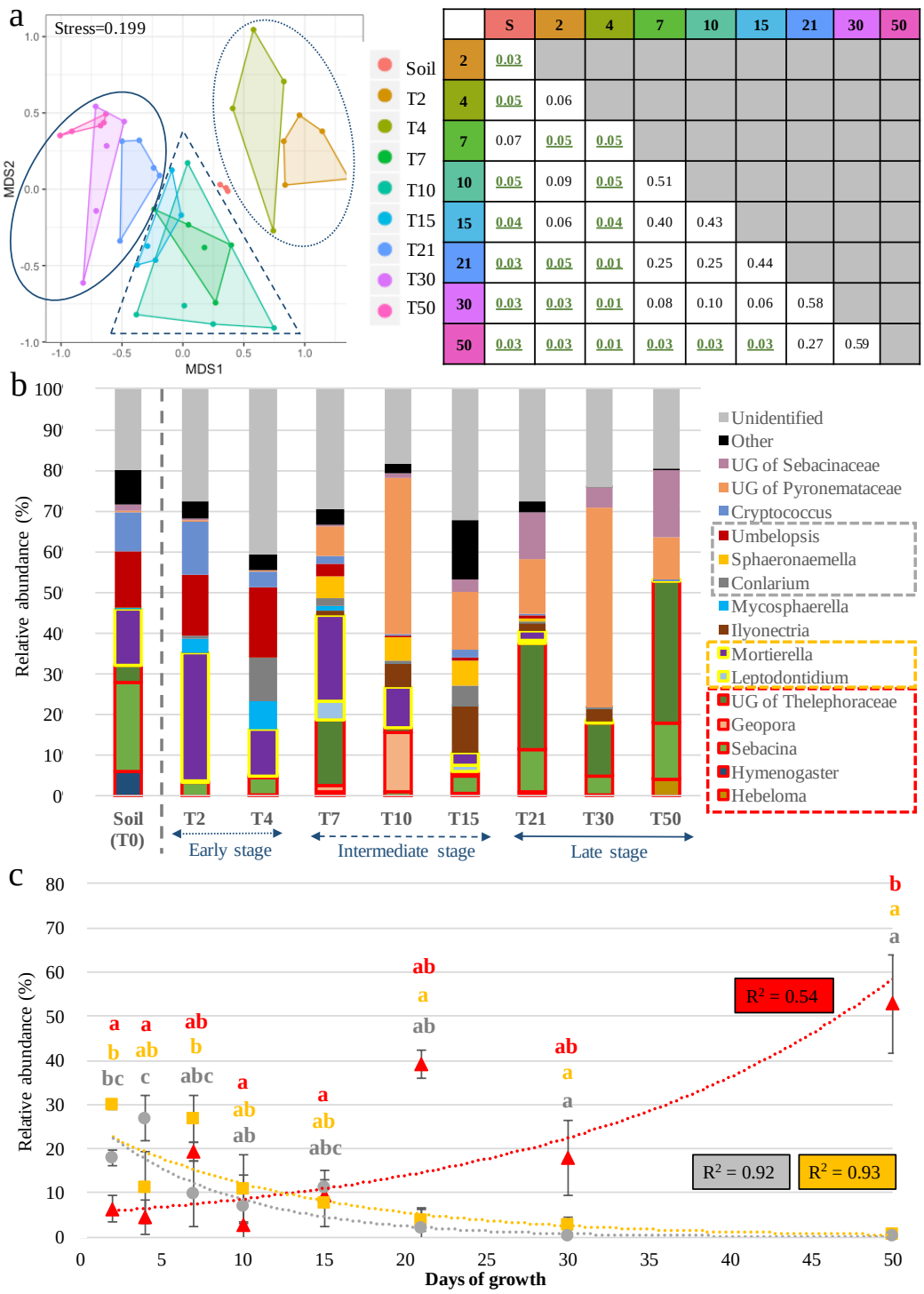
Like bacteria, we detected the presence of fungi on *Populus* roots after two days of growth in soil (T2) by Illumina Miseq sequencing of ITS amplicons. At the OTU level, the structure of fungal communities significantly shifted between roots samples collected from T2 to T50 (except for root samples collected at T7) compared to bulk soil samples collected at T0 (PERMANOVA, $P<0.05$, Figure 20 a). In addition, significant shifts in the structure of fungal communities were also observed between roots samples collected from T2 to T50 although close time points not statistically different (PERMANOVA test, $P<0.05$; Figure 20 a).

The number of fungal OTUs increased until 15 days of growth in natural soil then decreased until 50 days (Table 3). Diversity was stable between T2 and T10 and slowly tended to decrease over time between T10 and T50 as indicated by Shannon index values (Table 3).

A detailed analysis of fungal community composition across the different sampling times revealed a later selection of fungal communities colonizing *Populus* roots than for bacterial communities. As for bacteria, we observed a high variability in fungal colonization of the root system of *Populus*. This observation was made especially for the most abundant fungal genera in roots collected. However, we could distinguish three stages of root colonization: an early from T2 to T4, an intermediate from T7 to T15 and a late stage from T21 to T50 (Figure 20 b). The early fungal community was dominated by few saprotrophic fungi such as the yeast *Umbelopsis* and *Cryptococcus* and the filamentous saprotrophe/endophyte *Mortierella* that all together made

more than 50 % at T2 and 38 % at T4 of the reads (Table S3). Reads corresponding to EcM fungi were already detected at this stage but were in low abundance compared to the saprotrophs'ones and corresponded to 6 % of the reads at T2 and 4 % of the reads at T4 (for 9 genera detected). We detected the first Glomeromycota reads (*Claroideoglomus_sp*) after 4 days, even though their relative abundance remained very low (< 0.1 %, data not shown). The rhizosphere effect was also visible at this stage with a significant number of difference between soil and root composition. Indeed, we did not detect the fungal genera *Amylostereum*, *Daedaleopsis* and *Geopora* in the bulk soil, although these genera were found in the roots. A shift in the fungal community composition was observed at T7 with the significant increase of the relative abundance of some EcM fungi (e.g *Thelephoraceae* and *Hydnotrya*, Table S2) that all together made more than 19 % of the reads in T7 samples (Table S3). In addition, we observed that the relative abundance of saprotrophes and endophytes remained stable (37 % of the reads, Table S2) compared to the early stage of root colonization.

The relative abundance of AM fungi also increased from T7 (< 0.1 %) to T10 (around 0.5 %). *Archaeosporales_sp*, *Rhizophagus irregularis* and *Glomeromycota_sp* were present in a similar relative abundance at T7 while *R. irregularis* dominated at T10 (data not shown). At T10 and T15, the relative abundance of EcM fungi belonging to *Thelephoraceae* decreased while the relative abundance of EcM fungi of the *Sebacina, Hebeloma and Geopora* genera increased compared to T7 samples (Table S2). The total reads of EcM fungi reached 3 % at T10 and 10 % at T15 while the relative abundance of saprotrophes and endophytes reached 18 % at T10 and T15 (Table S3). *Paraglomus laccatum* was the only OTU belonging to the Glomeromycota detected in very low abundance in root samples (< 0.05 %) at T15 (data not shown). Finally, the relative abundance of Zygomycota decreased while the relative abundance of Basidiomycota increased in roots collected at T21, T30 and T50 (Table S2). The relative abundance of the fungal endophytes *Mortierella* and the saprotrophic fungi *Umbelopsis* significantly decreased at T21 and T30 and almost disappeared in roots collected at T50 while the relative abundance of the EcM fungi *Sebacina* and the EcM fungi belonged to *Thelephoraceae* significantly increased at T30 and T50 compared to the other sampling times (Figure 20 b, Table S2). In accordance with these observations, EcM fungi were significantly more abundant in roots collected at the late stage (52 % of the reads at T50, Table S3) while endophytes and saprotrophes were significantly more abundant during the early and the intermediate stages of colonization (Figure 20 c). From T21 to T30, we detected in very low abundance, two OTUs belonging to the Glomeromycota (*Paraglomus laccatum* and *Archaeosporales_sp*) before they vanished at T50 (data not shown).



	S	2	4	7	10	15	21	30	50
2	0.03								
4	0.05	0.06							
7	0.07	0.05	0.05						
10	0.05	0.09	0.05	0.51					
15	0.04	0.06	0.04	0.40	0.43				
21	0.03	0.05	0.01	0.25	0.25	0.44			
30	0.03	0.03	0.01	0.08	0.10	0.06	0.58		
50	0.03	0.03	0.01	0.03	0.03	0.03	0.27	0.59	

Figure 20 – Structure and composition of fungal communities colonizing Populus roots across time. Non-metric multidimensional scaling (NMDS) ordinations of fungal OTU across compartments (soil and roots) and sampling times (from T0 to T50). P-values of variances explanation based on permutational multivariate analysis using Euclidean dissimilarity matrix for fungal communities. P-values < 0.05 appear in green (a). Distribution of most abundant fungal genera (> 4 % in relative abundance in at least one sampling time) detected in bulk soil and in Populus roots at each sampling time grouped according to the stage of colonization: early, intermediate or late (b). Grey, yellow and red boxes highlight saprotrophic, endophytes and ectomycorrhizal fungi, respectively (b). Relative abundances of saprotrophic fungi (grey), fungal endophytes (yellow) and ectomycorrhizal fungi (red) detected in Populus roots at each sampling time (c). Each given value is the average of 4 to 5 replicates +/- SE. The dotted curves represent the exponential trend curves for each trophic. Letters denote significant difference between each sampling time (One-way ANOVA, factor=sampling time, P<0.05).

Table 2 – Diversity of the bacterial community detected in roots across time. Number of bacterial OTUs and Shannon indexes calculated for each root samples collected at the different sampling time from T2 to T50. Each given value is the average value of 4 or 5 replicates +/- SE. Different letters denote significant difference between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05).

Sampling time	Number of bacterial OTUs	Shannon index
T2	1019.3 ± 147.1 abc	3.5 ± 0.4 a
T4	828.4 ± 108.8 b	3.7 ± 0.2 a
T7	970.0 ± 120.0 abc	3.9 ± 0.2 ab
T10	906.2 ± 93.4 ab	3.5 ± 0.3 a
T15	963.2 ± 108.5 abc	4.3 ± 0.2 abc
T21	1477.8 ± 116.1 acd	4.4 ± 0.4 abc
T30	1507.4 ± 207.0 cd	4.9 ± 0.2 bc
T50	1738.5 ± 52.8 d	5.3 ± 0.1 c

Table 3 – Diversity of the fungal community detected in roots across time. Number of fungal OTUs and Shannon indexes calculated for each root samples collected at the different sampling time from T2 to T50. Each given value is the average value of 4 or 5 replicates +/- SE. Different letters denote significant difference between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05).

Sampling time	Number of bacterial OTUs	Shannon index
T2	43.0 ± 6.1 ab	2.1 ± 0.2 ab
T4	42.8 ± 4.3 abc	2.1 ± 0.2 ab
T7	65.8 ± 9.6 bc	2.4 ± 0.3 b
T10	53.0 ± 4.3 abc	2.1 ± 0.3 ab
T15	73.0 ± 4.9 c	2.6 ± 0.1 b
T21	68.0 ± 7.6 bc	2.3 ± 0.2 ab
T30	32.2 ± 2.1 a	1.4 ± 0.2 a
T50	32.4 ± 5.1 a	1.4 ± 0.3 a

Monitoring of fungal colonization in *Populus* roots by CLSM

MiSeq results brought global information about the structure and composition of microbial communities without knowledge about their spatial distribution. In order to deepen our understanding of the dynamic process of root colonisation, samples were also observed by CLSM.

The first fungal presence was detected by CLSM between 2 and 4 days of culture (Figure 21 a). We observed spores and extracellular hyphae colonising the surface of root system mainly from the apex (Figure 21 b). These colonisations were very heterogeneous from one sample to the other with some root apexes fully surrounded by fungal mycelia while other were only presenting few hyphae (Figure 21 c). The fungal extracellular hyphae were septed with a diameter under 1 µm and we observed a very low diversity of morphologies. During this time lap, we identified by light microscopy, as being melanised septed hyphae not stained by WGA-Alexa fluor 488, the early presence of dark septate endophytes (DSE) after 4 days of growth (Figure 22). Their hyphae were either extracellular or intercellular, but it was difficult to assess if they were located in the intracellular or apoplastic region. We detected an increased density of fungal morphologies by CLSM after 7 days of culture, in both the intercellular and intracellular root compartments. Fungal hyphae either developed between root cells, propagating in the apoplastic compartments particularly around epidermic regions or directly into root cells (Figure 23 a, b).

This apoplastic colonisation stayed heterogeneous along the root, and was dominantly present at the apex and in the root elongation zone. After 10 days, we observed an increase of the apoplastic and intracellular colonisation. Indeed, fungal hyphae were propagating from cell to cell by going through the root cell-walls and we were even able to see the pressure of the hyphae on the cell walls (Figure 23 b). Even though the global fungal diversity of morphologies remained poor at this stage of development, we noted the presence of septed and non-septed hyphae with diameters either inferior or superior to 1 µm and we still observed the presence of DSE. After 15 days of culture, we observed an important increase of fungal density and morphological diversity in the root systems. We identified within the same root region the occurrence of distinct fungal morphologies with the dominance of two major structures (Figure 23 c). We detected the first dominant morphology in the intracellular compartment propagating from cell to cell and displaying an « arbuscular mycorrhizal like » shape (Figure 23 d). Its hyphae diameter was inferior to 1 µm and developed going through the cell wall from the epidermic to the central cell forming a grid shaped network. The second dominant morphology was propagating in both the intracellular and intercellular compartments, with hyphae diameter closer to 5 µm and exhibiting « hand glove-like » structures (Figure 23 c). When in the intercellular compartment, this structure seemed to surround the root cell. The development of lateral roots after 15 days of culture was correlated with the establishment of the first distinct ectomycorrhizal structures (Figure 24). Most EcM root tips already exhibited a mantle and a Hartig net (Figure 24), however some EcM did not have a fully formed mantle and hyphal colonization originated either from the apical region or from the bottom of the main root system. The density of colonization and the occurrence of ectomycorrhizal structures were heterogeneous among the different root systems. Nevertheless, many fungal morphologies were present within the same region, both on lateral and main roots (Figure 24).

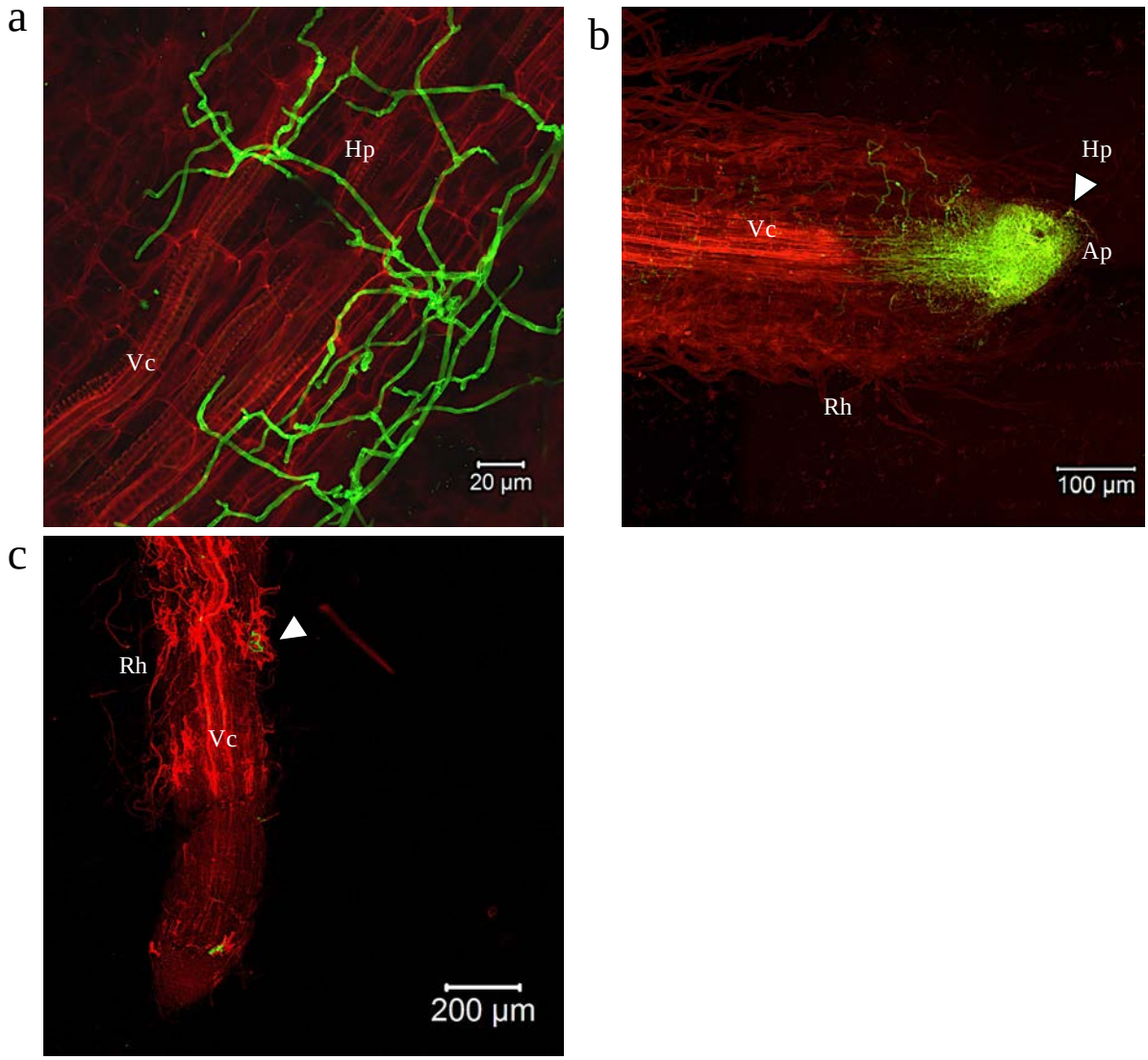


Figure 21 - Early stage of the fungal colonization dynamic. Confocal microscopy images of poplar roots colonized by fungi after 4 days of culture. (a) Extracellular hyphae surrounding a root after 4 days of culture. (b) Hyphae accumulation at the apex of the root after 4 days of culture. Hyphae on root hairs after 4 days of culture (c). Fungal structures appear in green through WGA-Alexa Fluor 488 staining while root cell-walls were stained with propidium iodide and appear in red. Ap, Apex; Vc, Vascular cylinder; Hp, Hyphae; Rh, Root hair.

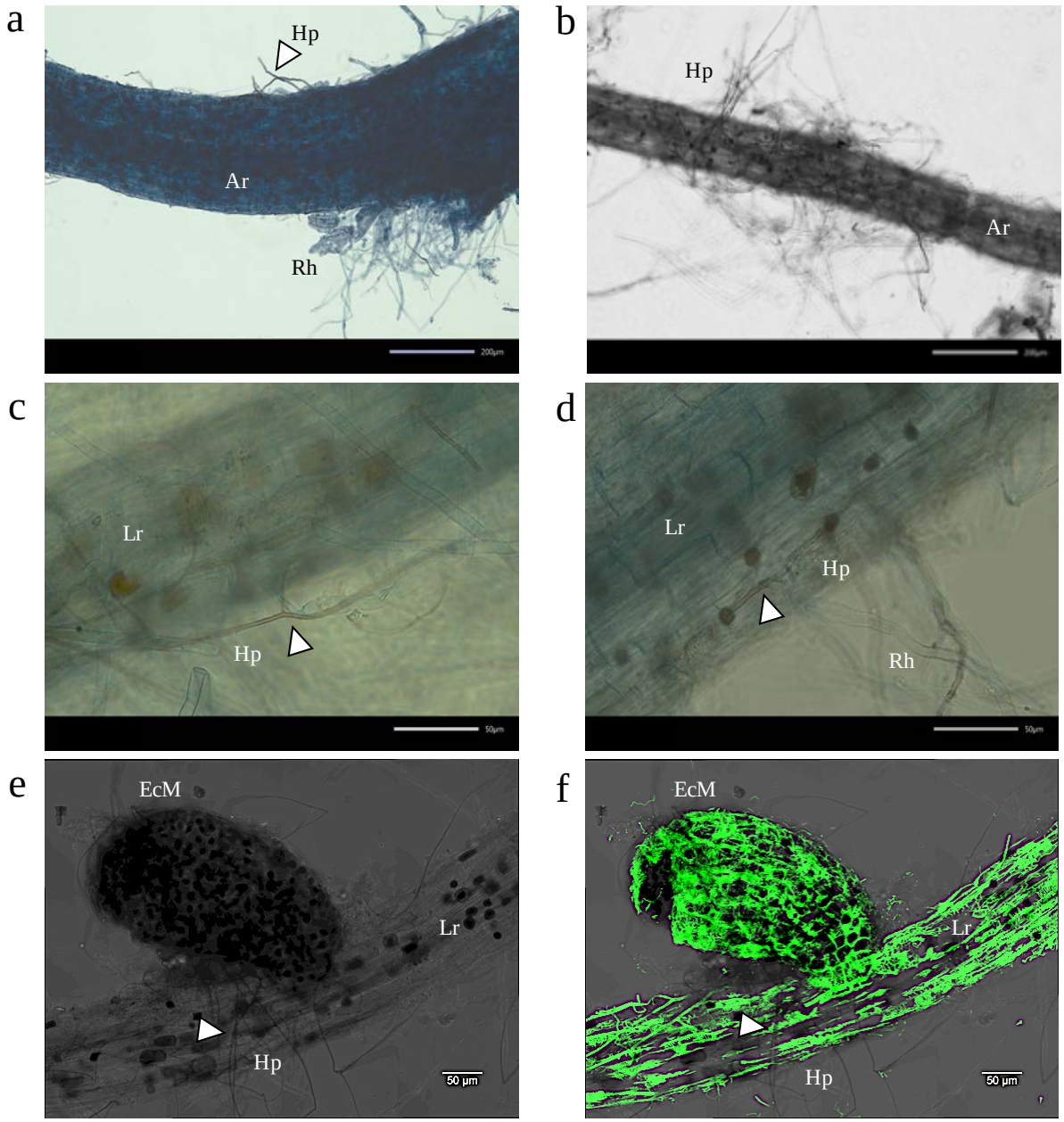


Figure 22 - Dark Septate Endophyte (DSE) colonizing poplar roots from 4 to 30 days of culture. Optic and confocal microscopy images of poplar roots colonized by DSE. (a) External colonization of roots by DSE after 4 days of culture. (b) Extracellular DSE hyphae surrounding an adventive root at 10 days of culture. (c) Extracellular DSE hyphae after 21 days of culture. Arrow indicates the DSE septa. (d) Intracellular DSE hyphae propagating in the apoplastic compartment after 21 days of culture. Arrow indicates the DSE septa. (e) Extracellular DSE hyphae surrounding an EcM forming on a lateral root after 30 days of culture. Arrow indicated the DSE hyphae. (f) Overlay of image (e) and the green track in order to visualize both melanized and non-melanized fungal structures. Non-melanized fungal structures appear in green through WGA-Alexa Fluor 488 staining. Ar, Adventive root; Hp, Hyphae; Lr; Lateral root; Rh, Root hair.

From 21 to 50 days of growth, we observed via CLSM a global increase of the fungal diversity and density within the same root region with some roots systems being colonised from the apex to the top of the root at 50 days, even though it remained heterogeneous within the different root systems. We still observed DSE, both inter or intracellular and we detected two new abundant fungal morphologies that were sometimes located within the same root region. The first structure was developing in the intracellular compartment in both adventive and lateral roots, displaying a globular shape with hyphae diameter inferior to 1 µm (Figure 25 a). The second morphology was only present in lateral and mycorrhized roots, with hyphae diameter superior to 1 µm and displaying a « maze like » structure (Figure 25 a). Its location between the inter/intra compartments, as well as its origin were difficult to determine, but it is noteworthy that it was often associated and seemed to develop within ectomycorrhizal structures (Figure 25 b). In addition, we observed fungal structures developing between the adventive and the lateral root forming a potential EcM (Figure 25 c). The apex of the lateral root was not colonized by any fungal structure suggesting that the EcM forming originated from pre-existing fungal structures on the adventive root. We also detected the presence of germinating spores with emerging hyphae colonizing the root cells (Figure 25 c). We observed an increase of EcM establishment (Figure 25 d) and we assessed by mycorrhizal counts under CLSM that 37 % of lateral roots were forming ectomycorrhizal structures at the end of the experiment, even if their presence was also variable depending on the root systems (Figure 17 b). We observed DSE until the end of the experiment at 50 days and we detected some of them in the same region where ectomycorrhization was taking place (Figure 22).

Regarding the lateral roots, we observed the successional replacement of the « arbuscular like » structures to the benefit of the « hand gloves like » structures and EcM. We did not observe this pattern in the main roots, where the « arbuscular like » morphologies continued to develop among the « hand gloves like » structures.

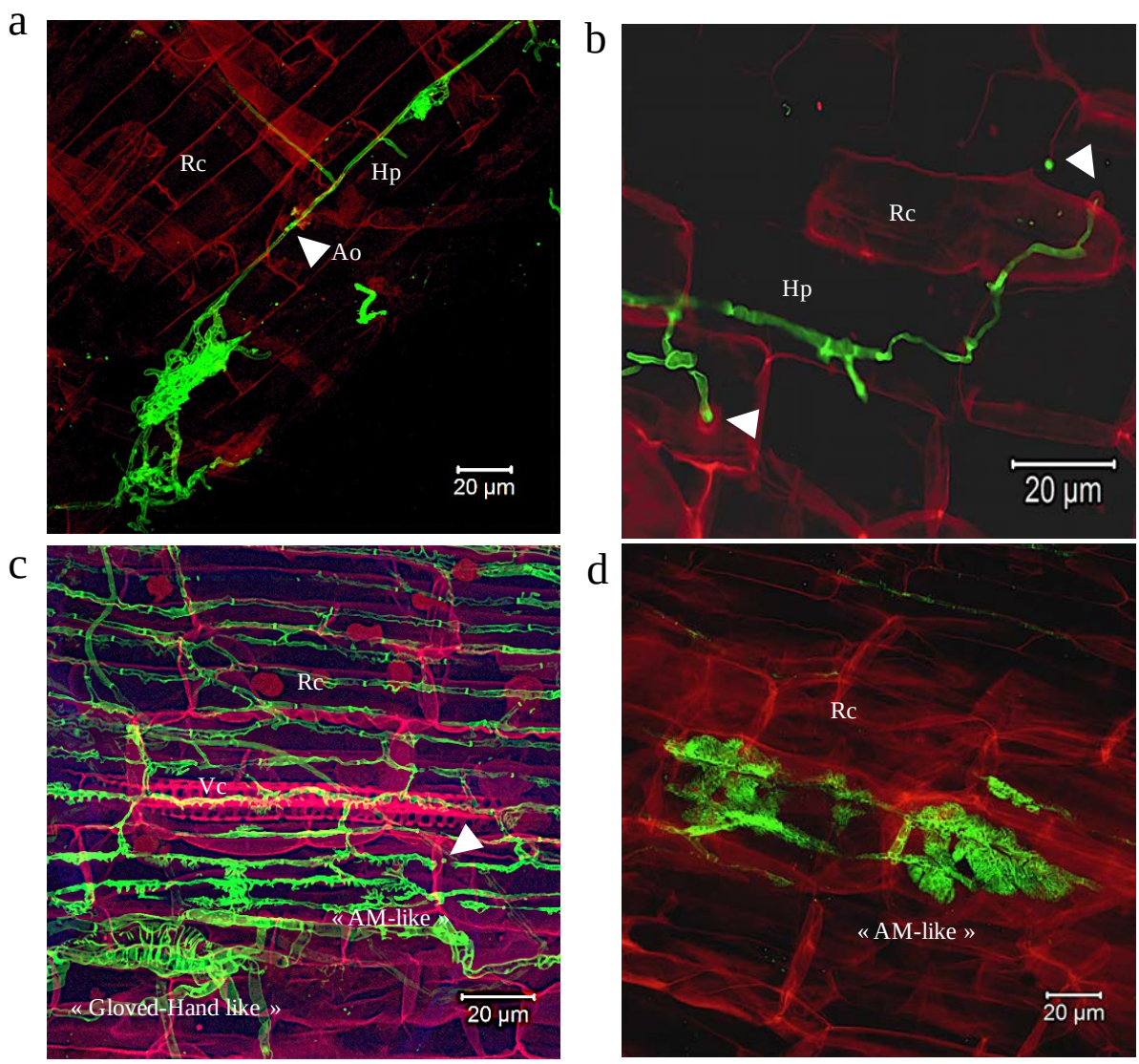


Figure 23 - Intermediate stage of the fungal colonization dynamic. Confocal microscopy images of poplar roots colonized by fungi after 7 to 15 days of culture. (a) Development of fungal hyphae in the apoplastic space of cortical cells of poplar after 7 days of culture. (b) Intracellular hyphal penetration in root cell after 7 days of culture. Arrows indicate the deformation of the root cell under the hyphal pressure. (c) Co-existing fungal morphologies (« arbuscular like » and « glove-Hand like »), within the same root region after 15 days of culture. Arrows indicate hyphal intracellular penetration. (d) « Arbuscular like » morphology observed in poplar root after 15 days of culture. Fungal structures appear in green through WGA-Alexa Fluor 488 staining while root membrane was stained with propidium iodide and appear in red. Ao, Apoplastic space; Hp, Hyphae; Rc, root cell; Vc, Vascular cylinder

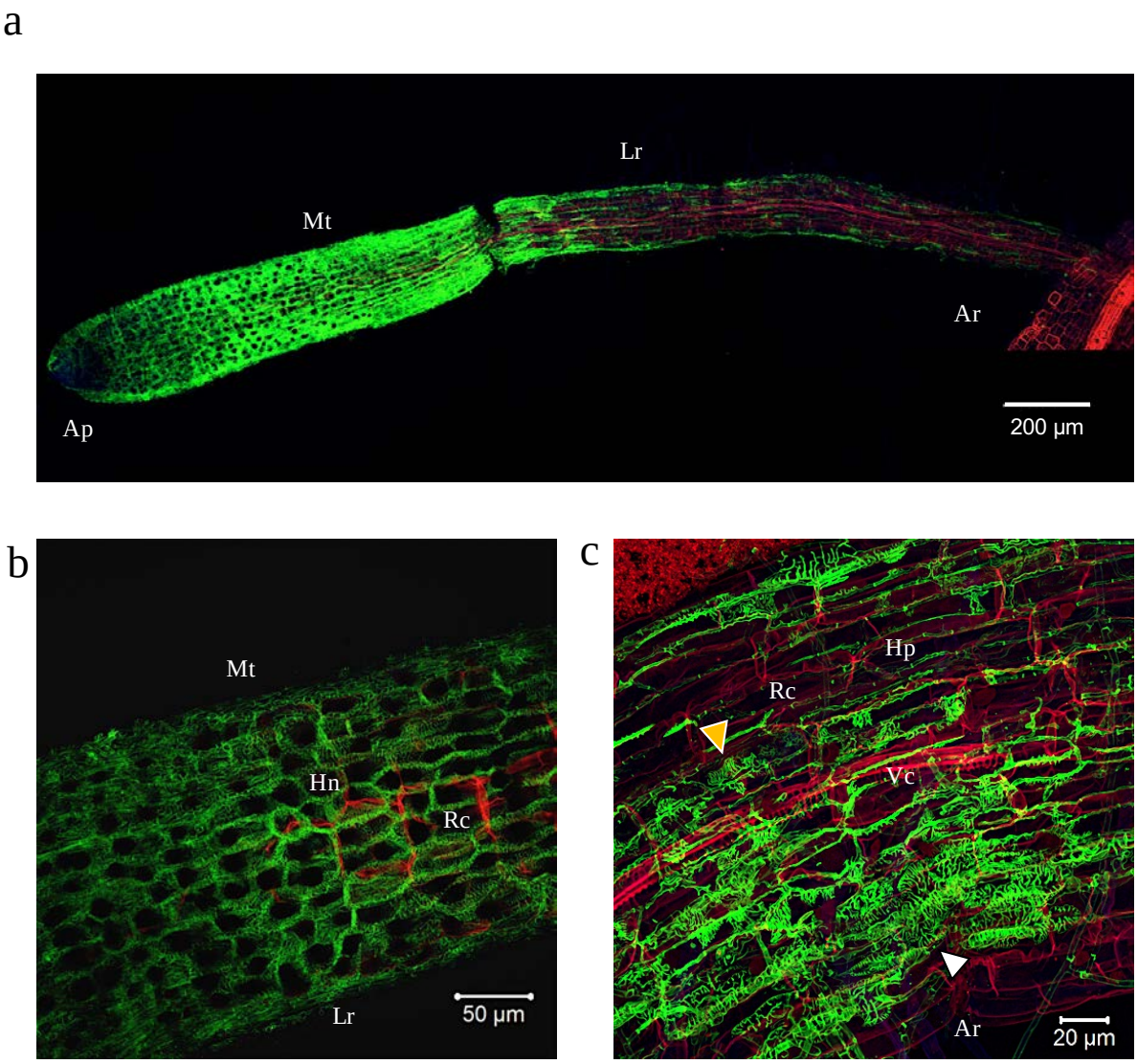


Figure 24 - Intermediate stage of the fungal colonisation dynamic. Confocal microscopy images of poplar roots colonised by fungi after 7 to 15 days of culture. (a) EcM formation on a lateral root after 15 days of culture. (b) Hartig net formation on EcM after 15 days of culture. (c) Co-existing and abundant fungal morphologies within the same root region after 15 days of growth. Orange arrow indicates the « arbuscular-like » and white arrow indicates the « hand glove-like » fungal structures. Fungal structures appear in green through WGA-Alexa Fluor 488 staining while root cell-walls were stained with propidium iodide and appear in red. Ar, Adventive root; Ap, Apex; Hn, Hartig net; Hp, Hyphae; Lr; Lateral root, Mt, Mantle; Rc, Root cell; Vc, Vascular cylinder

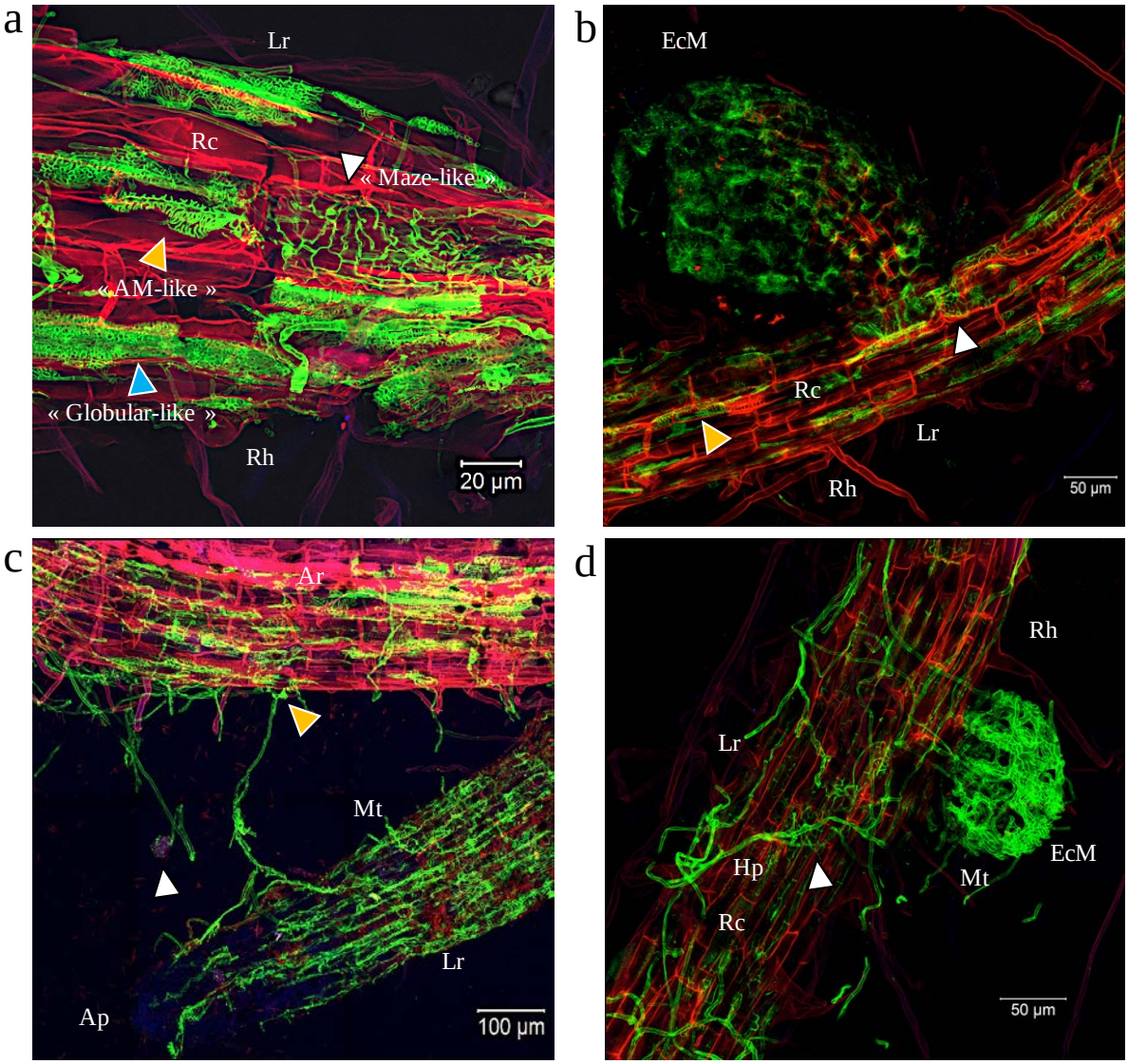


Figure 25 - Late stage fungal of the colonization dynamic. Confocal microscopy images of poplar roots colonized by fungi after 21 to 50 days of culture. (a) At least three different morphologies are co-existing within the same root region after 21 days of culture. White arrow indicates the « maze like » structure, the blue arrow shows the « globular like » and the orange arrow indicates « arbuscular like » structure. (b) Mycorrhizal formation with co-existing fungal morphologies after 30 days of culture. Orange arrow indicates the « arbuscular-like » structure and the white arrow the presence of the « maze like » fungal morphology, that seemed to be linked with the « glove-Hand like » morphology and the EcM forming structure. (c) Hyphal propagation at T30 between the adventive root to the lateral root forming a probable EcM. White arrow indicates a germinated spore and the orange arrow the « hand glove like » morphology. (d) EcM formation after 30 days of culture. Arrows indicate the « maze like » structure that seems to originate the EcM. Fungal structures appear in green through WGA-Alexa Fluor 488 staining while root cell-walls were stained with propidium iodide and appear in red. Ar, Adventive root; Ap, Apex; Hp, Hyphae; Lr, Lateral root; Mt, Mantle; Rc, Root cell; Rh, Root hair

Discussion

The establishment of the plant root microbiome is a dynamic process involving complex communities of microorganisms with distinct trophic mode and functions. These interactions, both between plant-microbes and microbes-microbes lead to microbial species turn over along with the tree development. If studies of the colonisation dynamic of roots by specific stains of bacteria or of single fungi have been performed in different plant species, especially to develop biocontrol strategies (Bueno de Mesquita et al., 2018; Noirot-Gros et al., 2018; Mesanza et al., 2019) , no study has been done so far to understand the colonisation dynamic of tree roots by complex microbial communities, so far. Here, we developed a microcosm to grow axenic Poplar in natural soil and to track the colonization of the root system by microorganisms. To do so, we used two complementary methods, CLSM and metabarcoding in order to investigate how the bacterial and fungal colonisation occurs in the root system of *Populus tremula x alba* grown in natural soil. The transfer of plantlets from axenic conditions to the microcosm did not induce visible stress to plants as they grew normally and developped short roots and ECM symbiosis at same rates and timing than in other systems (Vayssière et al. 2015). In addition, we observed a rapid but dynamic colonisation of the root system by both fungi and bacteria. We were able to track both arbuscular and ectomycorrhizal fungi, suggesting that our microscom allowed a normal development and colonization of the root system. To our knowledge, this is the first study investigating the primary steps of both bacterial and fungal colonization of axenic roots grown in a natural soil.

The dynamics of the rhizosphere effect was different between fungal and bacterial communities

Previous studies suggest that the root microbiome would be selected from the surrounding soil in a two steps process in which rhizodeposition would fuel an initial selection followed by a fine tuning of the communities of the rhizoplane and the endosphere by the plant (Bulgarelli et al. 2013). Our results are in accordance with this model but they suggest that this selection would occur through a different timing and a different process for bacterial and fungal communities. The structure and the composition of both fungal and bacterial communities were already clearly different from the one of the soil two days after planting poplar plantlets, suggesting that a very early selection is operating on both bacterial and fungal communities from the soil surrounding the roots. However, the degree of selection and the pattern of evolution differed between bacterial and fungal communities. A massive shift of the bacterial community composition already took place at day 2 while the fungal composition, although significantly different from the bulk soil, was still quite close to the one of the soil, at this stage. A gradual replacement of fungal species associated to a reduction of the richness and the diversity led to the domination of ECM species in the roots. By contrast, the bacterial richness and diversity increased over time and the change in the community composition was less pronounced as for fungi. Fungi and bacteria are already known to react differently to abiotic factors such as edaphic parameters and litter chemistry (Uroz et al. 2016). Our results would suggest that they also react differently to plant selection factors or that the plant would select the two communities through different processes. Such differencies may be explained by the existence of multiple signals

(e.g. flavonoids, nutrients, strigolactones) that would not be perceived by the same microorganisms (Bulgarelli et al. 2012, Martin et al. 2016).

Early fungal and bacterial communities are dominated by copiotrophs

Proteobacteria and particularly *Burkholderiaceae* dominated the early root microbial community. This is in accordance with previous studies on Poplar and other tree root microbiomes that showed a significant enrichment of OTUs from Proteobacteria and from *Burkholderia* in roots of trees (Gottel et al. 2011, Shakya et al., 2013, Uroz et al., 2010, Marupakula et al. 2016, Colin et al. 2017). Representative members of the *Burkholderiaceae* family have a high ability to develop on root exudates (Compant et al. 2008, Haicher et al. 2008). Similarly, dominant fungi at early time points were yeasts and filamentous saprotrophs/endophytes such as *Mortierella* that are likely able to quickly grow on root exudates. Members of the *Mortierella* genus are commonly detected in soils of forests and poplar plantation (Shakya et al., 2013; Bonito et al., 2014; Cregger et al., 2018). Although their ecological role is poorly understood, those fungi are charcterized by their rapid growth when encouterning rich media (Uehling et al. 2017). Yeast are commonly found in both natural and agricultural soil (Mestre et al. 2011, Yurkov et al., 2018). Some of the most frequently detected soil yeast belong to the genus *Cryptococcus* and have already been isolated from *Populus trichocarpa* roots (Gottel et al., 2011). Although their role remains largely unknown, plant growth promotion by soil yeast has been reported in different crops including maize (Sarabia et al., 2018), tomatoes, pepper and squash (Aziz Khan et al, 2012). This dominance of copiotrophs among both fungal and bacterial communities at early time points advocates for an important role of root exudates and particularly primary metabolites in the selection of the early root microbiome. In accordance with this hypothesis, fungal colonisation of the roots occurred mainly at the tip of roots (Figure 19), in the area where most of primary metabolites are exudated (Canarini et al. 2019). Preliminary analyses permitted to detect several sugars susceptible to attract microorganisms including mannitol, sucrose, glucose and arabinose, in root exudates of axenic *Populus tremula x alba* (data not shown). More unexpected is the very early detection reads corresponding to ECM fungi in the roots, long time before short roots start to develop. Similar early colonisation of roots by ECM fungi was found in *in vitro* experiments when inoculating eucalyptus roots with the ectomycorrhizal fungi *Pisolithus tinctorius* and *Paxillus involutus* (Horan et al., 1988) and between *Betula pendula* and *Paxillus involutus* (Brun et al., 1995). Indeed, both studies found evidence of hyphal attachment to the roots after two days of inoculation with an accumulation of hyphae at the root apex. These observations challenges the current model of the molecular dialog between ECM and roots leading to the establishment of the ECM symbiosis. In this model, the early molecular dialog between plant roots and ECM mycelium that lead to the formation of short roots and ECM colonization of the short roots rely on the production by both partner of diffusible compounds that act without physical contact between the root cells and the mycelium (Daguerre et al. 2016). Instead, our data would suggest that ECM fungi could colonize the main root before the formation of short roots and ectomycorrhizae. Further microscopy analyses will be needed to confirm this hypothesis.

The composition of bacterial communities colonizing *Populus* roots slowly evolved after seven days of root growth

We observed a significant shift in the structure and composition of the most dominant bacterial phyla and genera detetected in roots across time. Althought a strong selection of the bacterial communities from the soil occurred early on, the composition evolved slowly after seven days of root colonization. Marupakula et al. (2016) also observed that the bacterial colonization of single ECM root tips of *Pinus sylvestris* was highly dynamic and that bacterial community structure significantly differed depending on the sampling time. However, the time step chosen in this study was longer and main differences were visible between four and 24 weeks. This suggests that the bacterial communities may not have reached equilibrium by 50 days and that they could have evolved more if we had continued to sample longer. We observed the decrease of the relative abundance of members of the *Burkholderiaceae* family for the benefit of other well known tree root colonizers such as *Bradyrhizobium Rhizobacter* or *Sphingomonas* (Marupakula et al., 2016 ; Colin et al., 2017; Foulon et al., 2016; Bonito et al., 2019; Wang et al., 2019). Several phenomona could be involved in such evolution of the microbial communities: competition between microorganisms, slow growth of late comers, selection by the tree, cross-kingdom interactions with fungi… It is noteworthy the relatively high abundance of reads from *Collimonas* in some roots at intermediate time points (Figure 21). The genus *Collimonas* regroups a number of mycophagous bacteria (Leveau et al., 2010, Mela et al 2012). One could wonder if this increase is linked to specific interactions with fungi colonizing the roots. Further investigations will be needed to determine the mechanisms driving these dynamics.

The dominance of fungal endophytes and saprotrophs vs EcM fungi was reversed over time in *Populus* roots

The rhizosphere effect observed for fungal communities was not an isolated event and was followed by a time effect resulting in the successionnal replacement of fungal species within the root system over time. We distinguished three stages of root colonization: early from T2 to T4, intermediate from T7 to T15 and late from T21 to T50.

The relative abundance of endophytic and saprotrophic fungi tended to decrease over time, as mentionned in previous studies (Danielsen et al., 2012; Castano et al., 2019), in contrast to the relative abundance of EcM fungi which increased during the early and the intermediate stages of root colonisation (Figure 19). Both intra and intercellular colonisations by fungal hyphae were detected, with an increase of the density and diversity of fungal morphologies from 7 to 15 days. We observed the dominance and co-occurence of two major fungal morphologies “AM-like” and “Hand gloved-like” both in the adventive and lateral roots. Despite CLSM observations of AM morphologies within poplar root systems, their relative abundance was low in Miseq data, even if we detected their increase in roots collected after 10 days. However, these results must be confirmed by real-time PCR quantification of AM fungi (Voriskova et al., 2017). Indeed, our results as well as those of other studies showed that MiSeq high-throughput sequencing is not optimal for obtaining quantitative information on

AM fungi (Karlinski et al., 2010, Danielson et al., 2012, Bonito et al., 2014). We also detected by CLSM typical structures of dark septate endophyte in the roots system collected during the intermediate and late stage of colonization. These observations are in accordance with the detection of *Phialocephala*, *Cadophora* and *Leptodontidium* in poplar roots by amplicon sequencing. Berthelot et al, (2016) already isolated these DSE from poplar grown on a metal-polluted phytomanagement site and suggested their role in improving plant growth. As reported Jumpponen & Trappe (1998), we noted after CLSM observations that some DSE were often associated with EcM. Furthermore, we detected *Phialocephala* and *Leptodontidium* at the intermediate stage of colonisation whereas *Cadophora* was only present at the late stage according to amplicon sequencing. As recently described by Thoen et al. (2019), we also observed within a single ectomycorrhizal structure, a diverse range fungal morphologies and communities. These concurrent and successional occupation by distinct fungi also highlight the dynamics of the root colonising fungal communities.

From 15 to 21 days of *Populus* growth, CLSM observations and root DNA metabarcoding suggest a shift in the fungal composition, with the replacement of “AM-like” morphologies by the “Hand gloved-like” morphologies in lateral roots while “AM-like” morphologies remained present on adventive roots. This well known successional replacement of AM by EcM has been documented in different tree species such as eucalyptus (Lapeyrie et al., 1985; Chilvers et al., 1987; Castano et al., 2019) and poplar (Lodge et al., 1990). Nevertheless, these studies have been done on a long time scale, from 5 months to years, looking at the fungal colonisation dynamic of already grownup trees. According to the litterature, this is the first study attempting to characterise the fungal preliminary root colonisation from uncolonised root systems. The relative abundance of both saprotrophic and endophytic fungi decreased after 21 days, whereas the relative abundance of EcM fungi (e.g *Sebacina* and *Hebeloma*) tended to increase until the end of our root colonization monitoring. This correlates with the significant increase of the rate of ectomycorrhization until 50 days of root growth. The co-occurrence of distinct EcM fungi within the same root system has already been described in previous studies. For instance, Bahram et al. (2010) characterised the poplar EcM communities and recovered more than 122 EcM species within the same aspen root system. Furthermore, we observed the successional turnover of distinct EcM fungi in poplar root systems, going from *Sebacina* at the early stage of colonisation, replaced by Thelephoraceae and *Geopora* at an intermediate stage and dominated by Thelephoraceae, *Hebeloma*, and *Sebacina* in the late stage of colonisation. Interestingly, *Hebeloma* and *Telephora* have been discribed as “early-stage” EcM fungi colonisers in birch roots, with *Hebeloma* having the specificity to exclude colonisation by other mycorrhizal fungi (Fleming, 1985). Our results contrast with this priority concept for EcM colonisation as most changes in the EcM composition were due to fungal species present in soil that became more competitive over time (Kennedy et al., 2009). No changes in climatic or edaphic factors could explain these variations, thus plant-microbe related factors are responsible for the shift in EcM communities. Finally, we detected two new abundant fungal structures at the later time-point. The first one was intracellular in both adventive and lateral roots while the second one was only detected in mycorrhized roots. Further analysis using specific probes targeting a particular fungal species (such as Fluorescence In Situ Hybridization FISH) might help resolving the identification of the morphologies observed.

These observations infer the successional replacement of saprotrophic and endophytic fungi to the benefit of EcM fungi across the root development. It also points the important selection pressure undergoing between the plant root system and the soil microorganisms.

Conclusions

In conclusion, our data show that microbial communities of the natural soil successively colonized *Populus* roots punctuated by significant difference in the structure and composition of both fungal and bacterial communities. Bacteria were selected by the tree roots at a very early stage compared to fungi that were selected at a later stage. Our observations constitute a first phase of exploration of the establishment of tree-microbes' interactions as soon as roots appear and come into contact with the soil. Future studies on the interaction mechanisms of root microbiome could help us to understand how plant selection (and the mechanisms involved) and competition between microorganisms play a role in this dynamic of tree root colonization.

Acknowledgments

We wish to thank Milena Gonzalo (INRAE Nancy) for her helping in cultivating poplar vitroplants and Léa Jacquot (INRAE Nancy) for her helping in growing, maintaining and harvesting poplars.

Funding

FF was supported by "Contrat Doctoral" from the "Lorraine Université d'Excellence. LMP was supported by "Contrat Doctoral" from the French Ministère de l'éducation nationale et de la recherche. This research was sponsored by the Genomic Science Program, US Department of Energy, Office of Science, Biological and Environmental Research as part of the Plant-Microbe Interfaces Scientific Focus Area (http://pmi.ornl.gov), LABEX ARBRE and the "Méta-omiques & écosystèmes microbiens" INRAE metaprogram.

IV. Conclusions

Nos données montrent que les communautés microbiennes issues du sol naturel de peupleraie ont colonisé les racines des jeunes plantules de peupliers de manière successive. Cette colonisation successive est ponctuée par des différences significatives en termes de structure et de composition des communautés bactériennes et fongiques entre les différents points de prélèvement. Les bactéries ont été sélectionnées par les racines à un stade très précoce par rapport aux champignons qui ont été sélectionnés à un stade plus tardif.

Nos résultats constituent une première phase d'exploration dans l'établissement des interactions entre les arbres et les micro-organismes dès l'apparition des racines et les premiers contacts avec le sol environnant. De futurs études sur la colonisation racinaire du peuplier et d'autres espèces d'arbres par les micro-organismes du sol pourraient aider les chercheurs à développer un inoculum microbien efficace pour améliorer la croissance et la santé des arbres et des plantes pérennes.

Chapitre III

Effet du type de sol, du génotype et du climat sur le microbiote racinaire du Peuplier noir (*Populus nigra* L.)

I. Contexte général

En comparaison avec le microbiote des plantes de grandes cultures qui ont fait l'objet d'études approfondies au cours des dernières années, peu de travaux ont été réalisés concernant le microbiote des arbres. Ceci peut notamment s'expliquer par le fait que les recherches en foresterie soient moins développées qu'en agriculture et par le cycle de vie des arbres très différents de celui des plantes herbacées.

Selon les études qui ont été menées sur le microbiote racinaire de plusieurs espèces d'arbres tels que le hêtre, le chêne, le peuplier et le pin, nous savons que les principaux facteurs qui influencent les communautés de micro-organismes associées aux racines sont les propriétés physico-chimiques du sol, la physiologie de l'arbre hôte qui affectent directement la qualité et la quantité des exsudats racinaires, les champignons ectomycorhiziens et la saison (Uroz et al., 2016). De plus, nous savons que le génotype est également un facteur de structuration et de composition du microbiote racinaire (Cregger et al., 2018 ; Gallart et al., 2018).

Ces principaux facteurs peuvent être régulés par les conditions climatiques. Par conséquent, nous pouvons nous attendre à ce que le changement climatique actuel ait un impact conséquent sur le microbiote racinaire des arbres.

Le rapport de conférence suivant, publié dans la revue *New Phytologist* et auquel j'ai contribué, montre l'importance de développer des projets de recherches multidisciplinaires pour améliorer notre connaissance sur les effets globaux du changement climatique.

New Phytologist

Forum

Meetings

Facing global change: the millennium challenge for plant scientists

41st New Phytologist Symposium 'Plant sciences for the future', Nancy, France, April 2018

Introduction

We entered the Anthropocene with the industrial revolution. This geological era is defined by the unprecedented impact of human activities on the planet's geochemical cycles, making us the main driving force of Earth environmental changes (Crutzen, 2002; Steffen *et al.*, 2011). Since the middle of the twentieth century the human population has tripled, reaching seven billion today and probably 10 billion by 2050 (United Nations, 2015). This dramatic increase, associated with the improvement in the welfare of the population, has led to the overexploitation of natural resources. Intensive agriculture and industrialization has resulted in global warming, modification of nutrient cycles, pollution and reduction of wilderness; and endangering the preservation of eco- and agro-systems (Tilman *et al.*, 2002; Steffen *et al.*, 2011; Ehrlich & Harte, 2015). Today, the challenge is not only to intensify agro-productions to feed, fuel and shelter the growing population; but to do so in spite of the consequences of climate change while lessening our impact on the supporting ecosystems (Godfray *et al.*, 2010; Ehrlich & Harte, 2015; Byrne *et al.*, 2018).

Plant sciences can play an important part in mitigating both the causes and consequences of the pressure population growth imposes on the environment. As the primary producer of eco- and agro-systems, plants are essential to assess and understand human-driven environmental changes (Loreau *et al.*, 2001; Lin *et al.*, 2008). They are also central tools to develop sustainable production methods (Godfray *et al.*, 2010; Ehrlich & Harte, 2015; Byrne *et al.*, 2018).

In this context, the 41st New Phytologist Symposium 'Plant sciences for the future' was set as an experimental interdisciplinary platform. Bringing together early career and leader scientists from different fields of plant sciences, it aimed to promote the development of transdisciplinary research projects to build a better understanding of the multiple aspects of the upcoming environmental challenges; and to produce robust solutions for society. A special debate chaired by Marc-André Selosse (Natural History Museum of Paris, France) and Richard Norby (Oak Ridge National Laboratory, TN, USA) highlighted the critical topics and knowledge gaps the scientific community needs to fill in order to harness plant sciences to solve these societal issues. This event, held in Nancy, France on 11–13 April 2018, hosted researchers from 70 universities, research institutes and companies representing 29 countries in the fields of Developmental biology, Evolutionary biology, Ecology, Plant–microorganism interactions, Physiology and Genetic engineering (Fig. 1). In this article we outline how all plant science fields contribute to understand the effects of global change and to developing innovative solutions to maintain agro-productions, promote sustainability and counteract climate change.

Exploring biogeochemical cycles

Human activities have altered global biogeochemical cycles. Colin Brownlee (Marine Biological Association, Plymouth, UK) illustrated the role of marine phytoplankton in the carbon (C) cycle, reminding that coccolithophores are responsible for much of the calcium carbonate formation on Earth. The increasing input of CO_2 into the atmosphere since the industrial revolution, which is responsible for ocean warming and acidification, is compromising the ability of coccolithophores to form calcium carbonate and therefore affecting the completion of the global C cycle (Orr *et al.*, 2005). Brownlee demonstrated the role of proton channels in the calcification process of calcite coccoliths. Elucidating the cellular mechanisms involved in biomineralization is essential to minimize human impact on these critical species.

Forests represents a major C sink (Pan *et al.*, 2011). Björn Lindahl (Swedish University of Agricultural Sciences, Uppsala, Sweden) highlighted the importance of plant–fungi interactions in nutrient cycling and soil fertility in boreal forests. Using high-throughput sequencing to elucidate boreal forest mycobiome and combining it with climatic, edaphic and forest productivity parameters, Lindahl's group showed that the composition of the fungal community is the principal driver of organic matter storage in those environments. Lindahl proposed that intensification of forest practices by changing soil fungal communities could improve the soil C stock in boreal forest but presents long-term soil fertility risks.

From the boreal forest to the steppe, Amy Austin (University of Buenos Aires, Argentina) demonstrated that photodegradation is a dominant force controlling C losses in semi-arid ecosystems (Austin & Vivanco, 2006). Recent findings of her team suggests that photodegradation of the leaf litter promotes its subsequent biotic degradation by increasing accessibility of labile C compounds to microbes (Austin *et al.*, 2016). Land-use or climate change altering vegetation cover could largely influence the effect of sunlight on C cycling in these ecosystems. Croplands

© 2018 The Authors
New Phytologist © 2018 New Phytologist Trust

are an anthropogenic biome that we could manage to increase potential C sequestration. Carbon dioxide reaction with minerals naturally moderates atmospheric CO_2 and this effect has been enhanced since the emergence of land plants (Berner, 1997). David Beerling (University of Sheffield, UK) proposed to exploit this natural phenomenon by adding fast-reacting silicate rocks on croplands to trap CO_2. Eventually, weathering products could run-off in to oceans and enhance alkalinity, counteracting acidification, and sustaining the growth of marine phytoplankton that we presented as crucial for the completion of C cycle earlier in this paragraph. Together, these results highlight the importance of expanding our knowledge about C and nutrient turnover on Earth to predict and actively minimize our impact on climate.

Assessing the effects of climate change on plant physiology

Global warming has increased the intensity and frequency of extreme climactic events. High amplitude of temperature variation is the major cause of important plant losses in eco- and agro-systems (Eiche, 1966; Boyer, 1982; Hatfield & Prueger, 2015).

Plant pre-adaptation to climate variations could limit losses (Wikberg & Ögren, 2007; Yordanov *et al.*, 2000). Drought acclimation of trees involves structural changes in wood formation and abscisic acid (ABA) is a key plant regulator of this acclimation (Gupta *et al.*, 2017). Andrea Polle (University of Göttingen, Germany) showed the importance of ABA signal perception and response in wood formation of drought-stressed trees. Cecilia Brunetti (CNR, Sesto Fiorentino, Italy) demonstrated how trees limit xylem conduits embolism by modulating their carbohydrate metabolism and how ABA is involved in restoring xylem transport ability.

Limiting water loss by modulating stomatal aperture is another plant survival response to drought. Predicting plant responses to different levels of drought is still difficult. Belinda Medlyn (University of Western Sydney, Australia) reviewed recent advances in 'optimal stomatal theory' and presented a new *in silico* model to understand and predict stomatal responses to drought and heat.

Environmental stresses such as changes in temperature can affect plant metabolism and growth (Sampaio *et al.*, 2016). Shuhua Yang (China Agricultural University, Beijing, China) showed that stomatal conductance and, in consequence, leaf photosynthesis and respiration are affected by cold stress via the regulation of the CBF-dependent cold signalling pathway in Arabidopsis (Zhou *et al.*, 2011). Owen Atkin (Australian National University, Canberra, Australia) suggested that, by boosting plant respiratory metabolism, global warming could increase CO_2 release and influence the future atmospheric CO_2 concentrations.

Understanding plant physiological and metabolic adaptive responses to climate change are key factors for the production of efficient prediction models. These models are necessary to improve or develop novel management methods of eco- and agro-systems that could limit plant losses in the future.

Maintaining plant productivity

In our demographic context, maintaining population welfare depends on our ability to intensify agro-production. Environmental changes are threats to the maintenance of crop yields in both agricultural and forests agro-systems. They have direct impacts on plant mortality and biomass (Lobell & Field, 2007; Schlenker & Roberts, 2009), and indirectly affect plant productivity by altering population dynamics of plant pests, symbiotic microorganisms and competitive species (Gregory *et al.*, 2009; Lindner *et al.*, 2010). Biotechnological or agronomic solutions are necessary to lessen the consequences of global change on plant production.

In this frame, understanding the genetic basis of wood production in different tree lineages may help to mitigate the repercussions of abiotic stress on forest productivity through adapted management plans. Andrew T. Groover (US Forest Service and University of California, Davis, CA, USA) reviewed the genetic basis of evolution of woody plants and highlighted species-specific or conserved gene modules regulating the development of dicot and monocot cambium (Zinkgraf *et al.*, 2017).

Environmental changes are modifying development and distribution of plant pests, threatening crop and forest productivity (Porter *et al.*, 1991; Logan *et al.*, 2003). Plant diseases are now responsible for *c.* 25% of crop losses (Martinelli *et al.*, 2015). Controlling their outbreak is crucial to maintain plant productivity. A strategy to contrast future pest spread is to engineer crops resistant to a wide variety of pathogens. Ralph Panstruga's team (University of Aachen, Germany) explores the role of the MILDEW RESISTANCE LOCUS O genes (Jørgensen, 1992) – encoding members of a family of membrane integral proteins conserved in plants – in conferring multiple resistances. They showed that mutations in MLO genes improved *Arabidopsis thaliana* resistance to several leaf epidermal cell penetrating pathogens, but increased susceptibility to microbes with different invasion strategies (Acevedo-Garcia *et al.*, 2017). Stella Cesari (INRA, France), 2017 Tansley Medal winner, proposed to exploit the complex mechanistic and structural variability of nucleotide-binding domain and leucine-rich repeat-containing proteins (NLRs) to increase sensitivity or extend specificity of pathogen effector recognition (Cesari, 2017).

Understanding plants adaptive strategies to global change

Plants are increasingly exposed to new environmental stresses such as habitat degradation, climate change and the expanding range of invasive species and pests (Anderson *et al.*, 2011). To predict the consequences of global change on ecosystems, it is necessary to understand the different levels of plant adaptation (phenotypic plasticity, dispersion capacity and evolution) to new threats.

Plants can modulate the phenotypic plasticity of their neighbours by emission of volatile organic compounds (VOCs). André Kessler (Cornell University, Ithaca, NY, USA) showed that VOCs emitted by *Solidago altissima* upon herbivore attack alter herbivore dispersal and feeding behaviour through the modification of the

© 2018 The Authors
New Phytologist © 2018 New Phytologist Trust

Fig. 1 Group photograph of the attendees of the 41st New Phytologist Symposium 'Plant sciences for the future' in the entrance of the Hôtel de Ville, Nancy (France). Photograph by Steven White, Leeds Media Services.

metabolism of non-attacked plants. This indicates spreading the risk of herbivory to neighbours as a fitness-optimizing strategy. The high variability of VOC types and levels in the field suggests the possibility of herbivore-driven natural selection on chemical communication (Morrell & Kessler, 2017). This might modulate crop adaptability to newly introduced pests.

Linda F. Delph (Indiana University, Bloomington, IN, USA) reminded the audience that the phenotype is the direct interface between the organism and its environment and therefore at the centre of evolution. She showed that genetic selection on key fitness traits such as flower number and height was strongly influenced by the environmental conditions in *Silene latifolia*. In-depth investigation of environmental factors influencing plant evolution may help predict phenotypic traits and fitness of plants in changing ecosystems.

Flower development is one of the most intricate and finely tuned processes influencing plant reproductive success. The floral organ must acquire specialized structures, bloom at the right time of the year and bear coevolving traits with its pollinators. By taking advantage of the -omics technologies, several groups found that specific transcription factors (TFs) evolved to allow the formation of elaborate and diverse floral petals. Elena Kramer (Harvard University, Cambridge, MA, USA) presented the role of the AqJAGGED gene, a TF involved in multiple key aspects in *Aquilegia* flower morphogenesis (Min & Kramer, 2017), while Hongzhi Kong (Institute of Botany, Beijing, China) showed that NpLMI1 and NpYAB5-1 are involved in the control of *Nigella* petal shape.

Since the first observations of pollination systems by Darwin (1862), researchers have been seeking for evidence of pollinator-promoted selection for diverse floral shapes. Babu Ram Paudel (Yunnan University, China) showed how two alpine gingers (*Roscoea purpurea* and *R. tumjensis*) occur sympatrically and have similar morphology, but are reproductively isolated through a combination of phenological displacement of flowers and different attracted pollinators. Global change might reshape these evolutionary boundaries and modify population or speciation dynamics.

Human impacts on the environment will influence plant traits and drive their evolution by modulating plant fitness (resistance to pathogens, pollination, population dynamics). However, plant plasticity might provide a key for plant adaptability on the short term.

Innovative plant technology: a role for basic and applied science

Understanding the genetic and molecular basis of phenotypes is key to groundbreaking biotechnological applications; hence the importance of tight coordination and synergy between basic and applied sciences. The Symposium hosted researchers interested in fundamental biological mechanisms, scientists involved in both basic and applied research and developers employed in biotechnology companies, aiming to bridge their complementary mindsets.

Understanding the molecular aspects of nutrient uptake and storage by plants is crucial to improving the yield or nutritional

© 2018 The Authors
New Phytologist © 2018 New Phytologist Trust

properties of crops. By investigating the developmental biology of rooting systems in early land plants, Liam Dolan (University of Oxford, UK) showed that the development of rooting structures in land plants is tightly controlled by some conserved TF networks (Breuninger *et al.*, 2016; Proust *et al.*, 2016). Such highly conserved key regulators can be used to enhance crops ability to access nutrients (Dolan *et al.*, 2011; US Patent Application no. 12/451,574). The fine-tuning of lateral root emergence is another central aspect of root systems development. Keith Lindsey (Durham University, UK) showed how the 36-aa peptide POLARIS, orchestrating the auxin–ethylene crosstalk, modulates lateral root emergence (Chilley *et al.*, 2006). These signalling mechanisms affect plants' access to water and nutrients and mediate plant plasticity in a changing environment. The regulation of the level of reserves is also fundamental to plant nutrition. Alison M. Smith (John Innes Centre, Norwich, UK) highlighted the importance of clock genes, which modulate starch production and degradation for efficient plant sustainment (Graf *et al.*, 2010; Scialdone *et al.*, 2013). Arabidopsis leaves modulate the rate of starch degradation according to the duration of the night, in order not to starve before dawn (Fernandez *et al.*, 2017). A better understanding of the dynamics of plant nutrient reserves may help engineering stress-resistant or nutrient-rich crops.

Examples of basic sciences translated into innovative plant technologies were given at the symposium. As presented earlier, David Beerling is exploiting silica weathering to counter accumulation of excess atmospheric CO_2. These results involved integrative studies spanning through geology, chemistry, economy and plant sciences, demonstrating once more the inestimable power of transdisciplinary research. Anne Osbourn (John Innes Centre) showed that through coexpression, evolutionary co-occurrence and epigenomic coregulation genomes can be mined for biosynthetic gene clusters involved in production of secondary metabolites (Medema & Osbourn, 2016). Their genetic manipulation allows the production of specific chemicals at a lower cost than conventional synthetic chemistry (Owen *et al.*, 2017). Technical platforms and start-ups are being born in the exciting field of plant chemistry (Reed *et al.*, 2017).

In conclusion, the symposium highlighted the need of integrative research to (1) understand, model, predict the consequences of global change on ecosystems and plant physiology, productivity, epidemiology; (2) create innovative solutions to future challenges in the fields of food security, sustainable crop management and efficient production; (3) diffuse knowledge and know-how among specialists and the general public. To this purpose, the symposium was closed by a public talk on plant–microorganism interactions, given by Marc-André Selosse with the beautiful background of the Hôtel de Ville of Nancy.

Acknowledgements

The authors thank the New Phytologist Trust and the Lab of Excellence ARBRE for organizing this symposium. The authors are grateful to all attendees who contributed to the stimulating atmosphere of this conference. A special acknowledgement to M-A. Selosse and R. Norby for leading the debate on the role of plant sciences in solving societal issues. The authors thank A. Austin, A. Polle, B. Medlyn, L. Wingate and A. Kessler for their time and advice during the mentoring session on Gender equality in plant sciences. The authors are grateful to F. Martin and the New Phytologist Trust for their support and helpful comments on the report. This work was supported by the LabEx ARBRE (ANR-11-LABX-0002-01), the Région Grand-Est, the University of Lorraine, the Genomic Science Programme of the US Department of Energy as part of the Plant–Microbe Interfaces Scientific Focus Area and, the ANPCyT (PICT 2015-1231, PICT 2016-1780), the University of Buenos Aires (UBACyT) and the CONICET of Argentina.

Veronica Basso[1], Maíra De Freitas Pereira[2], François Maillard[1], Julieta Mallerman[3], Lauralie Mangeot-Peter[1], Feng Zhang[1] and Clémence Bonnot[1]*

[1]Laboratoire d'excellence Recherches Avancées sur la Biologie de l'Arbre et les Ecosystemes Forestiers (LabEx ARBRE), UMR Interactions Arbres/Microorganismes (IAM) 1136, INRA, Université de Lorraine, Centre INRA Grand-Est, Champenoux 54280, France;
[2]UMR Silva 1434, AgroParisTech, INRA, Université de Lorraine, Nancy F-54000, France;
[3]Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad de Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina
(*Author for correspondence: tel +33 (0) 383393315; email clemence.bonnot@inra.fr)

All authors contributed equally to this work.

References

Acevedo-Garcia J, Gruner K, Reinstädler A, Kemen A, Kemen E, Cao L, Takken FLW, Reitz MU, Schäfer P, O'Connell RJ *et al.* 2017. The powdery mildew-resistant Arabidopsis *mlo2 mlo6 mlo12* triple mutant displays altered infection phenotypes with diverse types of phytopathogens. *Scientific Reports* 7: 1–15.

Anderson JT, Willis JH, Mitchell-Olds T. 2011. Evolutionary genetics of plant adaptation. *Trends in Genetics* **27**: 258–266.

Austin AT, Méndez MS, Ballaré CL. 2016. Photodegradation alleviates the lignin bottleneck for carbon turnover in terrestrial ecosystems. *Proceedings of the National Academy of Sciences, USA* **113**: 1–6.

Austin AT, Vivanco L. 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. *Nature* **442**: 555–558.

Berner RA. 1997. The rise of plants and their effect on weathering and atmospheric CO_2. *Science* **276**: 544–546.

Boyer JS. 1982. Plant productivity and environment. *Science* **218**: 443–448.

Breuninger H, Thamm A, Streubel S, Sakayama H, Nishiyama T, Dolan L. 2016. Diversification of a transcription factor family led to the evolution of antagonistically acting genetic regulators of root hair growth report diversification of a transcription factor family led to the evolution of antagonistically acting genetic regulators of root hair growth. *Current Biology* **26**: 1622–1628.

Byrne PF, Volk GM, Gardner C, Gore MA, Simon PW, Smith S. 2018. Sustaining the future of plant breeding: the critical role of the USDA-ARS National Plant Germplasm System. *Crop Science* **58**: 1–18.

© 2018 The Authors
New Phytologist © 2018 New Phytologist Trust

Cesari S. 2017. Multiple strategies for pathogen perception by plant immune receptors. *New Phytologist* **219**: 17–24.

Chilley PM, Casson SA, Tarkowski P, Hawkins N, Wang KL, Hussey PJ, Baele M, Ecker JR, Sandberg K, Lindsey K. 2006. The POLARIS peptide of Arabidopsis regulates auxin transport and root growth via effects on ethylene signaling. *Plant Cell* **18**: 3058–3072.

Crutzen PJ. 2002. Geology of mankind. *Nature* **415**: 23.

Darwin C. 1862. *On the various contrivances by which British and foreign orchids are fertilized by insects.* London, UK: Murray.

Dolan L, Menand B, Yi K. 2011. *Composition and method for modulating plant root hair development.* US Patent Application no. 12/451,574.

Ehrlich PR, Harte J. 2015. Opinion: To feed the world in 2050 will require a global revolution. *Proceedings of the National Academy of Sciences, USA* **112**: 1–2.

Eiche V 1966. *Cold damage and plant mortality in experimental provenance plantation with Scots Pine in Northern Sweden.* Stockholm, Sweden: Studia Forestalia Suecica 0039-3150.

Fernandez O, Ishihara H, Georges MG, Mengin V, Flis A, Sumner D, Arrivault S, Feil R, Lunn JE, Zeeman SC *et al.* 2017. Leaf starch turnover occurs in long days and in falling light at the end of the day. *Plant Physiology* **174**: 2199–2212.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C. 2010. Food security: the challenge of feeding 9 billion people. *Science* **327**: 812–819.

Graf A, Schlereth A, Stitt M, Smith AM. 2010. Circadian control of carbohydrate availability for growth in Arabidopsis plants at night. *Proceedings of the National Academy of Sciences, USA* **107**: 9458–9463.

Gregory PJ, Johnson SN, Newton AC, Ingram JSI. 2009. Integrating pests and pathogens into the climate change/food security debate. *Journal of Experimental Botany* **60**: 2827–2838.

Gupta A, Hisano H, Hojo Y, Matsuura T, Ikeda Y, Mori IC, Senthil-Kumar M. 2017. Global profiling of phytohormone dynamics during combined drought and pathogen stress in *Arabidopsis thaliana* reveals ABA and JA as major regulators. *Scientific Report* **4**: 4017.

Hatfield JL, Prueger JH. 2015. Temperature extremes: effect on plant growth and development. *Weather and Climate Extremes* **10**: 4–10.

Jørgensen JH. 1992. Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica* **63**: 141–152.

Lin BB, Perfecto I, Vandermeer J. 2008. Synergies between agricultural intensification and climate change could create surprising vulnerabilities for crops. *AIBS Bulletin* **58**: 847–854.

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M *et al.* 2010. Climate change impacts, adaptive capacity and vulnerability of European forest ecosystems. *Forest Ecology and Management* **259**: 698–709.

Lobell DB, Field CB. 2007. Global scale climate-crop yield relationships and the impacts of recent warming. *Environmental Research Letters* **2**: 014002.

Logan JA, Réginière J, Powell JA. 2003. Assessing the impacts of global warming on forest pest dynamics. *Frontiers in Ecology and the Environment* **1**: 130–137.

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B *et al.* 2001. Biodiversity and ecosystem functioning: current knowledge and future challenge. *Science* **294**: 804–808.

Martinelli F, Scalenghe R, Davino S, Panno S, Scuderi G, Ruisi P, Villa P, Stroppina D, Boschetti M, Goulard LR. 2015. Advanced methods of plant disease detection. *Agronomy for Sustainable Development* **35**: 1–25.

Medema MH, Osbourn A. 2016. Computational genomic identification and functional reconstitution of plant natural product biosynthetic pathways. *Natural Product Reports* **33**: 951–962.

Min Y, Kramer EM. 2017. The *Aquilegia JAGGED* homolog promotes proliferation of adaxial cell types in both leaves and stems. *New Phytologist* **216**: 536–548.

Morrell K, Kessler A. 2017. Plant communication in a widespread goldenrod: keeping herbivores on the move. *British Ecological Society* **41**: 1049–1061.

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishika A, Joos F *et al.* 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**: 681–686.

Owen C, Patron NJ, Huang A, Osbourn A. 2017. Harnessing plant metabolic diversity. *Current Opinion in Chemical Biology* **40**: 24–30.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG. 2011. A large and persistent carbon sink in the World's forest. *Science* **333**: 988–993.

Porter JH, Parry ML, Carter TR. 1991. The potential effects of climatic change on agricultural insect pests. *Agricultural and Forest Meteorology* **57**: 221–240.

Proust H, Honkanen S, Jones VAS, Morieri G, Prescott H, Kelly S, Ishizaki K, Kohchi T, Dolan L. 2016. RSL Class I genes controlled the development of epidermal structures in the common ancestor of land plants. *Current Biology* **26**: 93–99.

Reed J, Stephenson MJ, Miettinen K, Brouwer B, Leveau A, Brett P, Goss RJM, Goossens A, O'Connell MA, Osbourn A. 2017. A translational synthetic biology platform for rapid access to gram-scale quantities of novel drug-like molecules. *Metabolic Engineering* **42**: 185–193.

Sampaio BL, Edrada-Ebel R, Batista F, Costa D. 2016. Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants. *Scientific Report* **6**: 1–11.

Schlenker W, Roberts MJ. 2009. Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. *Proceedings of the National Academy of Sciences, USA* **106**: 15594–15598.

Scialdone A, Mugford ST, Feike D, Skeffington A, Borrill P, Graf A, Smith AM, Howard M. 2013. Arabidopsis plants perform arithmetic division to prevent starvation at night. *eLife* **2**: 1–24.

Steffen W, Grinevald J, Crutzen P, Mcneill J. 2011. The Anthropocene: conceptual and historical perspectives. *Philosophical Transactions. Series A: Mathematical, Physical, and Engineering Sciences* **369**: 842–867.

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. 2002. Agricultural sustainability and intensive production practices. *Nature* **418**: 671–677.

United Nations. 2015. World population prospects: the 2015 revision. *United Nations Department of Economic and Social Affairs.* **33**: 1–66.

Wikberg J, Ögren E. 2007. Variation in drought resistance, drought acclimation and water conservation in four willow cultivars used for biomass production. *Tree Physiology* **27**: 1339–1346.

Yordanov I, Velikova V, Tsonev T. 2000. Plant responses to drought, acclimation and stress tolerance. *Photosynthetica* **38**: 171–186.

Zhou MQ, Shen C, Wu LH, Tang KX, Lin J. 2011. CBF-dependent signaling pathway: a key responder to low temperature stress in plants. *Critical Reviews in Biotechnology* **31**: 186–192.

Zinkgraf M, Gerttula S, Groover A. 2017. Transcript profiling of a novel meristem, the monocot cambium. *Journal of Integrative Plant Biology* **59**: 436–449.

Key words: biochemical cycles, climate change, food security, holobiont, phenotypic plasticity, plant adaptive strategies, plant productivity, plant sciences.

© 2018 The Authors
New Phytologist © 2018 New Phytologist Trust

II. Objectifs

D'après la littérature existante sur les plantes herbacées, il est désormais clairement établi qu'une diminution de la disponibilité en eau et une augmentation de la température modifie la composition et la structure du microbiote racinaire (Wallenstein & Hall, 2012). Chez les arbres, le lien entre les propriétés physico-chimiques du sol, les besoins physiologiques de l'arbre et les communautés microbiennes associées aux racines demeure encore peu étudié.

Parmi les espèces d'arbres qui sont naturellement confrontées aux effets du changement climatique, le peuplier noir d'Europe (*Populus nigra* L.) est un bon exemple. Il s'agit d'une espèce pionnière colonisant les sédiments alluviaux le long des fleuves dans les zones climatiques tempérées du continent Asie-Europe et d'Afrique du Nord. Avec d'autres espèces d'arbres riverains, *P. nigra* occupe une position clé dans l'écosystème riverain, mais sa pérennité et sa diversité génétique sont menacées pour deux raisons principales : l'absence de sites de régénération en raison de l'activité humaine sur les plaines inondables et l'impact du changement climatique. L'augmentation de la fréquence des événements naturels extrêmes liés aux changements climatiques est susceptible d'entraîner des variations plus fréquentes et plus intenses des nappes phréatiques et des régimes d'écoulement des cours d'eau qui affectent le développement des semis et la physiologie des arbres. Il existe de grandes variations dans la capacité de réaction au stress climatique au sein des populations de *P. nigra* et entre elles, ce qui suggère un important potentiel d'adaptation (Chamaillard et al., 2011). On pourrait supposer que le microbiote de la racine du peuplier noir contribue à cette adaptation, mais aucune étude n'a été réalisée jusqu'à présent pour évaluer son rôle relatif. Des études sur le microbiote des racines de peuplier d'autres espèces (e.g. *P. deltoides*, *P. trichocarpa*) ont déjà été faites mais jamais sur des écosystèmes riverains (Hacquard & Schadt, 2015).

Les travaux présentés dans ce chapitre font partie du projet POPMICROCLIM (soutenu par le métaprogramme ACCAF). Les objectifs de ce projet sont de caractériser le microbiote des sédiments de deux rivières françaises, habitats naturels du peuplier noir, le microbiote racinaire du peuplier noir, l'impact du climat sur ces microbiotes et le rôle potentiel du microbiote dans l'adaptabilité des jeunes plants aux variations climatiques. Ce projet est réalisé en collaboration avec les équipes du Dr Lional Ranjard (INRAE UMR Agroécologie Dijon) en charge de l'analyse du microbiote du sol, du Dr Marc Villar (INRAE UMR BioForA) et du Dr Régis Fichot (Université d'Orléans) en charge des analyses génétiques et écophysiologiques. Au moment du dépôt de mon manuscrit, l'ensemble des données expérimentales a été acquis mais les analyses couplant études écophysiologiques et études microbiologiques n'ont pas encore été réalisées. Ne seront donc présentées ici que les données concernant l'étude du microbiote.

Nous avons essayé de répondre à plusieurs questions

- Quelle est la composition du microbiote racinaire du peuplier noir ?
- Y a-t-il un effet de l'origine du sol sur la composition du microbiote racinaire et du microbiote du sol ?
- Y a-t-il un effet de la transplantation de sol dans un nouveau climat sur le microbiote racinaire ?
- Le microbiote du sol et le microbiote racinaire du peuplier noir sont-ils influencés par les conditions climatiques de la Loire et de le Drôme ?

III. Démarche expérimentale

Afin de répondre à ces questionnements, nous avons profité d'une expérience de transplantation réciproque mise en place par l'UAGPF (Unité Mixte de Recherche Biologie intégrée pour la valorisation de la diversité des arbres et de la forêt, INRAE Val de Loire) au printemps 2017. Dans le but d'étudier les mécanismes génétiques d'adaptation des plants de peupliers noirs au changement climatique, des graines de peupliers noirs génétiquement caractérisés (via des marqueurs SSR et SNP) provenant de deux régions de France climatiquement contrastées (Drôme et Loire) ont été prélevées sur 2 x 10 arbres mères et plantées dans des conteneurs contenant les sédiments bruts des deux rivières. Des copies de chaque conteneur ont été faites et transportées dans les deux sites aux climats contrastés.

Les paramètres de développement (croissance, mise en dormance) et des paramètres éco-physiologiques pertinents pour l'acquisition de l'eau (efficacité de l'utilisation de l'eau, répartition de la biomasse, architecture des racines, anatomie du xylème) ont été mesurés sur deux saisons de croissance. En novembre 2017, les systèmes racinaires ont été échantillonnés afin de mesurer leur développement et d'évaluer la composition du microbiome racinaire des différents génotypes.

Les résultats de cette étude sont décrits sous la forme d'un article scientifique actuellement en préparation.

Les tableaux et tableaux supplémentaires sont disponibles en Annexe (Annexe 2 de la page 9 à la page 26).

A mesocosm transplant experiment to investigate how climate, soil properties and plant genetics determine the structure of the root microbiome of *Populus nigra* seedlings

L. Mangeot-Peter[1], S. Uroz[1*], M. Lefebvre[2], M. Villar[2], F. Martin[1,3], A. Deveau[1*]

[1] Université de Lorraine, INRAE, UMR IAM, 54280 Champenoux, France
[2] INRAE, UR 0588 AGPF, Centre INRAE Val de Loire, 2163 avenue de la Pomme de Pin, CS 40001 – Ardon, 45075 Orléans, France
[3] Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Haisian District, Beijing, China

∗Corresponding author: Mailing address: UMR1136 INRAE Université de Lorraine, Interactions Arbres Micro-organismes, 54280 Champenoux, France. Phone: +33 3 83 39 40 41, Fax: +33 3 83 39 40 69. E-mail: aurelie.deveau@inrae.fr, stephane.uroz@inrae.fr

Abstract

Trees and their root-associated microorganisms are tightly interconnected. They play important roles in each other's nutrition and protection against stresses. Host factors such as tree genotype but also environmental factors particularly soil matrix and climate are key determinants of root microbiome structure and composition. Our understanding of the effects of soil origin and climate on the soil and the root microbiome and on tree physiology is incomplete. In the current context of climate change, it is not clear whether the soil and root microbiome react directly to the climatic variations and to the soil physico-chemical properties and indirectly to the tree physiology alteration. To address this question, we analysed the sediments of two French rivers located in two climatically contrasted regions Drôme and Loire and the root microbiome structure and composition of a pioneer tree from riparian ecosystem, the European black poplar (*Populus nigra* L.) cultivated in mesocosms in different sets of soil and climate conditions. Seeds were collected in the two contrasted regions and belonged to genetically characterized *Populus nigra* trees in order to study the relative contribution of host genotype on the root microbiome. After a season of growth, the above-ground size of seedlings was measured and sediment-, rhizosphere- and root-associated fungal and bacterial communities were characterized by high throughput MiSeq sequencing of rDNA ITS and 16S rRNA amplicons. Significant shifts of bacterial and fungal community composition were observed between native and transplant conditions of the seedlings culture. Enrichment of specific microbial communities in the rhizosphere and roots were correlated with increase of the aerial part growth of seedlings.

Keywords : Microbiome, metabarcoding, climate change, *Populus nigra*, sediments, genotype

Introduction

The European black poplar, *Populus nigra* L., is a natural and pioneer tree species colonizing alluvial sediments along rivers, where it often exists as a series of metapopulations (Villar & Forestier, 2009). *Populus nigra* occupies a keystone position within the riparian ecosystem thanks to its highly developed root system that allow it to be an efficient sediment trapper, riverbank fixative and natural nutrient purifier (Ruffinoni et al., 2003). However, its position is currently hindered by the lack of regeneration due to human pressure on flood plains and the impact of climate change (Lefevre et al., 1998; Cottrell, 2004). It is important to remind that for most riparian tree species, the regeneration is achieved through the colonization of river sediment along the riverbank according to the natural periodic flooding of the ecosystem and the lateral movement of the river bed. Seed dispersal combines an initial wind-mediated phase with the transport of seeds from the maternal tree to the ground or the water, followed by a secondary hydrochorous phase (Barat-Segretain, 1996; Imbert & Lefèvre, 2003). The *P. nigra* offprings are therefore often found several kilometres downstream of the mother tree on river bank or sediments islands subject to very variable environmental conditions. *Populus nigra* is considered as a fast-growing and opportunistic species, with a good tolerance to submersion, sediment burial and high temperature (Chamaillard, 2011; Corenblit et al., 2014). These abilities are important to adapt to the fluvial environment, which differs in space and time. Indeed, the place where *Populus nigra* seedlings begin their life cycle is more frequently disturbed during annual flood events compared to the place where they reached maturity (Corenblit et al., 2014). Climate change such as rising temperature and modifications of precipitation patterns could therefore have consequences on natural regeneration and genetic structuration of the populations of black poplars. The juvenile stage (i.e., seedling) is a key step in the development of trees but our knowledge of the parameters that determine the success of the installation and development remains limited.

In most of plant and tree species, extrinsic factors such as soil properties and climate, but also intrinsic factors such plant genotype are important determinants of the adaptation of tree seedlings to their environment. In addition, the root-associated microbiome, which corresponds to the complex microbial communities occurring on the surface and inside the roots, is known to increase nutrient and water acquisition and to protect host tree against biotic and abiotic stresses, improving by these ways the growth of the trees (Hacquard & Schadt, 2015; Timm et al., 2018). As several *Populus* species represent ecologically important species (e.g., *P. trichocarpa* or *P. deltoides* in the USA, *P. nigra* in Europe) and/or are used in plantations to produce wood biomass, recent efforts have been made to characterize their root microbiome and their potential role in the promotion of the growth of poplar (Germaine et al., 2004; Gottel et al., 2011; Danielsen et al., 2012; Shakya et al., 2013; Beckers et al., 2017; Durand et al., 2017; Durand et al., 2018; Cregger et al., 2018; Veach et al., 2019). Notably, Bonito et al., (2014) showed that the soil origin had a stronger effect on the fungal root community composition than on the bacterial communities, which were more tightly structured by host species (*Populus*, *Quercus* and *Pinus*) than by the soil origin. Considering mature trees of *Populus deltoides*, another riparian poplar tree species, Shakya et al., (2013) revealed that both the soil type, the season and the geographic distance (i.e., several tens of kilometres)

between trees were important drivers shaping bacterial and fungal communities in the rhizosphere and the endosphere. Comparatively to *P. trichocarpa* or *P. deltoides*, the composition and the structure of the root-associated microbiome of *P. nigra* has never been or rarely (Tesar et al., 2002) investigated nor how the *P. nigra* holobiont (i.e., the assemblage formed by the tree host and its associated microbiome; Hacquard & Schadt, 2015), could adapt to changing environments. The ecology of *Populus nigra* is currently well-known. The large-scale analysis of its distribution revealed that this species colonizes naturally alluvial sediments along rivers in several temperate climate zones of the Asian-European continent, such as in France, but also in Northern Africa. Those studies evidenced that *P. nigra* is able to adapt to different edaphic properties and to variable climates. Studies on phenotypic plasticity have been less discussed for black poplar, but this process has been demonstrated to be inheritable and has provided an evolving short-term response to climate change (Aitken et al., 2008; Nicotra et al., 2010). In this context, we can wonder whether the development of the tree seedlings of this species is linked to the selection of specific poplar populations (i.e., genetic adaptation), phenotypic plasticity and/or to the recruitment of particular microorganisms (i.e., the root-associated microbiome).

As, *P. nigra* is widespread in France along different rivers under different climates, we took the opportunity to study the factors that may explain their adaptation to a changing environment at the seedling stage. To do it, we used a mesocosm transplant experiment approach considering two different river sediments (i.e., Loire vs Drôme) as soil substrate, two different climates and several different tree progenies. Sediments of the Drôme and the Loire were conditioned in mesocosms, planted with different *P. nigra* progenies coming from the Drôme or the Loire region and incubated in natural conditions under the Drôme and the Loire climates with a constant irrigation. In this experiment, we evaluated the impact of these different parameters (sediment type, plant progeny, and climate) on the composition and structure of the root-associated microbiome of black poplar and on tree phenology and development. We characterized the microbial communities (*i.e.*, bacteria and fungi) colonizing sediment (BS), rhizosphere (R) and endosphere (E) of the three *Populus nigra* progenies per origin (i.e., 3 from the Drôme and 3 from the Loire) using amplicon 16S rRNA and ITS rDNA gene-targeted Illumina sequencing. In each location, the 3 progenies (i.e., the offsprings of *P. nigra* mother trees originating from Drôme and Loire) considered have been selected due to their contrasted above ground growth (i.e., low, medium and high) in their native sediment. In our experiment, their growth (roots and shoots) was monitored under each climate and in the two sediments considered and the chemical characteristics of the sediments were determined. We hypothesized that the growth of black poplar seedlings is influenced by the microbiome recruited in the root system. To test, this hypothesis, we organized our analyses to answer to three main questions: (i) Is there an effect of the soil origin on the microbiome composition occurring in the black poplar root system and in the surrounding soil? (ii) Do the genetic characteristics (i.e., progeny) influence the composition and structure of the root-associated microbiome? (iii) Are the black poplar soil and root-associated microbiome influenced by the contrasted climates and/or the soil conditions?

Material & Methods

Seed collection

For our transplant experiment, seeds from *Populus nigra* trees have been collected in two climatically contrasted regions of France (Drôme and Loire), along the Loire and Drôme rivers. In each location, seeds were specifically collected on *Populus nigra* mother trees previously genetically characterized based on single nucleotide polymorphism (SNP) or microsatellite analyses and presenting a flowering phenology out of step with the black poplar cv. Italica (Faivre-Rampant et al., 2016). The sampling period was determined to avoid as much as possible the hybridization with black poplar cv. Italica and to maximize the genetic differentiation of the *Populus nigra* seedlings progenies. In each region, the progeny of 10 mother trees has been collected.

For the experiments presented in this study, only 3 progenies per site have been considered. The Loire progenies were selected to present contrasted growth (low, medium and high) in the Loire sediment and under the Loire climate. The Drôme progenies were selected to present contrasted growth (low, medium and high) in the Drôme sediment and under the Drôme climate.

In the Loire region, the seeds from L04 progeny were collected from a mother tree located in the Natural reserve of Saint Pryvé Saint Mesnin (1.8415°/ 47.8824°) while the seeds from L06 and L08 progenies were collected from a mother tree located Guilly (2.2877°/ 47.8069°).

In the Drôme region, the seeds from D11, D13 and D15 were collected from mother trees located in Natural reserve of Les Ramières (4.9488°/ 44.7427°). Seeds collections were performed in May 2017. Seeds were stored in 4°C until germination assays in Petri dish with filter paper and water in order to check the seed viability.

Plant and soil material

In each location, raw sediments were collected using an excavator in the river bed. Although, these sediments did not follow paedogenesis steps, we will refer in our manuscript to BS as bulk sediment, in the sense of bulk soil (i.e., soil without roots). In the Loire region, sediment sampling was done in Saint Père sur Loire (2.3667°/47.7667°) in October 2016. In the Drôme region, the sampling was done in Livron (4.8399°/44.7682°) in December 2016. After the sampling of the sediments were transferred to the INRA Center of Orléans and conserved 4 months under ambient conditions and below a canvas sheet to let the sediments dry. After 4 months, the sediments were homogenised and conditioned in mesocosms (1m x 1m x 1m). A total of 12 mesocosms (6 containing the Drôme sediment and 6 containing the Loire sediment) has been prepared to permit our transplant experiment. A sediments sample (called “T0”) was collected in each mesocosm and conserved at -20°C until DNA extraction. After conditioning, half of the mesocosms was installed at the INRAE Center of Orléans (1.5452°/47.4942°; Loire region), while the other mesocosms were installed in the Natural reserve of Les Ramières (RMN) in Allex (4.9151°/44.7621°; Drôme region). The details of the experimental design are presented in Figure 26.

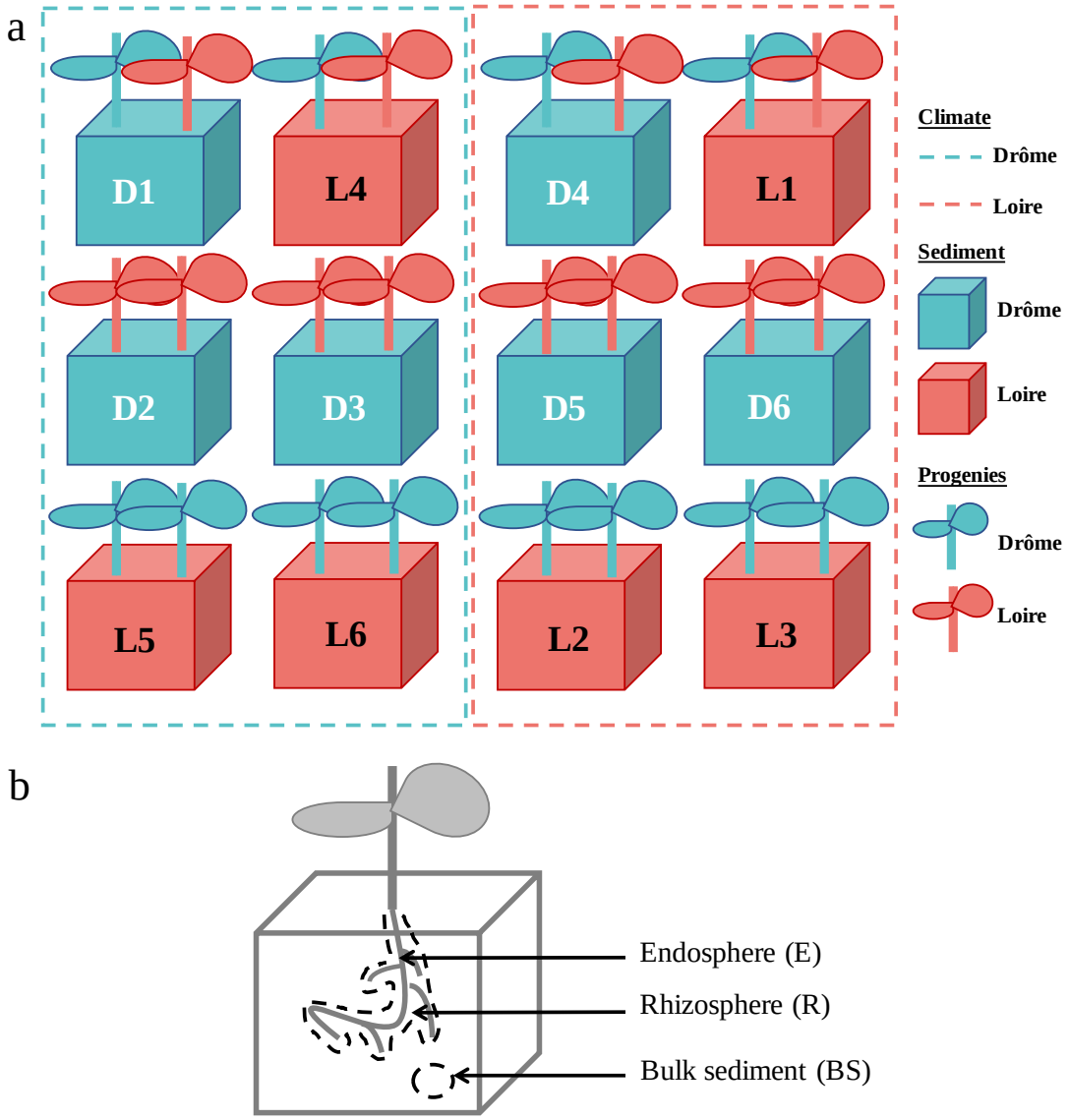


Figure 26 - Experimental design. In function of the question, the number of mesocosms treated varied from 1 to 4. To allow analysis, a minimum of n=3 samples have been collected for each sample type (compartment, progenie, climate; 3<n<6) (a). Comparmentalization of the different samples (BS, R and E) collected in each Populus nigra seedlings (b).

To avoid seedlings mortality or an absence of germination, seeds were first planted in soil plugs and incubated in greenhouse (20°C, 16 h light) at the end of the spring in 2017 with an unlimited watering. After two weeks of germination, seedlings were acclimated by shading with watering for one week. These seedlings were then transferred in the different mesocosms (Figure 26). Mesocosms were heavily watered along the full length of the experiment. Mesocosms located in Loire were watered 5 times a day for 5 minutes while mesocosms located in Drôme were watered 5 times a day for 10 minutes due to a lower pressure of the pump. Mesocosms were sprinkled from above with 5 sprinklers in unlimited quantities.

Physico-chemical analyses of the sediments and temperature and luminosity monitoring

The physico-chemical properties of the two sediments used in our study were determined by the Laboratoire d'Analyses des Sols (INRAE Arras, France) according to standard procedures. Briefly, exchangeable cations were extracted in either 1M KCl (Magnesium, Calcium, Sodium, Iron, Manganese) or 1M NH4Cl (Potassium) and determined by ICP-AES (JY180 ULTRACE). The 1M KCl extract was also titrated using an automatic titrimeter (Mettler TS2DL25) to assess exchangeable H+ and aluminium cations (Al^{3+}). The pH of the soil samples was measured in water at a soil to solution ratio of 1:2 (pH meter Mettler TSDL25). Exchangeable acidity was calculated by taking the sum of H^+ and Al^{3+}. The cation-exchange capacity (CEC) was determined by using cobaltihexamine chloride. Titration of the cobaltihexamine chloride soil extract was performed at 472 nm and compared to a reference of 0.05 N cobaltihexamine chloride extract.

Temperature and luminosity monitoring was performed on each site. The temperature and the Photosynthetically Active Radiation (PAR) of each site was taken once an hour from the beginning of the experiment (June 2017) until the seedlings were harvested (October 2017).

Seedlings aerial growth monitoring

In each location (Loire or Drôme) and after 10 weeks of growth in the mesocosms, the aerial growth of *Populus nigra* seedlings of each progeny from Loire (L04, L06, L08) and Drôme (D11, D13 and D15) was measured on September the 4th (Table S1).

Sampling strategy

Before the transplant experiment, a total of 3 spatially distant BS samples were collected in each mesocosm from 3 spatially distant areas free of seedlings roots. The BS samples of each mesocosm were pooled, giving a total of 12 BS samples corresponding to the T0 in our study.

After five months of growth in the Loire and Drôme regions, all the mesocosms were transferred to the INRAE Center of Orléans. Each mesoscosm was opened to collect: bulk sediment (BS), root adherent soil (i.e the rhizosphere; R) and root sample (i.e. the rhizoplane and the endosphere; E). In each mesocosm, a total of 3 spatially distant BS samples were collected in each mesocosm from 3 spatially distant areas free of seedlings roots. The BS samples were pooled, giving a total of 12 BS samples. For each seedling considered, the root system was harvested, shaken over a sieve of 2 mm to remove non-adherent soil. The rhizosphere (R) was

recovered by washing the root system with 40 mL of a sterile solution of NaCl (10 mM), as previously described by Gottel et al., 2011. Finally, the washed roots were conserved for each seedling to access to the endosphere (E). After conditioning, all the sample types were stored at -20°C until DNA extraction.

DNA extraction

Total soil DNA was extracted using the DNeasy PowerSoil Kit following the protocol provided by the manufacturer (Qiagen, Venlo, the Netherlands). For each DNA extraction, 250 mg of BS and R samples have been used. For the endopshere (E), 50 mg of root tissues were crushed in liquid nitrogen and DNA was extracted using the DNAeasy PowerPlant Kit (Qiagen, Venlo, the Netherlands). DNA was quantified with a NanoDrop 1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA).

DNA amplification and Illumina MiSeq sequencing

A two-step PCR approach was chosen to barcode tag templates with frameshifting nucleotide primers (Lundberg et al., 2013). Primer mixtures for tagging bacterial amplicons were composed of 4 forward 515F (Universal, Chloroflexi, TM7, Nano; Table S2) and 2 reverse 806R (Universal, Nano; Table S2) primers covering the 16S rRNA V4 gene region mixed in equal concentrations (0,1 μM) (Cregger et al., 2018). Primer mixtures for tagging fungal amplicons were composed of six ITS3 forward primers (ITS3NGS1, ITS3NGS2, ITS3NGS3, ITS3NGS4, ITS3NGS5 and ITS3NGS10; Table S2) and one ITS4 reverse primer (ITS4NGS; Table S2) for ITS2 rRNA region mixed in equal concentrations (0.1 μM; Cregger et al., 2018). To inhibit plant material amplification (i.e., mitochondria and chloroplast), PCR reaction mix was implemented by a mixture of peptide nucleotide acids (PNA, Panagene Korea) blockers. These PNA blockers targeted plant mitochondrial and chloroplast 16S rRNA genes (mtPNA_717-1B4, pPNA_717-1B4; Lundberg et al., 2013; Table S2) and plant ITS nuclear rRNA gene (ITSspacePNA_717-1B4; Cregger et al., 2018; Table S2). The mitochondrial PNA blocker (mtPNA_717-1B4; Table S2) of Lundberg et al., 2013 was adjusted for a 1 bp mismatch. Although these PNA blockers have been designed to block mitochondrial and chloroplastic sequences of *Populus tremula x alba*, they can also be used for *Populus nigra*.

Polymerase chain reactions (PCR) were performed for two replicates of each sample by mixing 12 µl of 2.5x Phusion flash high fidelity master mix (ThermoScientific) with 1.5 µl of forward and reverse primer mix (5 nM final concentration) and 20 ng of total DNA in a final reaction volume of 30 µl. For bacteria, 0.75 µl of PNA probe (5 nM) was added. PCR, primer and probes dilutions were performed in DNA free water (0.2 µm filtered and UV treated; Carl Roth, France). For the first amplification of bacterial 16S rRNA, the following cycle parameters were used for bacterial amplification were 30 cycles of 98°C for 5s, 78°C for 10s, 52°C for 20s and 72°C for 15s. Primary PCR condition for fungal amplification were 30 cycles of 98°C for 5s, 78°C for 10s, 55°C for 20s and 72°C for 15s. PCR products without addition of microbial DNA (negative control) or corresponding to mock communities of known fungal or bacterial compositions were added as quality controls. After checking concentration of PCR products and amplicons size (350 pb for 16S rRNA amplicon and 420 pb for ITS amplicon) on agarose electrophoresis gel, samples of 50 µl (30 ng DNA per µl) were sent for tagging and MiSeq Illumina

Next Generation Sequencing (GeT PlaGe INRAE sequencing platform, Toulouse, France). Sequencing was done on MiSeq 2500 system.

Bioinformatic analyses

Bacterial sequences were further processed with FROGS (Find Rapidly OTU with Galaxy Solution) (Escudié et al., 2018) based on the Galaxy analysis platform (Afgan et al., 2016). The 16S rRNA amplicon sequences were demultiplexed, dereplicated and sequence quality was checked. The oligonucleotides, linker, pads and barcodes were removed from sequences. In addition, sequences were removed from the dataset, if non-barcoded, if they exhibited ambiguous bases or did not match expectations in amplicon size. The remaining sequences were clustered into operational taxonomic units (OTUs) based on the iterative Swarm algorithm. Chimeras and singletons (OTUs supported by one sequence) were removed. Bacterial double affiliation was performed by blasting OTUs against the SILVA database v132 (Quast et al., 2012) and the ribosomal database project (RDP) classifier (Wang et al., 2007). OTUs with affiliation <100% at the phylum level (indicated by a RDP bootstrap value <1) and corresponding to chloroplasts or mitochondria were removed from the data set. OTUs at lower taxonomic ranks than the phylum level were considered as “unidentified”, when the RDP bootstrap value was < 0.70 OTUs with high relative abundances in negative controls were excluded from further analysis. The quality of the sequencing, and of the affiliation were evaluated based on the results obtained for the bacterial mock community.

Fungal sequences were processed as following. After demultiplexing and quality checking (QC quality score = 30, minimal size = 200 bp), bioinformatics analyses were performed using standard procedures as described in Pérez-Izquierdo et al. (2017) by using USEARCH. Briefly, the ITS2 was extracted with the Fungal ITSx v1.0.3 and partial ITS sequences were discarded. After de-replication, sequences were shorted by decreasing relative abundance and singletons discarded. OTUs were generated from abundance-sorted sequences with 97 % similarity threshold. Extracted sequences were then mapped against the OTU representative sequences. Taxonomic assignation of these representative sequences for each OUT was done by using the Basic Local Alignment Search Tool (BLAST) algorithm against the UNITE database.

For both fungal and bacterial data, per-sample rarefaction curves were calculated to assess sampling completeness, using function *rarecurve()* in package Vegan v3.5-1 (Oksanen et al., 2015) in R (version 3.4.3 ; R Core Team, 2016). Based on these, subsequent analyses of diversity and community structure were performed on datasets where samples have been rarefied with the Phyloseq (McMurdie, PJ. and Holmes, S., 2013) package to achieve equal read numbers according to the minimum number of total reads in any sample. In our study, the samples were rarefied to 10,733 sequences per sample for the bacteria and 4,162 sequences per sample for the fungi.

FUNGuild (Nguyen et al., 2016) was used to classify each fungal OTU into an ecological guild. We followed the same procedure as Cregger et al., 2018. OTUs identified to a guild with a confidence ranking to “highly probable” or “probable” were conserved in our analysis, whereas those ranking to “possible” or with multiple assignations were considered as unclassified.

Statistical analysis

Statistical analyses and data representations were performed using R software (R Core Team, 2016). After checking normal distribution of each dataset with Shapiro-Wilk test, Student t-tests were used to determine if the relative abundance of fungal guilds differed between the different compartments considered [sediments (BS), the rhizosphere (R) and the endosphere (E)] and/or according to the different treatments. A One-way ANOVA followed by Tukey HSD post-hoc test was used to determine if the relative abundance of dominant bacterial and fungal phyla and genera detected in the different compartments considered [sediments (BS), the rhizosphere (R) and the endosphere (E)] differed between the different treatments (sediments and climate conditions). Comparison of the relative abundance of bacterial and fungal OTUs in R and in E between two conditions was based on one-way ANOVA followed by Tukey HSD post-hoc test. Venn diagrams were created on http://bioinformatics.psb.ugent.be/webtools/Venn/. Microbial community structures were analysed using nonmetric multidimensional scaling analysis (NMDS) and permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrices.

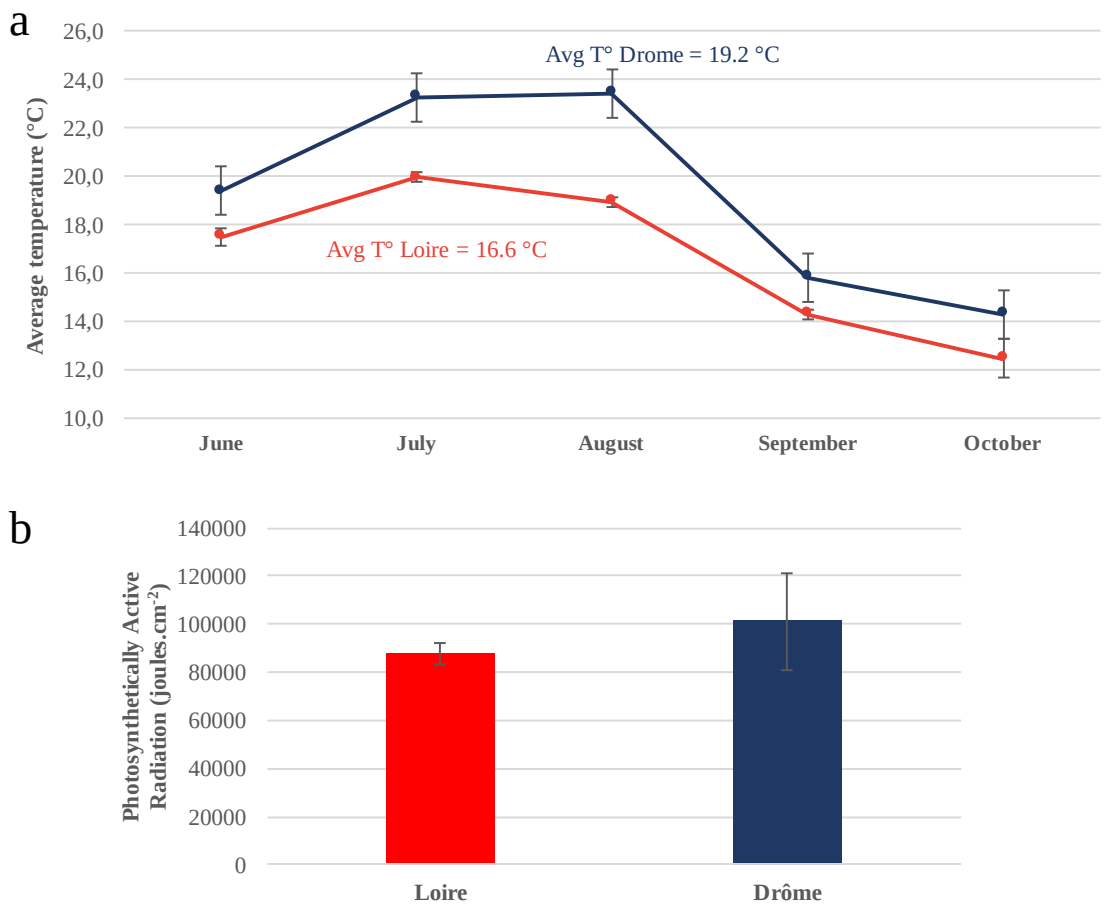


Figure 27 - Monitoring of temperature in Loire and Drôme regions. Average monthly temperature recorded by the meteorological station of INRAE (Loire, in red) and RMN (Drôme, in blue) from June to October 2017 (a). Cumulated PAR measures recorded from June to October 2017 in the Loire (red) and in the Drôme (blue) sites (means of daily measure ± SE) (b).

Results

Physico-chemical analyses of the sediment, temperature and luminosity monitoring and plant growth

Based on the physico-chemical analyses performed, the two sediments were characterized as silty-clayey for the Drôme and sandy for the Loire (Table S3) with alkaline pH. The two sediments strongly differed in terms of texture, cationic exchange capacity and amount of available nutrients. Estimated fertility was much higher in Drôme soil than Loire one.

The temperature monitoring performed from June to October 2017 revealed a warmer temperature (+2.6 °C) in the Drôme site compared to the Loire site (Figure 27). The luminosity (cumulated PAR measure from June to October 2017) was not significantly different between the Loire (87.5 ± 4.4 joules/cm^2) and the Drôme (101.2 ± 20.3 joules/cm^2) sites (Figure 27).

The measures done on the aerial parts of the poplar seedlings of the 3 progenies considered per site (i.e., Drôme and Loire) revealed significant differences of growth (ANOVA, P<0.05, Figure 28). Under their native conditions of culture (i.e., sediment and climate), D11 and L08 were the taller progenies, while L04 and D15 were the smaller (P<0.05), giving a specific pattern of distribution of the progenies according to their above ground size and for each site (Drôme: D11>D13>D15; Loire: L06>L08>L04).

We then considered the potential effect of the two sediments on the growth of the seedlings. When the measures were done considering the seedlings growing under their native climate, but in the transplanted sediment, both the Drôme and Loire progenies presented a different growth pattern than in their native conditions. For the Drôme seedlings we observed the following pattern (D11>D15>D13), while for the Loire seedlings no difference was observed (L04=L06=L08). Notably, the D15 progeny grew better in Loire sediment than in their native soil (+ 41 %; % expressed according to the native conditions; P<0.05), while the two other Drôme progenies grew less on the Loire sediment than in their native sediment (- 39 % for both D11 and D13; P<0.05). Similarly, the L06 seedlings grew better in Drôme sediments than their native sediments (+ 70 %; P<0.05), while no sediment effect was observed for the growth of the two other progenies.

The potential effect of climate on the growth of the seedlings was then considered. When the measures were done considering the seedlings growing under their non-native climate, but in their native sediment, the initial patterns were conserved (Drôme: D11>D13>D15; Loire: L06>L08>L04). However, the D15 seedlings grew better under their native climate than under the Loire climate (+ 75 %), while there was no climate effect for the two other Drôme progenies (D11 and D13).

Finally, when the combined effects of the transplant conditions (non-native climate + non-native sediment) were tested, the same patterns of growth were observed than under the native climate and the non-native sediment (D11>D15>D11; L04=L06=L08; Figure 28). All the Loire seedlings presented a significantly increased growth when transplanted in the Drôme soil and climate (L04 = + 69 %, L06 = + 48 %, L08= + 54 %), while D13 seedlings had a reduced aerial development when transplanted in the Loire soil and climate (- 200 %).

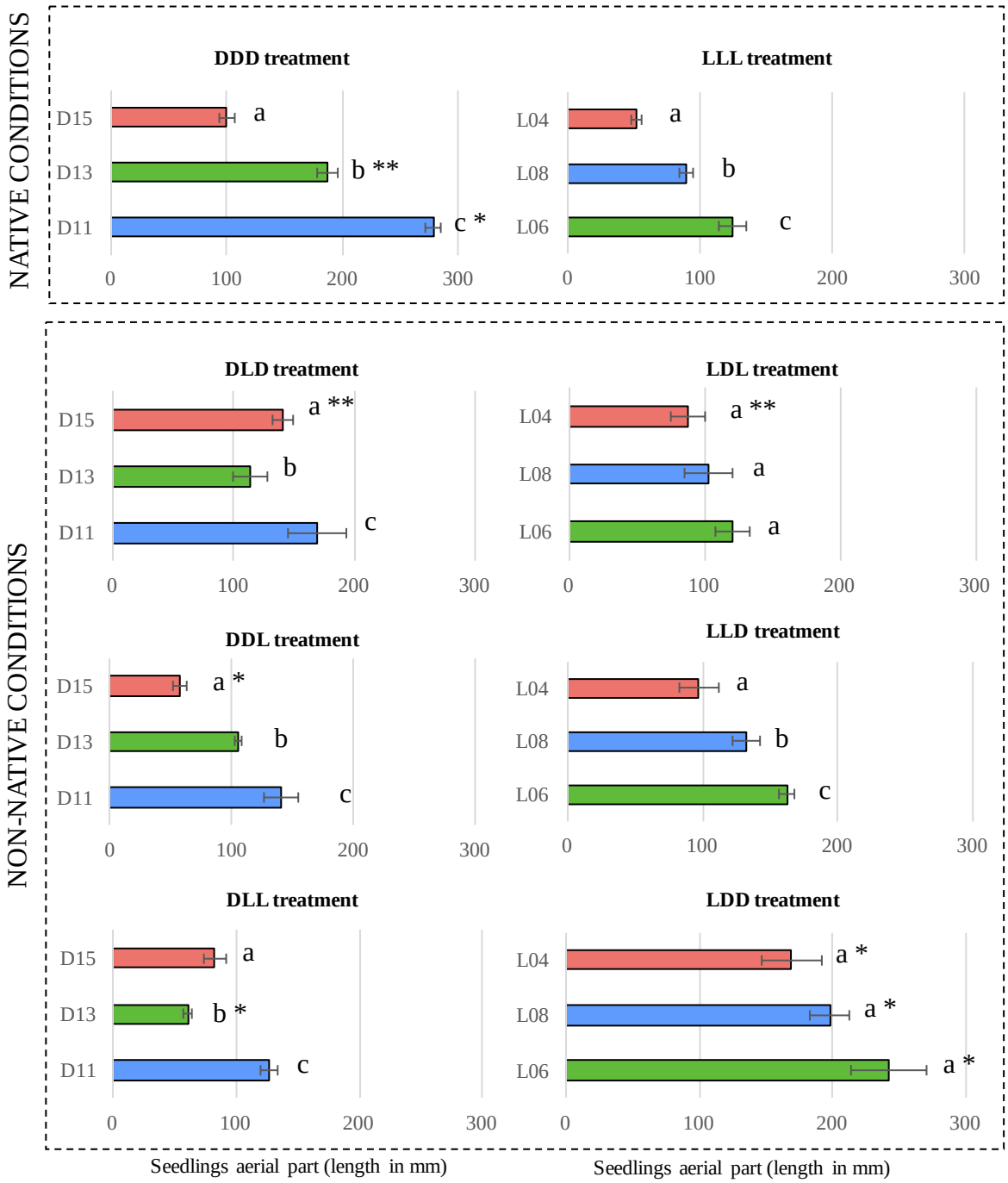


Figure 28 - Monitoring of the growth of the aerial part of Populus nigra seedlings. Length of aerial part of the seedlings of the Drôme (D11, D13, D15) and the Loire (L04, L06, L08) progenies cultivated in different climate and sediments conditions after 10 weeks of growth. The letters denote significant different in aerial part length of seedlings according to origin (i.e, Loire or Drôme) (ANOVA, P<0.05). The asterisks denote significant different in aerial part length of seedlings between treatment (ANOVA, P<0.05).

Sequencing results

After quality filtering, and chimera and singleton removals a total of 7,370,000 bacterial and 2,740,000 fungal reads with an average of 25,000 bacterial reads per sample (± 234 reads SE) and 9,830 fungal reads per sample (± 135 reads SE) remained. After taxonomy assignment, elimination of contaminants and singletons and completion of rarefactions, 2,479 bacterial and 3,197 fungal Operational Taxonomic Units (OTUs) with an average of 1,253 bacterial OTUs (± 21 SE) and 1,609 fungal OTUs (± 41 SE) per sample were considered for further analysis.

Structure and composition of the microbial communities of the Loire and Drôme sediments

The NMDS and PERMANOVA analyses revealed that in both sediments (*i.e.*, Loire and Drôme) the fungal and bacterial community structures varied significantly (P<0.05) between the preconditioning stage of the sediment in mesocosms and after the 5 months-incubation period (Figure 29, Table S4).

The same analyses only done on the 5 months-incubation period-related samples revealed that the Loire and Drôme BS microbiome were significantly different. In addition, the alpha-diversity indices (i.e., number of OTU observed and Shannon index) appeared significantly higher in the Drôme sediment than in the Loire sediment, for both the bacteria and fungi (One-way ANOVA, P<0.05, Figure 30 a, b). Our analyses also revealed that the climate did not significantly affect the structure of the Loire and Drôme BS microbiomes when the native and transplant conditions were compared (P>0.05, Table S4).

A detailed analysis evidenced that the differences between the two sediments (after the 5 months-incubation period) were significant only for the bacteria. Indeed, several phyla showed significantly different relative abundances between the Loire and Drôme sediments [Proteobacteria (26.4 ± 1.5 % in Loire vs 34.0 ± 1.7 % in Drôme), Acidobacteria (20.4 ± 0.6 % in Loire vs 16.3 ± 1.3 % in Drôme), Actinobacteria (14.8 ± 1.5 % in Loire vs 10.2 ± 2.4 % in Drôme), Chloroflexi (11.8 ± 1.5 % in Loire vs 16.1 ± 2.0 % in Drôme) and Firmicutes (5.5 ± 0.5 % in Loire vs 0.1 ± 0.0 % in Drôme)] (Figure 30 c). For the fungi, Ascomycota tended to be more abundant in the $BS_{Drôme}$ than in the BS_{Loire}. (P>0.05; Figure 30 d). The Loire and Drôme sediments appeared dominated by ectomycorrhizal fungi (EcM; 25.9 ± 18.8 % in Loire and 6.3 ± 2.0 % in Drôme), saprotrophic fungi (3.3 ± 0.9 % in Loire and 8.0 ± 3.0 % in Drôme) and fungal plant pathogen (2.7 ± 1.4 % in Loire and 1.8 ± 0.5 % in Drôme, Figure 30 e).

At the genus level (>1 % relative abundance), no significant difference was observed between the two sediments for both the bacteria and the fungi (P>0.05). However, at the OTU level, a high proportion of the OTUs appeared significantly enriched in only one sediment, as indicated by Venn diagram analyses. Indeed, only 61 % of the OTUs (=72 % of the 16S rRNA sequences) for bacteria and 17 % of the OTUs (=24 % of the ITS sequences) for the fungi appeared common to the two sediments (Figure 30 f).

Structure and composition of the rhizosphere and endosphere microbial communities in the native conditions

The NMDS and PERMANOVA analyses revealed the rhizosphere ($R_{Drôme}$ vs R_{Loire}) and the endosphere ($E_{Drôme}$ vs E_{Loire}) microbial (fungal and bacterial) community structures varied significantly ($P<0.05$) between the two sites (Table 1). A detailed analysis evidenced that these differences were explained by significant variations of the abundances of several bacterial (Chloroflexi [$R_{Drôme} > R_{Loire}$], Verrucomicrobia [$E_{Loire} > E_{Drôme}$] and Proteobacteria [$E_{Drôme} > E_{Loire}$]) (Figure 31 a) and fungal (Glomeromycota [$R_{Drôme} > R_{Loire}$], Chytridiomycota [$E_{Loire} > E_{Drôme}$], Ascomycota [$E_{Loire} > E_{Drôme}$] and Basidiomycota [$E_{Drôme} > E_{Loire}$]) (Figure 31 b) phyla. At the genus level, *Acidibacter*, *Azohydromonas* and *Steroidobacter* were significantly more abundant in $R_{Drôme}$ and $E_{Drôme}$ than in the Loire samples, while *Ohtaekwangia* was significantly more abundant in R_{Loire} and E_{Loire} than in the Drôme samples (ANOVA $P<0.05$, Table S5).

For the fungi, *Tomentella* and *Tetracladium* were significantly more abundant in Drôme samples (R and E) than in the related Loire samples (ANOVA $P<0.05$, Table S5). The funguild analyses revealed that the arbuscular mycorrhizal (AM) fungi were significantly more abundant in the R_{Loire} than in the $R_{Drôme}$ (ANOVA, $P<0.05$, data not shown), while saprotrophic fungi and plant pathogen were more abundant in the E_{Loire} than in the $E_{Drôme}$ (ANOVA, $P<0.05$, Figure 31 c).

Based on OTU analyses, the rhizosphere samples harboured the highest diversity (Shannon) and richness (OTUobs) values than the endosphere. In addition, the Drôme samples showed significantly higher values than the Loire samples (R and E). Notably, Venn diagram analyses revealed that 84 % and 36 % of the bacterial and fungal OTUs (= 92 % of the 16S rRNA 16S and 80 % of the ITS sequences) were common to the two rhizospheres ($R_{Drôme}$ vs R_{Loire}). Similarly, 54 % and 43 % of the bacterial and fungal OTUs (= 88 % of the 16S rRNA and 93 % of the ITS sequences) were common to the two rhizospheres ($E_{Drôme}$ vs E_{Loire}).

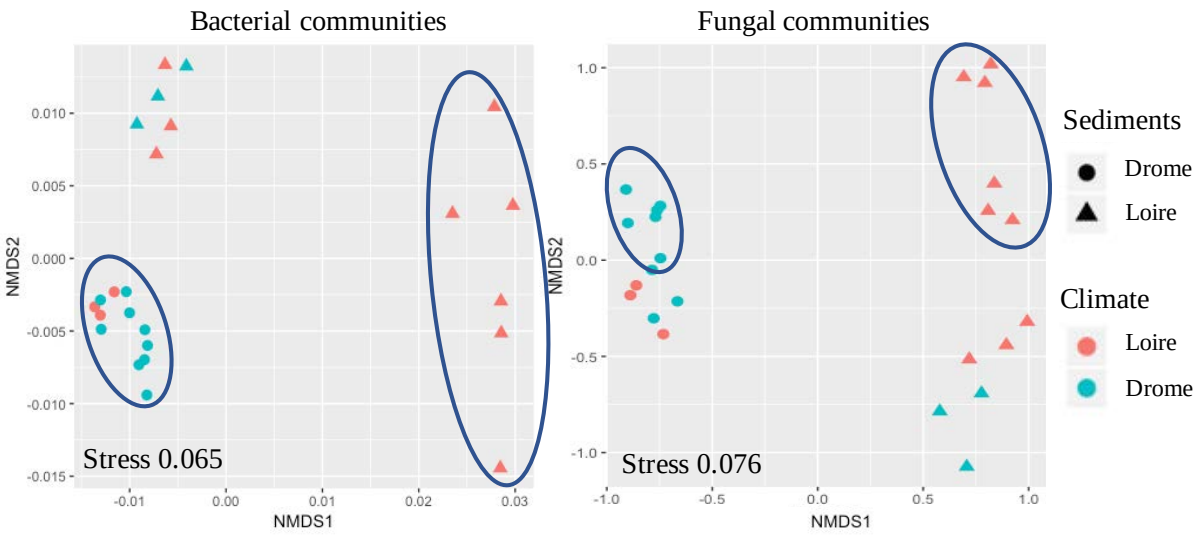


Figure 29 - Impact of pre-conditioning stage on the Loire and Drôme sediments microbiome. NMDS ordinations of bacterial and fungal OTUs across sediment type (Drôme and Loire) and climate (Drôme and Loire). Blue circles correspond to BS_T0 samples. Variances explanation based on permutational multivariate analysis using Euclidean dissimilarity matrix for bacterial and fungal OTUs are available in Table S4.

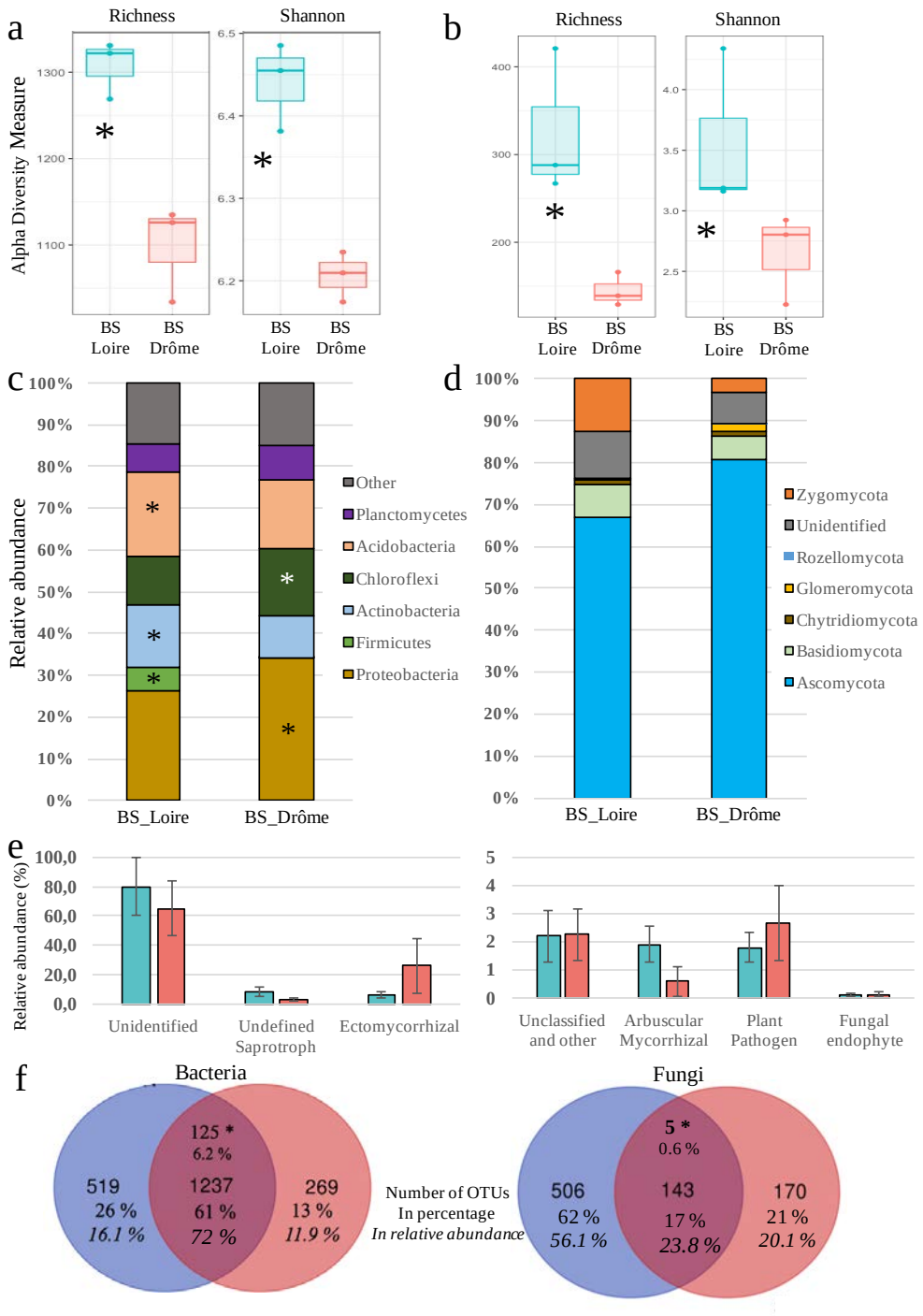


Figure 30 - Composition and structure of sediment microbiome. Alpha diversity (Richness and Shannon index) of the bacterial (a) and fungal (b) communities detected in BS samples collected in Loire (red) and Drôme (blue). The asterisks denote significant difference in each alpha diversity measure between Loire and Drôme BS (ANOVA, P<0.05). Distribution of most dominant bacterial (> 5 % in relative abundance) (c) and fungal (d) phyla detected in BS samples from Drôme and Loire. The asterisks denote significant difference in relative abundance of each bacterial and fungal phyla between Loire and Drôme BS (ANOVA, P<0.05). Venn diagrams of the bacterial and fungal OTUs only detected in BS samples from Drôme and Loire or shared by both (http://bioinformatics.psb.ugent.be). The number in bold represents the number of OTUs whose relative abundance is significantly different between the two types of sediments. This value is converted in percentage in bold and in relative abundance of total OTUs in brackets. The asterisks denote significant difference in relative abundance of each bacterial and fungal OTUs between Loire and Drôme sediments (ANOVA, P<0.05) (e). Relative abundance of fungal guilds detected in the Loire (in red) and in Drôme (in blue) sediments (f).

Analysis of the compartment effect (BS vs R and R vs E) in each treatment (seedling origin, sediment and climate)

For each treatment (i.e., LLL, Loire seedlings cultivated in the Loire sediment and under Loire climate; LDL, Loire seedlings cultivated in the Drôme sediment under Loire climate; LDD, Loire seedlings cultivated in the Drôme sediment and under Drôme climate; LLD, Loire seedlings cultivated in the Loire sediment and under Drôme climate; DDD, Drôme seedlings cultivated in the Drôme sediment and under Drôme climate; DLL, Drôme seedlings cultivated in the Loire sediment and under Loire climate; DLD, Drôme seedlings cultivated in the Loire sediment and under Drôme climate; DDL, Drôme seedlings cultivated in the Drôme sediment and under Loire climate) analyses were performed to determine whether i) the rhizosphere microbiome differed from the BS microbiome and ii) the rhizosphere microbiome differed from the endosphere microbiome.

The NMDS and PERMANOVA analyses revealed for most of the treatments that the bacterial community structure varied significantly ($P<0.05$) between BS and R compartments (*i.e.*, the rhizosphere effect) and between R and E compartments (*i.e.*, the host root filtering effect) whatever the sediments and climate conditions (*i.e.*, Loire and Drôme) (Table 2). A single exception was observed (BS=R) for the LDL treatment ($P=0.246$, Table 2). Concerning the fungal community structure, no difference was observed between R and BS, while the R and E compartments (*i.e.*, the host root filtering effect) significantly differed ($P<0.05$) (Table 2).

The comparisons done on the taxonomic composition revealed significant differences between the different compartments (BS vs R and R vs E) in the native conditions (*i.e.*, DDD and LLL) for both bacteria and fungi. For the LLL treatment, a significant gradient of enrichment was observed from BS to E for several bacterial phyla such as Actinobacteria, Bacteroidetes and Verrucomicrobia (BS<R<E, $P<0.05$). Proteobacteria appeared only enriched in R compared to BS. For the DDD treatment, a significant gradient of enrichment was also observed from BS to E for the Bacteroidetes phylum. Chloroflexi appeared more abundant in R than in BS and E, while Actinobacteria were significantly enriched in E (Figure 32 a). Among the dominant bacterial genera detected in our study, several appeared enriched in rhizosphere (R>BS; *Niastella*, *Ohtaekwangia* and *Steroidobacter*) and/or in the endosphere (E>R; *Streptomyces*, *Actinoplanes*, *Niastella* and *Steroidobacter*) (Table S6).

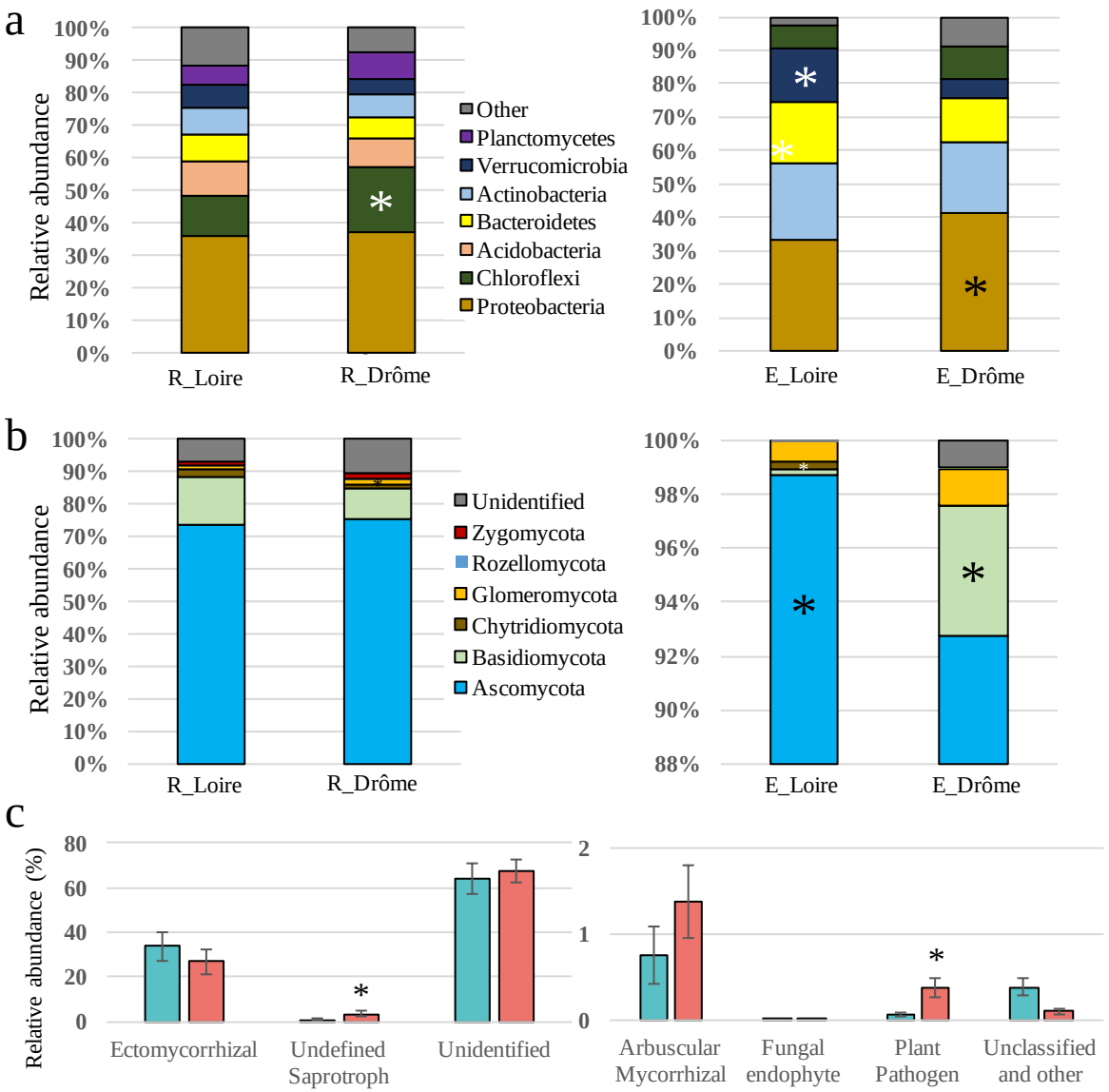


Figure 31 - Composition and structure of Populus nigra root microbiome. Distribution of most dominant bacterial (> 5 % in relative abundance) (a) and fungal (b) phyla detected in the rhizosphere (R) and in the endosphere (E) of the seedlings of Drôme and Loire progenies cultivated in their native conditions of sediments and climate (i.e, DDD and LLL treatment). The asterisks denote significant difference in relative abundance of each microbial phyla detected in the R or E compartments between the seedlings of the DDD or the LLL treatment (ANOVA, P<0.05). Relative abundance of fungal guilds detected in the endosphere of the seedlings of the Loire (in red) and the Drôme (in blue) cultivated in their native conditions (c). The asterisks denote significant difference in relative abundance of each fungal guild between the DDD and LLL treatment (ANOVA, P<0.05).

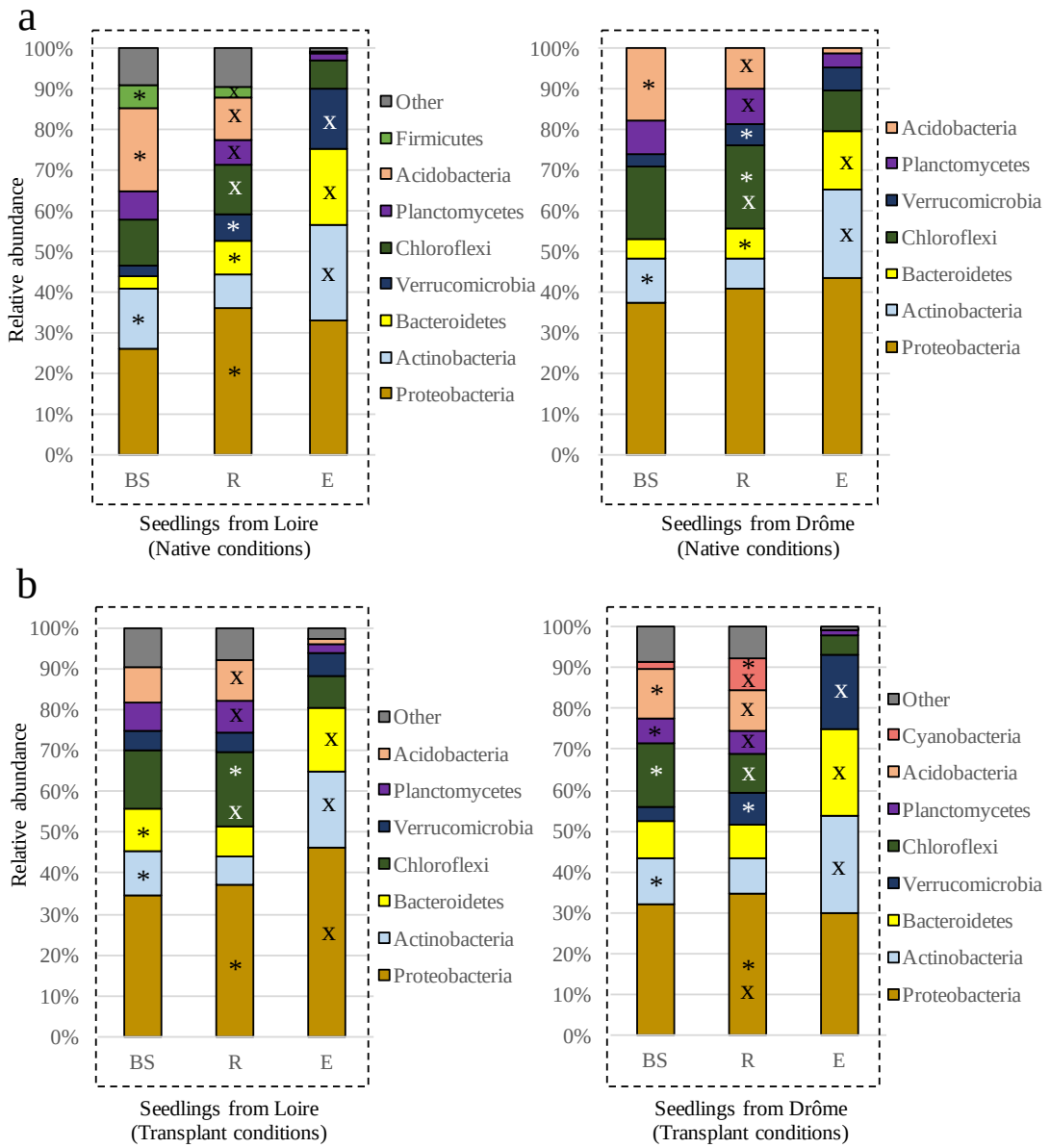


Figure 32 - Rhizosphere and root filtering effects in the native and transplant conditions. Distribution of most dominant bacterial phyla (> 2 % in relative abundance) detected in the bulk soil (BS) and in the rhizosphere (R) and the endosphere (E) of Populus nigra seedlings of the Loire and the Drôme cultivated in their native conditions (i.e., LLL and DDD treatments) (a) and in the transplant conditions (LDD and DLL treatments) (b). The asterisks denote significant difference in relative abundance of bacterial phyla between BS and R compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

For the fungi, the stronger modifications of composition were observed between the R and the E compartments, and in a lower extent, between the BS and the R. For instance, *Cladosporium* (plant pathogen), *Tetracladium* (saprotroph) and *Corallomycetella* (saprotroph) were significantly more abundant in the R compared to the BS, while *Geopora* (EcM) was significantly more abundant in the E compared to the R (Table S7). These observations were confirmed by the distribution of the fungal guilds identified by Funguild analyses across the three studied compartments. The EcM fungi were significantly more abundant in the endosphere (E) of the Loire and Drôme seedlings cultivated in their native conditions (34.3 ± 7.1 % in E_{Loire}, 28.9 ± 5.2 % in $E_{Drôme}$).

Significant modifications were also observed when only one parameter varied (*i.e.*, sediment [LDL and DLD] or climate [LLD and DDL]). When the seedlings were cultivated in another sediment (LDL and DLD) than their native sediment, several significant differences were observed at the phylum and genus levels (Figure 35, Table S6, Table S7). Notably, Proteobacteria presented a significantly higher relative abundance in E than in the other compartments whatever the origin of the seedlings (ANOVA, $P<0.05$). Verrucomicrobia tended to be increased in the root compartments (BS<R<E), especially in the DLD treatment. When the seedlings were cultivated in another climate (LLD and DDL) than their native climate, several significant differences were observed at the phylum and genus levels between compartments (Figure 35). Notably, Actinobacteria and Bacteroidetes, were enriched in E compared to the other compartment in LLD and DDL. Verrucomicrobia and Proteobacteria were only enriched in the endosphere of the LLD treatment. For the fungi, no difference was observed at the phylum and only few genera appeared affected such as *Geopora* (BS<R<E), and *Alternaria*, *Corallomycetella*, *Cladosporium* (R >BS=E) (Figure 35).

In the complete transplant conditions (i.e., LDD and DLL; climate + sediment), we observed that Proteobacteria, Chloroflexi and Cyanobacteria were enriched in the R of the Loire and Drôme seedlings (R>BS), while Proteobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia were significantly enriched in the E (E>R) (Figure 32 b). Several genera appeared more abundant in the endosphere (*Niastella, Streptomyces, Cellvibrio, Actinoplanes*) compared to the other compartments, while *Allorhizobium* was only significantly enriched in the rhizosphere (Table S6). For the fungi, no difference was observed at the phylum compared to the native conditions (Table S7). The funguild analyses revealed that EcM fungi significantly dominated the endosphere (E_{Loire} =32.7 ± 3.5 % vs R_{Loire} =15.7 ± 2.1 %; $E_{Drôme}$ 22.6 ± 4.0 % vs $R_{Drôme}$ 9.2 ± 1.9 %).

Comparative analysis of the compartments between the native conditions and transplant conditions

For each transplantation treatment (i.e., LLD, LDD, LDL, DLL, DDL and DLD), analyses were performed to determine whether i) the sediment origin, ii) the climate and iii) the sediment and the climate affected the composition of the rhizosphere and/or the endosphere microbiomes compared to native conditions (i. e., LLL and DDD). The NMDS and PERMANOVA analyses done directly on the OTUs revealed that the fungal and bacterial community structures varied significantly ($P<0.05$) in the two studied compartments (*i.e.*, R or E) between the seedlings cultivated in their native conditions and the seedlings cultivated in the transplant conditions of sediment and/or climate. Exceptions were observed in the endosphere for fungal communities for the LLD and DDL treatments ([E_{LLD}=E_{LLL}; P=0.377]; [E_{DDL}=E_{DDD}; P= 0.052]) (Table 3).

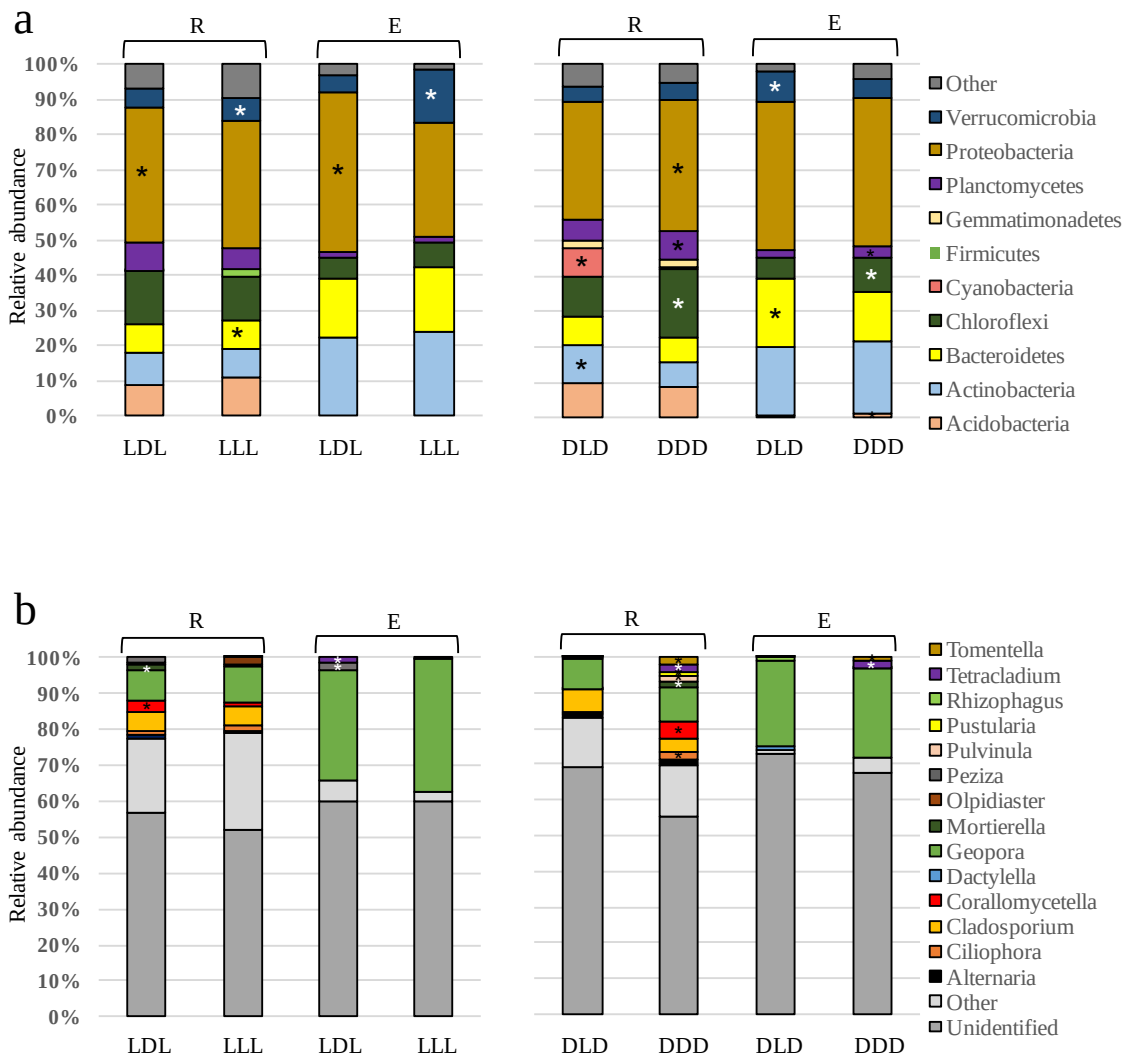


Figure 33 - Impact of sediment origin on the Populus nigra root microbiome. Distribution of most dominant bacterial phyla (> 1 % in relative abundance) (b) and fungal genera (> 1% in relative abundance) (c) detected in the rhizosphere (R) and in the endosphere (E) of Populus nigra seedlings of the Loire and the Drôme cultivated in their native sediment conditions (i.e., LLL and DDD treatments) and in the other type of sediments (i.e., LDL and DLD treatments). The asterisks denote significant difference in relative abundance of each bacterial phyla and fungal genera between the two conditions of seedlings culture (ANOVA, P<0.05).

The taxonomic composition of the microbial communities colonizing the R or the E compartments varied significantly between the native conditions (*i.e*., LLL and DDD) and the transplant conditions of sediment (i.e, LDL and DLD) and of climate (i.e, LLD and DDL) for both bacteria and fungi (ANOVA; P<0.05).

For the bacteria, the LDL treatment, Proteobacteria were significantly more abundant in the two compartments (i.e, R and E) compared to their related native compartment (LLL) (P<0.05; Figure 33 a). For the DLD treatment, Cyanobacteria and Actinobacteria appeared only enriched in R (R_{DLD}>R_{DDD}), while Chloroflexi, Plantomycetes and Acidobacteria were significantly enriched in E (E_{DLD}>E_{DDD}) (P<0.05; Figure 33 a). For the LLD treatment, Cyanobacteria and Actinobacteria were significantly more abundant in the R, while Proteobacteria appeared significantly enriched in the E than in the LLL treatment (P<0.05; Figure 34 a). For the DDL treatment, Chloroflexi, Plantomycetes and Acidobacteria were significantly enriched in E compared to the native treatment (DDD) (P<0.05; Figure 34 a). Among the dominant bacterial genera detected in our study, several appeared enriched in rhizosphere (R) of the seedlings cultivated in the non-native sediment than in the native condition ((i.e., *Acidibacter*, *Allorhizobium, Azohydromonas Bacillus, Gaiella, Niastella, or Ohtaekwangia);* P<0.05; Table S8). Several dominant bacterial genera appeared also significantly enriched in the endosphere (E) of the seedlings cultivated in the non-native sediment (i.e., *Acidibacter, Allorhizobium, Actinocorallia, Actinoplanes, Azohydromonas*, *Bradyrhizobium, Cellvibrio, Lechevaliera, Ohtaekwangia*, *Rhodomicrobium, Steroidobacter*) (P<0.05; Table S8).

For the fungi, *Corallomycetella* (saprotroph) and *Mortierella* (endophytes) were significantly enriched in the R of the non-native treatments (LDL>LLL; DLD>DDD). *Tetracladium* (saprotroph) and *Tomentella* (EcM) were significantly enriched in the E of the LDL and the DLD treatments than in their related native treatments (P<0.05; Figure 33 b). *Tomentella* were significantly more abundant in the E of the seedlings of the DDL treatment compared to the DDD treatment (P<0.05; Figure 34 b). The funguild analyses revealed significant differences between the R compartments for the plant pathogens and of AM fungi (P<0.05; [Pathogen; R_{DDD} > R_{DLD}, R_{LLL} > R_{LDL}]; [AM fungi; R_{LLL} > R_{LDL}, R_{DDD} > R_{DLD}] (Figure 37).

In the complete transplant conditions (i.e., LDD and DLL), Chloroflexi and Planctomycetes appeared significantly enriched in the R compartment compared to the native treatments (i.e., LDD>DDD and DLL>LLL), while Proteobacteria, Planctomycetes and Acidobacteria were significantly enriched in the E of the LDD treatment compared to native treatment (LLL). For the DLL treatment, Verrucomicrobia and Bacteroidetes were significantly enriched in the R and E compartments compared to the native treatment (DDD; P<0.05; Figure 36 a). Several genera appeared more abundant in the rhizosphere and the endosphere of the seedlings of the LDD treatment compared to the native treatment (LLL; *Acidibacter* and *Steroidobacter*). For the DLL treatment, *Bacillus* and *Nodosilinea* appeared enriched in the rhizosphere (R_{DLL}>R_{DDD}), while *Ohtaekwangia* and *Streptomyces* were enriched the endosphere (E_{DLL}>E_{DDD}).

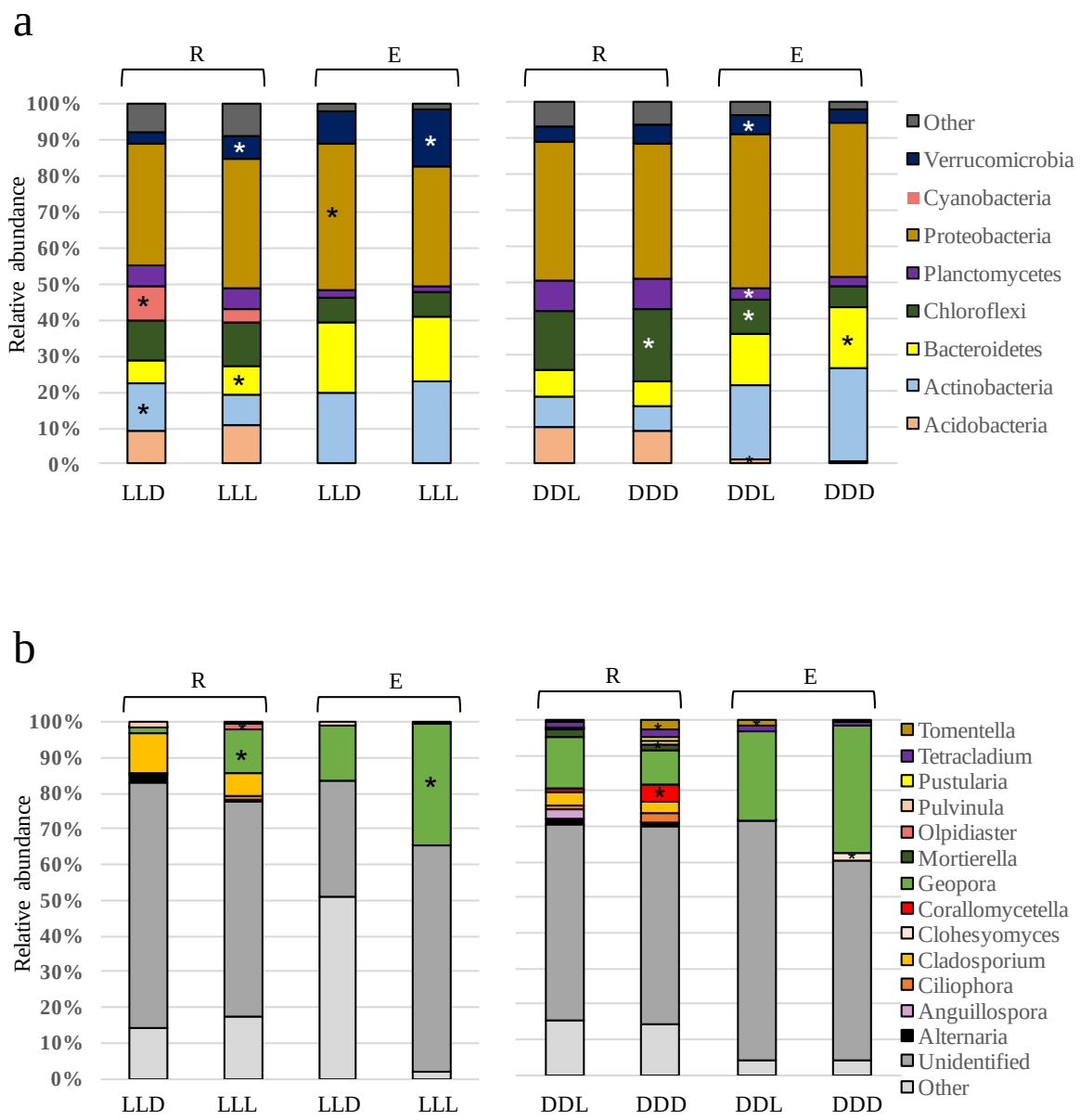


Figure 34 - Impact of climate on the Populus nigra root microbiome. Distribution of most dominant bacterial phyla (> 1 % in relative abundance) (a) and fungal genera (> 1 % in relative abundance) (b) detected in the rhizosphere (R) and in the endosphere (E) of Populus nigra seedlings of the Loire and the Drôme cultivated under their native climate (i.e., LLL and DDD treatments) and under the opposite climate (LLD and DDL treatments). The asterisks denote significant difference in relative abundance of each bacterial phyla and fungal genera between the two conditions of seedlings culture (ANOVA, P<0.05).

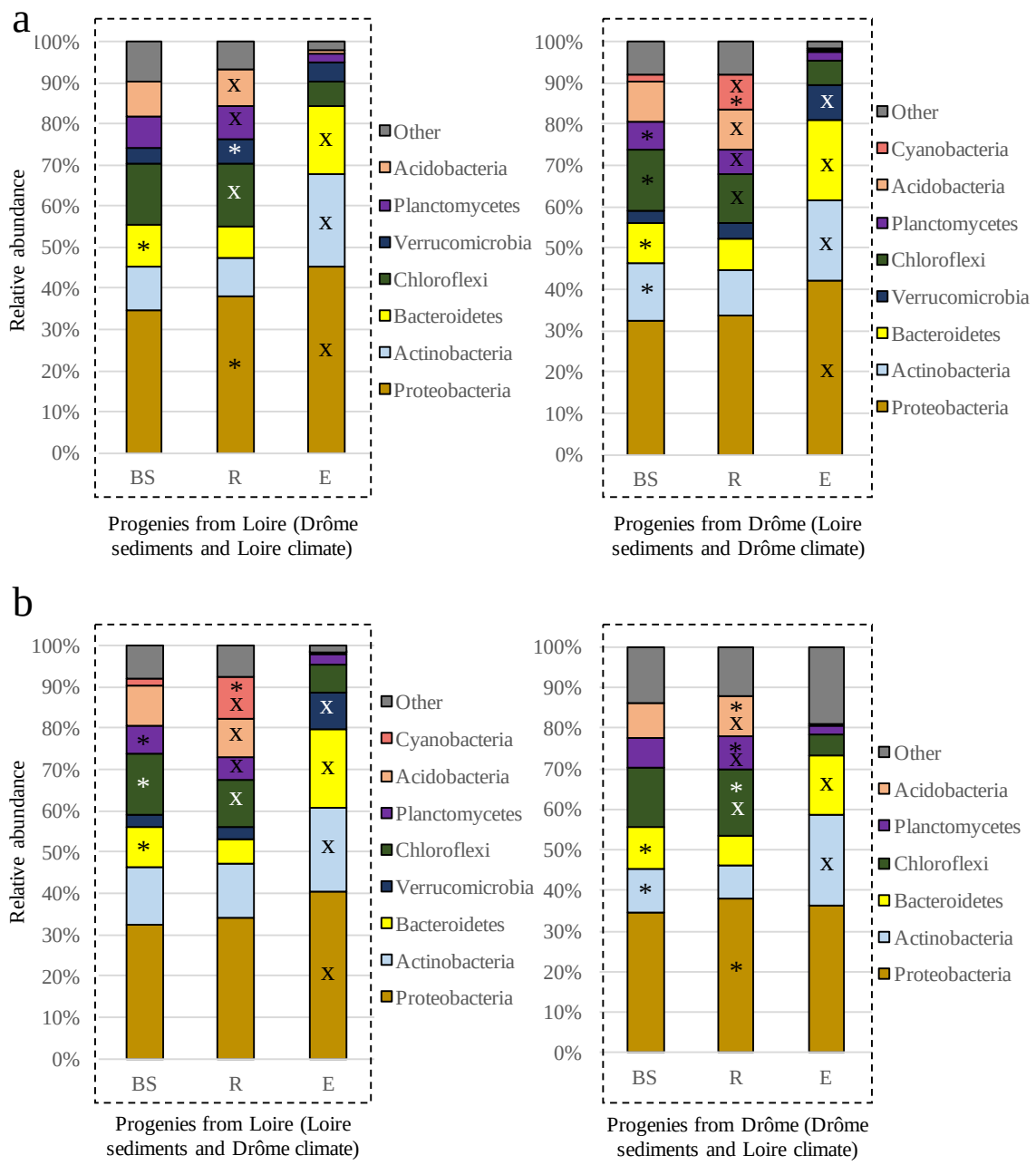


Figure 35 - Rhizosphere and root filtering effects in the non-native conditions of culture. Distribution of most dominant bacterial phyla (> 2 % in relative abundance) detected in the bulk soil (BS) and in the rhizosphere (R) and the endosphere (E) of Populus nigra seedlings of the Loire and the Drôme cultivated in the non-native sediments (i.e., LDL and DLD treatments) (a) and in the non-native climate (i.e, LLD and DDL treatments) (b). The asterisks denote significant difference in relative abundance of bacterial phyla between BS and R compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

For the fungi, no difference was observed at the phylum compared to the native conditions. *Tomentella* were significantly enriched in the R and E compartments of the LDD treatment compared to the native treatment (LLL). *Verrucladosporium* were significantly enriched in the R of the DLL treatment compared to the native treatment (DDD; P<0.05; Figure 36 b). The funguild analyses revealed that EcM fungi, the saprotrophs and the AM fungi were significantly enriched in the R of the native treatment (DDD > DDL) (Figure 37).

Focus on the microbiomes of the different progenies

The global analyses (NMDS and PERMANOVA) done on the OTUs revealed no significant shift in the fungal and bacterial community structures between the 3 progenies of each site (i.e., Loire and Drôme) when the different treatments were compared (Table 4).

However, detailed analyses done on the taxonomic composition revealed significant differences between the rhizosphere microbiomes of the different progenies (*i.e.,* Drôme or Loire), but no effect for the endosphere. In native condition, Chloroflexi appeared significantly enriched in the rhizosphere of D11 compared to the other Drôme progenies (ANOVA, P=0.003; D11 (22.6 ± 1.9 %) > D13 (17.4 ± 1.1 %)= D15 (19.2 ± 0.6 %)), while Actinobacteria appeared significantly enriched in the rhizosphere of L08 than in the other Loire progenies cultivated under Drôme climate (i.e, LLD treatment) (P=0.005; L08 (20.3 ± 5.1 %) > L06 (10.8 ± 1.7 %) = L04 (9.8 ± 1.3 %)). At the genus level, *Niastella* (member of Bacteroidetes) was the single genus significantly enriched in the rhizosphere of the D11 progeny in the DDL treatment (P=0.002; D11 (2.1± 0.3 %) >D13 (1.1 ± 0.2 %) = D15 (0.68±0.25 %)).

Concerning the fungal communities, no effect was observed at the phylum level. At the genus level, *Geopora* (one of the main genus detected in our study) appeared significantly enriched in the rhizosphere of the L08 progeny only in the LLL treatment (L08 (19.3 ± 10.1 %)>L06 (11.1 ± 4.6 %)> L04 (3.0 ± 1.5 %) (ANOVA; R_{L04}, P=0.002). The same genus appeared also significantly enriched in the rhizosphere of the D11 progeny in the DDD treatment (R_{D11}, P=0.003; D11 (15.6 ± 4.7 %) >D15 (9.1 ± 2.7 %)>D13 (5.1 ± 1.0 %)), in the D15 progeny in the DLD treatment (P=0.006; D15 (15.0 ± 3.9 %) > D13 (4.3 ± 2.4 %) = D11 (3.9 ± 3.2 %)) and in D15 in the DDL treatment (P=0.002; D15 (25.5 ± 10.7 %) > D13 (10.1 ± 2.3 %) = D11(11.1 ± 3.3 %)). The funguild analyses evidenced a significant enrichment of plant pathogens under the Drôme climate (*i.e*, LLD treatment) for the Loire progeny L08 progeny (P=0.04; L08 (23.5 ± 8.7 %) > L06 (9.6 ± 3.3 %) = L04 (4.7 ± 1.9 %)).

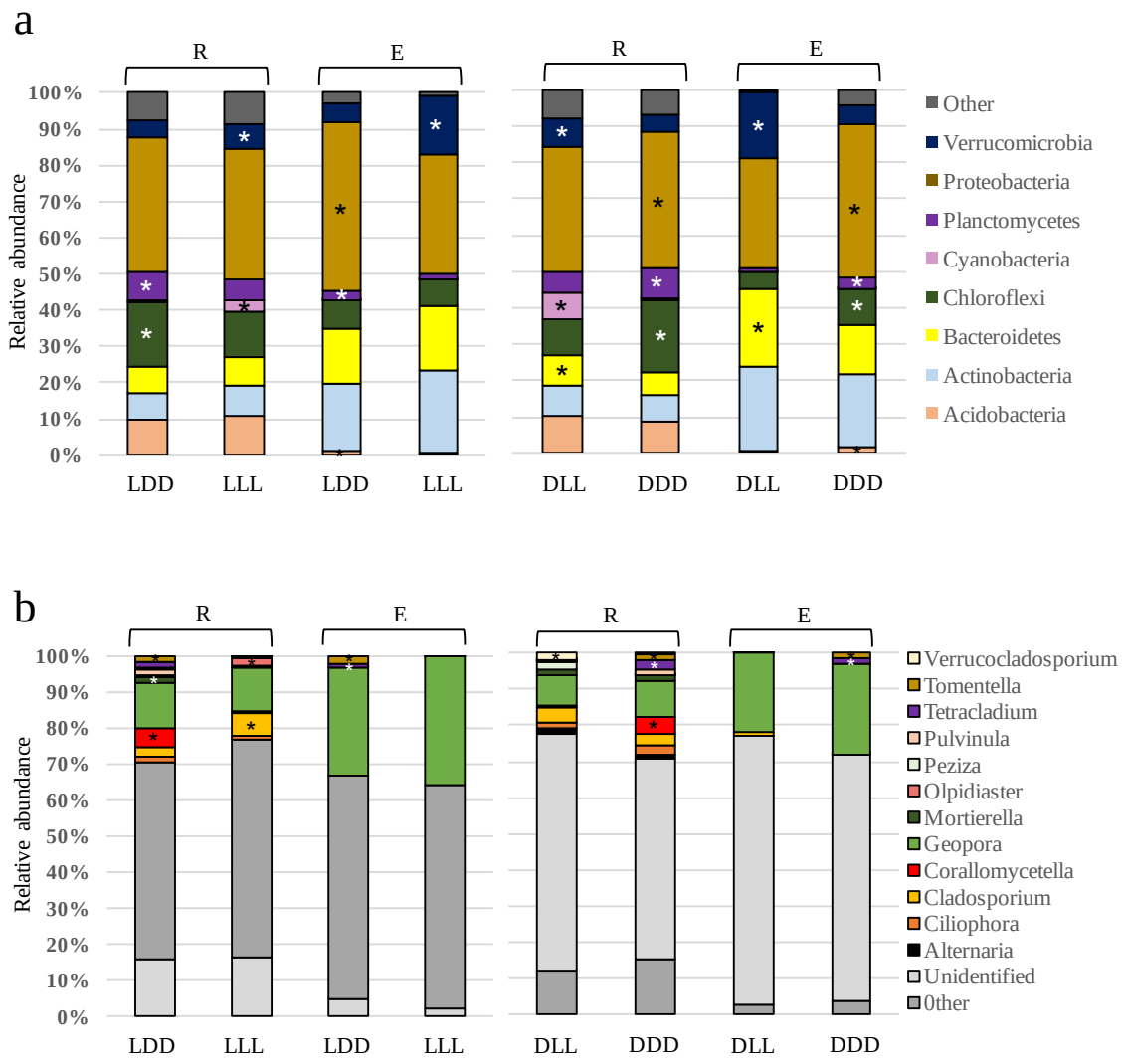


Figure 36 - Combined impact of sediment origin and climate on the Populus nigra root microbiome. Distribution of most dominant bacterial phyla (> 1 % in relative abundance) (a) and fungal genera (> 1 % in relative abundance) (b) detected in the rhizosphere (R) and in the endosphere (E) of Populus nigra seedlings of the Loire and the Drôme cultivated in their native sediment and under their native climate conditions (i.e., LLL and DDD treatments) and in transplant conditions (LDD and DLL treatments). The asterisks denote significant difference in relative abundance of each bacterial phyla and fungal genera between the two conditions of seedlings culture (ANOVA, P<0.05).

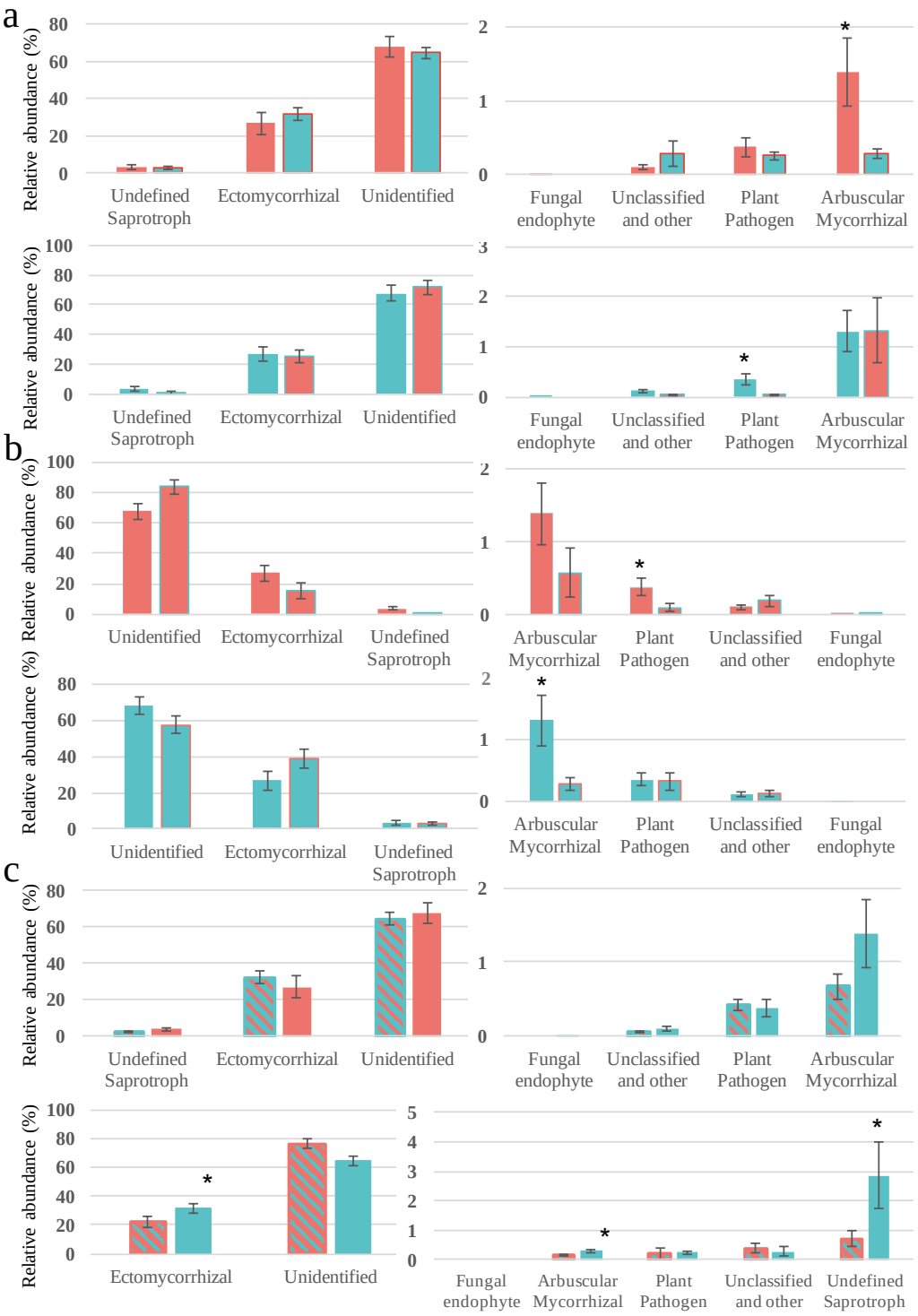


Figure 37 - Impact of the transplant conditions of sediment and climate on the relative abundance of fungal guilds detected in the endosphere of Populus nigra seedlings. Relative abundance of fungal guilds detected in the endosphere of Populus nigra cultivated in their native sediment (Loire in red and Drôme in blue) and in the other type of sediments (Loire sediments in red frame and Drôme sediment in blue frame) (a). Relative abundance of fungal guilds detected in the endosphere of Populus nigra cultivated under their native climate (Loire in red and Drôme in blue) and under the opposite climate (Loire sediments in red frame and Drôme sediment in blue frame) (b). Relative abundance of fungal guilds detected in the endosphere of Populus nigra cultivated in their native conditions of sediments and climate (Loire in red and Drôme in blue) and in the transplant conditions of sediments and climate (Loire sediments in red frame and Drôme sediment in blue frame) (c). The asterisks denote significant difference in relative abundance of each fungal guild between the two different tested conditions (ANOVA, P<0.05).

Discussion

In this study, we used a transplant mesocosm approach to decipher how the structure and composition of the root-associated microbiome of *Populus nigra* are influenced by the soil properties, the climate and the plant traits. To do it, we compared the offspring of two progenies of *P. nigra* collected in two contrasted climate regions (Loire vs Drôme) using their related sediments as soil matrix and source of microbial inoculum and grow them in their native or transplant conditions. The combination of our culture-independent approach on the surrounding bulk soil and the root-associated microbiome (ie., the endosphere and the rhizosphere) with the measure of aerial growth of the *P. nigra* progenies in the different conditions allowed us to demonstrate that the adaptation of *P. nigra* seedlings in non-native conditions of culture (i.e, sediments and climate) strongly correlates with the enrichment of specific microbial communities in the rhizosphere and the endosphere.

Growth of *Populus nigra* is strongly depending on soil fertility but also on temperature

It is well established that plant growth is conditioned by the physico-chemical properties of the soil matrix (i.e., fertility, water content), the selection of beneficial microorganisms and the climate, but also by the genetic properties of the plants (Letey, 1958; Ryan, 2010; Martinez-Graza et al., 2016; Quan & Liang, 2017). In our study, the impact of the plant genetic was apparent as the three progenies considered per site showed significant differences in their growth (aerial part) when cultivated in their native conditions (Loire, L04<L08<L06; Drôme, D15<D13<D15, Figure 28). However, the level of growth and the progeny patterns appeared modified when cultivated in their non-native sediment. The Drôme progenies presented a higher growth in their native conditions of soil and climate than in the other treatments, while the Loire progenies presented a higher growth in the complete transplant treatment (i.e., Drôme climate x Drôme sediment). Based on the nutritive content of the two sediments considered and the temperatures measured on each site, such results were expected as the Drôme sediment is characterized by a higher fertility and warmer climate than the Loire sediment. This assumption was confirmed by the lower growth of the Drôme progenies in the Loire sediment (less fertile) and under a lower temperature than the one of the Drome region. Warmer temperature significantly enhanced the growth of deciduous trees such as beech, oak and ash as well as the soil fertility, especially C/N, and soil moisture (Levesque et al., 2016). This last factor was avoided in our study with the constant watering of the mesocosms. However, the differences in the progeny patterns between the native and transplant treatments suggest the existence of interactions between the different factors (genetic x soil x climate) and potentially the microbiome selected in *P. nigra* root system as previously suggested (Classen et al., 2015; Bonito et al., 2019). This hypothesis is in accordance with the work of Gungale et al. (2013) who showed that whatever the difference in soil fertility, pine trees (*Pinus cordata*) grew better in their introduced ranges (Swedish sites) compared to their native ranges (Canadian sites) thanks to the soil microbiome, especially fungi and bacteria.

Colonization of *Populus* nigra rhizosphere by fungi and bacteria is mainly driven by sediments origin and climate

The composition of the soil microbiome significantly shifted between Drôme and Loire sediments. This significant change could be linked to the important difference in physico-chemical properties observed between the two sediment types. It is likely an important driver of such differences, as previously demonstrated in other soils (Lauber et al., 2008; Fierer, 2017; Terrat et al., 2017; Nicolitch et al., 2019). By contrast, difference in average temperature between Drôme and Loire (2.6°C in average) did not seem to influence the microbiome of the sediments. Indeed, in our study, no significant difference in both microbial community structure and composition was observed between sediments placed under their native climate and those cultivated under the opposite climate. This result contrasts with the one obtained by Liang and colleagues who showed that soils transplanted to warmer regions caused more significant change in the microbial community composition and structure compared to the soil transplantation in colder region (Liang et al. 2015). These discrepancies may be explained by the fact that the experiment of Liang et al. was run on a longer period (several years) and with higher temperature gradients (up to 6°C). Nevertheless, the difference between temperature of Drôme and Loire could impact the physiology and notably the photosynthesis of the seedlings. Such correlation was already made in *Populus simonii* (Song et al., 2014) and other trees such as subtropical forest trees (Slot et al., 2017; Wu et al., 2018) and pine (Hari & Nöjd; 2009).

We hypothesized that the change in the composition of sediment microbiome was responsible for the change in the composition of microbial communities colonizing the rhizosphere. The rhizosphere corresponds to the area of sediment directly under influence of roots where host factors (e.g., physiology, root exudates and genotype) as well as environmental factors (e.g., soil properties and climate) shape microbial community composition and structure while microbial communities of the bulk soil, the area of the soil free of tree roots is only influenced by environmental factors. By comparing both bacterial and fungal communities between the bulk soil and the rhizosphere compartments, we demonstrated that bacterial and fungal community composition varied significantly between BS and R, no matter the treatment (i.e. soil, climate …). However, this well-known phenomenon so-called the rhizosphere effect (Hiltner, 1904; Hartmann et al., 2008) was different according to the treatment. The most important variations were observed between LLL and LDL treatment (soil effect) and between DDD and DDL treatment (climate effect). These important shifts when seedlings were transplanted in the non-native sediment could be linked to the difference in physico-chemical properties (Lauber et al., 2008; Fierer, 2017; Terrat et al., 2017; Nicolitch et al., 2019). In addition, these important shifts when climate was reversed could be due to modification of tree physiology (e.g., photosynthesis, transpiration, root exudation quality and quantity) which indirectly modify the composition of microbial communities of the rhizosphere (Lau et al., 2017; Mercado-Blanco et al., 2018; Compant et al., 2019). In different tree species, root exudation, notably of carbon, is significantly affected by increased temperature. Indeed, increased of the temperature was responsible of the increase of root exudation of organic carbon by a factor of 1.7 in *Robinia* (Uselman et al., 2000). In seedlings of *Populus tremuloides* exposed to colder soils, the concentration of non-structural carbon increased in roots (Karst et al., 2016). These different observations could suggest that the significant enrichment of Cyanobacteria and the

copiotrophic bacteria belonged to the *Cellvibrio* genus (Spring et al., 2015) in DLL, DLD and LLD treatments could be linked to a modification of root exudation. Further analyses will be required to test this hypothesis. Concerning fungal communities, the plant pathogen *Cladosporium* and the saprotrophes *Tetracladium* and *Corallomycetella* were significantly more abundant in the R compared to the BS in seedlings cultivated in transplant conditions of sediments and/or climate. This observation was in accordance with the modification of the relative distribution of plant pathogens and AM fungi in the rhizosphere of Drôme and Loire seedlings cultivated in non-native conditions of sediment or of climate compared to the native conditions. Because abiotic factors such as temperature can affect host susceptibility to pathogens (Sturrock et al., 2011), the increase of root colonization by potential fungal plant pathogens (e.g *Alternaria* and *Cladosporium*) observed in the Drôme seedlings cultivating under Loire climate could suggest that alteration of average temperature could increase the proliferation of fungal pathogens. By contrast, the relative abundance of the EcM fungi *Geopora* was significantly enriched in the rhizosphere of Drôme and Loire seedlings cultivated in Drôme sediment suggesting that only sediment origin affect the colonization of this fungus.

Taken together, these observations showed that the soil matrix and temperature could have a strong impact on bacterial and fungal community colonizing the rhizosphere of trees via the alteration of the reservoir of microorganisms and/or modifications of the quality and the quantity of tree root exudation.

Populus nigra endosphere colonization is independent of the soil origin and the climate

The microbial colonization of tree roots is a highly dynamic process in which bacterial and fungal communities, mainly originating from the soil reservoir, colonize the endosphere from the rhizosphere (root filtering effect, Bulgarelli et al., 2013). In *Populus*, the root filtering effect is well known (Gottel et al., 2011; Cregger et al., 2018) but the relative impact of soil origin and climate on the microbial communities which colonize the endosphere are poorly known. Detailed analysis of the microbial composition revealed that cultivating *P. nigra* seedlings of Drôme and Loire in their non-native conditions of sediment and/or climate significantly affected the composition of the microbial communities of the endosphere. Proteobacteria have been described as the predominant bacterial phylum present in root endophytic communities of *Populus tremula x alba* and *P. deltoides* (Gottel et al. 2011, Beckers et al., 2017). Surprisingly, this phylum was not significantly enriched in the endosphere of seedlings cultivated in their native conditions of sediments and climate although they were dominant members of both rhizospheric and endospheric compartments. However, we observed a significant enrichment of this phylum in the endosphere for each treatment except DLL and DDL. Four bacterial genera were particularly impacted by the transfer of the seedling on their non-native soil and climate: *Actinocorallia* (Actinobacteria), *Terrimonas (*Bacteroidetes*), Phytohabitans* (Actinobacteria) and *Rhodomicrobium (*Proteobacteria*)*. We could hypothesize that these specific bacterial endophytes are favoured by non-native culture conditions (i.e, soil origin and/or climate) in the rhizosphere and belonged to the small set of rhizosphere microbiome able to enter within the endosphere which represent a unique niche for these endophytes (Gottel et al., 2011; Beckers et al., 2017; Liu et al., 2017). By contrast, Verrucomicrobia were significantly enriched in the endosphere of Loire and Drôme seedlings only when cultivated in Loire sediments (i.e., LLL; DLD; LLD; DLL treatments). The relative abundance

of Verrucomicrobia was equal between Drôme and Loire sediments (BS_{Loire} = 2.7 ± 0.5 %, $BS_{Drôme}$ =2.8 ± 0.1 %) suggesting that the higher proportion of Verrucomicrobia colonizing *P. nigra* root was not linked to their dominance in the sediments of Loire. One could wonder if changes in the physiology of the seedlings when cultivated in Loire maybe responsible for this effect. Little is known about functional abilities of members of the Verrucomicrobia as very few representatives are cultivable up to now (Nunez de Rocha et al. 2011). However, Verrucomicrobia are expected to be oligotrophs whose growth depends on the nature of the carbon source in the rhizosphere (Aguirre von Woboser et al. 2018, Fierer et al. 2013). Such behaviour would fit with a potential change in the access to nutrients in the endosphere of roots grown in Loire.

Regarding fungi, the EcM fungi *Geopora* dominated the fungal community of the endosphere. This fungus was also detected in the endosphere of other *Populus* species (Danielsen et al., 2012; Foulon et al., 2016; Durand et al., 2017; Gehring et al., 2017; Bonito et al., 2019). It was the only fungal genus significantly enriched in this compartment whatever the considered treatment (>25 % in relative abundance) and their relative abundance in the rhizosphere. As EcM fungi massively colonize the inner tissue of roots while the Hartig to exchange nutrients, such dominance in the endosphere is not unexpected. Long and colleagues proposed that the EcM *Geopora* could be defined as an important mutual partner for host tree resisting to the different environmental conditions (Long et al. 2016). More unexpected is the absence of effect of soil origin on the fungal community of the endosphere. Indeed, Bonito and colleagues found that the soil origin was the major determinant of fungal assembly in roots of *P. deltoides*. However, the EcM fungus *Geopora* was not detected in the roots of *Populus* studied in this work (Bonito et al., 2014). In addition, we could imagine that Loire sediments slow the colonization of EcM fungi *Geopora* in the rhizosphere of the *P. nigra* seedlings while roots favor this colonization whatever the origin of the seedlings.

These different observations suggest that the selection of bacterial endophytes has already taken place in the rhizosphere under the influence of the soil and the climate. By contrast, the colonization of *Populus* roots by the EcM *Geopora* is independent of the rhizosphere selection and the host tree.

Specific members of the rhizosphere of *Populus nigra* are affected by the progeny properties

Plant fitness is known to influence microbial community structure through the modification of plant signalling and the composition of root exudates (Lau et al., 2017; Mercado-Blanco et al., 2018; Compant et al., 2019). In our experimental conditions, a global analysis through a PERMANOVA did not permit to evidence significant differences in the microbial community structure between the different progenies of Drôme and Loire despite their different growth patterns. However, detailed analyses revealed that the relative abundance of several taxa was significantly affected in the rhizosphere but not in the endosphere. Notably, our analyses revealed significant effects of the sediment, or of the climate, but not by the combination of these two factors in the rhizosphere. For the bacteria, 16S rRNA sequences affiliated to the *Niastella* genus tended to be enriched in the rhizosphere of the D11 progeny cultivated in their native conditions (> 2 % in relative abundance) which is the progeny presenting the higher aerial growth. It was also significantly enriched in the rhizosphere of the D11 progeny cultivated under Loire climate compared to D13 and D15 progenies cultivated in the same conditions (DDL

treatment) which had a smaller size, suggesting a potential role of *Niastella* in the promotion of *P. nigra* growth. Interestingly, this genus was reported in the rhizosphere of plants such as maize (Visioli et al., 2018) and in the rhizosphere of various *Populus* species (Beckers et al., 2017; Bonito et al., 2019).

For the fungi, ITS sequences affiliated to the EcM fungus *Geopora* appeared significantly enriched in the rhizosphere of D11 progeny cultivated in their native conditions (DDD, 16 % in relative abundance) and in D15 progeny cultivated under Loire climate (DDL, 15 % in relative abundance). D11 seedlings showed the highest aerial part in the DDD treatment compared to D13 and D15. By contrast, D15 seedlings had the smallest aerial part in the DDL treatment compared to D11 and D13 seedlings of the same treatment and to D15 seedlings cultivated in the DDD treatment. Variations of the root-associated microbiome structure according to the plant genotype have been reported for several plants (*Boechera stricta*, maize; Wagner et al., 2016; Gomes et al., 2018) and different poplar species (Bonito et al., 2014; Lamit et al., 2016; Cregger et al., 2018; Bonito et al., 2019). In *Populus* roots, Bonito and colleagues found significant differences in fungal-root associated communities that could be attributed to the genotype but they could not associate the effect to specific OTUs, suggesting that the host effect occurred at the community level (Bonito et al., 2019). The dominance of *Geopora* in the root systems of *P. nigra* may explain the discrepancies with results obtained on *P. deltoides and P. trichocarpa* x *deltoides*. No specific enrichment of *Geopora* was detected in the endosphere of our seedlings suggesting that the modification of the growth of the aerial part is not due to a direct effect of the most dominant EcM fungus on the nutrition of the seedlings as previously shown for other trees (Quoreshi et al., 2008; Kipfer et al., 2012; Nylund & Wallander, 1989).

Taken together, these observations could partially explain the adaptation of young seedlings in new environmental conditions of sediments and temperature. Indeed, our results suggest that environmental factors (i.e, sediments and climate) could improve the root exudation and then, the colonization of the rhizosphere by specific taxa of plant-growth-promoting bacteria. However, we provide first evidences that specific fungal taxa such as EcM fungi was not responsible of the modification of the aerial part growth. Future works is needed to confirm these observations by taking into account other physiological parameters of *P. nigra* ssedlings as root growth.

Conclusion

Data from our experimentation in mesocosm provide new assessments on impacts of sediment origin, climate and *Populus nigra* progenies on fungal and bacterial communities from the bulk sediment to the root endosphere. For the same young black poplar belonging to the same progeny, the aerial growth pattern was unchanged or, on the contrary, modified by the different extrinsic parameter tested. The two sediment types showed differences in microbiome composition and structure related to the different physico-chemical properties observed in the two matrices. These differences were responsible of significant differences in the composition and structure of the rhizosphere and the root microbiome. In addition, climate was also correlated with these significant modifications

especially in the rhizosphere, the soil area richest in root exudates. Finally, the progeny effect was responsible of enrichment of specific microbiome members on the rhizosphere suggesting that modification of plant physiology by environmental factors was the key driver of the microbiome composition and structure.

Acknowledgments

We wish to thank Kenza Benkirane (INRAE Nancy) for her help in harvesting poplars.

Funding

LMP was supported by “Contrat Doctoral” from the French Ministère de l’éducation nationale et de la recherche. This work was funded by the Metaprogram ACCAF (project POPMICROCLIM) and the French National Research Agency through the Laboratory of Excellence ARBRE [ANR-11-LABX-0002-01].

IV. Conclusions

Les données de nos expériences menées en mésocosmes fournissent de nouvelles informations concernant les impacts de l'origine des sédiments, du climat et du génotype sur les communautés bactériennes et fongiques de la rhizosphère et de l'endosphère du peuplier noir (*Populus nigra* L.).

Pour chaque progénie de peuplier noir originaire de la Drôme ou de la Loire observée, le taux de croissance aérienne reste inchangé ou, au contraire, modifié par les différents paramètres extrinsèques testés (le type de sédiments et/ou le climat). Les sédiments de la Drôme et ceux de la Loire présentent des microbiotes différents en termes de composition et de structure des communautés microbiennes. Ces différences peuvent être corrélées aux différentes propriétés physico-chimiques des deux types de sédiments. De plus, ces différences de composition et de structure observées entre le microbiote des deux principaux réservoirs de micro-organismes entraînent une modification de la composition des communautés microbiennes sélectionnées dans la rhizosphère et l'endosphère des peupliers noirs de la Drôme et de la Loire. Le climat semble également être responsable de modifications importantes du microbiote, surtout dans la rhizosphère, la zone du sol la plus riche en exsudats racinaires. Enfin, l'effet progénie est à l'origine de l'enrichissement de certains taxa bactériens et fongiques dans la rhizosphère, ce qui suggère que la modification de la physiologie des plantes par les facteurs extrinsèques est le facteur clé de la composition et de la structure du microbiote racinaire.

Chapitre IV

Impact des variations de la composition et de la structure du microbiote du sol sur la colonisation racinaire et le métabolome du Peuplier (*Populus tremula x alba*)

I. Contexte général

La plante et son microbiote racinaire forment un écosystème complexe dans lesquel plusieurs organismes contribuent au fonctionnement général et à la stabilité de l'écosystème (Hacquard & Schadt, 2015).

La composition et la structure du microbiote racinaire sont très dynamiques et sont influencées par la disponibilité en nutriments, les paramètres environnementaux et les interactions d'origine biotiques (Llado et al., 2017 ; Haas et al., 2018). Si les paramètres édaphiques et l'activité de la plante hôte sont connus pour être fortement impliqués dans la structure du microbiote racinaire, les avis restent partagés concernant l'importance relative de ces deux facteurs dans la composition du microbiote racinaire (Lareen et al., 2016). D'une part, les facteurs dépendants de la plante hôte tels que les exsudats racinaires et les métabolites secondaires affectent la colonisation des racines par les micro-organismes. Ces facteurs peuvent dépendre du génotype de l'hôte mais aussi de facteurs extrinsèques tels que l'état de la plante et du sol ou encore les variations environnementales. D'autre part, les facteurs environnementaux tels que les propriétés du sol et le climat sont connus pour avoir un impact sur les communautés de micro-organismes du sol, principal réservoir de micro-organismes pour les racines (Fierer, 2017).

Il a été démontré que le microbiote racinaire de la plante évolue rapidement en réponse aux variations abiotiques qui correspondent à des stress responsables de la réduction de la croissance de la plante (Lau & Lennon, 2012). De telles capacités sont très intéressantes dans le domaine de l'ingénierie microbienne pour optimiser la croissance des plantes tout en réduisant les effets néfastes des stress liés au changement climatique (Farrar et al., 2014; Meena et al., 2017). Pour ce faire, il est nécessaire d'identifier comment manipuler et contrôler le microbiote du sol, de comprendre comment la composition et les fonctions du microbiote racinaire sont régulées et, enfin, de comprendre quel est l'impact de la composition de ce microbiote racinaire sur la biologie de l'hôte. Si ces questions font aujourd'hui l'objet de travaux de recherches sur les plantes annuelles d'intérêts agronomiques (Chaparro et al., 2012), peu d'études concernent les arbres malgré un cycle de vie très différents et des capacités d'interactions plus larges par rapport aux plantes herbacées et annuelles (Nehls, 2008; Uroz et al., 2016; Liao et al., 2019).

Une importante variabilité existe entre les capacités fonctionnelles des micro-organismes avec des redondances, des synergies et des antagonismes (Chaparro et al., 2012). Bien que les paramètres influençant la composition taxonomique du microbiote du sol et, dans une moindre mesure, ceux influençant la composition taxonomique du microbiote racinaire sont aujourd'hui bien connus, peu d'informations sont disponibles concernant l'ampleur de la redondance fonctionnelle de ces variations.

II. Objectifs

Dans ce contexte, les objectifs de ce chapitre sont, d'une part, de caractériser la composition et la structure du microbiote d'un sol de peupleraie durant deux années successives, et, d'autre part, d'évaluer si les potentielles modifications de la composition du microbiote du sol sont également visibles au niveau du microbiote et du métabolome racinaire du peuplier.

Plusieurs questions se posent alors :

- Y a-t-il des changements de composition du microbiote du sol entre les deux années successives ?
- Ces potentiels changements sont-ils également visibles au niveau des communautés fongiques et bactériennes associées aux racines du peuplier ?
- Les changements observés au niveau de la composition du microbiote racinaire sont-ils responsables de modifications du métabolisme primaire et secondaire des racines du peuplier ?

III. Démarche expérimentale

Afin de répondre à ces questionnements, le sol issu d'une peupleraie âgée de dix-huit ans et dominée par *Populus trichocarpa x deltoides* a été prélevé durant deux années consécutives. Plus précisément, le sol a été collecté sous le même arbre en juin 2016 (Sol 1) et en juillet 2017 (Sol 2) afin de caractériser la composition et la structure des communautés bactériennes et fongiques du sol d'une année à l'autre. Ensuite, en cultivant des boutures de peuplier dans le Sol 1 et le Sol 2 en serre, nous avons caractérisé et comparé la composition et la structure des communautés microbiennes associées aux racines ainsi que le métabolome primaire et secondaire du Peuplier gris (*Populus tremula x alba*) successivement les deux années.

Les communautés bactériennes et fongiques du sol et des racines ont été caractérisées par séquençage Illumina MiSeq haut débit des amplicons d'ADNr ITS et d'ARNr 16S tandis que le métabolome racinaire a été analysé par Chromatographie en phase Gazeuse couplée à la Spectrométrie de Masse (en anglais, Gas Chromatography-Mass Spectrometry ou GC-MS).

Les résultats de cette étude sont décrits sous la forme d'un article scientifique publié dans la revue *Phytobiomes* en mars 2020.

Les tableaux supplémentaires sont disponibles en Annexe (Annexe 3 de la page 27 à la page 52).

Phytobiomes Journal • 2020 • 4:142-155 https://doi.org/10.1094/PBIOMES-08-19-0042-R

OPEN ACCESS phytobiomesjournal.org APS

Phytobiomes Journal A Transdisciplinary Journal of Sustainable Plant Productivity

RESEARCH

e-Xtra*

Impacts of Soil Microbiome Variations on Root Colonization by Fungi and Bacteria and on the Metabolome of *Populus tremula* × *alba*

L. Mangeot-Peter,[1] T. J. Tschaplinski,[2] N. L. Engle,[2] C. Veneault-Fourrey,[1] F. Martin,[1,3] and A. Deveau[1,†]

[1] Université de Lorraine, INRAE, UMR IAM, 54280 Champenoux, France
[2] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, U.S.A.
[3] Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Haisian District, Beijing, China

Accepted for publication 12 March 2020.

ABSTRACT

Trees depend on beneficial interactions between roots and soil microbes for their nutrition and protection against stresses. The soil microbiome provides the main reservoir of microbes for root colonization and is subject to natural variations that can affect its composition. It is not clear whether the tree's root system is able to buffer the natural variations occurring in the soil microbiome to capture a stable and effective microbiome or whether these variations affect its microbiome to impact its physiology. To address this question, we planted cuttings of Gray Poplar (*Populus tremula* × *alba* clone 717-1B4) in natural soil taken from a poplar stand under the same tree over two consecutive years and grew them in a greenhouse. We analyzed the soil and root microbiomes by high throughput Illumina MiSeq sequencing of fungal rDNA internal transcribed spacer and bacterial 16S rRNA amplicons and we characterized the root metabolome by gas chromatography-mass spectrometry. Soil and root microbial communities significantly shifted over the 2 years. A modification of the balance between endophytes, saprophytes, and mycorrhizal fungi occurred in the roots as well as a replacement of some dominant operational taxonomic units by others. These modifications were correlated with a significant alteration of the levels of about 10% of primary and secondary metabolites, suggesting that natural fluctuations in soil microbial communities can have a profound impact on tree root metabolism and physiology. Tree roots functioning may thus be indirectly strongly affected by the effects of future extreme climatic variations on the soil microbiome.

Keywords: endophyte, ectomycorrhizal fungi, metabarcoding, metabolome, microbiome, *Populus*, symbiosis

Roots are heavily colonized by complex communities of microorganisms (the so-called root microbiome) as a result of the massive release of carbon compounds in the rhizosphere that provides energy sources to a multitude of soil microbes. Among the numerous members of the root-associated microbiome, fungi and bacteria dominate in terms of abundance and diversity, and play key roles in plant development and health (Lladó et al. 2017). Some of these microorganisms, such as mycorrhizal symbionts, benefit plants by promoting plant nutrition (Jacoby et al. 2017) and resistance to biotic and abiotic stress (Meena et al. 2017; Naylor and Coleman-Derr 2018), while other microorganisms, such as pathogens and parasites, are detrimental to plants (Raaijmakers et al. 2009). The host plants and their microbiomes form a complex ecosystem in which many interacting species contribute to the overall functioning and stability of the system (Hacquard and Schadt 2015).

The composition (i.e., identity of taxa) and the richness (i.e., number of taxa) of the root microbiome are highly dynamic and vary according to nutrient availability, physico-chemical environmental parameters, and biotic interactions (Haas et al. 2018; Lladó et al. 2017). If edaphic parameters and the activities of the host plant are both involved in the structuring of the root microbiome, opinions remain divided on the relative importance of these two factors in driving the root microbiome composition (Lareen et al. 2016). On one hand, host factors such as root exudate quality and quantity, and plant secondary metabolites affect the colonization of roots by microorganisms (Berg and Smalla 2009). Host factors depend on the genotype of the plant, but are also modulated by extrinsic factors, such as plant and soil management or environmental

†Corresponding author: A. Deveau: aurelie.deveau@inrae.fr

Funding: This work was supported by the Agence Nationale de la Recherche (ANR-11-LABX-0002-01) through the Laboratory of Excellence ARBRE and by the Plant-Microbe Interfaces Scientific Focus Area in the Genomic Science Program, the Office of Biological and Environmental Research in the U.S. Department of Energy Office of Science. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy Office of Science (DE-AC05-00OR22725). L. Mangeot-Peter was supported by "Contrat Doctoral" from the French Ministère de l'éducation nationale et de la recherche.

*The *e*-Xtra logo stands for "electronic extra" and indicates that three supplementary figures and supplementary tables are published online.

The author(s) declare no conflict of interest.

© 2020 The American Phytopathological Society

variation. For instance, atmospheric CO_2 enrichment stimulates tree physiology and modifies the quantity and quality of root exudates (Pritchard 2011). On the other hand, environmental factors such as soil properties (e.g., pH and nitrogen availability) and climate (e.g., humidity and temperature) affect the survival and the activities of microorganisms present in the surrounding bulk soil that serve as a reservoir, and thus their ability to colonize roots (Fierer 2017). Finally, stochastic processes including ecological drift and colonization history can also affect the root microbiome composition (Emerson and Gillespie 2008).

As a complex community, the plant root microbiome has been shown to adapt rapidly to abiotic limitations, to alleviate host stress and to modify plant growth (Lau and Lennon 2012). Such abilities open opportunities for microbial engineering to optimize plant growth and reduce the negative effects of stressors linked to climate change (Farrar et al. 2014; Meena et al. 2017). Yet this requires (i) identifying how we can manipulate and manage the soil microbiome, (ii) understanding how the root microbiome composition and activities are regulated, and (iii) understanding how its composition impacts the plant host biology. Such questions are currently the subjects of extensive studies on agronomic annual crop species (Chaparro et al. 2012), but much less is known regarding woody perennials, including trees and shrubs, despite their importance for terrestrial ecosystems, climate change mitigation, and forestry. Trees are long-lived woody perennial plants that manage nutrient allocations, notably to the roots, in a very different way compared with *Arabidopsis thaliana*, the model herbaceous species, or annual crop species (Nehls 2008). Moreover, in contrast to crops, ectomycorrhizal fungi are a major component of tree microbiome in temperate and boreal forests (Uroz et al. 2016), while most studies of the plant microbiome currently focus on bacterial communities (Mendes et al. 2013; Van Der Heijden et al. 2008). Endophytic and saprophytic fungi also colonize trees but their roles remain elusive with a few exceptions (Liao et al. 2019). In addition, the mutualistic symbiosis between tree roots and mycorrhizal fungi shape specific communities of bacteria that tend, on the whole, to be beneficial to their hosts and that differ from those of nonmycorrhizal roots (Frey-Klett et al. 2007).

There is an important variability between microorganisms in their functional capacities with redundancies, synergies, and antagonisms (Chaparro et al. 2012), as well as in their ability to resist stress (Gehring et al. 2017; Naylor and Coleman-Derr 2018). For instance, species of ectomycorrhizal fungi differ in their abilities to provide nitrogen to their hosts (Pena and Polle 2014). Nevertheless, a shift in the taxonomic composition of microbial communities can be functionally neutral, due, in part, to functional redundancies of species as shown in the rhizosphere of beech (*Fagus sylvatica* L.) (Colin et al. 2017). In this study, it has been shown that the operational taxonomic unit (OTU) composition of bacterial communities associated to beech trees along a toposequence substantially vary, but the nutrition-related functions remain stable. While there is a good understanding of the parameters influencing the taxonomic composition of the soil, and to a lesser extent of the root microbiome, how much functional redundancy buffers these variations is poorly known. Neither do we know the extent to which the degree of change in the soil microbiome affects the root microbiome and therefore tree metabolism. In the present study, we aimed to assess whether local shifts in soil microbiome composition potentially due to year-to-year environmental variations influence the composition of the root-associated microbiome, and consequently, the root metabolism. We used the Gray Poplar, *Populus tremula* × *alba*, as a model system. *Populus* has become a model to study genetics and biology of woody perennials (Tuskan et al. 2006), and more recently, tree microbiome interactions (Cregger et al. 2018; Gottel et al. 2011; Shakya et al. 2013; Veach et al. 2019). *Populus* have the capacity to form both arbuscular mycorrhizal and ectomycorrhizal symbioses. In addition to mycorrhizal symbionts, *Populus* is also host to a variety of beneficial bacterial and fungal endophytes (Gehring et al. 2006; Hacquard and Schadt 2015; Timm et al. 2018). Microbial community members from *Populus* have been shown to enhance tree growth and development (Timm et al. 2016). The composition and structure of the root microbiome shift across environmental gradients (Gottel et al. 2011; Shakya et al. 2013; Veach et al. 2019) but also between *Populus* genotypes and species (Bonito et al. 2014; Veach et al. 2019). Differences in bacterial and fungal community composition and structure varied across season due to climatic fluctuations and tree physiology (Shakya et al. 2013).

In the present work, we hypothesized that potential natural shift in soil microbiome composition and structure could be also observed in roots, and therefore, alter the tree root metabolome. To test this hypothesis, we sampled soil in the same location near the same tree in a poplar plantation over 2 years and planted cuttings of *Populus tremula* × *alba* clone 717-1B4 in pots filled with this soil. Cuttings were grown in a greenhouse under controlled conditions for one and a half months. We characterized the initial soil microbial communities as well as the soil- and root-associated fungal and bacterial communities after 10 days and 6 weeks of growth, using amplicon 16S and internal transcribed spacer (ITS) rRNA gene-targeted Illumina MiSeq sequencing. In parallel root metabolomes were assessed by gas chromatography-mass spectrometry (GC-MS) at the two time points of sampling.

MATERIALS AND METHODS

Plant and soil material. We used cuttings of *Populus tremula* × *alba* INRA 717-1B4 clone (Mader et al. 2016) harvested from a mother tree cultivated in the INRA poplar nursery located in Champenoux, France (48°45′3′′N/6°20′6′′E). The mother tree was pruned at the end of winter 2016 and 2017, and cuttings were stored at 4°C until further use. Soil (clayey loamy soil type) was collected from an 18-year-old poplar stand planted with *Populus trichocarpa* × *deltoides* and located in Champenoux, France (48°45′24′′N/6°21′24′′E) after pruning of brambles and adventitious plants and litter removal with a rake. About 100 liters of top soil (0 to 15 cm) were taken each year in the same location under the same tree on 9 June 2016 and on 3 July 2017. The tree was located in the heart of the stand and separated from the neighboring trees by a minimum of 3 m. The same procedure was followed for two consecutive years: soil was stored in a greenhouse at room temperature for 3 days before being sieved at 2 mm and adjusted to 75% humidity. Three samples of soil of 5 g were collected and stored at –20°C until DNA extraction. One sample of 50 g of soil was collected, dried at 30°C for 3 days, sieved at 2 µm, and stored at room temperature in an airtight container until physico-chemical analysis.

Soil physico-chemical properties. Soil analyses were performed using the LAS (Laboratoire d'Analyses des Sols) technical platform of soil analyses at INRA Arras, according to standard procedures, detailed online (https://www6.hautsdefrance.inrae.fr/las/Methodes-d-analyse). Exchangeable cations were extracted in either 1 M KCl (magnesium, calcium, sodium, iron, and manganese) or 1 M NH_4Cl (potassium) and determined by inductively coupled plasma atomic emission spectroscopy (JY180 ULTRACE). The 1 M KCl extract was also titrated using an automatic titrimeter (Mettler TS2DL25) to assess exchangeable H^+ and aluminum cations (Al^{3+}). Total carbon, nitrogen, and phosphorus contents were obtained after combustion at 1,000°C and were determined using a Thermo Quest Type NCS

2500 analyzer. The pH of the soil samples was measured in water at a soil to solution ratio of 1:2 (pH meter Mettler TSDL25). Exchangeable acidity was calculated by taking the sum of H^+ and Al^{3+}. The cation-exchange capacity (CEC) was calculated by taking the sum of both extracted exchangeable base cations and exchangeable acidity. Soils collected at the beginning of the 2 years of experiment were called bulk soil T0 in this study (Supplementary Fig. S1).

Cultivation and sampling strategies. *Populus tremula* × *alba* INRA 717-1B4 cuttings were maintained at 4°C until transfer to a greenhouse (20°C, 16 h light), where they were cultivated in a hydroponic system containing the full-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) for 1 month in order to induce root formation and to homogenize root length of all cuttings. Cuttings with homogeneous root systems were then transferred in early summer (13 June 2016, seven cuttings and 6 July 2017, 12 cuttings, Supplementary Fig. S1) to 1-liter pots filled with soil previously taken from the poplar plantation (described previously). Experiments performed in 2016 and 2017 are further referred to as soil 1 and soil 2. Humidity in pots was monitored and maintained at approximately 75% during the experiments. Two samplings were conducted both years to take into account the dynamic of root colonization by different microorganisms: the first after 10 days of growth (T1) and the second after 6.5 weeks of growth (T2). Indeed, bacteria, saprophytic fungi, and pathogenic fungi are able to colonize roots within a few days, while ectomycorrhizal fungi often require several weeks to establish a functional symbiosis (Marupakula et al. 2016; Smith and Read 2008).

Bulk soil samples were collected from each pot from an area free of roots and stored at −20°C until DNA extraction. Soil collected at T1 and T2 were called bulk soil T1 and bulk soil T2 (Supplementary Fig. S1). Two representative parts of the total root system of each tree were harvested, cleaned with an NaCl 10 mM solution and then with sterile water, frozen in liquid nitrogen, and stored at –20°C until DNA extraction or at –80°C until metabolomics analyses. Three trees were sampled at T1 in soil 1, four at T2 in soil 1, and six at both T1 and T2 in soil 2 (Supplementary Fig. S1).

DNA extraction, amplification, and Illumina MiSeq sequencing. To minimize DNA extraction bias, DNA was extracted in triplicate from the bulk soil. Approximatively 250 mg of soil sample was used for each individual DNA extraction. DNA was extracted using the DNeasy PowerSoil Kit following the protocol provided by the manufacturer (Qiagen, Venlo, the Netherlands). Fifty milligrams of root tissue was crushed in liquid nitrogen, and DNA was extracted using the DNAeasy PowerPlant Kit (Qiagen). DNA were quantified with a NanoDrop 1000 spectrophotometer (NanoDrop Products, Wilmington, DE, U.S.A.).

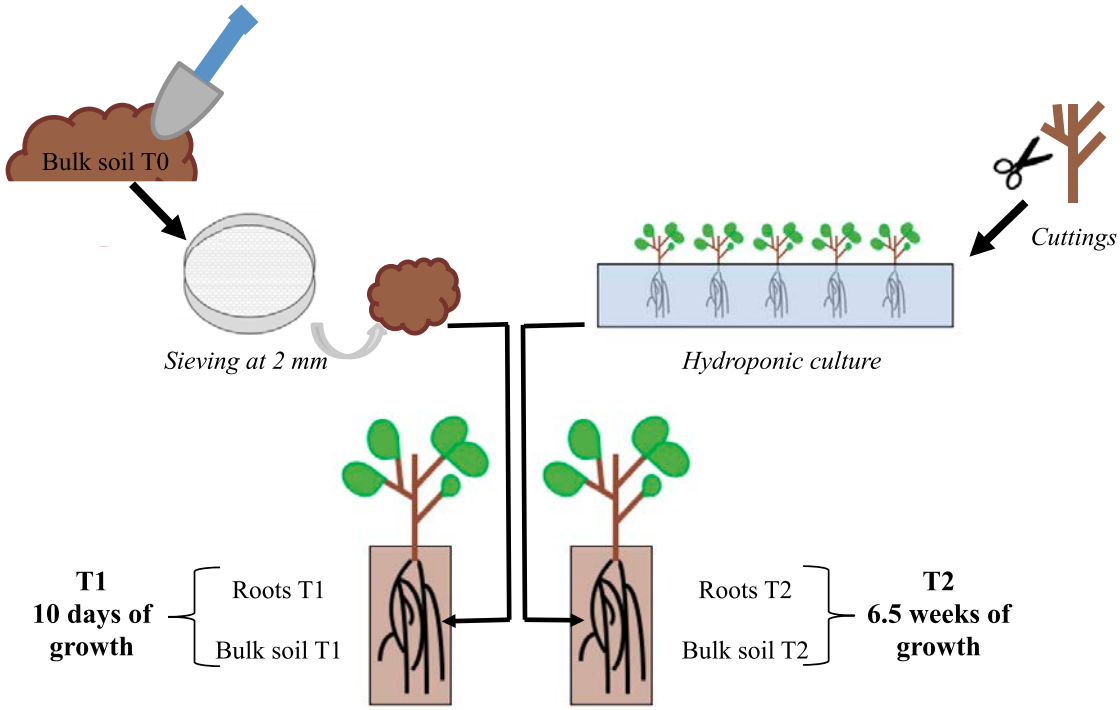
A two-step PCR approach was chosen to barcode tag templates with frameshifting nucleotide primers (Lundberg et al. 2013). Primer mixtures for tagging bacterial amplicons were composed of four forward 515F (Universal, Chloroflexi, TM7, Nano; Supplementary Table S1) and two reverse 806R (Universal, Nano; Supplementary Table S1) primers screening the 16S rRNA V4 gene region in equal concentration (0.1 μM) previously described by Cregger et al. (2018). Primer mixtures for tagging fungal amplicons were composed of six forward (ITS3NGS1, ITS3NGS2, ITS3NGS3, ITS3NGS4, ITS3NGS5, and ITS3NGS10; Supplementary Table S1) and one reverse (ITS4NGS; Supplementary Table S1) for ITS3-ITS4 rRNA region at equal concentration (0.1 μM; Cregger et al. 2018). To inhibit plant material amplification, a mixture of peptide nucleotide acid (PNA) (Panagene, Korea) blockers were added in PCR reaction mixes. These PNA blockers targeted plant mitochondrial and chloroplast 16S rRNA genes (mtPNA_717-1B4, pPNA_717-1B4; Lundberg et al. 2013; Supplementary Table S1) and plant ITS nuclear rRNA gene (ITSspacePNA_717-1B4; Cregger et al. 2018; Supplementary Table S1). The mitochondrial PNA (mtPNA_717-1B4; Supplementary Table S1) of Lundberg et al. (2013) was adjusted for a 1 bp mismatch in *Populus alba* × *tremula*. PCR were performed for three replicates of each sample (2 μl of isolated DNA at about 10 ng/μl) using 2.5× Phusion flash high fidelity master mix (ThermoScientific) with 1.5 μl of forward and reverse primer mix, 0.75 μl of PNA probe, and 8.5 μl of 0.2 μm filtered UV-treated DNA-free water (Carl Roth, France) in a total reaction volume of 30 μl per sample. Thermal cycler conditions for the primary PCRs for bacterial amplification were 30 cycles of 98°C for 5 s, 78°C for 10 s, 52°C for 20 s, and 72°C for 15 s. Primary PCR condition for fungal amplification were 30 cycles of 98°C for 5 s, 78°C for 10 s, 55°C for 20 s, and 72°C for 15 s. PCR products without addition of microbial DNA (negative control), mock communities of known fungal or bacterial compositions were added as quality controls. Samples of 50 μl (30 ng of DNA per μl) were sent for tagging and MiSeq Illumina Next Generation Sequencing (GeT PlaGe INRA sequencing platform, Toulouse, France). The raw data were deposited in the NCBI Sequence Read Archive (SRA) website (https://www.ncbi.nlm.nih.gov/sra) under the SRA study accession numbers PRJNA548249 for 16S data and PRJNA548250 for ITS data.

Amplicon data processing and analysis. Bacterial sequences were further processed with FROGS (Find Rapidly OTU with Galaxy Solution) (Escudié et al. 2018) based on the Galaxy analysis platform (Afgan et al. 2016). Sequences were demultiplexed, dereplicated, sequence quality was checked, oligonucleotides, linker, pads and barcodes were removed from sequences, and sequences were filtered on additional criteria. Sequences were removed from data set, if nonbarcoded, if sequences exhibited ambiguous bases or did not match expectations in amplicon size. Remaining sequences were clustered into OTUs based on the iterative Swarm algorithm, then chimeras and singletons (OTUs containing only one sequence) were removed. Bacterial double affiliation was performed by blasting OTUs against SILVA database (Quast et al. 2013) and the ribosomal database project (RDP) classifier (Wang et al. 2007). OTUs with affiliation <100% at the phylum level (indicated by an RDP bootstrap value of <1) and corresponding to chloroplasts or mitochondria were removed from the data set. OTUs at lower taxonomic ranks than the phylum level were considered as "unidentified" below when the RDP bootstrap value was <0.70. OTUs with high abundances in negative controls were excluded from further analysis and sequencing, and affiliation quality was evaluated based on the results obtained for the bacterial mock community.

Fungal sequences were processed as following. After demultiplexing and quality checking (QC quality score = 28, minimal size = 180 bp), bioinformatics analyses were performed using standard procedures as described in Pérez-Izquierdo et al. (2017).

For both fungal and bacterial data, per-sample rarefaction curves were calculated to assess sampling completeness, using function *rarecurve*() in package Vegan v3.5-1 (Oksanen 2015) in R (version 3.4.3; R Core Team 2016). Based on these, subsequent analyses of diversity and community structure were performed on datasets where samples had been rarefied with the Phyloseq (McMurdie and Holmes 2013) package to achieve equal read numbers according to the minimum number of total reads in any sample (8,982 bacteria, 8,005 fungi). Microbial community composition and structure in bulk soil and roots data were further analyzed by using Phyloseq package (McMurdie and Holmes 2013). Alpha diversity was characterized at the OTU level by measuring richness and Shannon index for each sample. Package Metacoder was used for graphical representation of the relative abundances of microbial taxa detected

EXPERIMANTAL DESIGN



SAMPLING

	Soil 1 (2016)	Soil 2 (2017)
Bulk soil T0	3 samples	3 samples
T1	3 samples (roots/soil)	4 samples (roots/soil)
T2	6 samples (roots/soil)	6 samples (roots/soil)

ANALYSES

Soil physico-chemical characteristics

Fungal and bacterial community composition in soil and *Populus* roots (Miseq Illumina sequencing)

Root metabolomes

Figure S1. Scheme of the experimental design and sampling strategy.

in T0 soils and in roots (Foster et al. 2017). Venn diagrams were created on http://bioinformatics.psb.ugent.be/webtools/Venn/.

FUNGuild (Nguyen et al. 2016) was used to classify each OTU into an ecological guild. OTUs identified to a guild with a confidence ranking to highly probable or probable were conserved in our analysis, whereas those ranking to probable or with multiple assignation were unclassified.

Metabolome analyses. Root samples for metabolomic analysis were lyophilized for 24 h (Alpha 1-2 LI Plus, Christ Martin Osterode am Harz, Germany). Then root metabolome extraction and analyses were carried out as previously described by Tschaplinski et al. (2014). Briefly, between 20 and 100 mg of fresh weight of roots samples was ground to a fine powder in liquid nitrogen, weighed, and then transferred to an acid washed glass scintillation vial containing 2.5 ml of 80% ethanol. Sorbitol was used before extraction as an internal standard to correct potential extraction efficiency difference and to normalized final concentrations of metabolites in each sample. After overnight extraction and the solvent transferred into another vial, each sample pellet was re-extracted with another 2.5 ml of 80% ethanol overnight and the supernatant combined with the prior extract. Using a nitrogen stream, 2 ml of this solution was dried and stored at –80°C until analysis. The samples were dissolved in 750 µl of aqueous 80% ethanol, from which 500 µl was dried in a nitrogen stream. Metabolites were trimethylsilyl derivatized and analyzed using GC-MS, as previously described (Tschaplinski et al. 2014). The peaks of known metabolites were extracted using a key mass-to-charge (m/z) ratio and scaled back up to the total ion current using predetermined scaling factors. The scaling factor for sorbitol was used for unidentified metabolites. Unidentified metabolites were denoted by their retention time as well as key m/z fragments. Peaks were quantified by area integration and were normalized to the quantity of the internal standard (sorbitol) recovered, accounting for the volume of sample processed, derivatization volume, and injection volume.

Statistical analyses. Statistical analyses and data representations were performed using R software (R Core Team 2016). Averaged relative abundances (RAs) of taxonomic groups were achieved by averaging across all samples from a particular sampling time and soil inoculum. These mean RA values were used to estimate the differential abundance of taxonomic groups depending on soil origin using Student's *t* test followed by Benjamini-Hochberg false discovery rate (FDR) correction, after verifying normality of data with Shapiro-Wilk test. Differences in RAs of the different fungal guilds between soil inocula were assessed with a Student's *t* test followed by Bonferroni correction.

Microbial community structures were analyzed using nonmetric multidimensional scaling analysis and permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrices.

The *P* values of root metabolite differences were calculated using a one-way ANOVA and post-hoc honest significant difference tests between the means of sets of soil 1 root samples and the means of sets of soil 2 root samples for each sampling time (T1 and T2), with the data expressed as fold changes relative to soil 1 results. Projection to latent structure (PLS) models were used to look for associations between microorganisms and metabolites (mixOmics package, Rohart et al. 2017). To do so, two independent matrices were built: one assembling T1 data and one assembling T2 data. Each matrix contained OTUs and root metabolites showing significant differences in relative abundance between soil 1 and 2, as well as microbial OTUs specifically detected in roots cultivated in soil 1 or soil 2. Each matrix was used independently to PLS graphic representations of metabolite and OTU associations at T1 and T2.

RESULTS

Soil physico-chemical analysis and climate. Physico-chemical analyses of T0 bulk soils collected under the same poplar tree during early summer 2016 (soil 1) and 2017 (soil 2) were performed to assess potential differences in soil properties over the experiment (Supplementary Table S2). Total organic content, C:N:P stoichiometry, and soil pH remained relatively stable over the 2 years and were typical of a forest acidic soil with poor phosphorous content and a low cation-exchange capacity (as indicated by CEC values, Supplementary Table S2). By contrast, available amounts of sodium and manganese changed by 3- and 1.6-fold, respectively. Note that Winter and Spring climates differed between 2016 and 2017, with 2017 (263 mm/9.7°C) being unusually dryer than 2016 (544 mm/9.3°C), despite a similar average temperature (Supplementary Fig. S2).

Microbial sequencing. To characterize the natural variation of soil- and root-associated microbiomes, Illumina MiSeq sequencing of rDNA 16S and ITS amplicons were performed on soils and root DNA. After quality filtering, and chimera and singleton removal, a total of 770,000 fungal reads (17,500 ± 4,500 reads) and 970,000 bacterial reads (23,000 ± 8,800 reads) per sample were kept for further analyses. After taxonomic assignment, elimination of contaminants and rarefactions of data to achieve equal number of reads, 1,521 fungal OTUs (315 ± 143 per sample) and 6,136 bacterial OTUs (1,210 ± 413) were identified and analyzed.

Microbial community composition of the initial bulk soils differed. Six fungal phyla were detected in bulk soil T0 in soil 1 and soil 2. Basidiomycota and Zygomycota dominated fungal soil communities at T0 (Fig. 1A) followed by Glomeromycota, Chytridiomycota, and Rozellomycota (Supplementary Table S3). Nine dominant bacterial phyla (>1% relative abundance) were detected in bulk soil T0 in soil 1 and soil 2: *Acidobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, *Planctomyces*, *Actinobacteria*, *Gemmatimonadetes*, and WPS-2 (Fig. 1B, Supplementary Table S3).

Comparison of the structure of fungal and bacterial communities indicated a significant shift in both microbial communities between the two soils (Fig. 2). The soil parameter was responsible for 54 and 62% of the variability of the bacterial and fungal communities between soil samples (PERMANOVA test, $P < 0.05$). However, the response of bacterial OTUs was more pronounced than that of fungi as illustrated by alpha-diversity indexes and the relative compositions of the communities. Bacterial, but not fungal, richness and evenness were slightly higher in soil 1 compared with soil 2 (Student's *t* test, $P < 0.05$; Fig. 2C). In addition, although significant variations of relative abundance were measured for both fungi and bacteria at all taxonomic levels, the number of bacterial OTUs that varied between the two soils was higher than that of fungi (Figs. 1 and 2A and B). In all, 61 and 58%, representing 25 and 20% of total abundance of fungal and bacterial OTUs, respectively, were detected only in one of the two soils. Furthermore, 0.2% of fungal and 0.8% of bacterial OTUs (2 and 30, respectively) that were shared between the two soils significantly differed in relative abundance between the two soils (Student's *t* test, $P < 0.05$, Fig. 2A and B). This significant change in community composition did not modify the relative abundance of the different soil fungal trophic guilds: the relative proportion of predicted mycorrhizal fungi, saprotrophs, and potential pathogens was similar in the two soils (data not shown). Instead, we observed a replacement of fungal species within each trophic guild. The ectomycorrhizal fungal community was dominated by *Hymenogaster* in soil 1, whereas *Tuber* and *Tomentella* were prominent in soil 2 (Fig. 1A, Supplementary Table S4). Some bacterial OTUs were specifically detected in a single soil (e.g.,

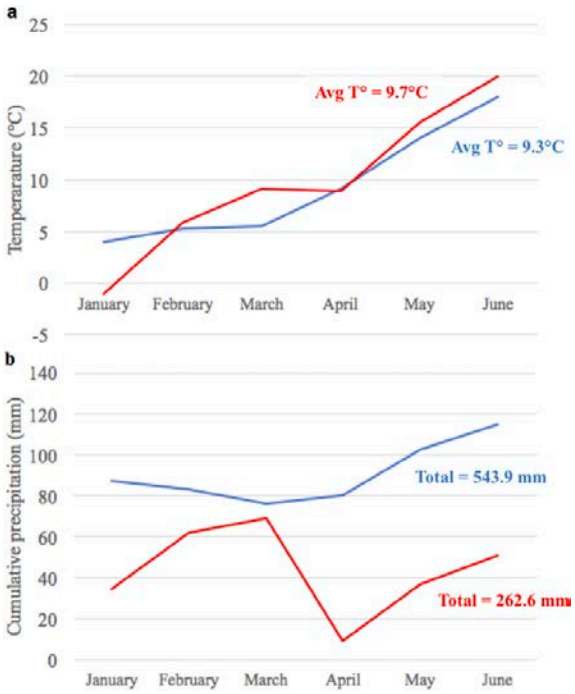


Figure S2. Average monthly temperatures (**a**) and cumulative precipitations (**b**) recorded by the meteorological station of Essey located 12 km from the sampling site from January to June 2016 (Soil 1; https://www.infoclimat.fr/climatologie/annee/2016/nancy-essey/valeurs/07180.html) and 2017 (Soil 2; https://www.infoclimat.fr/climatologie/annee/2017/nancy-essey/valeurs/07180.html).

Bdellovibrio, *Acidicapsa*, and *Terrimonas*; Supplementary Table S5), while other OTUs were significantly more abundant in one of the two soils (Student's *t* test, $P < 0.05$; Fig. 2B). For instance, the relative abundance of *Candidatus Udaeobacter* was 3.5-fold higher in bulk soil T0 collected in soil 1 compared with soil 2, while *Bryobacter* and *Acidothermus* were more abundant in soil 2 (Student's *t* test, $P < 0.05$; Fig. 1B; Supplementary Table S6).

***Populus* root microbiome structure and composition varied depending on bulk soil inoculum.** To test whether natural variations in the composition of soilborne microorganisms may impact

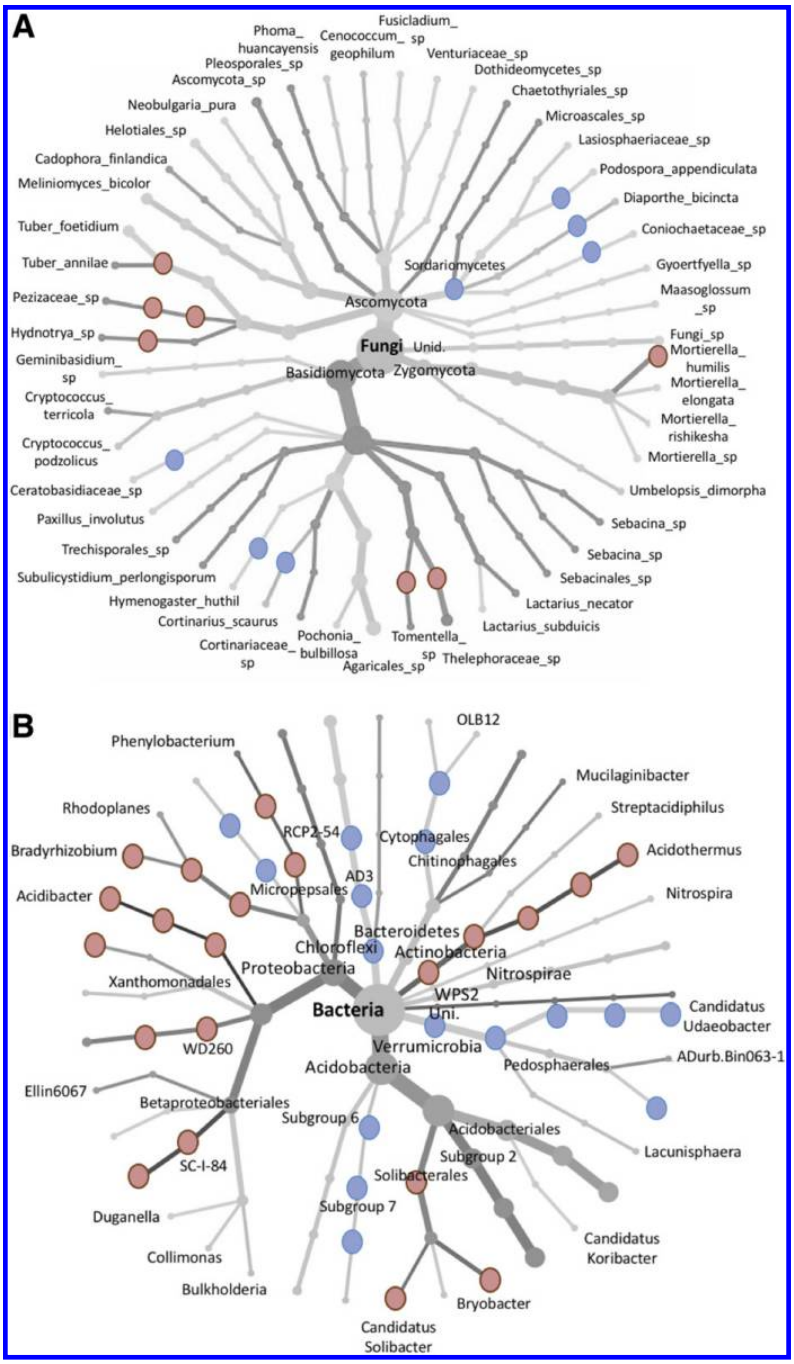


Fig. 1. Taxonomic representation of the most abundant **A,** fungal and **B,** bacterial communities (>0.1% in total relative abundance) detected in bulk soil T0. The thickness of tree branches represents the value of $\log_2$ ratio between soil 1 and soil 2 at each taxonomic rank. The thicker the line, the more important the value. Blue circles represent microbial taxonomic rank significantly more abundant in soil 1, whereas red circles represent microbial taxonomic rank significantly more abundant in soil 2 (Student's *t* test, $P < 0.05$).

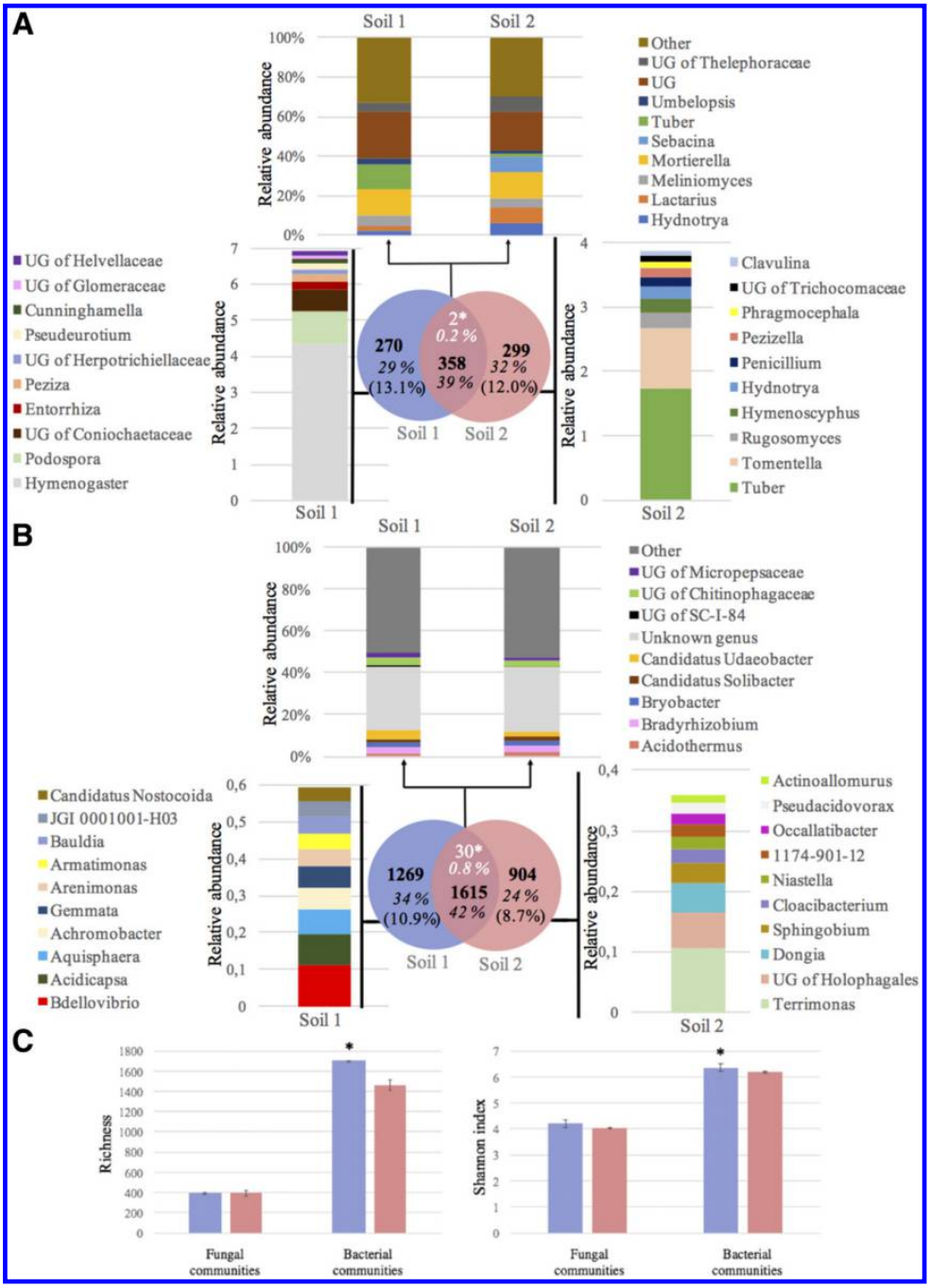


Fig. 2. Differences in the composition and diversity of fungal and bacterial communities between T0 bulk soils. Venn diagrams indicate the number of the **A,** fungal and **B,** bacterial operational taxonomic units (OTUs) detected only in one soil of the experiment or retrieved in both soils. The number in bold represents the number of OTUs detected only in one soil of the experiment or retrieved in both soils. This value is converted in percentage in italic and in relative abundance of total OTUs in brackets. The number in white followed by an asterisk represents the number of OTUs whose relative abundance is significantly different between the two soils (* = $P < 0.05$, Student's *t* test). This value is converted in percentage in italic. **C,** Compared alpha diversity of the fungal and bacterial communities from the bulk soil T0 collected in soil 1 and in Soil 2 (richness and Shannon index). Diversity indices are given as means ± standard error. The asterisks denote significant difference in each alpha-diversity measure between soil 1 and soil 2 (* = $P < 0.05$, Student's *t* test; $n = 3$).

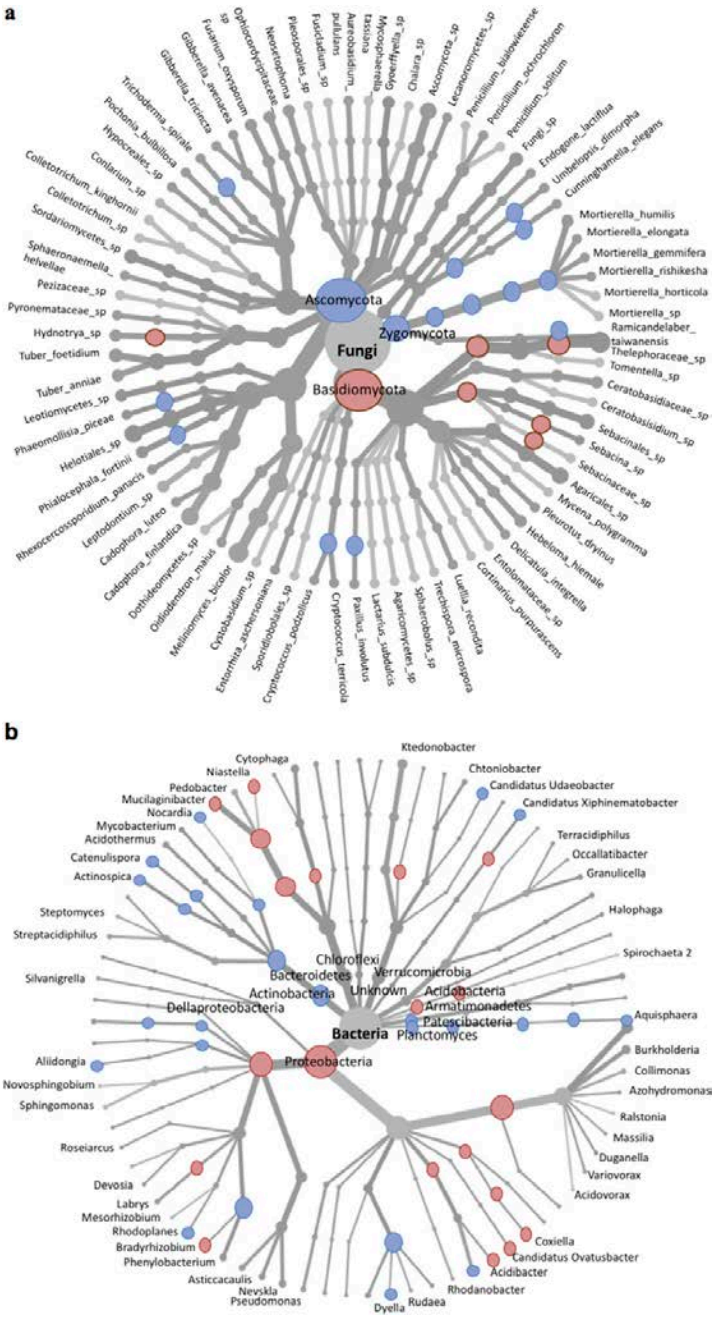


Figure S3. Taxonomic representation of the most abundant fungal (**a**) and bacterial (**b**) communities (>0.1% in total relative abundance) detected in Populus roots. The thickness of tree branches represents the value of log2 ratio between Soil 1 and Soil 2 roots at each taxonomic rank. The thicker the line, more important the value is. Blue circles represent microbial taxonomic rank significantly more abundant when Poplar were grown in Soil 1 whereas red circles represent microbial taxonomic rank significantly more abundant when Poplar were grown in Soil 2 (Student t-test, $p<0.05$).

the root microbiome, we characterized bacterial and fungal communities in roots of poplar cuttings grown in greenhouse in soil 1 and soil 2.

Overall, six fungal phyla and 13 dominant (>1% relative abundance) bacterial phyla were detected in roots of cuttings grown in soil 1 and soil 2. Like in soil, Ascomycota, Basidiomycota, and Zygomycota dominated the root communities, while *Proteobacteria*, *Actinobacteria*, *Patescibacteria*, *Acidobacteria*, and *Verrucomicrobia* were the most abundant bacterial phyla (Supplementary Fig. S3). Fungi identified in the roots mainly belonged to the trophic groups of ectomycorrhizal fungi (28.2 ± 3.8%), fungal endophytes (12.3 ± 1.5%), and saprotrophic fungi (5.5 ± 1.0%).

The structures of fungal and bacterial communities colonizing roots clearly differed depending on the soil for both sampling times, as illustrated by nonmetric multidimensional scaling analyses (Fig. 3A and B). The soil parameter was responsible for 31% of the variability of the bacterial and fungal communities between root samples according to the PERMANOVA test, while the stage of colonization (T1 versus T2) explained 16 and 17% of the variability, respectively. By contrast, no significant difference was observed in microbial community structure of the bulk soils in the pots along the different sampling times of the experiment, suggesting that neither the greenhouse parameters (e.g., temperature and humidity) nor the tree seedlings impacted the soil microbial community structure (Fig. 3A and B).

In *Populus* roots harvested at T1, 68%, representing 22.7 and 22.6% of total abundance of fungal and bacterial OTUs, respectively, were detected only in the roots collected in one of the two soils. Furthermore, 0.5% of fungal and 0.03% of bacterial OTUs (3 and 1, respectively) that were shared between the two soils significantly differed in relative abundance (Student's *t* test, $P < 0.05$, Fig. 3C and D). In *Populus* roots harvested at T2, 67 and 66%, representing 19 and 14% of total abundance of fungal and bacterial OTUs, respectively, were detected only in the roots collected in one of the two soils (Supplementary Table S7 and S8). Furthermore, 0.4% of fungal and 0.2% of bacterial OTUs (2 and 4, respectively) that were shared between roots grown in the two soils significantly differed in relative abundance (Student's *t* test, $P < 0.05$, Fig. 3C and D). These changes in root community composition associated with soil variation resulted in a modification of the relative abundance of fungal trophic guilds, particularly at T1. Ectomycorrhizal fungi tended to be more abundant in roots grown in soil 2 than in soil 1, while endophytes, plant pathogens, and arbuscular mycorrhizal fungi tended to be more abundant in roots grown in soil 1 than in soil 2 (Student's *t* test, $P < 0.05$; Fig. 4). At T2, the relative abundance of fungal endophytes was significantly higher in soil 1

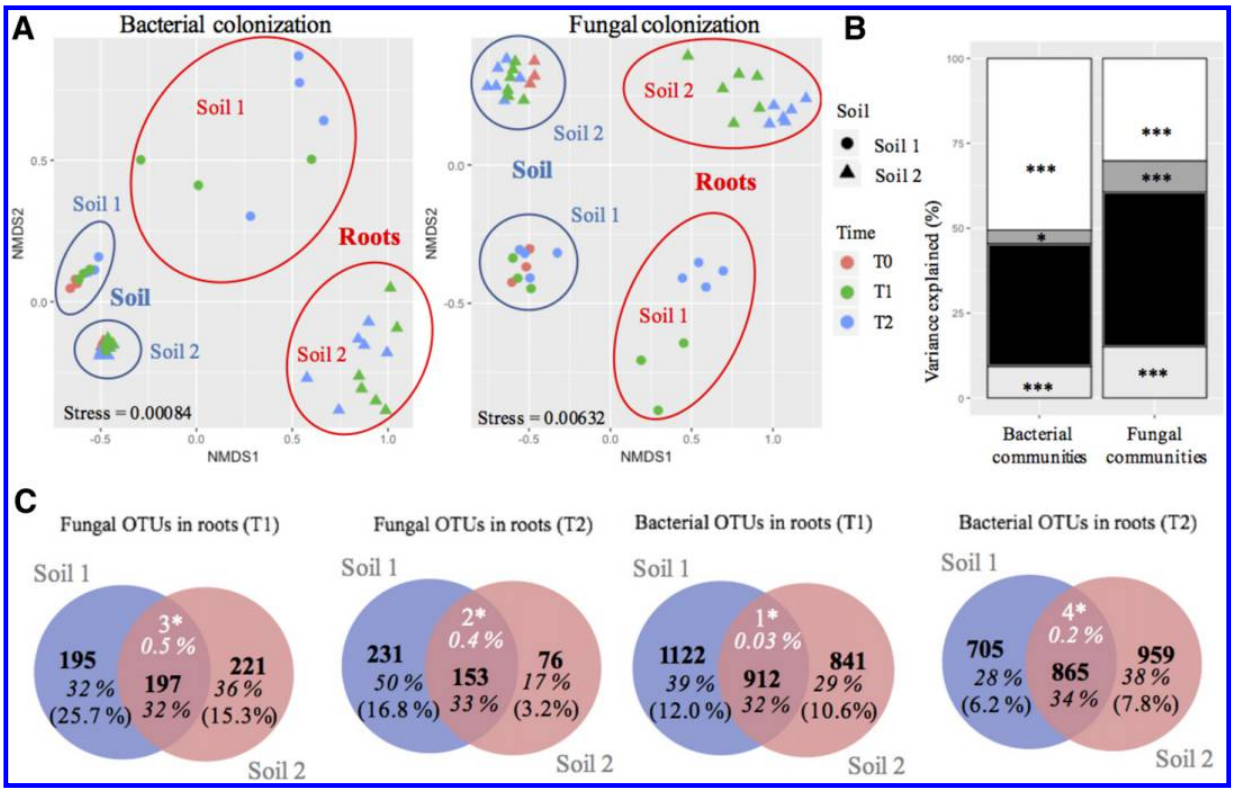


Fig. 3. Differences in the structure and composition of root fungal and bacterial communities of cuttings grown in soil 1 and soil 2. **A,** Nonmetric multidimensional scaling (NMDS) analysis ordinations of fungal and bacterial communities across compartments (soil and roots), sampling time (T0, red; T1, green; and T2, blue), and soil origin (soil 1, circles; soil 2, triangles). **B,** Variance explanation based on permutational multivariate analysis using Euclidean dissimilarity matrix for bacterial and fungal communities (white, compartment; gray, time; black, unexplained; light gray, year; *, $P < 0.05$; and ***, $P < 0.001$). **C,** Venn diagrams of the fungal and bacterial operational taxonomic units (OTUs) detected in roots grown in the two soils. The number in bold represents the number of OTUs detected in roots only in one soil condition or retrieved in both soil conditions. This value is converted in percentage in italic and in relative abundance of total OTUs in brackets. The number in white followed by an asterisk represents the number of OTUs whose relative abundance is significantly different between the two soil conditions (* = $P < 0.05$, Student's *t* test). This value is converted in percentage in italic.

roots compared with soil 2 roots. Indeed, we observed that the relative abundance of the fungal endophytes *Mortierella* and *Phialocephala* was, respectively, 23 and 25 times higher in soil 1 roots compared with soil 2 roots. While the relative abundance of the other fungal trophic guilds was maintained over the two conditions, we observed that the saprotrophes *Phaemollisia* and *Ramicandelaber* were significantly more abundant in soil 1 roots, whereas the ectomycorrhizal fungus belonging to the family *Thelephoraceae* were 6.5 times higher in roots of *Populus* harvested in soil 2 (Student's *t* test, $P < 0.05$, Supplementary Table S9).

Similarly, some bacterial OTUs were specifically detected in roots grown in one of the two soils (e.g., *Pedobacter*, *Amycolatopsis*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and *Lacunisphaera*; Supplementary Table S8), while others were significantly more abundant in roots grown in one of the two soils. At T1, the relative abundance of bacteria belonging to *Gammaproteobacteria Incartae Sedis*, *Coxiellales*, and *Acetobacterales* were three times higher in roots collected in soil 2, while *Xanthomonadales* relative abundance was significantly enriched in roots collected in soil 1 (Student's *t* test, $P < 0.05$, Supplementary Table S6). At the genus level, *Rhodanobacter* relative abundance was higher in roots of soil 1, whereas relative abundance of *Acidibacter*, *Coxiella*, and *Phenylobacterium* were significantly enriched in roots collected in soil 2 (Student's *t* test, $P < 0.05$, Supplementary Table S9). At T2, significant differences in relative abundance were only observed at the bacterial order level. *Xanthomonadales*, *Saccharimonadales*, and *Catenulisporales* were significantly more abundant in soil 1 roots, while *Ktedobacterales*, *Chitinophagales*, *Pedosphaerales*, *Rhizobiales*, and *Sphingobacteriales* were significantly more abundant in soil 2 roots (Student's *t* test, $P < 0.05$, Supplementary Table S9).

Root metabolome varies according to soil inoculum and correlates with microbiome composition. Since the composition of the root-associated microbiome could impact by the metabolism of the host plant (Berg and Smalla 2009), we characterized by GC-MS the root metabolome of poplar cuttings grown for 10 days and 6.5 weeks in soils of the poplar plantation sampled on 2016 (soil 1) and 2017 (soil 2). A total of 90 metabolites were quantified in roots

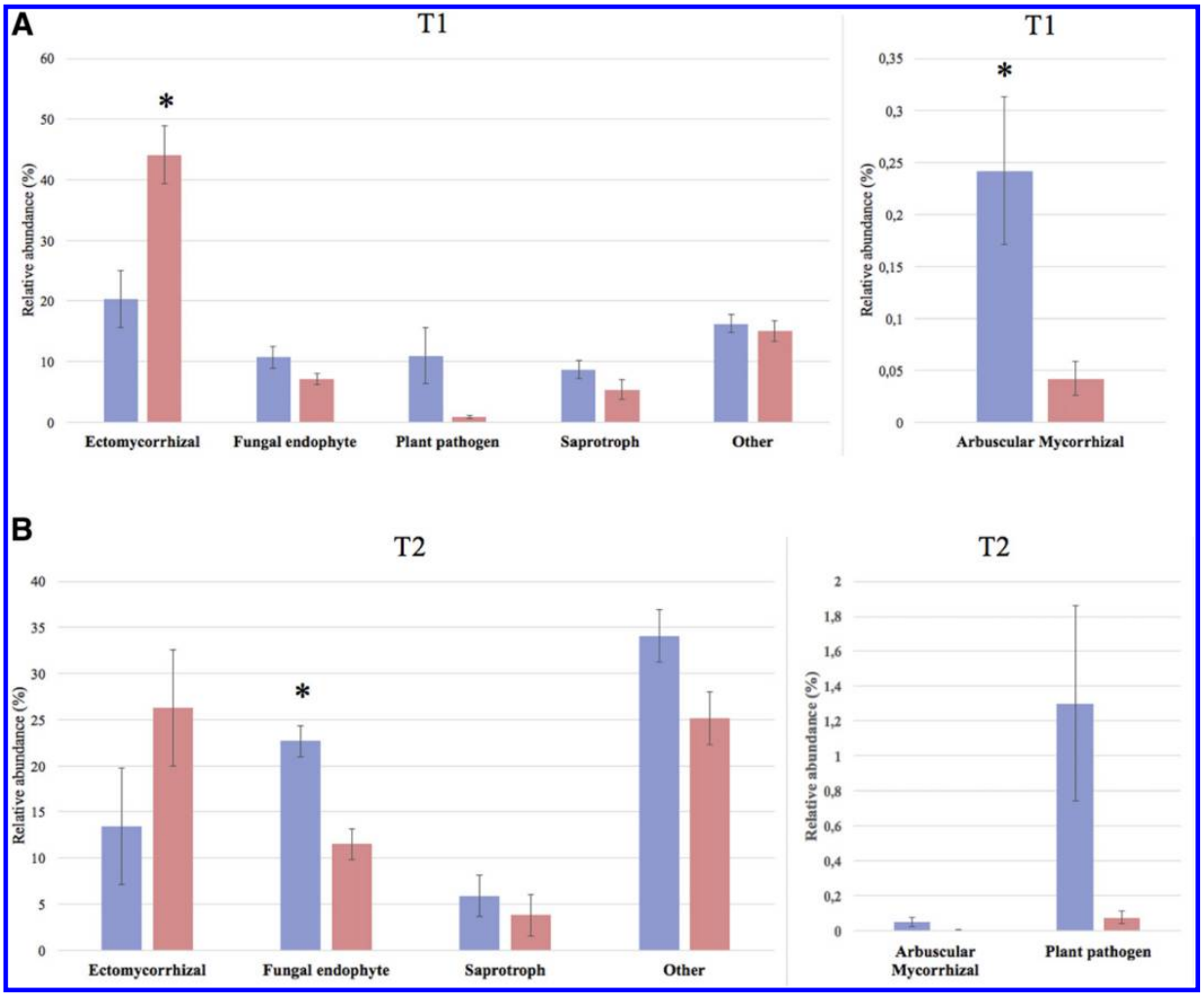


Fig. 4. Relative abundance of fungal functional guilds of OTUs detected in roots of *Populus tremula* × *alba* harvested at **A,** T1 and **B,** T2 grown in soil 1 (blue) and soil 2 (red). The asterisks indicate significant differences in relative abundance of fungal guilds between soil 1 roots and soil 2 roots (* = $P < 0.05$; Student's *t* test).

harvested at T1 and T2. Some of these metabolites are known to be only produced by *Populus* while others can also be of microbial origin (Supplementary Table S10). Thirty-four of the detected metabolites were involved in primary metabolism, while 38 were linked to secondary metabolism. In addition, 18 detected metabolites corresponded to unidentified compounds. The main primary metabolites (>1% of the total metabolites amount) were sucrose (30.7%), malic acid (9.7%), glucose (8.1%) and fructose (3.7%), palmitic acid (3.3%), and phosphate and galactose (1.3%), while secondary metabolites were dominated by salicylic acid derivatives (α-salicyloylsalicin, 14.1%; salicin, 6.7%; and tremuloidin, 3.9%), as well as β-sitosterol (2.1%) and catechin (1.7%) (Supplementary Table S10).

Differential root colonization by soil fungal and bacterial communities was correlated with changes in 10 to 20% of root metabolites collected from soil 1 and soil 2. At T1, only seven metabolites differed significantly between soil 1 and soil 2, out of the 76 compounds quantified in roots extracts. Alanine, salicylic acid, and an unidentified compound (10.68 min, m/z 217 391 411) significantly increased with a fold change of 9, 2.5, and 2, respectively, in roots collected from soil 1 compared with soil 2, whereas mannitol, glycerol (16.11 min, m/z 297), guaiacyl lignan, and another unidentified compound (14.09 min, m/z 375 292 217) significantly increased with a fold change of 13, 8, 2, and 3, respectively, soil 2 roots compared with soil 1 roots (one-way ANOVA, $P < 0.05$; Table 1).

At T2, 18 metabolites differed between soil 1 and soil 2 roots, out of the 84 compounds quantified in root extracts. Alanine was the most responsive metabolite, exhibiting a 20-fold increase in concentration in soil 1 roots compared with soil 2 roots, followed by oxalomalic acid (eightfold increase) (one-way ANOVA, $P < 0.05$; Table 1). The concentrations of some metabolites involved in carbon metabolism, such as fructose and glucose, also varied (one-way ANOVA, $P < 0.05$; Table 1).

A PLS combining amplicon sequencing and metabolite data was conducted to investigate the existence of potential associations between changes in abundance in the roots of microbial OTUs and metabolites (Fig. 5).

At T1, the enrichment in the bacterial genera *Cytophaga*, *Acidibacter*, *Candidatus Ovatusbacter*, *Coxiella*, and *Silvanigrella*,

TABLE 1
List of metabolites detected in roots of *Populus tremula* × *alba* harvested after 10 days (T1) and 6.5 weeks (T2) of growth for which a significant difference of concentration has been measured between soil 1 and soil 2 (* = $P < 0.05$, one-way analysis of variance)

Metabolite (RT-m/z)	Plant metabolite	Bacterial or fungal metabolite	*Populus* roots collected in soil 1 versus soil 2: 10 days of growth (T1)[a]	*Populus* roots collected in soil 1 versus soil 2: 6.5 weeks of growth (T2)[a]	Root metabolites of *P. tremula* × *alba* (%)[b]
Sucrose[c]	X	X	0.61	0.40*	30.72
Malic acid[c]	X	X	1.77	0.59*	9.74
Glucose[c]	X	X	4.87	3.13*	8.11
Fructose[c]	X	X	3.46	3.40*	3.72
Catechin[d]	X		1.00	0.42*	1.68
7.69 169 101 75 68		?	ND	3.16*	1.65
Mannitol[c]		X	0.08*	0.79	0.65
Salicylic acid[d]	X	X	2.55*	0.48	0.51
Glycerol[c]	X	X	0.13*	0.46	0.50
Threonic acid[c]	X		1.45	0.25*	0.33
Alanine[c]	X	X	9.15*	20.07*	0.21
Ethyl-phosphate		?	3.21	3.13*	0.09
Oxalomalic acid[c]	X	X	54.78	7.63*	0.08
10.68 217 391 411		?	2.15*	4.11*	0.06
Xylono-1,4-lactone[d]	X		2.72	2.09*	0.04
Maleic acid[c]	X	X	1.09	0.28*	0.032
14.09 375 292 217		?	0.34*	ND	0.030
16.11 guaiacyl lignan[d]	X		0.45*	0.85	0.028
Phluoroglucinol[d]	X	X	4.84	4.84*	0.021
Erythronic acid[c]	X		0.94	0.51*	0.014
6-Hydroxy-2-cyclohexenone-1-carboxylic acid[d]	X	X	0.78	0.25*	0.013
Cis-aconitic acid[c]	X	X	0.23	0.41*	0.012
11.22 450 dehydro sugar		?	1.08	0.34*	0.009

[a] Values indicate fold changes between soil 1 and soil 2.
[b] The last column indicates the relative abundance of each metabolite in the total root metabolome of *Populus tremula* × *alba* seedlings.
[c] Metabolites involved in primary metabolisms.
[d] Metabolites involved in secondary metabolisms.

the ectomycorrhizal fungi *Tuber* and *Hydnotrya* in soil 2 roots was associated with an accumulation of glycerol and mannitol in roots. In contrast, the enrichment of the bacterial genus *Methylophilus*, the fungal endophytes *Mortierella* and *Fusarium*, and the ectomycorrhizal fungus *Paxillus* in soil 1 roots was associated with the accumulation of salicylic acid (Fig. 5A).

At T2, associations between several microorganisms were evident (Fig. 5B). For instance, the associations of bacterial genera

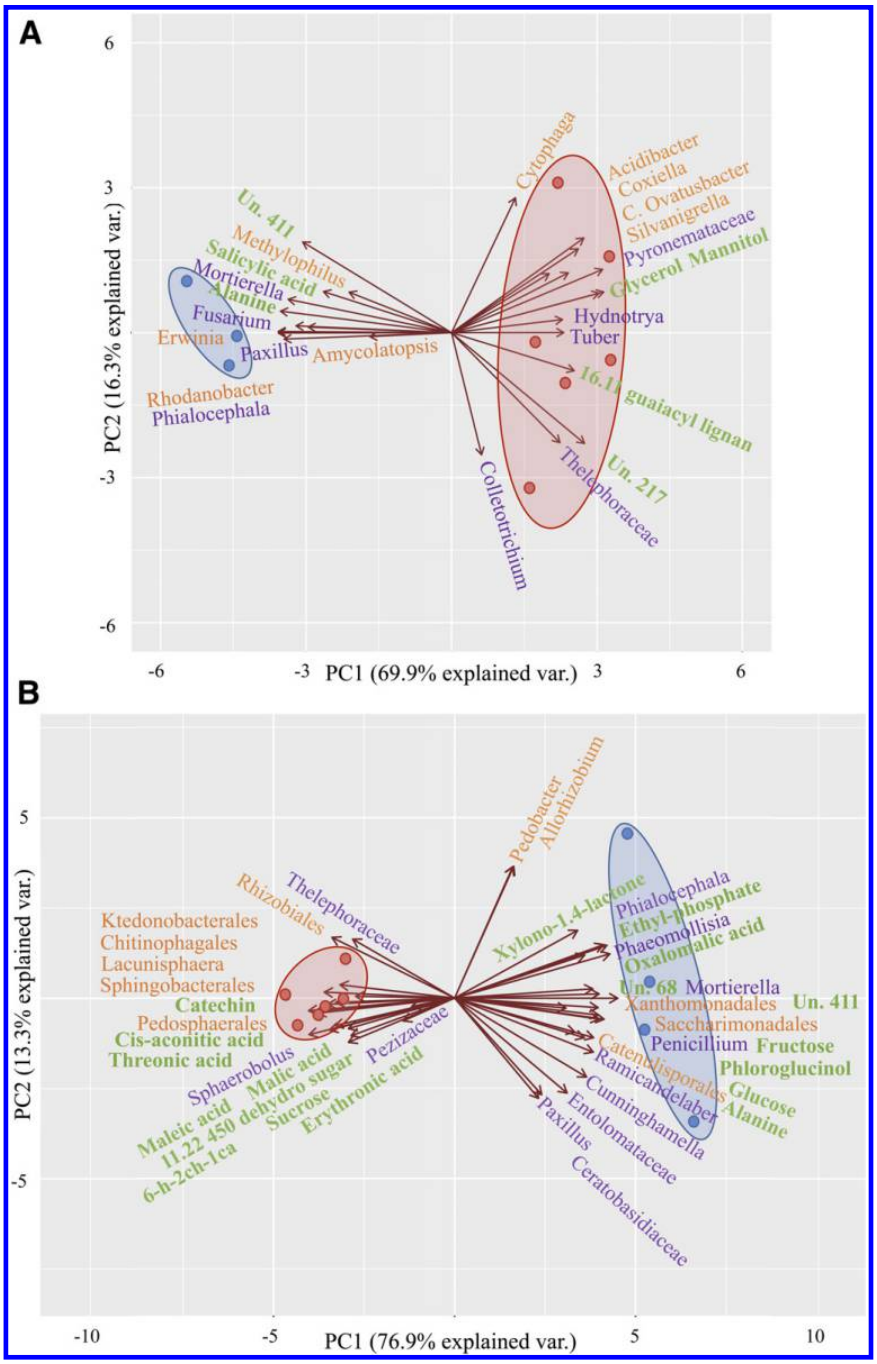


Fig. 5. Projection to latent structures of the dominant root microbial communities and root metabolites significantly varying between soil 1 (red) and soil 2 (blue) conditions at **A,** T1 and **B,** T2. Bacterial (>0.1% relative abundance), fungal (>0.5%) taxa, and metabolites are represented in orange, purple, and green, respectively. Un. 411 = unidentified 10.68 m/z, 217 391 411; Un. 217 = unidentified 14.09 m/z, 375 292 217; Un. 68 = unidentified 7.69 m/z, 169 101 75 68; and 6-h-2ch-1ca = 6-hydroxy-2-cyclohexenone-1-carboxylic acid.

Pedobacter and *Allorhizobium* or the ectomycorrhizal fungi of the family Telephoraceae and bacterial OTU from the order *Rhizobiales* were tightly linked. Associations between the presence of microorganisms and the accumulation of metabolites were also revealed. The enrichment of the dark septate endophyte *Phialocephala* and, the saprotrophic fungus *Phaomollisia* in soil 2 roots was associated with the accumulation of primary metabolites oxalomalic acid and xylono-1.4-lactone. The presence and/or enrichment of three bacterial orders (*Xanthomonadales*, *Catenulisporales*, and *Saccharimonadales*) three saprotrophic fungi (e.g., *Penicillium* and *Cunninghamella*), the ectomycorrhizal fungus *Paxillus* was associated with increased levels of primary metabolites, including fructose, glucose, and alanine, and the secondary metabolite, phloroglucinol. Conversely, in soil 1 roots, the presence of five bacterial orders (e.g., *Pedosphaerales* and *Sphingobacterales*), the saprotrophic fungus, *Sphaerobolus*, and members of the *Pezizaceae* were associated with the accumulation of several primary metabolites (e.g., malic acid and sucrose) and the secondary metabolite, catechin.

DISCUSSION

The composition of the root microbiome and the host metabolism are tightly interrelated: the type and level of carbon and nitrogen compounds accumulating and released by roots strikingly influence the nature and abundance of soilborne microorganisms colonizing the rhizosphere and root tissues (Berg and Smalla 2009; Jacoby et al. 2017). Abiotic stresses experienced by trees lead to major physiological alterations triggering dramatic shifts in root microbial communities (Timm et al. 2018). Conversely, the composition of the microbial communities colonizing roots can modulate tree nutrition and their sensitivity to diseases and stresses as microorganisms can differ in their abilities to capture nutrients or restrict pathogen colonization (Chaparro et al. 2012; DeAngelis et al. 2009). However, a high level of functional redundancy exists among root-colonizing microorganisms and the replacement of a species by another can be functionally neutral (Colin et al. 2017). Soil is the major reservoir of microorganisms from which plants draw their rhizospheric and endophytic microbiomes (Edwards et al. 2015; Mercado-Blanco et al. 2018). This pool of soil microorganisms is likely to greatly vary locally over time due to environmental changes, such as drought, flooding, or heat waves (Brockett et al. 2012). Here, we asked whether natural variations in the soil microbial reservoir influence the root-associated microbiome of poplar and its metabolome, or whether such variations are buffered by functional redundancies in microbial communities. We observed that only ~40 and 30% of the fungal and bacterial OTUs, respectively, were conserved in the soil and roots over the 2 years of the experiment. This important turnover of microbial taxa was accompanied by changes in the relative proportion of fungal guilds, i.e., symbionts, pathogens, and endophytes, colonizing roots. Alterations in microbial communities were correlated to a shift in the level of 10% of the root metabolites, including carbohydrates, amino acids, and secondary metabolites.

Changes in bulk soil microbiome are accompanied by a change in the composition and structure of the root-associated microbiome. The soil microbiome of the poplar plantation was typical of a forest soil with low pH (Lladó et al. 2017; Uroz et al. 2016): *Acidobacteria* and *Proteobacteria* dominated the bacterial communities, while fungal communities were mainly made of a mix of ectomycorrhizal fungi (e.g., *Tuber*, *Hymenogaster*, and *Hydnotrya*) and saprophytes (e.g., *Podospora*) as previously observed in soils of other *Populus* genotype plantations (Beckers et al. 2017; Bonito et al. 2014). However, soil microbiome composition and structure were significantly different between the 2 years of the experiment with more than half of fungal and bacterial OTUs being replaced from 1 year to the other. Many factors can be responsible for this difference; local heterogeneity in soil microbial community, disturbing effect of the first sampling, and climate differences between the 2 years of sampling (winter drought in 2017). In any cases, the changes in the bulk soil communities were large enough to result in an alteration of the root microbiome.

Root bacterial and fungal communities were similar to those previously described in other *Populus* genotypes (Beckers et al. 2017; Bonito et al. 2016, 2019; Durand et al. 2017; Shakya et al. 2013): bacteria of the genera *Actinospica* (*Actinobacteria*), *Mucilaginibacter* (*Sphingobacteria*), and *Bradyrhizobium* (*Rhizobiales*), the ectomycorrhizal fungi, *Paxillus*, and members of *Thelephoraceae*, and the endophytes *Mortierella*, dominated in the microbial communities.

As observed in soil, the composition and structure of root microbial communities varied depending on the soil inoculum. Some bacterial and fungal genera shifted in relative abundance between roots grown in soil 1 and soil 2, but we also observed that 17 to 50% of microbial OTUs were soil specific. This is in accordance with the fact that soil microbiome is a more important driver of bacterial and fungal community assemblage in *Populus* and other trees than genetic factors (Bonito et al. 2014; Uroz et al. 2016; Veach et al. 2019). However, this contrasts with the hypothesis, forwarded from other studies on other plant types, that roots exert a recruitment effect on microbial consortia independent of the microbiome source (Edwards et al. 2015; Pérez-Jaramillo et al. 2016). Thus, the relative importance of soil microbiome versus plant factors likely varies depending on plant type and species.

While the relative abundance of particular taxa of ectomycorrhizal fungi (e.g., *Tuber*, *Thelephoraceae*, and *Hydnotrya*), endophytes, and saprotrophes (e.g., *Mortierella*) changed in soil, the relative abundance of each fungal trophic guild was maintained in soil over the 2 years. By contrast, in the *Populus* roots, changes in the relative abundance of specific taxa (e.g., *Tuber*, *Thelephoraceae* and *Hydnotrya* at T1, and *Phialocephala* and *Mortierella* at T2) was accompanied by change in the relative abundance of trophic guilds. Similarly, the inoculation of *Populus* roots with the endophyte *Mortierella* led to a change in the entire fungal community colonizing the root system (Liao et al. 2019). Changes in the relative contribution of trophic guilds also take place over development stages in poplars and other trees (Gehring et al. 2006; Hacquard and Schadt 2015). Altogether, these findings suggest that the balance between ectomycorrhizal fungi, endophytes, and saprophytes, is unstable and not entirely controlled by rootborne factors. It is likely affected by the composition of the soil microorganism pool and environmental fluctuations. Since these different types of fungi have different roles in terms of nutrition and protection against stresses (Baum et al. 2018; Van Der Heijden et al. 2008), one could expect that such changes would impact *Populus* tree nutrition and physiology or possibly limit stress resistance of trees to biotic and abiotic stress. For instance, the endophytic *Mortierella* can stimulate the growth of *Populus* and modify the activity of ectomycorrhizal fungi, but also other saprotrophic fungi and fungal endophytes (Bonito et al. 2016; Liao et al. 2019).

Changes in the composition and structure of the root-associated microbiome are accompanied by a change in the root metabolome. In accordance with this hypothesis, the changes in the root microbiome were concomitant with a modification of 10% of the measured root metabolites. Changes were mainly observed in cuttings after 6.5 weeks of growth in plantation soil. Major differentially accumulated metabolites were related to primary carbon metabolism (15%). Of note, a number of the responsive

compounds are those that are often found in tree root exudates (e.g., malate, maleate, and alanine) and/or involved in nutrient fluxes between ectomycorrhizal fungi and their host roots. The observed decrease in sucrose level was correlated with an increased level of fructose and glucose as observed in *Pisolithus-Eucalyptus* ectomycorrhizae (Martin et al. 1998). Concomitantly, the level of alanine involved in nitrogen transfer from *Paxillus* to the roots (Chalot et al. 1995) significantly increased in roots. Similarly, the accumulation of the storage sugar mannitol correlated with the presence of the ectomycorrhizal *Hydnotrya* and *Tuber*. Ectomycorrhizal fungi produce mannitol which serves as a storage compound but also as an osmo-protectant to preserve fungi of abiotic stresses such as drought (Solomon et al. 2007). Altogether, these results suggest that the replacement of some specific ectomycorrhizal fungal species by others within the microbiome could significantly affect nutrient fluxes and metabolism of *Populus* roots, as previously suggested by functional comparison of ectomycorrhiza of different species (Mello and Balestrini 2018). Further in situ functional analyses are needed to characterize these relationships.

It is also possible that modification of the colonization by fungal endophytes (e.g., *Phialocephala*) led to the alteration of the primary metabolism in the roots. In raygrass, fungal endophytes colonization induced important changes in the host cells, notably in the expression of genes encoding for proteins involved in the tricarboxylic acid cycle (TCA) cycle (Dupont et al. 2015). Organic acids that varied in concentration between poplar roots grown in soil 1 and soil 2 also participate in the TCA cycle, in addition to be found in root exudates. This shift in the concentration of TCA cycle metabolites was positively or negatively correlated with significant shifts of a part of the fungal root microbiome.

Secondary metabolism was also affected over the 2 years, although to a lesser extent than primary metabolism. The accumulation of several secondary metabolites, such as salicylic acid (T1) and phloroglucinol (T2), was associated with an increased presence of some microorganisms. For instance, bacterial OTUs from the genus *Methylophilus*, the fungal endophytes *Mortierella* and the saprotrophes *Penicillium* tended to be more abundant at higher levels of salicylic acid at T1. Increases in the relative abundance of the latter two fungi were also associated with increased level of phloroglucinol at T2. Phloroglucinol is involved in plant defense against soilborne pathogenic microbes, in addition to its role as growth regulator (Haas and Keel 2003). Salicylic acid modulates the colonization of the root by specific bacterial taxa in *Arabidopsis* (Lebeis et al. 2015) and by pathogenic fungi (Dieryckx et al. 2015). Its impact on the fungal symbiont is currently poorly known, but it is likely that salicylic acid affects fungal symbiont root colonization (Basso et al. 2020). At the same time, some microorganisms trigger salicylic acid production as part of a defense response (Liao et al. 2019; Martínez-Medina et al. 2017). Whether specific microorganisms have induced the accumulation of these secondary metabolites or whether the secondary metabolites have promoted colonization by specific microorganisms will require further study.

Conclusion. In conclusion, the root microbiome of *Populus* and its metabolome are strongly influenced by variations in the composition of the surrounding bulk soil microbiome. On one hand, as such variations are expected to be amplified in the future by the frequency of extreme climatic events (e.g., winter and summer droughts, heat waves, etc.), it can be predicted from our results that these events will indirectly affect *Populus* trees through the recruitment of their root microbiome. On the other hand, one could imagine taking advantage of such process for engineering soil microbiomes in order to improve the resistance of *Populus* to environmental stresses. Future works are needed to assess if such applications are feasible.

ACKNOWLEDGMENTS

We thank Lucas Auer (INRAE Nancy) for his help in implementing PLS analyses; and Patrice Vion, Yoann Guignet, and Mehdi Maaroufi (INRAE Nancy) for their help in growing, maintaining, and harvesting poplars.

LITERATURE CITED

Afgan, E., Baker, D., Van den Beek, M., Blankenberg, D., Bouvier, D., Čech, M., et al. 2016. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res.: W3-W10.

Basso, V., Kohler, A., Miyauchi, S., Singan, V., Guinet, F., Šimura, J., Novák, O., Barry, K. W., Amirebrahimi, M., Block, J., Daguerre, Y., Na, H., Grigoriev, I. V., Martin, F., and Veneault-Fourrey, C. 2020. An ectomycorrhizal fungus alters sensitivity to jasmonate, salicylate, gibberellin, and ethylene in host roots. Plant Cell Environ. 43:1047-1068.

Baum, C., Hrynkiewicz, K., Szymanska, S., Vitow, N., Hoeber, S., Fransson, P. M. A., and Weih, M. 2018. Mixture of *Salix* genotypes promotes root colonization with dark septate endophytes and changes P cycling in the mycorrhizosphere. Front. Microbiol. 9:1012.

Beckers, B., De Beeck, M. O., Weyens, N., Boerjan, W., and Vangronsveld, J. 2017. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. Microbiome 5: 25.

Berg, G., and Smalla, K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68:1-13.

Bonito, G., Benucci, G. M. N., Hameed, K., Weighill, D., Jones, P., Chen, K. H., Jacobson, D., Schadt, C., and Vilgalys, R. 2019. Fungal-bacterial networks in the *Populus* rhizobiome are impacted by soil properties and host genotype. Front. Microbiol. 10:481.

Bonito, G., Hameed, K., Ventura, R., Krishnan, J., Schadt, C. W., and Vilgalys, R. 2016. Isolating a functionally relevant guild of fungi from the root microbiome of *Populus*. Fungal Ecol. 22:35-42.

Bonito, G., Reynolds, H., Robeson, M. S., Nelson, J., Hodkinson, B. P., Tuskan, G., Schadt, C. W., and Vilgalys, R. 2014. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Mol. Ecol. 23: 3356-3370.

Brockett, B. F. T., Prescott, C. E., and Grayston, S. J. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol. Biochem. 44: 9-20.

Chalot, M., Kytöviita, M. M., Brun, A., Finlay, R. D., and Söderström, B. 1995. Factors affecting amino acids uptake by the ectomycorrhizal fungus *Paxillus involutus*. Mycol. Res. 99:1131-1138.

Chaparro, J. M., Sheflin, A. M., Manter, D. K., and Vivanco, J. M. 2012. Manipulating the soil microbiome to increase soil health and plant fertility. Biol. Fertil. Soils 48:489-499.

Colin, Y., Nicolitch, O., Van Nostrand, J. D., Zhou, J. Z., Turpault, M. P., and Uroz, S. 2017. Taxonomic and functional shifts in the beech rhizosphere microbiome across a natural soil toposequence. Sci. Rep. 7:9604.

Cregger, M. A., Veach, A. M., Yang, Z. K., Crouch, M. J., Vilgalys, R., Tuskan, G. A., and Schadt, C. W. 2018. The Populus holobiont: Dissecting the effects of plant niches and genotype on the microbiome. Microbiome 6:31.

DeAngelis, K. M., Brodie, E. L., DeSantis, T. Z., Andersen, G. L., Lindow, S. E., and Firestone, M. K. 2009. Selective progressive response of soil microbial community to wild oat roots. ISME J. 3:168-178.

Dieryckx, C., Gaudin, V., Dupuy, J. W., Bonneu, M., Girard, V., and Job, D. 2015. Beyond plant defense: insights on the potential of salicylic and methylsalicylic acid to contain growth of the phytopathogen *Botrytis cinerea*. Front. Plant Sci. 6:859.

Dupont, P. Y., Eaton, C. J., Wargent, J. J., Fechtner, S., Solomon, P., Schmid, J., Day, R. C., Scott, B., and Cox, M. P. 2015. Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. New Phytol. 208:1227-1240.

Durand, A., Maillard, F., Foulon, J., Gweon, H. S., Valot, B., and Chalot, M. 2017. Environmental metabarcoding reveals contrasting belowground and aboveground fungal communities from *Poplar* at a Hg phytomanagement site. Microbiol. Ecol. 74:795-809.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., and Bhatnagar, S., Eisen, J. A., and Sundaresan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl. Acad. Sci. 112(8):E911-E920.

Emerson, B. C., and Gillespie, R. G. 2008. Phylogenetic analysis of community assembly and structure over space and time. Trends Ecol. Evol. 23:619-630.

Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., et al. 2018. FROGS: Find, rapidly, OTUs with galaxy solution. Bioinformatics 34: 1287-1294.

Farrar, K., Bryant, D., and Cope-Selby, N. 2014. Understanding and engineering beneficial plant-microbe interactions: Plant growth promotion in energy crops. Plant Biotechnol. J. 12:1193-1206.

Fierer, N. 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15:579-590.

Foster, Z. S. L., Sharpton, T. J., and Grünwald, N. J. 2017. Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. PLOS Comput. Biol. 13:1-15.

Frey-Klett, P., Grabaye, J., and Tarkka, M. 2007. The mycorrhizal helper bacteria revisited. New Phytol. 176:22-36.

Gehring, C. A., Mueller, R. C., and Whitham, T. G. 2006. Environmental and genetic effects on the formation of ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. Oecologia 149:158-164.

Gehring, C. A., Sthultz, C. M., Flores-Rentería, L., Whipple, A. V., and Whitham, T. G. 2017. Tree genetics defines fungal partner communities that may confer drought tolerance. Proc. Natl. Acad. Sci. 114: 11169-11174.

Gottel, N. R., Castro, H. F., Kerley, M., Yang, Z., Pelletier, D. A., Podar, M., Karpinets, T., Uberbacher, E., Tuskan, G. A., Vilgalys, R., Doktycz, M. J., and Schadt, C. W. 2011. Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. Appl. Environ. Microbiol. 77:5934-5944.

Haas, D., and Keel, C. 2003. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. Annu. Rev. Phytopathol. 41:117-153.

Haas, J. C., Street, N. R., Sjödin, A., Lee, N. M., Högberg, M. N., Näsholm, T., and Hurry, V. 2018. Microbial community response to growing season and plant nutrient optimisation in a boreal Norway spruce forest. Soil Biol. Biochem. 125:197-209.

Hacquard, S., and Schadt, C. W. 2015. Towards a holistic understanding of the beneficial interactions across the *Populus* microbiome. New Phytol. 205: 1424-1430.

Hoagland, D. C., and Arnon, D. I. 1950. The water culture method for growing plant without soil. California Agric. Exp. Circ. 337. The College of Agriculture University of California, Berkeley.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., and Kopriva, S. 2017. The role of soil microorganisms in plant mineral nutrition—Current knowledge and future directions. Front. Plant Sci. 8:1617.

Lareen, A., Burton, F., and Schäfer, P. 2016. Plant root-microbe communication in shaping root microbiomes. Plant Mol. Biol. 90:575-587.

Lau, J. A., and Lennon, J. T. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc. Natl. Acad. Sci. 109:14058-14062.

Lebeis, S. L., Herrera Paredes, S., Lundberg, D. S., Breakfield, N., Gehring, J., McDonald, M. Malfatti, S., Glavina del Rio, T., Jones, C. D., Tringe, S. G., and Dangl, J. L. 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science 349:860-864.

Liao, H. L., Bonito, G., Rojas, J. A., Hameed, K., Wu, S., Schadt, C. W., Labbe, J. L., Tuskan, G., Martin, F. M., Grigoriev, I. V., and Vilgalys, R. 2019. Fungal endophytes of *Populus trichocarpa* alter host phenotype, gene expression and rhizobiome composition. Mol. Plant-Microbe Interact. 32: 853-864.

Lladó, S., López-Mondéjar, R., and Baldrian, P. 2017. Forest soil bacteria: Diversity, involvement in ecosystem processes, and response to global change. Microbiol. Mol. Biol. Rev. (MMBR) 81:e00063-16.

Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., and Dangl, J. L. 2013. Practical innovations for high-throughput amplicon sequencing. Nat. Methods 10:999-1002.

Mader, M., Le Paslier, M. C., Bounon, R., Berard, A., Faivre Rampant, P., Fladung, M., Leple, J.-C., and Kersten, B. 2016. Whole-genome draft assembly of *Populus tremula* × *Populus alba* clone INRA 717-1B4. Silvae Genet. 65:74-79.

Martin, F., Boiffin, V., and Pfeffer, P. E. 1998. Carbohydrate and amino acid metabolism in the *Eucalyptus globulus-Pisolithus tinctorius* ectomycorrhiza during glucose utilization. Plant Physiol. 118:627-635.

Martínez-Medina, A., Appels, F. V. W., and van Wees, S. C. M. 2017. Impact of salicylic acid- and jasmonic acid-regulated defences on root colonization by *Trichoderma harzianum* T-78. Plant Signal. Behav. 12: e1345404.

Marupakula, S., Mahmood, S., and Finlay, R. D. 2016. Analysis of single root tip microbiomes suggests that distinctive bacterial communities are selected by *Pinus sylvestris* roots colonized by different ectomycorrhizal fungi. Environ. Microbiol. 18:1470-1483.

McMurdie, P. J., and Holmes, S. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8: e61217.

Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A., Singh, D. P., Prabha, R., Sahu, P. K., Gupta, V. K., Singh, H. B., Krishanani, K. K., and Minhas, P. S. 2017. Abiotic stress responses and microbe-mediated mitigation in plants: The omics strategies. Front. Plant Sci. 8:172.

Mello, A., and Balestrini, R. 2018. Recent insights on biological and ecological aspects of ectomycorrhizal fungi and their interactions. Front. Microbiol. 9: 216.

Mendes, R., Garbeva, P., and Raaijmakers, J. M. 2013. The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. 37:634-663.

Mercado-Blanco, J., Abrantes, I., Caracciolo, A. B., Bevivino, A., Ciancio, A., Grenni, P., Hrynkiewicz, K., Kredics, L., and Proenca, D. N. 2018. Belowground microbiota and the health of tree crops. Front. Microbiol. 9: 1006.

Naylor, D., and Coleman-Derr, D. 2018. Drought stress and root-associated bacterial communities. Front. Plant Sci. 8:2223.

Nehls, U. 2008. Mastering ectomycorrhizal symbiosis: The impact of carbohydrates. J. Exp. Bot. 59:1097-1108.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., and Kennedy, P. G. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20: 241-248.

Oksanen J. 2015. Multivariate Analysis of Ecological Communities in R: Vegan tutorial.

Pena, R., and Polle, A. 2014. Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. ISME J. 8: 321-330.

Pérez-Izquierdo, L., Zabal-Aguirre, M., Flores-Rentería, D., González-Martínez, S. C., Buée, M., and Rincón, A. 2017. Functional outcomes of fungal community shifts driven by tree genotype and spatial-temporal factors in Mediterranean pine forests. Environ. Microbiol. 19:1639-1652.

Pérez-Jaramillo, J. E., Mendes, R., and Raaijmakers, J. M. 2016. Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol. Biol. 90:635-644.

Pritchard, S. G. 2011. Soil organisms and global climate change. Plant Pathol. 60:82-99.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41:D590-D596.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., and Moënne-Loccoz, Y. 2009. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341-361.

Rohart, F., Gautier, B., Singh, A., and Lê Cao, K. A. 2017. mixOmics: An R package for 'omics feature selection and multiple data integration. PLOS Comput. Biol. 13:e1005752.

Shakya, M., Gottel, N., Castro, H., Yang, Z. K., Gunter, L., Labbé, J., Muchero, W., Bonito, G., Vilgalys, R., Tuskan, G., Podar, M., and Schadt, C. W. 2013. A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. PLoS One 8:e76382.

Smith, S. E., and Read, D. J. 2008. Mycorrhizal Symbiosis, 3rd ed. Academic Press, New York.

Solomon, P. S., Water, O. D., and Oliver, R. P. 2007. Decoding the mannitol enigma in filamentous fungi. Trends Microbiol. 15:257-262.

Timm, C. M., Carter, K. R., Carrell, A. A., Jun, S. R., Jawdy, S. S., Vélez, J. M., et al. 2018. Abiotic stresses shift belowground *Populus*-associated bacteria toward a core stress microbiome. mSystems 3:e00070-17.

Timm, C. M., Pelletier, D. A., Jawdy, S. S., Gunter, L. E., Henning, J. A., Engle, N., Aufrecht, J., Gee, E., Nookaew, I., Yang, Z., Lu, T. Y., Tschaplinski, T. J., Doktycz, M. J., Tuskan, G. A., and Weston, D. J. 2016. Two poplar-associated bacterial isolates induce additive favorable responses in a constructed plant-microbiome system. Front. Plant Sci. 7:497.

Tschaplinski, T. J., Plett, J. M., Engle, N. L., Deveau, A., Cushman, K. C., Martin, M. Z., Doktycz, M. J., Tuskan, G. A., Brun, A., Kohler, A. and Martin, F. 2014. *Populus trichocarpa* and *Populus deltoides* exhibit different metabolomic responses to colonization by the symbiotic fungus *Laccaria bicolor*. Mol. Plant-Microbe Interact. 27:546-556.

Tuskan, G. A., DiFazio, S., Grigoriev, I., Hellsten, U., Putnam, N., and Ralph, S. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313:1596-1604.

Uroz, S., Buée, M., Deveau, A., Mieszkin, S., and Martin, F. 2016. Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. Soil Biol. Biochem. 103:471-488.

Van Der Heijden, M. G. A., Bardgett, R. D., and Van Straalen, N. M. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296-310.

Veach, A. M., Morris, R., Yip, D. Z., Yang, D. K., Engle, N. L., Cregger, M. A., Tschaplinski, T. J., and Schadt, C. W. 2019. Rhizosphere microbiomes diverge among *Populus trichocarpa* plant-host genotypes and chemotypes, but, it depends on soil origin. Microbiome 7:76.

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73:5261-5267.

IV. Conclusions

Dans cette étude, nous souhaitions savoir si les variations naturelles du sol, principal réservoir de micro-organismes pour les racines, influence le microbiote racinaire ainsi que le métabolome du peuplier ou, si ces variations sont atténuées par des redondances fonctionnelles existantes entre les communautés microbiennes.

Nous avons observé que seulement 30 à 40 % des unités taxonomiques bacteriennes et fongiques étaient conservées dans le sol et les racines durant les deux années d'expériences. Ces changements de composition du microbiote du sol et des racines étaient accompagnés de changements au niveau de la distribution des guildes fongiques au sein des communautés et de changements dans le taux de métabolites produits par les racines incluant les glucides, les acides aminés et les métabolites secondaires.

Nos résultats suggèrent ainsi qu'une sécheresse hivernale peut avoir un impact significatif sur les micro-organismes du sol et, par conséquent, un impact sur le microbiote et le métabolome racinaire des arbres.

Chapitre V

Etude de l'expression hétérologue de l'effecteur fongique MiSSP7 sur la structuration et la composition du microbiote racinaire et sur le métabolome du Peuplier

I. Contexte général

Des mutations dans la voie de signalisation de l'AJ conduisent à des modifications de la composition du microbiote associé à la racine de la plante modèle herbacée, *Arabidopsis thaliana*, en modifiant la composition des exsudats racinaires (Carvailhais et al., 2015). L'activation de la voie de signalisation de l'AJ chez *A. thaliana* a également été liée à l'augmentation de l'abondance relative de certains membres de communautés bactériennes (Carvalhais et al., 2013). Ces études suggèrent que la voie de signalisation de l'AJ n'est pas seulement un élément clé du système de défense, mais qu'elle participe également à la formation du microbiote racinaire complet des plantes herbacées. Cependant, aucune étude explorant l'impact de la voie de signalisation de cette phytohormone sur le microbiote racinaire des arbres n'est, à l'heure actuelle, disponible dans la littérature scientifique. Il est connu que la modulation de la voie de signalisation de l'AJ serait nécessaire pour permettre l'établissement d'une symbiose ectomycorhizienne (EcM) entre *Laccaria bicolor* et les racines du peuplier (Plett et al. 2014). Le champignon ectomycorhizien sécrète une petite protéine de 7 kDa appelée MiSSP7 qui pénètre dans le noyau des cellules racinaires de l'hôte où elle interagit avec le répresseur PtJAZ6. Il a été démontré qu'en stabilisant le complexe PtJAZ6, MiSSP7 bloque le déclenchement de la voie de signalisation de l'AJ et, ainsi, limite les mécanismes de défense qui empêcheraient la colonisation des racines par *L. bicolor* (Martin et al. 2016). Néanmoins, nous ne savons pas si l'altération de la voie de signalisation de l'AJ module seulement l'établissement de la symbiose EcM ou si elle a un impact plus large sur le microbiote.

II. Objectifs

Dans ce contexte, les objectifs de ce chapitre sont, d'une part, de caractériser la composition et la structure taxonomique des communautés bactériennes et fongiques de la rhizosphère et de l'endosphère de peupliers exprimant de manière constitutive l'effecteur MiSSP7 en comparaison avec des peupliers sauvages (non génétiquement modifiés), et, d'autre part, d'évaluer si les potentielles modifications de la composition du microbiote racinaire sont également visibles au niveau du métabolome et du métatrancriptome racinaire des peupliers génétiquement modifiés.

Nous avons tenté de répondre à plusieurs questions :

- Est-ce que la répression de la voie de signalisation de l'AJ par MiSSP7 a un impact sur la physiologie du peuplier ?
- Est-ce que la répression de la voie de signalisation de l'AJ par MiSSP7 a un impact sur le métabolome racinaire du peuplier ?
- Est-ce que la répression de la voie de signalisation de l'AJ par MiSSP7 a un impact sur le métatranscriptome racinaire du peuplier ?

- Est-ce que les manipulations de la voie de signalisation de l'AJ par MiSSP7 influencent la composition et la structure de l'ensemble du microbiote racinaire du peuplier ou seulement la colonisation par *Laccaria bicolor* ?

III. Démarche expérimentale

Afin de répondre à ces questions, le sol de peupleraie précédemment décrit (Chapitre IV) a été utilisé comme substrat et inoculum naturel pour la culture de boutures de peupliers (*Populus tremula x alba*) exprimant de manière constitutive le gène fongique MiSSP7 (peupliers MiSSP7) et de boutures de peupliers non génétiquement modifiées (peupliers sauvages). Ces boutures ont été prélevées sur leur arbre mère en 2016 (Année 1) et en 2017 (Année 2), cultivées en hydroponie pour contrôler l'enracinement et ensuite, cultivées dans le sol naturel en serre durant 10 jours ou 6,5 semaines. La culture de ces boutures nous a permis de caractériser et comparer la structure et la composition taxonomique des communautés microbiennes (bactéries et champignons) associées aux racines ainsi que le métabolome racinaire des deux populations de peupliers (MiSSP7 et sauvages) cultivées l'année 1 et l'année 2.
Les communautés bactériennes et fongiques du sol et des racines ont été caractérisées par séquençage Illumina MiSeq haut débit des amplicons d'ADNr ITS et d'ARNr 16S tandis que le métabolome racinaire a été analysé par GC-MS. En plus de ces deux types d'analyses, nous avons comparé certains paramètres phénotypiques entre les deux populations de peupliers (MiSSP7 et sauvages) et réalisé une analyse des transcriptomes et du métatranscriptomes des racines de ces peupliers cultivés durant l'année 2.

L'ensemble des résultats de cette étude est présenté ci-après sous la forme d'un article scientifique. Les figures et tableaux supplémentaires sont disponibles en Annexe (Annexe 4 de la page 53 à la page 75).
Contrairement aux articles en préparation présentés dans les chapitres II et III, la publication de ce dernier chapitre n'est pas prévue à court terme.

Is Jasmonic acid signaling pathway a hub for controlling the *Populus tremula x alba* root microbiome colonization?

Introduction

The roots of terrestrial plants are an important habitat for microorganisms. Plants transfer up to 40 % of their photosynthetically-fixed carbon to their roots and 10 to 30 % of this carbon is allocates to the root microbiome (Dilkes et al., 2004; Kaiser et al., 2014). The root microbiome corresponds to the dynamic community of microorganisms associated with plant roots. It is mainly composed of bacteria and fungi recruited from the surrounding bulk soil, the area of soil outside the rhizosphere or vertically transmitted from generation to generation (i.e, the endophytes; Frank et al., 2017). Major interactions between micro-organisms and plant roots are known to be those between the microbial communities of the rhizosphere and the roots. The main players of the root microbiome are fungi and bacteria that can reach cell densities up to 10^8 cells, a number much greater than the number of plant cells (Mendes et al., 2013). These microorganisms play an important role for plant growth and health by increasing water and nutrient acquisition (Pii et al., 2015; Bowles et al., 2017) but also for plant resistance against biotic and abiotic stresses (Zelicourt et al., 2013). By contrast, some microbial members of the root microbiome are pathogenic and detrimental for plant growth and health (Raaijmakers et al., 2009). Additionally, thanks to its plasticity, its short-term dynamic and its large pool of genes, the tree root microbiome has a much higher potential capacity to adapt to environmental changes compared to the plants (Goh et al., 2013; Mendes et al., 2013) and thus offer potential interesting perspective of microbial-plant engineering.
Research on the interactions between tree roots and their microbiome has exponentially increased in the past 15 years. The development of next generation sequencing technologies and associated computational analysis tools allow more detailed investigation of factors which impact the composition and structure of root microbiome (Bulgarelli et al., 2012; Lundberg et al., 2012). Such analyses revealed the complexity of interactions between microorganisms and plant roots. This complexity, already perceptible in annual and crop plants, is exacerbated in trees and shrubs, where seasonal climatic variability, difference in life style compared to herbaceous plant in terms of nutrient allocation and root system development are known to differentially influenced microbial communities associated with roots.

Studies performed on tree species revealed that root microbiome of woody plant composition is influenced by both environmental factors such as soil properties and climate (Compant et al., 2010; Voriskova et al., 2014; Bonito et al., 2014; Mercado-Blanco et al., 2018) and host factors such as root exudates and root secondary metabolites (Tschaplinski et al., 2014; Hacquard & Schadt, 2015; Wagner et al., 2016; Gallart et al., 2018). In addition to these factors modulating and shaping root microbiome composition and functions, microbial communities may also have to deal with plant immunity and defence mechanisms. Indeed, plants have evolved a sophisticated immune system to detect and respond to potential microbial invaders (Jones & Dangl, 2006).

Cell-surface and intracellular localized pattern recognition receptors (PRR) can detect pathogens by recognizing microbe-associated molecular patters (MAMPs) which are molecules that are generally conserved through a wide range of microorganisms (Boller & Felix, 2009). Beneficial microorganisms possess MAMPs that are very similar to those of pathogens. During the initial contact with plant roots, beneficial microorganisms are also recognized by PRRs, activating plant immune system signalling pathways (Yu et al., 2019). Plant defence is partly mediated by phytohormones that trigger the activation of signalling pathways involved in defence mechanisms against pathogens. One of these defence phytohormone is jasmonic acid (JA). JA is involved in the control of cell development and cycling, of vegetative growth and in the mediation of plant defensive response against necrotrophic pathogens (McDowell & Dangl, 2000; Thomma et al., 2001). In addition, recent evidences suggest that these defence mechanisms could play important roles in helping plants to recruit microbiomes that enhance stress tolerance (Pineda et al., 2013). Furthermore, JA signalling influences the composition of the root-associated microbiome of the herbaceous plant model *Arabidopsis thaliana* by altering root exudate composition (Carvailhais et al., 2015). Activation of JA signalling pathway in *A. thaliana* was also linked to the increase of the relative abundance of bacterial communities closely related to taxa that are reported to supress phytopathogens colonisation (e.g., *Bacillus* population, *Paenibacillus amylolyticus* and *Lysinibacillus*-related population; Carvalhais et al., 2013). Altogether these studies suggest that JA signalling is not only a key element of the defence system but that it also participates to the shaping of the whole root microbiome of non-perennial plants.
Less is known regarding trees. However, the modulation of JA signalling would also be necessary to allow the establishment of ectomycorrhizal (EcM) symbiosis between *Laccaria bicolor* and poplar roots (Plett et al., 2014). The ectomycorrhizal fungus secretes a Mycorrhizal induced Small Secreted Protein of 7kDa (MiSSP7) that penetrates in the nucleus of *Populus* root cells where it interacts with PtJAZ6 and competes for the binding of jasmonate. By stabilizing PtJAZ6 complex, MiSSP7 is thought to block the triggering of JA signalling and thus to limit defence mechanisms that would preclude the colonization of *Populus* roots by *L. bicolor* (Martin et al., 2016). Whether the alteration of JA signalling modulates only the establishment of the EcM symbiosis or whether it has a broader impact on the microbiome is unknown.

In order to answer this question, we analysed the microbial communities of *Populus tremula x alba* lines constitutively expressing the fungal effector MiSSP7 and we compared it to the ones of wild type lines (WT). We hypothesized that, due to the alteration of the JA signalling pathway by MiSSP7 expression in *Populus*, the root microbiome composition and structure would vary compared to the control lines.
Rooted cuttings of WT and MiSPP7 lines were planted in pots containing natural soil taken from a poplar plantation and were grown in a greenhouse under controlled conditions. Bulk soil, rhizospheric and endospheric microbiomes were characterized using 16S and ITS rRNA gene-targeted amplicon Illumina MiSeq sequencing. Growth of each *Populus* line was monitored to observe the potential effect of MiSSP7 expression on tree development. Metabolomes of roots were also characterized by mass spectrometry (GC-MS) to determine the potential effects of MiSSP7 expression and the alteration of the microbiome on the physiology of the roots. Finally, a metatranscriptome analysis was performed on the roots of each *Populus* line to observe if MiSSP7

expression was responsible of potential differences in the root microbiome activity and in the activity of the root as well. Analyses of microbial community (i.e DNA metabarcoding) and root metabolome characterization was repeated over two consecutive years to control the reproducibility of the effects measured.

Material and Methods

Tree cutting origin and cultivation conditions

Different lines of *Populus tremula x alba* (clone INRA 717-1B4) were used in this study: wild type *P. tremula x alba* (WT) and four independent lines of *P. tremula x alba* constitutively expressing MiSSP7 (MiSSP7.1, MiSPP7.2, MiSSP7.3 and MiSSP7.6 lines, Plett et al., 2011). For each line, seven to 30 cuttings were taken from mother trees both years in February and were conserved at 4°C until being transferred in hydroponic solution to stimulate rooting and synchronize growth (Hoagland's nutrient solution; Hoagland & Arnon, 1950). Immersed parts of the cuttings were maintained in the dark and were aerated by air bubbling. Hydroponic cultivation was done in a class 2 GMO greenhouse at 20°C and with a 16h light period for a month. Rooted cuttings with similar root length were then transferred at the beginning of the summer of Year 1 and Year 2 (June 13th, 2016 and July 6th, 2017) to one litre pots containing natural soil taken from a poplar stand (see description below). Cuttings were further grown in the same glasshouse at 20°C and with a 16h light period for 6.5 weeks. Humidity in pots was maintained at approximately 75 % during all the experiments. At least 6 cuttings per line were planted independently each year (Table S1).

Seventy-two hours before the transplant of cuttings in pots in greenhouse, the top soil horizon used and characterized in our previous study (Mangeot-Peter et al., 2020) was collected. The soil was collected under the same tree in June 2016 and in July 2017 following the procedure described in Mangeot-Peter et al. (2020). Briefly, soil was stored at room temperature for 72 hours before being sieved at 2 mm and adjusted to 75 % of humidity. One litre of soil was transferred in each pot.

Monitoring of cutting growth

Phenotypic parameters of MiSSP7 and WT cuttings cultivated in Year 2 were monitored throughout the cultivation period, prior to sampling. Cutting diameters, length and fresh weight were measured before the establishment of the hydroponic culture. Number of roots and length of the main root were measured every week from the beginning of rhizogenesis to the end of the hydroponic culture. The number of leaves, the leaf surface and the stem length were monitored once a week from the transfer of cuttings in pots to the sampling time.

Sampling procedures

Samples were taken at three time points: at the transplanting time (T0), after 10 days (T1) and after 6,5 weeks of growth (T2) of growth in pots. At T0, samples of initial soils were collected in triplicates of 50 g, dried at 30 °C and stored at room temperature in an airtight container until physico-chemical analysis (see below). Samples of roots

collected after the hydroponic culture (T0) were freezed in liquid nitrogen and conserved at -20°C until DNA extraction.

At T1 and T2, five types of samples were collected on each *Populus* cuttings (i.e., WT and MiSSP7) in each pot. These samples correspond to the leaves, the bulk soil (BS, the soil in an area free of cuttings root in pot), the adherent soil of the roots (AS, the rhizosphere), the rhizoplane (Rh, the external surface of the roots) and the endosphere (E, the internal tissue of the roots).
BS, AS, Rh and E samples were collected according to the collection procedure detailed in Gottel et al., 2011 for microbiome analyses. Briefly, bulk soil samples were collected from each pot in an area free of roots and stored at -20°C until DNA extraction while five representative parts of the total root system were collected: (i) One part was cleaned with 10 mM NaCl solution, freezed in liquid nitrogen and stored at -80°C until being lyophilized then processed for metabolomic analysis. (ii) The second representative part of each root system was shaken to remove non-adherent soil, and washed in a centrifuge tube containing 35 mL of 10 mM NaCl solution to remove the root adherent soil (i.e, the rhizosphere). Then, tubes were centrifuged at 6,500 g for 15 min and the pellet was aliquoted in 2 mL tubes and stored at -20°C until DNA extraction. (iii) The surface of the third part of roots (i.e., the endosphere) were sterilized by using the sterilization steps described by Gottel et al., 2011: washed with sterile H_2O 3 times, 3% H_2O_2 for 30 s, 100% ethanol for 30 s, 6.15% NaOCl with 2 to 3 drops of Tween 20 per 100 mL for 3 min, 3% H_2O_2 for 30 s and finally 3 washed with sterile H_2O. Surface sterility was confirmed for all samples by touching a subsampled root from each collection onto LB plates and incubating overnight at 30°C. (iv) Another batch of roots were taken for qPCR assays and metatranscriptomic analyses. The roots were cleaned with sterile 10 mM NaCl solution and immediately dried freeze in liquid nitrogen and conserved at -80°C until RNA extractions. Finally, (v) the last sample of roots system collected in Year 2 at T2 was used to quantify the ectomycorhization rate according to the procedure detailed in Labbé et al., 2011. Briefly, each root system was rinsed with tap water and analysed under a dissecting microscope. For each root system, 100 short roots were randomly examined and assessed as mycorrhizal or non-mycorrhizal. In addition, ectomycorrhizae was harvested and conserved at -20°C until DNA extraction for identification.

All the leaves of each cuttings were sampled in each *Populus* cuttings, freezed in liquid nitrogen and stored at -80°C until RNA extraction. Detailed information about the number of each sample types collected in Year 1 and Year 2, at T1 and T2, and used for each experimental approach is available in Table 5. In total, thirteen tree cuttings (3 WT and 10 MiSSP7) were sampled at T1, 18 (4 WT and 14 MiSSP7) at T2 in Year 1, 14 (6 WT and 8 MiSSP7) at T1 in Year 2 and 22 (6 WT and 16 MiSSP7) at T2 in Year 2 (Table 5).

Table 5 - Number of samples used for each experimental approach performed in this study.

***Populus tremula x alba* cuttings used in Year 1 at T1**	**qpCR (Leaves samples)**	**16S amplification**	**ITS amplification**	**Root metabolome study (Roots samples)**	**Metatranscriptome study (Roots samples)**
WT	**0**	BS: 3 ; AS: 3 ; Rh: 3 ; E: 3	BS: 3 ; AS: 3 ; Rh: 3 ; E: 3	**3**	
MiSSP7 lines	0	BS: 10 ; AS: 10 ; Rh: 10 ; E: 8	BS: 10 ; AS: 10 ; Rh: 10 ; E: 9	6	
***Populus tremula x alba* cuttings used in Year 1 at T2**	**qpCR (Leaves samples)**	**16S amplification**	**ITS amplification**	**Root metabolome study (Roots samples)**	**Metatranscriptome study (Roots samples)**
WT	**4**	BS: 4 ; AS: 4 ; Rh: 4 ; E: 4	BS: 4 ; AS: 4 ; Rh: 4 ; E: 4	**4**	
MiSSP7_High exp	4	BS: 4 ; AS: 3 ; Rh: 4 ; E: 3	BS: 4 ; AS: 3 ; Rh: 4 ; E: 3	4	
MiSSP7_Low exp	8	BS: 8 ; AS: 7 ; Rh: 8 ; E: 8	BS: 8 ; AS: 8 ; Rh: 8 ; E: 8	7	
***Populus tremula x alba* cuttings used in Year 2 at T1**	**qpCR (Roots samples)**	**16S amplification**	**ITS amplification**	**Root metabolome study (Roots samples)**	**Metatranscriptome study (Roots samples)**
WT	**6**	BS: 6 ; AS: 6 ; Rh: 6 ; E: 6	BS: 6 ; AS: 6 ; Rh: 6 ; E: 6	**6**	
MiSSP7_High exp	3	BS: 3 ; AS: 3 ; Rh: 3 ; E: 3	BS: 3 ; AS: 3 ; Rh: 3 ; E: 3	3	
MiSSP7_Low exp	5	BS: 4 ; AS: 5 ; Rh: 3 ; E: 5	BS: 5 ; AS: 5 ; Rh: 5 ; E: 4	5	
***Populus tremula x alba* cuttings used in Year 2 at T2**	**qpCR (Roots samples)**	**16S amplification**	**ITS amplification**	**Root metabolome study (Roots samples)**	**Metatranscriptome study (Roots samples)**
WT	**6**	BS: 6 ; AS: 6 ; Rh: 6 ; E: 6	BS: 6 ; AS: 6 ; Rh: 6 ; E: 6	**6**	**6**
MiSSP7_High exp	9	BS: 8 ; AS: 9 ; Rh: 8 ; E: 6	BS: 8 ; AS: 8 ; Rh: 8 ; E: 8	7	9
MiSSP7_Low exp	5	BS: 4 ; AS: 4 ; Rh: 4 ; E: 3	BS: 5 ; AS: 5 ; Rh:5 ; E: 4	4	5

DNA extraction, amplification and Illumina MiSeq sequencing

To minimize DNA extraction bias, DNA was extracted in triplicate from the bulk soil. Approximately 250 mg of soil samples was used for each individual DNA extraction. DNA was extracted using the DNeasy PowerSoil Kit following the protocol provided by the manufacturer (Qiagen, Venlo, the Netherlands). Fifty mg of root tissues and isolated ectomycorrhizae were crushed in liquid nitrogen and pulverized tissue was extracted using the DNAeasy PowerPlant Kit (Qiagen, Venlo, the Netherlands). All extractions were quantified on a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA).

A two-step PCR approach was chosen in this study to barcode tag templates with frameshifting nucleotide primers (Mangeot-Peter et al., 2020). Forward and reverse primer mixtures were used to maximize phylogenetic coverage of bacteria and fungi. Primer mixtures for tagging bacterial amplicons were composed of 4 forward and 2 reverse 515F and 806R primers screening the 16S rRNA V4 gene region in equal concentration (0.1 μM). Primer mixtures for tagging fungal amplicons were composed of 6 forward and 1 reverse for ITS1 – ITS4 rRNA region at equal concentration (0.1 μM). To inhibit plant material amplification, a mixture of peptide nucleotide acid (PNA) blockers targeting plant mitochondrial and chloroplast 16S rRNA genes and plant 5.8S nuclear rRNA gene were added in PCR reaction mixes (Mangeot-Peter et al., 2020). Thermal cycler conditions for the primary PCRs for bacterial amplification in soil and root samples were 30 cycles of 98°C for 5s, 78°C for 10s, 52°C for 20s and 72°C for 15s. Primary PCR condition for fungal amplification in soil and root samples were 30 cycles of 98°C for 5s, 78°C for 10s, 55°C for 20s and 72°C for 15s. PCR products without addition of microbial DNA (negative control), mock communities of known fungal or bacterial compositions were added as quality controls. Samples of 50 µl (30 ng DNA per µl) were sent for tagging and MiSeq Illumina Next Generation Sequencing (GeT PlaGe INRAE sequencing platform, Toulouse, France).

Bacterial sequences were further processed with FROGS (Find Rapidly OTU with Galaxy Solution) (Escudié et al., 2018) based on the Galaxy analysis platform (Afgan et al., 2016). Sequences were demultiplexed, dereplicated, sequence quality was checked, oligonucleotides, linker, pads and barcodes were removed from sequences and sequences were filtered on the additional following criteria. Sequences were removed from data set, if non-barcoded, if sequences exhibited ambiguous bases or did not match expectations in amplicon size. Remaining sequences were clustered into operational taxonomic units (OTUs) based on the iterative Swarm algorithm, then chimeras and singletons (OTUs containing only one sequence) were removed. Bacterial double affiliation was performed by blasting OTUs against SILVA database (Quast et al., 2012) and the ribosomal database project (RDP) classifier (Wang et al., 2007). OTUs with affiliation <100% at the phylum level (indicated by a RDP bootstrap value <1) and corresponding to chloroplasts or mitochondria were removed from the data set. OTUs at lower taxonomic ranks than the phylum level was considered as "unidentified" below when the RDP bootstrap value was < 0.70. OTUs with high abundances in negative controls were excluded from further analysis, sequencing, and affiliation quality was evaluated based on the results obtained for the bacterial mock community.

For fungal reads, after demultiplexing and quality checking (QC quality score = 28, minimal size = 180 bp), bioinformatics analyses were performed using standard procedures as described in Pérez-Izquierdo et al. (2017). For both fungal and bacterial data, per-sample rarefaction curves were calculated to assess sampling completeness, using function *rarecurve()* in package Vegan v3.5-1 (Oksanen et al., 2015) in R (version 3.4.3 ; R Core Team, 2016). Based on these, subsequent analyses of diversity and community structure were performed on datasets where samples had been rarefied with the Phyloseq (McMurdie & Holmes, 2013) package to achieve equal read numbers according to the minimum number of total reads in any sample (8,982 bacteria, 8,005 fungi). Microbial community composition and structure in bulk soil and roots data were further analysed by using Phyloseq package (McMurdie & Holmes, 2013).

FUNGuild (Nguyen et al., 2016) was used to classify each OTU into an ecological guild. OTUs identified to a guild with a confidence ranking to "highly probable" or "probable" were conserved in our analysis, whereas those ranking to "probable" or with multiple assignations were called as "unclassified".

Molecular identification of EcM root tips

Ectomycorrhiza DNAs were amplified using the primer pair ITS1F and ITS4. PCR products (size and concentration) were controlled by gel electrophoresis, and successful amplifications were purified using the GeneMatrix PCR/DNA Clean-Up purification kit (EURx, Gdansk, Poland) as per the manufacturer's instructions. Purified products were then sequenced by the Sanger sequencing forward approach (Eurofins, Germany). Sequences were then blasted against UNIT database.

RNA extraction and analysis of the MiSSP7 expression in transformed lines

Approximately 80 mg of tissues (leaves collected in Year 1 and roots collected in Year 2) were used for total RNA extraction using the Plant RNAeasy kit (Qiagen) as per the manufacturer's instructions except the addition of 20 mg poplyethylene glycol 8000 per mL of RLC buffer. A on-column DNA digestion step was peformed with the DNase Removal kit (ThermoFisher Scientific, Carlsbad, CA, USA) to avoid DNA contamination. RNA were quantified by using Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and RNA quality was verified by Experion HighSens capillarity gels (Bio-Rad). Synthesis of cDNA from 400 ng of total RNA extracted from leaves and/or roots of each *Populus* cuttings was performed using the iScript kit (Bio-rad). Expression levels of *missp7* transcripts in transgenic poplars was compaired to the one of *missp7* in 2 weeks old ectomycorrhizae of *Laccaria bicolor* in *Populus tremula x alba* roots cultivated in axenic conditions. A Chromo4 Light Cycler Real-time PCR was used for real-time PCR analyses with two technical replicates per biological replicate using the SYBRGreen Supermix following the manufacturer's instructions (Bio-Rad; 5.5 μL of primers 10 μM [MiSSP7_Fwd: ATTGCTGCGTTAGCCATCTC and MiSSP7_Rev: GGAATTGGTCCTCTCTCAACC], 2 μL of cDNA at 400 ng, 7.5 μL of SYBRGreen Supermix). Thermal cycler conditions for qPCR were 40 cycles of 95°C for 15s and 60°C for 10s. Normalization in gene expression between the target gene MiSSP7 and the reference gene UBIQUITIN (Plett et al., 2011) were calculated. Relative expression of MiSSP7 in each cuttings and in the

control (i.e., 2 weeks old ectomycorrhizae of *Laccaria bicolor* in *Populus tremula x alba* roots) was normalized with the gene reference UBIQUITIN.

qPCR products were purified using the GeneMatrix PCR/DNA Clean-Up purification kit (EURx, Gdansk, Poland) as per the manufacturer's instructions. Purified products were then sequenced by the Sanger sequencing approach to check if all amplification products correspond to the sequence of MiSSP7.

All samples were then classified according to their degree of MiSSP7 expression : Not expressed, MiSSP7_Low expression and MiSSP7_Low expression.

Metabolome analysis

Root metabolome extraction and analyses were carried out as previously described by Tschaplinski et al. (2014). Briefly, between 20 and 100 mg fresh weight of roots and leaves samples were ground to a fine powder in liquid nitrogen, weighed and then transferred to an acid washed glass scintillation vial containing 2.5 mL 80% ethanol. Sorbitol was used before extraction as an internal standard to correct potential extraction efficiency difference and to normalized final concentration of metabolites in each sample. After overnight extraction and the solvent transferred into another vial, each sample pellets were re-extracted with another 2.5 ml of 80 % ethanol overnight and the supernatant combined with the prior extract. Using a nitrogen stream, 2 mL of this solution were dried and stored at -80°C until analysis. The samples were dissolved in 750 μL of aqueous 80% ethanol, from which 500 μL were dried in a nitrogen stream. Metabolites were trimethylsilyl derivatized and analysed using gas chromatography-mass spectrometry (GC-MS), as previously described (Tschaplinski et al., 2014). The peaks of known metabolites were extracted using a key mass-to-charge (m/z) ratio and scaled back up to the total ion current using predetermined scaling factors. The scaling factor for sorbitol was used for unidentified metabolites. Unidentified metabolites were denoted by their retention time as well as key m/z fragments. Peaks were quantified by area integration and were normalized to the quantity of the internal standard (sorbitol) recovered, accounting for the volume of sample processed, derivatization volume, and injection volume.

Meta-Trasncriptome analysis

Illumina sequencing was performed at the Joint Genome Institute (JGI; https://jgi.doe.gov). Stranded RNASeq libraries were created and quantified by qPCR using their standard protocol for low input RNA samples. Sequencing was performed using an Illumina NovaSeq. A read preprocessing was performed as following :raw fastq file reads were filtered and trimmed using the JGI QC pipeline (http://1ofdmq2n8tc36m6i46scovo2e.wpengine.netdna-cdn.com/wp-content/uploads/2013/11/Poster_Singan.pdf), raw reads were evaluated for artifact sequence by kmer matching (kmer=25), allowing 1 mismatch and detected artifact was trimmed from the 3' end of the reads. RNA spike-in reads, PhiX reads and reads containing any Ns were removed. Quality trimming was performed using the phred trimming method. Finally, following trimming, reads under the length threshold were removed (minimum length 25 bases). Filtered reads from libraries were aligned to the reference genome (*Populus trichocarpa* v3.0 assembly with v3.1 gene annotation) using HISAT2 version 2.1.0 (Kim et al., 2015). FeatureCounts (Liao et al., 2014) was used to generate the raw gene counts file

using gff3 annotations. Only primary hits assigned to the reverse strand were included in the raw gene counts. FPKM and TPM normalized gene counts are also provided. Raw gene counts were used to evaluate the level of correlation between biological replicates using Pearson's correlation and determine which replicates would be used in the DGE analysis.

DESeq2 (version 1.18.1, Love et al., 2014) was subsequently used to determine which genes were differentially expressed between pairs of conditions. For the strandedness estimation, features assigned to the forward strand were also tabulated. Strandedness of each library was estimated by calculating the percentage of reverse-assigned fragments to the total assigned fragments (reverse plus forward hits).

Read pairs from all samples were used to perform a *de novo* co-assembly using Megahit version 1.1.3 with default parameters (Li et al., 2015). Contigs under 500 pb length were discarded. For each sample, reads were then mapped on the selected contigs using bowtie version 2.3.0 (Langmead et al., 2012), and counts were determined using SAMtools version 1.7 (Li et al., 2009). Contigs supported by less than 3 samples with at least 5 counts were then discarded. Remaining contigs were annotated using Diamond version 0.9.19 (Buchfink et al., 2015) with the parameters --more sensitive --max-target-seqs 1 --max-hsps 1 --evalue 0.00001 and JGI-Mycocosm fungal genomes (deposited before July 2018) as reference database. To check for fungal false positives, Diamond annotation was also used with NCBI-NR database (March 2018 version). Comparison between Mycocosm and NR was based on best bit-scores. Count tables and annotations were then analyzed using R version 3.4.3 and packages dplyR and ggplot2.

Statistical analysis

Statistical analyses and data representations were performed using R software (R Core Team, 2016). After checking normal distribution of each dataset with Shapiro-Wilk test, one-way ANOVA followed by a Tukey HSD multiple comparison test were used to determine if the relative abundance of dominant (>1%) bacterial and fungal phyla and genera differed in soil samples (BS and AS) and in roots samples (Rh and E) collected in Year 1 and Year 2 from *Populus* WT, MiSSP7_High expression and MiSSP7_Low expression cuttings. Microbial community structures were analysed using nonmetric multidimensional scaling analysis (NMDS) and permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrices. Wilcoxon test were used to determine difference in transcripts frequency of the most active fungal genera detected between *Populus*–expressing MiSSP7 (MiSSP7_Low expression or MiSSP7_High expression) and *Populus* WT roots collected in Year 2 at T2. The p-values of root metabolite differences were calculated using a One-way ANOVA and post-hoc HSD tests between the means of sets of MiSSP7 root samples (MiSSP7_Low expression or MiSSP7_High expression) and the means of sets of WT root samples collected in Year 1 and in Year 2 for each sampling time (T1 and T2), with the data expressed as fold changes relative to a WT samples.

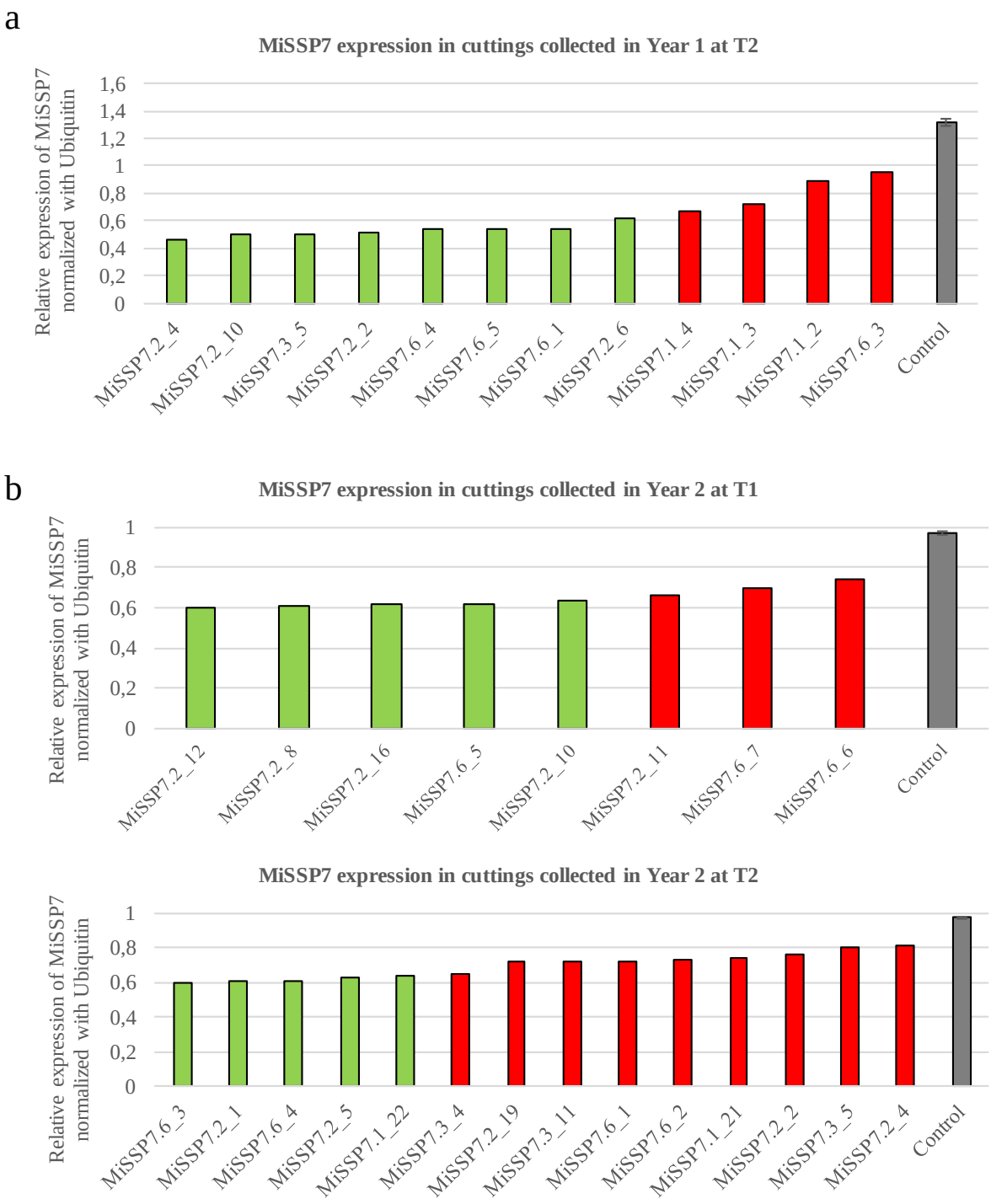


Figure 38 - The relative expression of MiSSP7 in each cutting used in this study. Relative expression of MiSSP7 normalized with Ubiquitin expression in the leaves of Populus-expressing MiSSP7 collected at T2 in Year 1 (a) and in the roots of Populus-expressing MiSSP7 collected at T1 and T2 in Year 2 (b). The green bars correspond to the cuttings with a low expression of MiSSP7 (between 0.40 and 0.60 in Year 1 and between 0.60 and 0.64 in Year 2) while the red bars correspond to the cuttings with a high expression of MiSSP7 (between 0.70 and 0.90 in Year 1 and between 0.65 and 0.80 in Year 2). The dark-grey bars correspond to the relative expression of MiSSP7 normalized with Ubiquitin in 2 weeks old ectomycorrhizae of Laccaria bicolor in Populus tremula x alba roots cultivated in axenic conditions (Control).

Results

The MiSSP7 expression in leaves and roots of *Populus* cuttings

qPCR analysis performed on the four MiSSP7 lines cultivated in natural soil revealed that several cuttings did not express the gene of interest anymore. In Year 1, only one cuttings of the MiSSP7.3 line collected at T2 did not express the gene of interest anymore. By contrast, 23% of the cuttings had lost the ectopic expression of MISSP7 on Year 2. Some lines were poorly impacted (e.g. MISSP7.1, MISSP7.6), while few cuttings kept expressing the gene in other lines (e.g. MISSP7.3, Table S1).

We observed that the expression of MiSSP7 in transgenic lines was lower compared to the one in the control (i.e, cDNA of 2 weeks-old *Laccaria*/*Populus* ectomycorrhizae) in Year 1 and in Year 2. (Figure 38). In addition, we observed an important variability in the expression level of MiSSP7 in each MiSSP7 cutting collected in Year 1 and Year 2, no matter the line considered (Figure 38). In Year 1, eight cuttings collected at T2 showed a low expression of MiSSP7 (between 0.40 and 0.60 after the normalization with the reference gene) and four cuttings showed a high expression of MiSSP7 (between 0.70 and 0.90 after the normalization with the reference gene). No qPCR analysis was performed on MiSSP7 cuttings collected at T1 in Year 1 because we could not successfully extract RNA from leaves and roots with these cuttings.

In Year 2, five cuttings collected at T1 showed a low expression of MiSSP7 (between 0.60 and 0.64 after the normalization with the reference gene) and three cuttings showed a high expression of MiSSP7 (between 0.66 and 0.75 after the normalization with the reference gene). At T2, five cuttings showed a low expression of MiSSP7 (between 0.60 and 0.64 after the normalization with the reference gene) and nine cuttings showed a high expression of MiSSP7 (between 0.65 and 0.80 after the normalization with the reference gene).

The impact of MiSSP7 ectopic expression on *Populus* growth and development

Phenotypic parameters were monitored before, during and after the hydroponic culture of MiSSP7 lines and WT to observe potential alterations of cuttings development and growth due to the constitutive expression of MiSSP7 in *Populus* (i.e. aerial parts and roots) in Year 2.

Significant differences were observed in phenotypic parameters monitored before the hydroponic culture of MiSSP7 and WT cuttings collected at T1. Indeed, the WT cuttings were longer and heavier than the MiSSP7 cuttings (ANOVA, $P<0.05$; WT > MiSSP7_Low > MiSSP7_High; Figure 39 a). In addition, we observed significant differences in the length of the main root between WT and MiSSP7 cuttings during the hydroponic culture for cutting collected at T1 (ANOVA, $P<0.05$; MiSSP7_Low > WT > MiSSP7_High; Figure 39 b). At T2, we also observed that WT cuttings were longer and heavier than the MiSSP7 cuttings (ANOVA, $P<0.05$; WT > MiSSP7_High > MiSSP7_Low; Figure 39 a). No significant differences were observed between WT and MiSSP7 cuttings collected at T1 and T2 for the phenotypic parameters monitored after the transfer in pots (i.e., number of leaves, leaf area, Figure 39 c) except for the length of the collected at T2 (ANOVA, $P<0.05$; WT < MiSSP_Low < MiSSP7_High, Figure 39 c).

*Table 6 - Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation in this study (compartments [BS, AS, Rh and E], sampling times [T1 and T2], treatment [MiSSP7 and WT cuttings] or expression level [WT, MiSSP7_Low exp and MiSSP7_High exp]) for fungal and bacterial communities at the OTU level. BS, AS, Rh and E samples of the WT and MiSSP7 cuttings collected in Year 1 were included in this analysis. Statistical significance (p-value) computed was based on sequential sums of square from 999 permutations (*** = P-value < 0.01, ** = P-value < 0.03, * = P-value < 0.05).*

Fungi_Year1			**PermANOVA**		
Compartment	**Time**	**Source of variation**	**F**	**R2**	**p-value**
BS, AS, Rh, E	T1 and T2	Compartment	12.45	0.220	***
		Time	14.16	0.080	***
		Treatment	3.19	0.018	***
BS	T1, T2 and T0	Time	9.37	0.215	***
		Treatment	2.04	0.094	*
BS	T1 and T2	Time	9.24	0.242	***
		Treatment	0.82	0.021	0.515
AS	T1 and T2	Time	11.38	0.280	***
		Treatment	1.26	0.030	0.227
Rh	T1 and T2	Time	5.04	0.145	***
		Treatment	2.34	0.067	*
E	T1 and T2	Time	2.44	0.084	**
		Treatment	1.69	0.058	0.060
BS	T1	Treatment	0.97	0.080	0.513
BS	T2	Expression	0.86	0.104	0.581
BS	T2	Treatment	0.78	0.048	0.520
AS	T1	Treatment	0.85	0.072	0.642
AS	T2	Expression	1.39	0.162	0.140
AS	T2	Treatment	1.36	0.079	0.197
Rh	T1	Treatment	1.96	0.151	*
Rh	T2	Expression	0.55	0.065	0.943
Rh	T2	Treatment	1.88	0.111	0.075
E	T1	Treatment	1.01	0.100	0.482
E	T2	Expression	1.01	0.121	0.432
E	T2	Treatment	1.59	0.096	0.095
Bacteria_Year1			**PermANOVA**		
Compartment	**Time**	**Source of variation**	**F**	**R2**	**p-value**
BS, AS, Rh, E	T1 and T2	Compartment	35.58	0.055	***
		Time	3.35	0.014	*
		Treatment	4.17	0.471	***
BS	T1, T2 and T0	Time	3.60	0.102	***
		Treatment	1.72	0.098	**
BS	T1 and T2	Time	3.59	0.118	***
		Treatment	0.84	0.027	0.684
AS	T1 and T2	Time	5.34	0.169	***
		Treatment	1.19	0.037	0.207
Rh	T1 and T2	Time	8.92	0.238	***
		Treatment	2.43	0.065	*
E	T1 and T2	Time	3.94	0.137	***
		Treatment	1.67	0.058	*
BS	T1	Treatment	0.72	0.061	0.836
BS	T2	Expression	1.23	0.081	0.135
BS	T2	Treatment	1.04	0.068	0.362
AS	T1	Treatment	0.85	0.072	0.662
AS	T2	Expression	1.21	0.082	0.170
AS	T2	Treatment	1.47	0.100	0.053
Rh	T1	Treatment	1.85	0.143	0.051
Rh	T2	Expression	2.45	0.146	*
Rh	T2	Treatment	1.27	0.076	0.197
E	T1	Treatment	1.87	0.172	*
E	T2	Expression	0.87	0.060	0.615
E	T2	Treatment	1.60	0.110	*

Concerning the ectomycorrhization rate calculated from MiSSP7 cuttings collected in Year 2 at T2, no significant differences were observed between *Populus* lines (WT vs. MiSSP7_Low vs. MiSSP7_High). However, the ectomycorrhization rate tended to be higher in MiSSP7_Low cuttings compared to WT and MiSSP7_High cuttings (ANOVA, P=0.07; Figure 40). Collected ectomycorrhizae mostly belonged to *Thelephora* sp. (Figure 40).

Microbial sequencing results

In order to characterize the structure and composition of soil- and root-associated microbiomes of *Populus* WT and *Populus*-expressing MiSSP7 lines, MiSeq sequencing of rDNA 16S and ITS rDNA were performed on DNA extracted from bulk soil, rhizosphere, rhizoplan and endosphere in two independent experiments performed over two years. After quality filtering, chimera and singleton removals, a total of 399,560 fungal reads (6,445 ± 16 reads per sample) and 1,777,000 (29,000 ± 814 reads per sample) for Year 1, and 363,130 fungal reads (5,044 ± 275 reads per sample) and 1,135,000 (15,500 ± 494 reads per sample) for Year 2 were kept for further analyses. After taxonomic assignment, elimination of contaminants and rarefactions of the sequences, 908 fungal OTUs (263 ± 13 OTUs per sample) and 1,938 bacterial OTUs (1087 ± 30 OTUs per sample) in Year 1, and 985 fungal OTUs (149 ± 8 OTUs per sample) and 2,030 bacterial OTUs (1,088 ± 24 OTUs per sample) in Year 2 were retained for further analyses.

Structure and composition of microbial communities of each compartment in *Populus* WT and *Populus*-expressing MiSSP7 line cultivated in Year 1

In Year 1, we observed a significant effect of the time, the treatment (WT vs MiSSP7) and the expression (WT, MiSSP7_Low exp and MiSSP7_High exp) on the structure of both fungal and bacterial communities associated to the soil (BS and AS) and roots (Rh and E) samples of the cuttings collected at T1 and T2 (Table 6). At T1, the structure of fungal communities associated to the rhizoplane of the cuttings was significantly impacted by the treatment at the OTU level. Concerning bacterial communities, the structure was significantly impacted by the treatment in the endosphere and tended also to be impacted by the treatment in the rhizoplane (P=0.051, PermANOVA, Table 6). At T2, the structure of fungal communities associated to the rhizoplane tended to be impacted by the treatment (P=0.075, PermANOVA, Table 6) while the structure of bacterial communities associated to the rhizoplane and the endosphere was significantly impacted by expression level of MiSSP7 and by the treatment (P<0.05, PermANOVA, Table 6).

Detailed analyses of the composition of fungal communities showed significant differences in the relative abundance of the most dominant fungal and bacterial genera colonizing the rhizosphere and the roots of WT and MiSSP7 cuttings at T1. For instance, the endophytes *Mortierella* and *Phialocephala* were significantly enriched in AS, Rh and E samples of the WT cuttings while *Cryptococcus* was significantly enriched in the Rh samples of the MiSSP7 cuttings (P<0.05, ANOVA, Figure S1, Table S2).

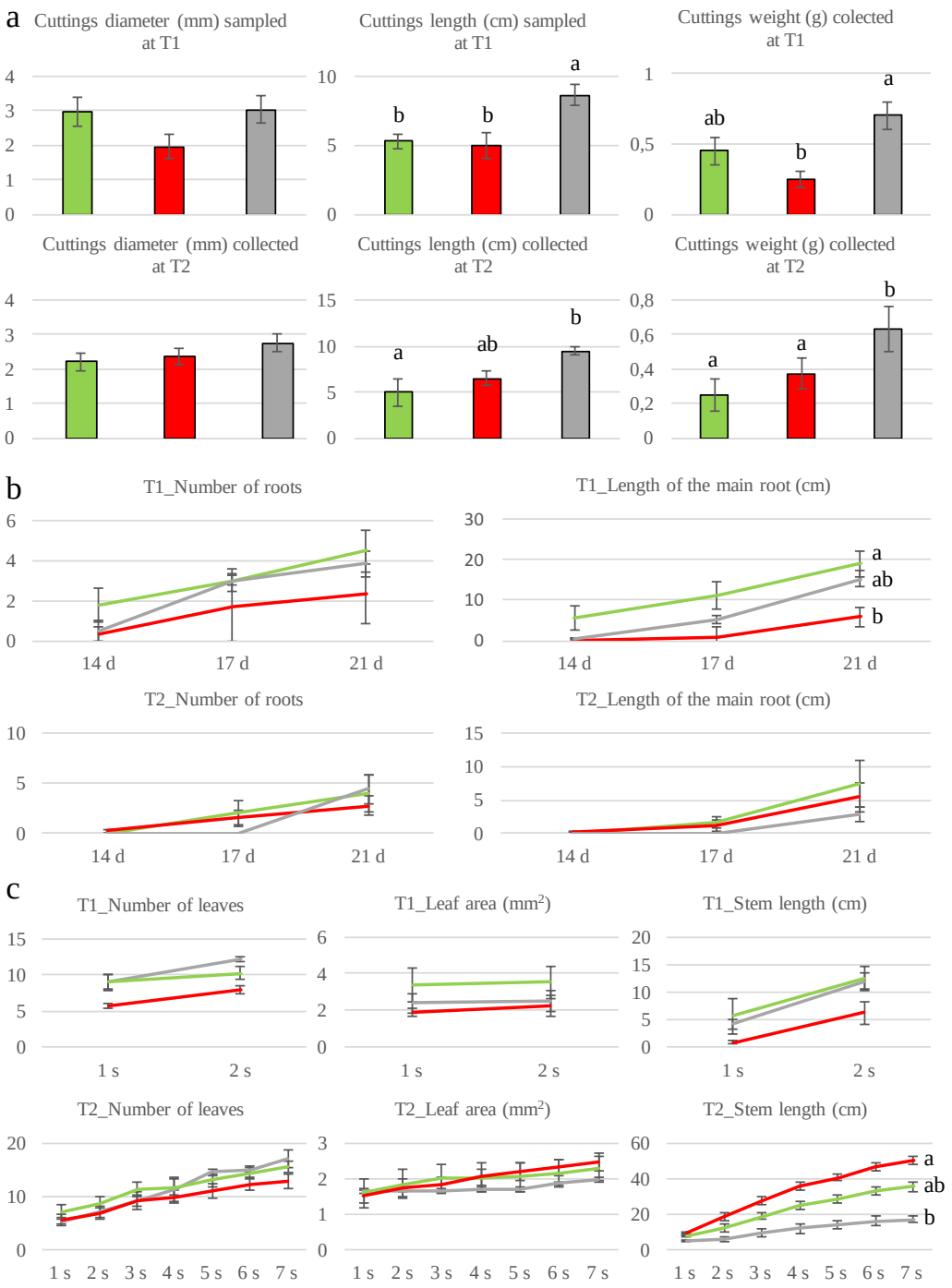


Figure 39 - The phenotypic parameters of Populus-expressing MiSSP7 and Populus WT monitored before, during and after the hydroponic culture in Year 2. Diameter, length and weight of Populus-expressing MiSSP7 and Populus WT cuttings collected at T1 and T2 measured before the culture in hydroponic system (a). Number of roots and length of the main root of Populus-expressing MiSSP7 and Populus WT collected at T1 and T2 monitored once a week during the culture in hydroponic system (b). Number of leaves, leaf area and stem length of Populus-expressing MiSSP7 and Populus WT collected at T1 and T2 monitored once a week during the culture of cuttings in pots (c). The green bars and lines correspond to the cuttings of Populus-expressing MiSSP7 with a low expression of MiSSP7 (average of 10 replicates ± SE) while the red bars and lines correspond to the cuttings of Populus-expressing MiSSP7 with a high expression of MiSSP7 (average of 12 replicates ± SE). The grey bars and lines correspond to the cuttings of Populus WT (average of 12 replicates ± SE). Letters denote significant differences in the phenotypic parameters between Populus-expressing MiSSP7 and Populus WT (P<0.05, ANOVA).

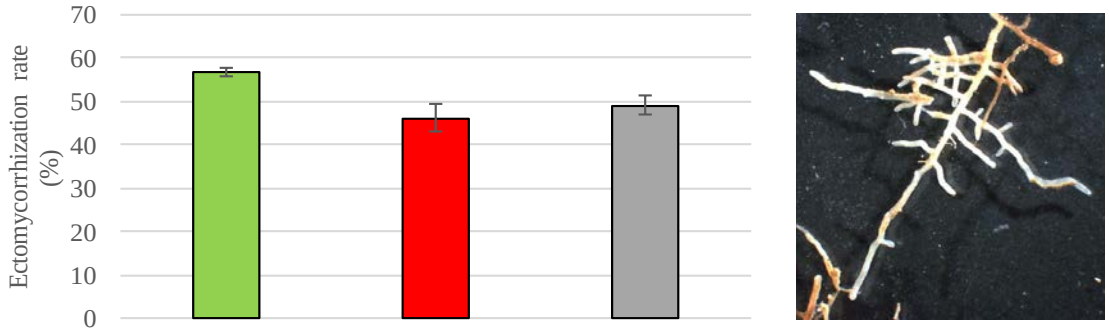


Figure 40 - Ectomycorrhization rate measured from the roots of Populus-expressing MiSSP7 and Populus WT collected at T2 in Year 2. The green bar corresponds to Populus-expressing MiSSP7 roots with a low expression of MiSSP7 (average of 5 replicates ± SE). The red bar corresponds to Populus-expressing MiSSP7 roots with a high expression of MiSSP7 (average of 9 replicates ± SE). The grey bar corresponds to Populus WT roots (average of 6 replicates ± SE). The picture corresponds to Thelephora ectomycorrhizae.

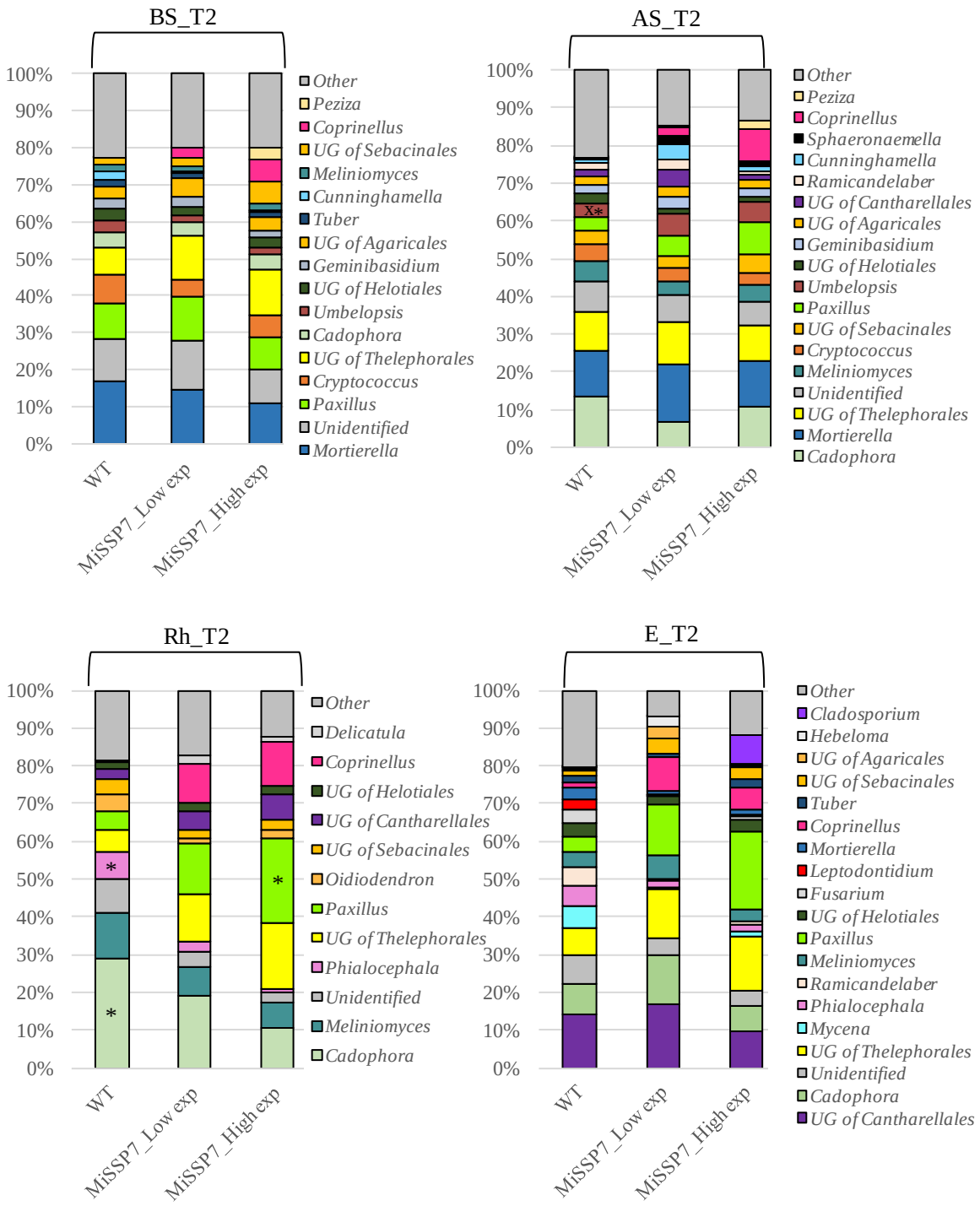


Figure 41 - The distribution of the most dominant fungal genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of Populus-expressing MiSSP7 and Populus WT cuttings collected at T2 in Year 1. The crosses (x) denote significant difference in the relative abundance of fungal genera detected between WT and MiSSP7_Low expression. The asterisks (*) denote significant difference in the relative abundance of fungal genera detected between WT and MiSSP7_High expression (P<0.05, ANOVA). Detailed information are available in Table S2.

Concerning bacterial communities, the relative abundance of Proteobacteria and Firmicutes was significantly enriched in the Rh and E of MiSSP7 cuttings while Saccharibacteria and Bacteroidetes were significantly more abundant in the endosphere of WT (P<0.05, ANOVA, Table S3). At the genus level, the relative abundance of *Massilia* (Proteobacteria) was significantly higher in the Rh and E samples of the MiSSP7 cuttings compared to the WT cuttings (P<0.05, ANOVA, Figure S2, Table S3).

At T2, we observed a significant enrichment of the saprotrophe *Umbelopsis* in the rhizosphere of WT cuttings compared to MiSSP7 cuttings. In the rhizoplane, the relative abundance of the endophytes *Cadophora* and *Phialocephala* was significantly higher in WT while the relative abundance of the EcM *Paxillus* was significantly higher in MiSSP7 cuttings with a high level of MiSSP7 expression (MiSSP7_ High exp, P<0.05, ANOVA, Figure 41, Table S2). These observations were confirmed by the relative distribution of fungal guilds in the rhizoplane of WT and MiSSP7 cuttings. Indeed, the relative abundance of fungal endophytes was significantly enriched in WT while the relative abundance of EcM fungi was significantly enriched in MiSSP7 cuttings (MiSSP7_Low exp and MiSSP7_High exp, P<0.05, ANOVA, Table S4). In addition, at the bacterial phylum level, we observed that the relative abundance of Acidobacteria, Planctomycetes and Proteobacteria (*Delproteobacteria* class) was significantly enriched in the Rh of MiSSP7_High exp compared to the WT (P<0.05, ANOVA, Table S3). At the genus level, the relative abundance of *Streptacidiphilus* was significantly enriched in the Rh of WT while the relative abundance of *Acidothermus* was significantly enriched in the Rh of MiSSP7_High exp cuttings (P<0.05, ANOVA, Figure 42, Table S3).

The root metabolome of *Populus* WT and *Populus*-expressing MiSSP7 lines cultivated in Year 1

We characterized by GC-MS the root metabolome of *Populus* WT and *Populus*-expressing MiSSP7 cuttings after 10 days and 6.5 weeks of growth in natural soil sampled on Year 1 to visualized if an alteration of the microbiome composition linked to MiSSP7 expression could induced alteration of the root metabolome. Some of the detected metabolites are known to be only produced by host tree while others can also be produced by bacterial and fungal members of root microbiome.

In roots of *Populus*-expressing MiSSP7 cuttings cultivating in Year 1, the main primary metabolites (>1 % of the total metabolites amount) were sucrose (22.4 %), palmitic acid (9.0 %), malic acid (7.6 %), glucose (6.4 %), succinic acid (1.5 %), phosphate (1.4 %) and galactose (1.1 %) while the main secondary metabolites (>1 % of the total metabolites amount) were α-salicyloylsalicin (12.1 %), tremulacin (6.8 %), salicin (6.7 %), tremuloidin (4.3 %), β-sitosterol (2.6 %) and catechin (1.4 %). All of these 15 metabolites were also found to be the main metabolites produced by *Populus* WT roots except for tremulacin and succinic acid which, respectively, corresponded to only 0.12 and 0.20 % of all root metabolites of *Populus* WT (Table S5).

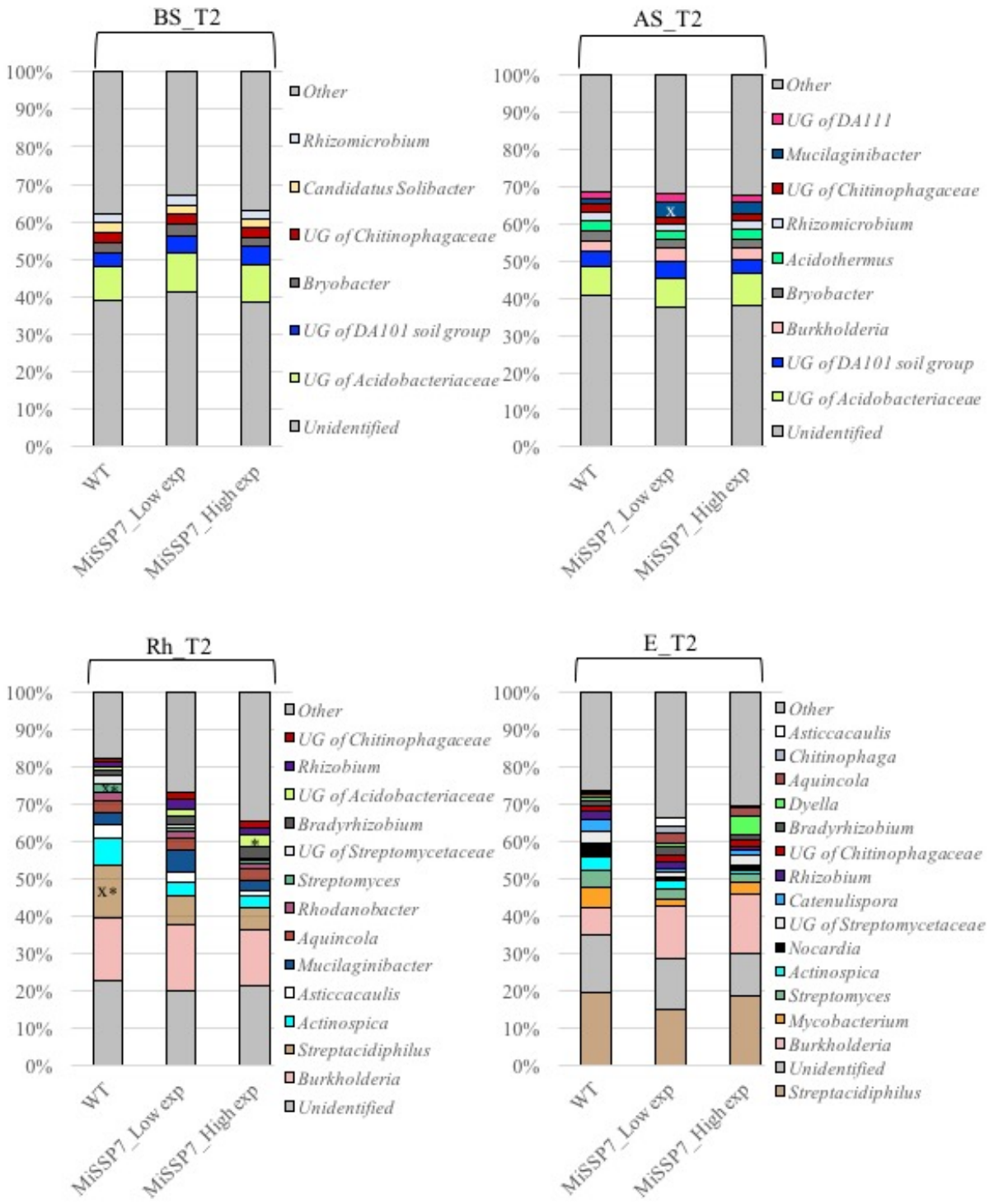


Figure 42 - The distribution of the most dominant bacterial genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of Populus-expressing MiSSP7 and Populus WT cuttings collected at T2 in Year 1. The crosses (x) denote significant difference in the relative abundance of bacterial genera detected between WT and MiSSP7_Low expression. The asterisks (*) denote significant difference in the relative abundance of bacterial genera detected between WT and MiSSP7_High expression (P<0.05, ANOVA). Detailed informations are available in Table S3.

Table 7 - List of metabolites detected in roots of Populus tremula x alba WT and Populus tremula x alba expressing MiSSP7 roots harvested in Year 1 after 10 days (T1) and 6.5 weeks (T2). Values indicate fold changes between WT and MiSSP7 line at each sampling time. The asterisks denote significant difference of concentration which has been measured between WT and MiSSP_High and between WT and MiSSP7_Low (= P<0.05, one way ANOVA). Metabolites highlighted in yellow and green are involved in primary, secondary metabolisms, respectively.*

Metabolite (RT-m/z)	Plant metabolite	Bacterial or fungal	WT/MiSSP7 (T1)	WT/MiSS7_Low (T2)	WT/MiSSP7_High (T2)
1,2,3-benzenetriol	X	X	ND	0.91	0.50
1,2,4-benzenetriol	X	X	ND	0.98	0.46
10.68 217 391 411	?		1.52	1.41	1.25
10.90 450 dehydro sugar	?		ND	1.40	2.29
11.22 450 dehydro sugar	?		0.77	0.97	0.58
11.29 393 303 257	?		ND	2.25	3.44
13.84 183 256 167	?		0.36	ND	ND
14.09 375 292 217	?		1.18	ND	ND
14.25 331 263 233 258 M+ glycoside	?		0.87	1.88	1.38
14.38 254 inositol conj	?		0.45	ND	ND
15.18 284 glycoside	?		0.82	0.93	0.35
15.24 284 glycoside	?		0.88	0.93	0.73
16.04 guaiacyl lignan	X		0.67	0.88	0.53
16.11 guaiacyl lignan	X		0.52	0.60 *	0.29
16.37 guaiacyl lignan	X		ND	0.64	0.56
17.65 418 179 193 91 glycoside	?		1.53	1.76	1.12
19.00 219 171 331	?		0.61	2.15	0.97
19.18 171 coumaroyl glycoside	?		0.63	1.51	0.79
19.69 171 caffeoyl glycoside	?		0.93	1.39	0.75
2-hydroxypentanedioic acid	X	X	0.89	0.61	0.14
2,5-dihydroxybenzoic acid-2-O-	X	X	ND	1.29	0.13
2,5-dihydroxybenzoic acid-5-O-	X		ND	1.17	0.18
4-hydroxybenzoic acid	X	X	0.69	0.73	0.29
5-oxo-proline	X	X	1.66	1.77	1.28
6-hydroxy-2-cyclohexenone alcohol	X		0.71	0.86	0.28
6-hydroxy-2-cyclohexenone-1-carboxylic acid	X	X	0.67	0.95	0.54
6.94 225 240 332 278	?		0.61	0.72	0.33
7.69 169 101 75 68	?		ND	1.27	0.79
8.34 256 167	?		ND	1.67	1.14
9.98 98 288 390	?		ND	0.00	0.0
a-linolenic acid	X	X	1.22	0.77	0.55
a-salicyloylsalicin	X		2.15	1.68	1.12
a-tocopherol	X	X	0.74	1.14	0.52
alanine	X	X	1.38	2.04	1.73
arabinose	X	X	1.15	ND	ND
arabitol	X	X	0.82	0.42	1.10
arbutin	X		0.63	0.59	0.77
B-sitosterol	X		0.73	0.93	0.51
caffeic acid	X		0.71	1.11	0.61
catechin	X		0.70	0.49 *	0.24
catechol	X		0.69	1.10	0.69
cis-aconitic acid	X	X	1.29	0.66	0.26
citric acid	X	X	1.19	0.73	0.21
digalactosylglycerol	X	X	0.56	1.09	1.38
erythronic acid	X		1.50	0.69 *	0.34
ethyl-phosphate	?		0.88	1.27	0.89
fructose	X	X	3.14	1.28	2.03 *
fumaric acid	X	X	1.03	0.53	0.22

Metabolite (RT-m/z)	Plant metabolite	Bacterial or fungal	WT/MiSSP7 (T1)	WT/MiSS7_Low (T2)	WT/MiSSP7_High (T2)
GABA	X	X	1.88	2.55	1.75
galactose	X	X	2.21	0.97	0.75
gallocatechin	X		0.62	0.72	0.32
glucose	X	X	2.29	0.98	1.17
glutamic acid	X	X	ND	3.26	1.44
glyceric acid	X	X	1.11	0.63 *	0.30
glycerol	X	X	0.62	0.64	0.44
glycerol-1/3-P	X	X	0.90	0.72	0.70
hydroquinone	X		0.68	0.72	0.11
lactic acid	X	X	0.79	0.62	0.52
linoleic acid	X	X	1.08	0.68	0.52
maleic acid	X	X	1.26	0.80	0.06
malic acid	X	X	1.56	0.74	0.15
mannitol		X	0.91	0.53	1.63
monogalactosylglycerol	X	X	1.08	0.91	0.86
myo-inositol	X		2.23	2.36	1.22
nonanoic acid	X		0.69	0.65	0.33
oxalomalic acid	X	X	1.21	0.74	0.52
palmitic acid	X	X	0.87	0.66	0.41
phluoroglucinol	X	X	0.62	0.67	0.09
phosphate	X	X	0.59	0.71	0.32
quinic acid	X	X	2.68	1.10	0.84
ribitol	X	X	ND	1.48	2.41
salicin	X		0.82	0.91	0.95
salicortin	X		1.52	1.00	0.51
salicyl alcohol	X		0.70	1.06	0.54 *
salicyl-salicylic acid-2-O-glucoside	X	X	2.43	1.16	0.59
salicylic acid	X	X	0.65	1.08	0.61
salicyltremuloidin	X		1.12	1.75	1.43
salireposide	X		1.25	1.58	1.09
shikimic acid	X	X	1.51	0.54	0.47 *
succinic acid	X	X	1.39	0.87	0.40
sucrose	X		1.59	0.67	0.60
threonic acid	X		3.50	0.42	0.24
threono-1,4-lactone	X	X	1.18	0.94	0.49
trehalose		X	2.08 *	0.24 *	0.42
tremulacin	X		0.89	1.52	1.09
tremuloidin	X		1.18	1.27	1.19
xylitol	X	X	0.84	1.00	0.85
xylono-1,4-lactone	X		1.26	0.82	0.58

At both T1 and T2, 33 of these detected metabolites were linked to primary metabolism while 36 were involved in primary metabolism while. In addition, 19 metabolites corresponded to unidentified compounds. In addition, 19 metabolites corresponded to unidentified compounds. At T1, the concentration of trehalose (primary metabolite, fungal disaccharide) was significantly higher in WT roots compared to MiSSP7 roots (P<0.05, Student-t-test, Table 7). In roots of cuttings collected at T2, the concentrations of the primary metabolites glyceric acid and of the secondary metabolite catechin were significantly higher in WT roots while the concentration of the primary metabolite trehalose were higher in the roots of MiSSP7_Low exp cuttings. The concentration of the secondary metabolite salicyl alcohol was significantly higher in the roots of MiSSP7_High exp cuttings while the concentration of fructose (primary metabolite) was significantly higher in the roots of WT cuttings (P<0.05, Student-t-test, Table 7).

Structure and composition of microbial communities of each compartment in *Populus* WT and *Populus*-expressing MiSSP7 line cultivated in Year 2

Experimental set-up used in Year 1 was conserved for a second experiment performed the following year (Year 2) to observe if the observations made in Year 1 were confirmed whatever the potential shifts in microbial community composition and structure of the natural soil.

Comparison of the structure of fungal and bacterial communities detected in soil (i.e., BS and AS samples) and in roots (i.e., Rh and E samples) of *Populus* WT and *Populus*-expressing MiSSP7 lines (i.e., MiSSP7_Low and MiSSP7_High) collected at T1 an T2 revealed significant differences between compartments and between sampling time at the OTU level (PermANOVA, P<0.05; Table 8). However, no significant shift in the microbial community structure was linked to the treatment effect (WT vs. MiSSP7_Low vs. MiSSP7_High; Table 8). These observations were confirmed in each compartment (i.e., BS, AS, Rh and E) and at each sampling time (i.e., T0, T1 and T2) (Table 8).

Detailed analyses of the fungal and bacterial community compositions in each compartment (i.e., BS, AS, Rh and E) revealed some significant differences between *Populus* WT and *Populus*-expressing MiSSP7 lines (i.e., MiSSP7_Low and MiSSP7_High) collected at T1 and T2 in Year 2 (ANOVA, P<0.05). Significant shifts in fungal community composition were only detected in the BS. At T2, Ascomycota were significantly enriched in the BS of *Populus* WT (74.4 ± 3.2 %) and MiSSP7_Low (75.3 ± 3.0 %) compared to MiSSP7_High (61.4 ± 2.9 %; Table S6) while Basidiomycota were significantly enriched in the BS of MiSSP7_High (18.1 ± 2.5 %) compared to WT (18.3 ± 1.9 %) and MiSSP7_Low (29.9 ± 2.2 %; Table S6). Concerning bacterial community, Proteobacteria (*Alphaproteobacteria* class) were significantly more abundant in the endosphere of MiSSP7_High (8.0 ± 1.2 %) compared to MiSSP7_Low (3.5 ± 0.6 %) and WT (3.7 ± 0.8 %) cuttings (Table S7). No significant change was observed between *Populus* WT and MiSSP7 lines collected at T1 at the fungal and bacterial phylum level.

At the genus level, the relative abundance of *Cryptococcus* was significantly enriched in the BS of WT compared to MiSSP7_Low cuttings collected at T1 (ANOVA, P<0.05; WT: 12.7 ± 1.0 % = MiSSP7_High: 10.5 ± 1.3 % > MiSSP7_Low: 9.0 ± 0.8 %, Figure S3, Table S6). In addition, we observed that the relative abundance of *Leptodontidium* was significantly enriched the Rh compartment of WT compared to MiSSP7_High line collected at

*Table 8 - Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation in this study (compartments [BS, AS, Rh and E], sampling times [T1 and T2] and expression [WT, MiSSP7_Low exp and MiSSP7_High exp]) for fungal and bacterial communities at the OTU level. BS, AS, Rh and E samples of the WT and MiSSP7 cuttings collected in Year 2 were included in this analysis. Statistical significance (p-value) computed were based on sequential sums of square from 999 permutations (*** = P-value < 0.01, ** = P-value < 0.03).*

Fungi_Year2			PermANOVA			Bacteria_Year2			PermANOVA		
Compartment	**Time**	**Source of variation**	**F**	**R2**	**p-value**	**Compartment**	**Time**	**Source of variation**	**F**	**R2**	**p-value**
BS, AS, Rh, E	T1 and T2	Compartment	45.13	0.486	***	BS, AS, Rh, E	T1 and T2	Compartment	31.56	0.397	***
		Time	7.15	0.025	***			Time	11.30	0.095	***
		Expression	1.44	0.087	0.110			Expression	1.08	0.009	0.326
		Treatment	1.10	0.003	0.318			Treatment	1.16	0.019	0.215
BS	T1, T2 and T0	Time	6.00	0.132	***	BS	T1, T2 and T0	Time	2.22	0.063	**
		Expression	1.18	0.087	0.0109			Expression	1.39	0.079	0.055
		Treatment	2.10	0.093	0.063			Treatment	1.45	0.087	0.065
Rh	T1, T2 and T0	Time	10.1	0.420	***	Rh	T1, T2 and T0	Time	15.43	0.488	***
		Expression	0.98	0.020	0.414			Expression	1.39	0.022	0.178
		Treatment	0.87	0.018	0.464			Treatment	1.25	0.036	0.209
BS	T1 and T2	Time	4.54	0.111	***	BS	T1 and T2	Time	2.19	0.069	***
BS	T1 and T2	Expression	1.44	0.142	*	BS	T1 and T2	Expression	1.42	0.045	0.053
BS	T1 and T2	Treatment	1.32	0.032	0.124	BS	T1 and T2	Treatment	1.37	0.135	0.067
AS	T1 and T2	Time	9.50	0.216	***	AS	T1 and T2	Time	3.53	0.108	***
AS	T1 and T2	Expression	1.05	0.047	0.342	AS	T1 and T2	Expression	1.00	0.066	0.405
AS	T1 and T2	Treatment	1.35	0.030	0.158	AS	T1 and T2	Treatment	0.91	0.030	0.618
Rh	T1 and T2	Time	8.40	0.197	***	Rh	T1 and T2	Time	6.65	0.193	***
Rh	T1 and T2	Expression	1.09	0.051	0.327	Rh	T1 and T2	Expression	0.79	0.023	0.635
Rh	T1 and T2	Treatment	0.94	0.022	0.411	Rh	T1 and T2	Treatment	0.66	0.023	0.845
E	T1 and T2	Time	3.87	0.111	**	E	T1 and T2	Time	4.00	0.137	**
E	T1 and T2	Expression	0.97	0.056	0.478	E	T1 and T2	Expression	1.16	0.040	0.260
E	T1 and T2	Treatment	1.00	0.028	0.428	E	T1 and T2	Treatment	0.94	0.073	0.521
BS	T1	Expression	1.01	0.094	0.082	BS	T1	Expression	1.07	0.089	0.125
BS	T1	Treatment	1.35	0.211	0.434	BS	T1	Treatment	1.00	0.167	0.421
BS	T2	Expression	1.08	0.157	0.313	BS	T2	Expression	1.35	0.077	0.086
BS	T2	Treatment	1.47	0.071	0.080	BS	T2	Treatment	1.12	0.194	0.166
AS	T1	Expression	0.91	0.125	0.558	AS	T1	Expression	0.98	0.082	0.562
AS	T1	Treatment	1.77	0.121	**	AS	T1	Treatment	0.90	0.153	0.940
AS	T2	Expression	1.33	0.129	0.182	AS	T2	Expression	0.98	0.057	0.462
AS	T2	Treatment	0.85	0.041	0.502	AS	T2	Treatment	1.01	0.118	0.373
Rh	T1	Expression	0.95	0.134	0.477	Rh	T1	Expression	0.79	0.078	0.732
Rh	T1	Treatment	1.29	0.091	0.181	Rh	T1	Treatment	0.85	0.085	0.645
Rh	T2	Expression	0.78	0.081	0.598	Rh	T2	Expression	0.81	0.048	0.716
Rh	T2	Treatment	0.75	0.038	0.676	Rh	T2	Treatment	0.72	0.087	0.959
E	T1	Expression	1.00	0.170	0.503	E	T1	Expression	0.77	0.066	0.673
E	T1	Treatment	0.73	0.062	0.843	E	T1	Treatment	0.92	0.156	0.572
E	T2	Expression	0.85	0.090	0.655	E	T2	Expression	1.25	0.094	0.202
E	T2	Treatment	1.29	0.067	0.215	E	T2	Treatment	1.08	0.164	0.363

T1 (ANOVA, P<0.05; WT: 7.2 ± 0.9 % > MiSSP7_High: 3.0 ± 1.1 % = MiSSP7_Low: 3.8 ± 1.0 % Figure S3, Table S6). *Cryptococcus* was also significantly enriched in the BS of MiSSP7_High line compared to WT and MiSSP7_Low line collected at T2 (ANOVA, P<0.05; MiSSP7_High: 27.4 ± 2.4 % > WT: 14.6 ± 1.8 % = MiSSP7_Low: 17.0 ± 1.8 %, Figure 43, Table S6). Significant changes were also observed in the distribution of fungal guilds at T1. Indeed, the fungal endophytes were significantly enriched in the R compartment of *Populus* WT compared to MiSSP7_High cuttings (ANOVA, P<0.05; WT: 8.0 ± 0.9 % > MiSSP7_Low: 4.6 ± 1.0 % = MiSSP7_High: 3.6 ± 0.8 %, Table S8). At T2, no significant change was observed in the distribution of fungal guild in the four-studied compartment between WT and MiSSP7 lines. Concerning bacterial community composition, no significant change was observed in the four studied compartments between WT and MiSSP7 lines collected at T1 (ANOVA, P<0.05; Figure S4, Table S8).

At T2, we observed that the relative abundance of *Candidatus Xiphinematobacter* and unknown genus of *Methylacidiphilaceae* was significantly higher in the rhizosphere of MiSSP7_High compared to WT cuttings (ANOVA, P<0.05; Figure 44, Table S7). In addition, the relative abundance of *Chthoniobacter* was significantly higher in the endosphere of MiSSP7_High cuttings compared to WT cuttings (ANOVA, P<0.05; Figure 44, Table S7).

The root metabolome of *Populus* WT and *Populus*-expressing MiSSP7 lines cultivated in Year 2

We detected the same main primary and secondary metabolites in the roots of *Populus*-expressing MiSSP7 cuttings sampled in Year 2 compared with those sampled in Year 1. However, the concentration of some of these metabolites was significantly different between Year 1 and Year 2. For instance, the concentration of sucrose and of four secondary metabolites (i.e., α-salicyloylsalicin, tremulacin, salicin and tremuloidin) was significantly higher in T1-samples collected in Year 2 while the concentration of glucose, fructose and galactose was significantly higher in T2-samples collected in Year 1 (Table S5).

At T1, the level of salicin, tremulacin and the unidentified compound 14.25 min (m/z 331 263 233 258 M+ glycoside) was significantly enriched in the roots of WT cuttings compared to MiSSP7_Low roots (ANOVA, P<0.05; Table S10). No significant difference was observed in the roots of WT and MiSSP7_High cuttings collected at T1 (Table S10). At T2, two primary metabolites (oxalomalic acid and threono-1,4-lactone) and three secondary metabolites (6-hydroxy-2-cyclohexenone-1-carboxylic acid, catechin and xylono-1,4-lactone) were significantly enriched in the roots of MiSSP7_Low compared to the roots of WT cuttings (ANOVA, P<0.05; Table S10). Comparison of the root metabolome of WT and MiSSP7_High revealed significant difference between the two lines of *Populus* collected at T2. Several secondary metabolites were more abundant in WT roots (e.g catechin, catechol, salicin and xylitol) while tremulacin was significantly more abundant in the roots of MiSSP7_High line with a fold change of 50 (ANOVA, P<0.05; Table S10). Concerning primary metabolites, we observed that the level of oxalomalic acid, cis-aconitic acid and sucrose was significantly enriched in WT roots compared to MiSSP7_High roots (ANOVA, P<0.05; Table S10).

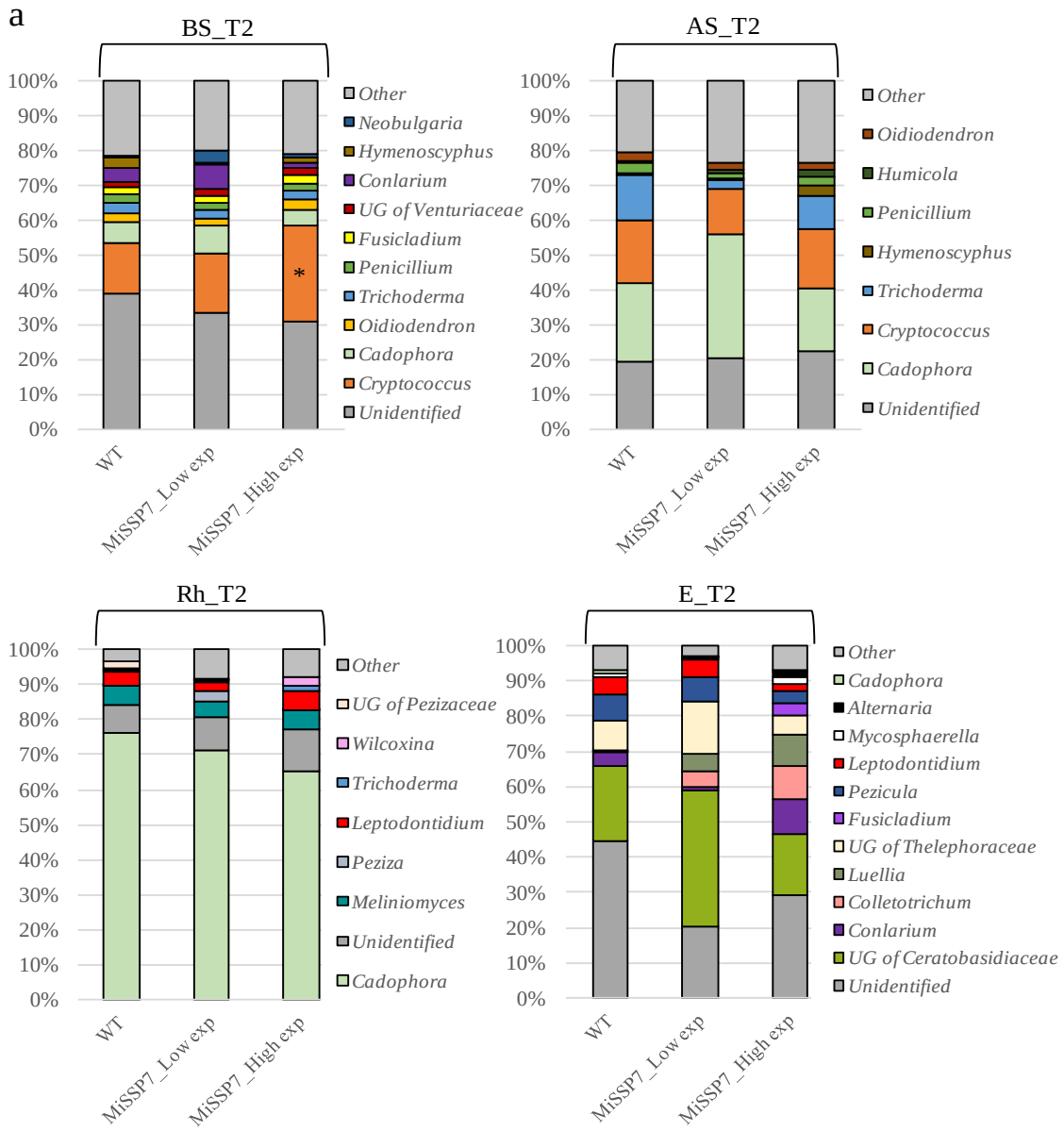


Figure 43 - The distribution of the most dominant fungal genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of Populus-expressing MiSSP7 and Populus WT cuttings collected at T2 in Year 2. The asterisks (*) denote significant difference in the relative abundance of fungal genera detected between WT and MiSSP7_High expression (P<0.05, ANOVA) (a). Detailed informations are available in Table S6.

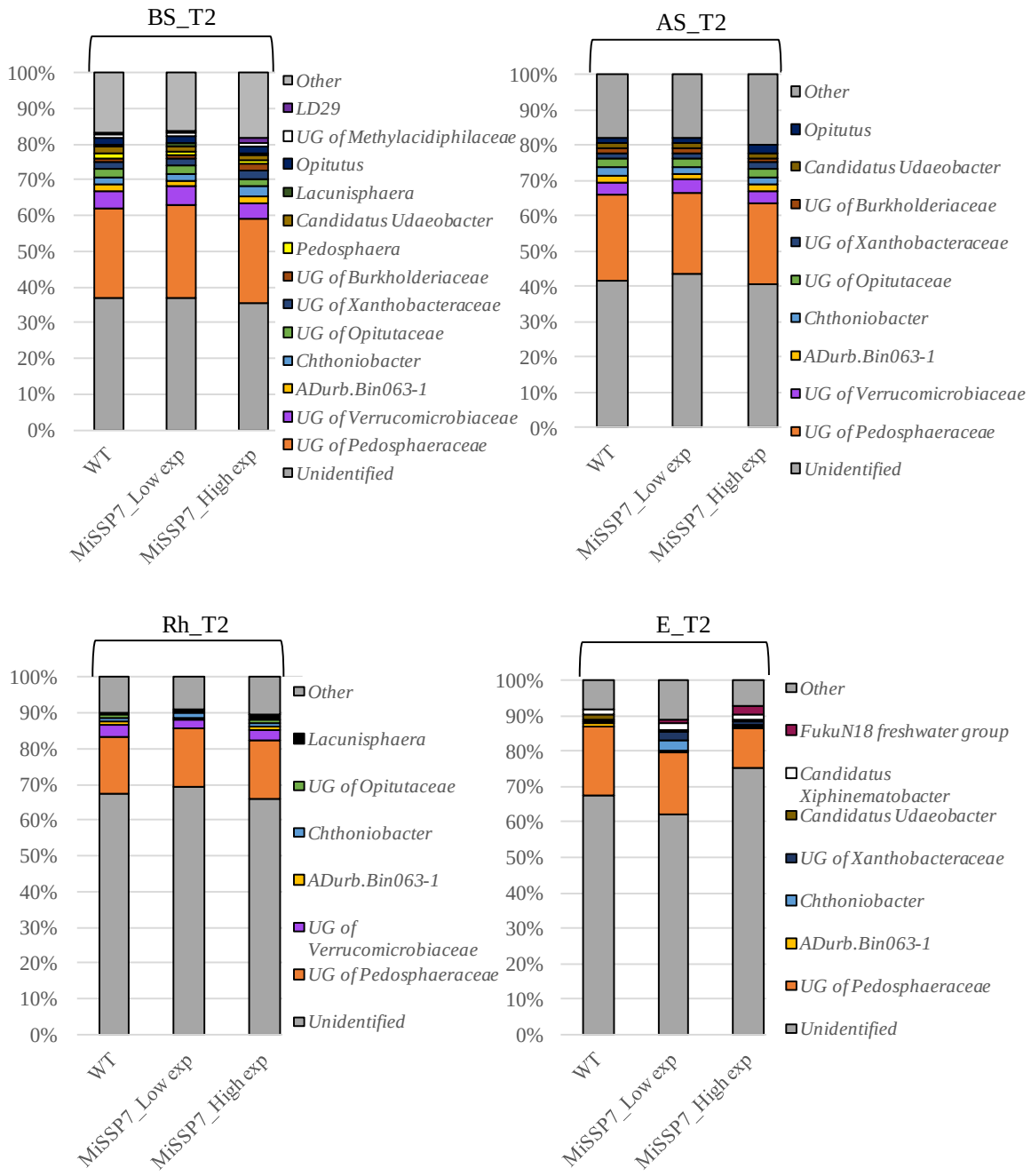


Figure 44 - The distribution of the most dominant bacterial genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of Populus-expressing MiSSP7 and Populus WT cuttings collected at T2 in Year 2. Detailed informations are available in Table S7.

The metatranscriptome of the *Populus* WT and *Populus*-expressing MiSSP7 roots collected in Year 2

A metatranscriptome analysis of the microbial communities colonizing the roots (i.e., both Rh and E compartments) of the WT and the *Populus*-expressing MiSSP7 lines (i.e., MiSSP7_Low exp and MiSSP7_High exp) was performed in order to highlight the active communities detected at T2.

We observed that the EcM fungi *Thelephora* were the most abundant fungal genus detected in the metatranscriptome of the roots of *Populus* cuttings (more than 50 % of the transcript detected in *Populus*-expressing MiSSP7 roots and 31 % of the transcript detected in the *Populus* WT roots, Figure 45 a, Table S11). No significant difference was observed in the proportion of *Thelephora*-related transcripts between the roots of *Populus*-expressing MiSSP7 and WT (Wilcoxon test, MiSSP7_Low exp vs WT, P=0.21; MiSSP7_High exp vs WT, P=0.23, Figure 45 b). In addition, we also did not detect any significant difference in the proportion of transcripts of the other most abundant fungal genera in the roots of *Populus*-expressing MiSSP7 and WT (Wilcoxon test, Figure 45 b). However, the abundance of *Tulasnalla*-related transcripts tended to be higher in the roots of MiSSP7_Low expression compared to WT (Wilcoxon test, P=0.06, Figure 45 b) while the abundance of *Meliniomyces*-related transcripts tended to be higher in the roots of WT compared to MiSSP7_High expression (Wilcoxon test, P=0.08, Figure 45 b). Although the dominance of the fungal endophytes *Phialocephala* and *Cadophora* and the yeast *Cryptococcus* in *Populus* roots, their transcripts were undetected.

Discussion

The composition of the root microbiome is influenced by the host physiology and metabolism (Berg & Smalla, 2009; Jacoby et al., 2017). Defence phytohormones (JA, SA and ET) are structurally diverse plant secondary metabolites involved in plant immune system but also in the regulation of plant growth and development. Previous studies examined the roles of defence hormones in shaping the root microbiome by using mutant lines defective in their biosynthesis or perception in combination with microbial culturing (Lebeis et al., 2015; Liu et al., 2017). Although these studies improved our knowledge concerning the effect of plant defence on root microbiome composition and structure, no study has been performed on woody perennial plant. Here, we asked if the ectopic expression of MiSSP7 in *Populus* impact the composition and the structure of the root microbiome. Based on the work of Plett et al., (2011), we hypothesized that alteration of JA signalling pathway by MiSSP7 influences the root-associated microbiome of *Populus* and if these potential modifications of the root microbiome are accompanied by alteration of root metabolism. For this, we took advantage of the ability of the action of MiSSP7, the most highly symbiosis-upregulated gene from the EcM fungi *Laccaria bicolor*, known for its interaction with the negative repressor JAZ6 maintaining repression of JA-induced genes (Plett et al., 2011; 2014).

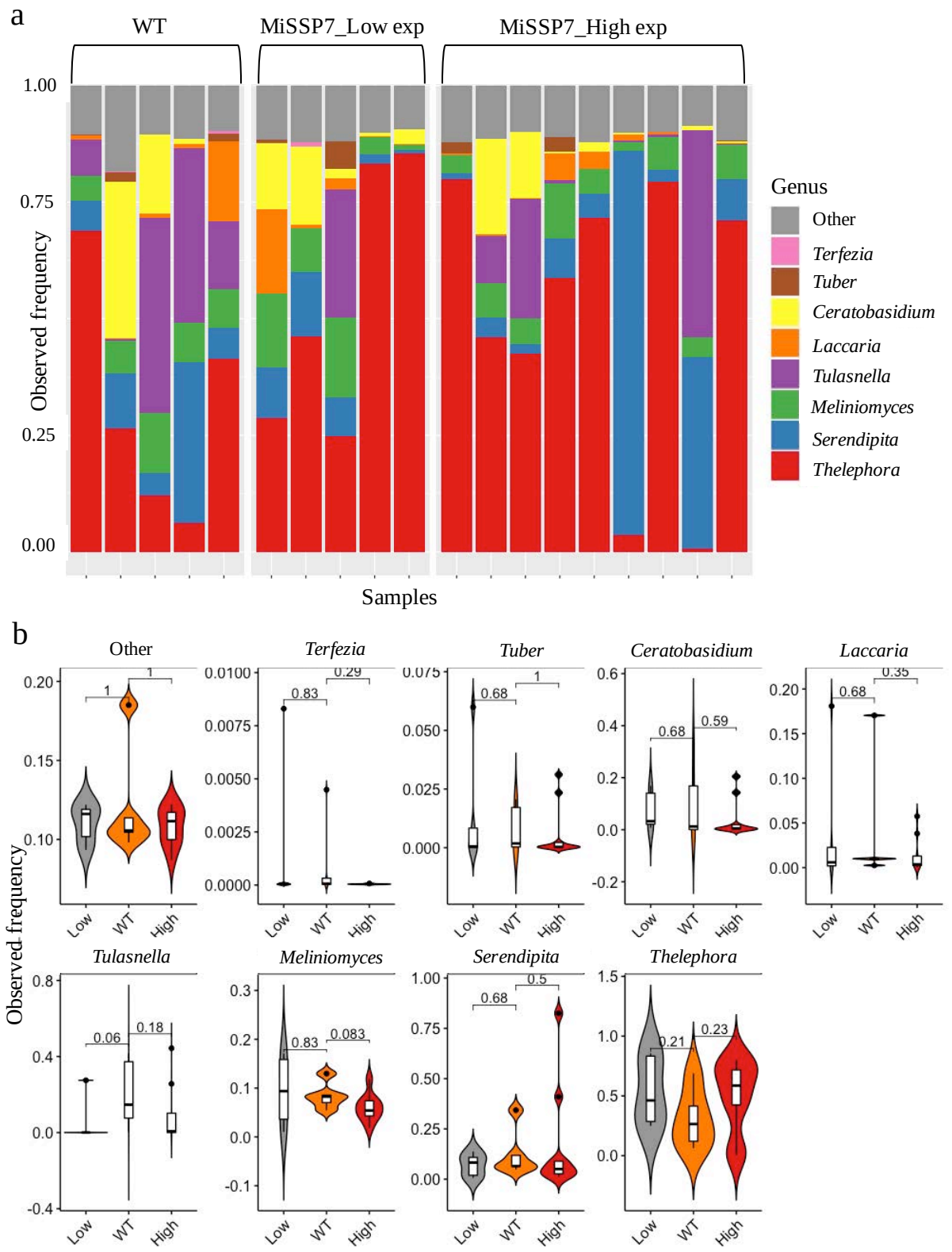


Figure 45 - Proportional distribution of the most active fungal genera detected in the roots of Populus-expressing MiSSP7 and Populus WT cuttings collected at T2 in Year 2. Each column corresponds to the observed frequency of the most active fungal genera based on metatranscriptomic rRNA data collected from the roots samples of WT, MiSSP7_Low expression and MiSSP7_High expression cuttings (a). Comparison of the observed frequency of the most active fungal genera detected in the roots of the different Populus lines (b). P-value are given for fungal genus and correspond to the comparison between WT (in orange) and MiSSP7_Low exp (in grey) cuttings and the comparison between WT and MiSSP7_High exp (in red) cuttings (Wilcoxon test).

We observed that MiSSP7 expression in *Populus* induced contrasted change in root microbiome composition according to the year of experiment (i.e., Year 1 and Year 2). Although we do not know the precise effect of MiSSP7 on the biosynthesis and the perception of JA in *Populus* roots, contrasted changes in the composition of bacterial and fungal communities associated with *Populus* roots detected between the two years of experiments was observed and was accompanied by contrasted changes in the root metabolome of *Populus* lines cultivated in Year 1 and Year 2. Indeed, the concentration of different metabolites was impacted by MiSSP7 expression in *Populus* roots between Year 1 and Year 2. Therefore, we could imagine that the root metabolome is more regulated by microorganisms than by MiSSP7 expression. Even if MiSSP7 expression in *Populus* significantly affected the root microbiome composition, microbial communities detected in the roots of *Populus*-expressing MiSSP7 were similar to those previously described in other *Populus* genotype (Gottel et al., 2011; Danielsen et al., 2012; Shakya et al., 2013; Bonito et al., 2014; Cregger et al., 2018). *Burkholderia* (Proteobacteria), *Mucilaginibacter* (Bacteroidetes) and *Streptacidiphilus* (Actinobacteria) dominated root bacterial communities while *Meliniomyces,* the fungal endophytes *Cadophora* and the EcM fungi *Paxillus* dominated the root fungal communities.

MiSSP7 expression in *Populus* was not responsible of modification of the host tree transcriptome

A transcriptome analysis of the roots of *Populus*-expressing MiSSP7 and *Populus* WT cuttings was performed with Year 2 samples in order to observed if the ectopic expression of MiSSP7 in Poplar could regulate the expression of other genes of interest (data not shown). Almost no significant differences were observed between the transcriptomes of the roots of *Populus*-expressing MiSSP7 and *Populus* WT cuttings. Indeed, only three and two genes were up-regulated (DHBP synthase RibB-like alpha [Potri.001G234900.1], kunitz trypsin inhibitor 1 [Potri.007G111500.1] and amino acid permease 2 [Potri.007G100100.1]) and down-regulated (cinnamate-4-hydroxylase [Potri.018G146100.1] and senescence-related gene 1 [Potri.001G355100.1]) with a maximum fold change of 1.2 between MiSSP7 and WT roots (data not shown). Similarly, no significant difference between the phenotype of *Populus*-expressing MiSSP7 and *Populus* WT was observed (Figure 39). Analysis of the transcriptome of poplar roots incubated with MiSSP7 protein induced the modulation of 200 transcripts mostly involved in the alteration of the root architecture (Plett et al., 2011). However, this study was performed on the roots of 2-weeks-old *Populus* cultivated in axenic conditions and incubated with MiSSP7 protein for 1 hour. In addition, transcriptome analysis was performed with only one replicates which could lead to misinterpretation. Therefore, we could easily imagine that after 6.5 weeks of growth, the ectomycorhization as well as the induction of other genes by MiSSP7 are already established in *Populus* roots.

MiSSP7 expression in Populus induces contrasted change in the composition and the structure of the root microbiome

We previously described that soil microbiome which is now considered as the main reservoir of microorganisms for tree roots (Lareen et al., 2016, Mangeot-Peter et al., 2020) was significantly altered in its composition and its

structure from year to year. These modifications were responsible for significant shifts of *Populus* root microbiome composition but also for changes in both primary and secondary metabolisms in the roots of the WT (Mangeot-Peter et al., 2020, Chapter 3). In the current study, we compared the composition and the structure of bacterial and fungal communities associated with the roots of genetically modified *Populus* line (*Populus*-expressing MiSSP7) and of non-genetically modified *Populus* (*Populus* WT) cultivated in the same soil across two following years. This natural soil was characterized in our previous work (Mangeot-Peter et al., 2020). Colonization of the roots by microorganisms is highly dynamic: bacterial communities as well as fungal communities assigned as saprotrophes and pathotrophes are able to colonize host roots in few days while the functional association of the roots with ectomycorrhizal fungi required several weeks (Smith & Reads, 2008; Marupakula et al., 2016, chapter 1 of this thesis). The sampling time (i.e, abiotic factor) affected OTU-level microbial soil and root community structure of the WT cuttings in Year 1 and Year 2 (Mangeot-Peter et al., 2020). In addition, the expression of MiSSP7 in *Populus* (i.e, biotic factor) significantly impacted fungal communities detected in soil and roots only in Year 1.

In our study, we expect that MiSSP7 expression was responsible of the alteration of the JA signalling pathway in *Populus*. Other studies were interested by the impact of SA manipulation on the bacterial communities colonizing *Arabidopsis* roots. Knowing that JA and SA signalling pathway are antagonistics (Koornbeef et al., 2008), the increase of SA could lead to the decrease of JA, mimicking the expected effect of MiSSP7 expression. Significant shifts in the relative abundance of the most dominant bacterial and fungal communities were observed but were contrasted according to the year of experiment (i.e., Year 1 and Year 2). In Year 1, the relative abundance of *Streptomyces* (Actinobacteria), *Acidovorax* and *Massilia* (Proteobacteria) was significantly enriched in the roots of *Populus*-expressing lines compared to the roots of *Populus* WT collected at T1 (Figure 42). These observations were in accordance with other studies which showed a significant increase of the relative abundance of Proteobacteria and a decrease of the relative abundance of Actinobacteria in roots of SA-deficient *Arabidopsis* plants (Lebeis et al., 2015). Exogenous SA application was responsible for the enrichment of *Streptomyces* in *Arabidopsis* roots (Lebeis et al., 2015). Although the antagonistically action of JA and SA (Koornneef et al., 2008), these two phytohormones seemed to have the same effect on bacterial members of the root microbiome. These results were in accordance with our study. However, the activation of JA signalling pathway had a more contrasted effect on *Streptomyces* populations in roots of wheat (*Triticum aestivum*) where some OTU increased and other decreased in relative abundance in response to JA. These observations performed on different plant species highlight that manipulation on JA signalling pathway could promote or prevent the root colonisation by bacteria. We could also hypothesize that the colonisation of the *Populus* roots by bacteria is mostly influence by fungal communities potentially impacted by MiSSP7 expression.

Concerning fungal communities, EcM fungi (e.g *Paxillus*) were more abundant in the roots of *Populus* expressing MiSSP7 line while fungal endophytes (e.g *Cadophora* and *Phialocephala*) were more abundant in the rhizoplane of *Populus* WT line in Year 1 at T2 (Figure 41). In addition, the same trend was observed in the endosphere suggested that MiSSP7 expression have the same effect on and in the roots. To our knowledge, it is the first work dealing with JA signalling pathway and fungal microbiome of tree roots. In Year 2, a significant enrichment of

fungal endophytes (e.g *Cadophora*) was observed in the roots of *Populus*-expressing MiSSP7 collected at T1 whereas no enrichment of EcM fungi was observed in *Populus*-expressing MiSSP7 (Table S8). However, the metatranscriptome analysis of *Populus* roots collected at T2 in Year 2 revealed that the EcM fungus *Thelephora* was the most active colonizers of the *Populus* roots (Figure 45). This result was in concordance with DNA metabarcoding approach which revealed that *Thelephoraceae* was the main EcM detected in *Populus* roots (10 % in relative abundance, Figure 43). It was also the fungus which formed the most identified ectomycorrhizae on the *Populus* roots (Figure 40). No significant difference was observed in the proportional distribution of *Thelephora* transcripts between WT and MiSSP7 roots suggesting that MiSSP7 expression had no effect on the colonization of this EcM fungus. Although JA signalling pathway seems to be the main defence pathway to protect tree the attack against necrotrophic pathogens and against *Laccaria bicolor* root colonisation (Plett et al., 2011; 2014), other signalling pathways or molecular mechanisms may also play this role. In addition, if this fungal effector of 7kDa involved in symbiosis establishment was specific of the EcM fungus *Laccaria bicolor* (Plett et al., 2014), other EcM fungi produced MiSSPs such as *Paxillus* (Kohler et al., 2015). Although the genome of *Thelephora* had just been sequenced from mycelium samples, no information is currently available regarding the ability of this EcM fungus to produce MiSSP. In vitro culture of *Populus* in the resence of this fungus may be carried out to allow the sequencing of ectomycorrhizae.

Taking together, these observations suggest that the alteration of JA signalling pathway induced by MiSSP7 expression in *Populus* was responsible of the modification of the balance between some EcM fungi and fungal endophytes only in Year 1. In addition, we could hypothesize that the EcM fungi *Paxillus* and *Thelephora* have different strategy to counterbalance defence mechanisms of the host to establishment symbiotic interactions which could be add to the initial effect of MiSSP7 in genetically-modified *Populus*.

Contasted change in the composition of the root microbiome induced by MiSSP7 expression was correlated with difference in the concentration of the main metabolites detected in *Populus* roots

We observed that MiSSP7 expression in *Populus* was correlated with changes in the distribution of most dominant fungal guilds in Year 1: EcM fungi colonizing root system was significantly enriched in *Populus*-expressing MiSSP7 line while fungal endophytes were significantly enriched in non-genetically modified *Populus*. These different types of fungi have different roles in terms of nutrition and protection against stresses (Van der Heijden et al., 2008; Baum et al., 2018). Analyses of the metatranscriptome revealed that the transports of amino acid, nucleotide, coenzyme and inorganic iron were significantly more active in EcM fungi while the transports of carbohydrate and lipid was significantly more active in endophytes. Secondary metabolites biosynthesis and transport tended to be increased in EcM fungi and endophytes compared to saprotrophes while defence mechanisms tended to be increased in EcM fungi compared to saprotrophes and endophytes (data not shown). In Year 2, the most dominant endophytes and EcM fungi detected in *Populus* roots were *Cadophora* and *Thelephoraceae* species. Bonito et al., (2016) showed that *Thelophoraceae* species interacted and compete for resources using a range of different strategy. These results highlight that specific members of the root

microbiome adopt different strategy to colonize tree roots which could also impact the assembly and the functioning of microbial communities (Bonito et al., 2019).

Significant differences were observed in the concentration of primary and secondary metabolites detected in *Populus*-expressing MiSSP7 roots collected in Year 1 and Year 2. This observation was concomitant with the significant shifts detected in root microbiome composition between Year 1 and Year 2. For instance, the concentration of fructose and glucose was significantly enriched in Year 1 compared to Year 2 while the concentration of sucrose remained stable between Year 1 and Year 2 in roots of *Populus*-expressing MiSSP7 harvested at T2 (Table S9). This is particularly interesting in the context of carbon fluxes occurring between host tree and EcM fungi; sucrose is excreted by plant root cells into the common apoplast of the host-fungus interface where it is hydrolysed into equimolar amount of glucose and fructose which could trigger fungal physiology in symbiosis (Nehls et al., 2004; 2008). By contrast, we observed that the concentration of tremulacin was significantly enriched in the roots of *Populus*-expressing MiSSP7 collected in Year 2 compared to those collected in Year 1 (Table S9). Tremulacin is a plant phenolic glucoside that may divert carbon flux through the lignin pathway and play a role in defence (Busov et al., 2006). According to these results, we could hypothesize that the root microbiome which significantly shifts in its composition from year to year, may be responsible of the differential accumulation of metabolites involved in nutrition or in defence mechanisms. In accordance with this hypothesis, the changes in the root microbiome between *Populus*-expressing MiSSP7 and *Populus* WT were concomitant with a modification of 15 % of the measured root metabolites, mostly observed after 6.5 weeks of growth in natural soil. The root metabolites significantly regulated between the two studied lines were different between Year 1 and Year 2: the root metabolites significantly regulated between the two *Populus* lines in Year 1 were involved in primary metabolism while the root metabolites significantly regulated between the two *Populus* line in Year 2 mostly belonged to secondary metabolism. In Year 1, the accumulation of a-linolenic acid and linoleic acid (i.e, the fatty acid substrates of JA biosynthesis; Vick & Zimmerman, 1984) in the roots of *Populus*-expressing MiSSP7 could be explained by the effect of MiSSP7 on the JA signalling and biosynthesis pathway (Plett et al., 2014). In addition, we observed the significant accumulation of SA in the roots of *Populus*-expressing MiSSP7 compared to *Populus* WT collected in Year 1 and the opposite in *Populus* lines collected in Year 2. The roots of *Populus* MiSSP7 cultivated in Year 1 being more colonized by EcM fungi compared to those cultivated in Year 2, these observations were in accordance with the work of Basso et al., (2019) who observed the accumulation of SA in ectomycorrhized roots of *Populus*. In Year 1, the significant accumulation of trehalose coupled with the significant decrease of fructose in the roots of *Populus*-expressing MiSSP7 was in accordance with the presence of EcM fungi (Lewis & Harley, 1965). In Year 2, the accumulation of mannitol, another sugar commonly produced by EcM fungi (Deveau et al., 2008), in the roots of *Populus*-WT could be correlated by the presence of EcM fungi (e.g, members of *Thelephoraceae*). In parallel, fungal endophytes are known to induce important changes in the expression of genes involved in TCA cycle in host cell of raygrass (Dupont et al., 2015). This information was concomitant with the increase of oxalomalic acid level in *Populus*-WT roots collected in Year 2, significantly more colonized by fungal endophytes compared to *Populus*-expressing MiSSP7 roots.

Taken together, these results show that modifications of the composition of the root microbiome observed between *Populus*-expressing MiSSP7 and *Populus* WT correlate with different change in the root metabolome. In Year 1, the significant increase of EcM fungi detected in *Populus*-expressing MiSSP7 roots was concomitant with the accumulation of metabolites, known to be highly regulated in the interactions between EcM fungi and tree host. In Year 2, the change in root metabolome potentially due to MiSSP7 expression was less clear as well as the change in the composition of root microbiome observed between *Populus*-expressing MiSSP7 and *Populus* WT.

Conclusions

The root microbiome of *Populus* cuttings with altered JA signalling pathway differed in the relative abundance of specific bacterial as well as specific fungal communities as compared with those of *Populus* WT. The repetition of our experimental approach over two consecutive years has allowed us to highlight the contrasting effect of JA on the root microbiome and host metabolism. Although the immune system of the tree is an important regulator of the composition and the structure of root microbiome, our study shows that the soil type and natural variations of its microbiome are the key factors involved in the formation of the tree root microbiome.

At this stage, it is difficult to conclude on the actual effect of the JA signalling pathway modulated by MiSSP7 expression on the root microbiome of *Populus*. Further work is needed to validate the observations made in Year 1 and understand those made in Year 2.

III. Conclusions

L'altération de la voie de signalisation de l'AJ via l'expression de MiSSP7 chez les peupliers génétiquement modifiés a engendré des changements au niveau de l'abondance relative des taxons fongiques dominants de la rhizosphère et de l'endosphère par rapport aux peupliers non génétiquement modifiés. Des résultats différents ont été obtenus à l'issue des deux répétitions de notre approche expérimentale sur deux années consécutives concernant l'effet de l'expression ectopique de MiSSP7 sur le microbiote et le métabolome de l'arbre hôte. Bien que le système immunitaire des plantes soit un régulateur important de la composition et de la structure du microbiote racinaire, notre étude montre que le type de sol et les variations naturelles de son microbiote sont les facteurs clés impliqués dans la mise en place et l'évolution du microbiote racinaire. Suite à l'obtention de ces résultats, il est difficile de conclure réellement sur l'effet de la voie de signalisation de l'AJ modulée par MiSSP7 sur la composition et la structure du microbiote racinaire du peuplier. D'autres travaux seraient nécessaires pour confirmer les observations et interprétations réalisées suite à l'expérimentation de l'Année 1 et comprendre celles réalisées suite à l'expérimentation de l'Année 2.

Chapitre VI

Conclusion générale, discussion et perpectives

I. Résultats majeurs de la thèse

Le microbiote racinaire de l'arbre a un rôle essentiel dans la vie de l'arbre et le fonctionnement des écosystèmes forestiers. En effet, les communautés de micro-organismes associées aux racines améliorent le développement et la croissance des arbres-hôtes, leurs résistances aux stress biotiques et abioques et participent aux cycles des nutriments. La composition et la structure taxonomique et fonctionnelle de ces communautés sont modulées par l'environnement et par l'arbre hôte.

Dans ce contexte, les objectifs de ma thèse étaient d'étudier :

- la dynamique de colonisation des racines du peuplier par les communautés de micro-organismes (bactéries et champignons) naturellement présentes dans le sol forestier ;
- l'impact des facteurs environnementaux tels que le type de sol (propriétés physico-chimiques et origine) et le climat (température, précipitations) sur la composition et la structure des communautés de micro-organismes (bactéries et champignons) du sol et des racines du peuplier ;
- l'impact des facteurs liés à la physiologie de l'arbre hôte tels que le métabolisme racinaire et la voie de signalisation d'une hormone de défense sur la composition et la structure des communautés de micro-organismes (bactéries et champignons) associées au système racinaire.

La stratégie utilisée pour ces travaux a reposé sur (i) une expérience en conditions contrôlées et en mésocosmes pour ne faire varier que les paramètre à tester (dans ce cas, le climat et l'origine de la matrice sol) et sur (ii) des expérimentations en pots et en serre afin d'observer la colonisation racinaire des jeunes boutures d'arbre par les micro-organismes naturellement présents dans le sol.
En raison de ses nombreux atouts en matière de manipulations expérimentales et de ses capacités d'interactions avec un large panel de micro-organismes détaillés dans le chapitre I de ce manuscrit, le Peuplier a été choisi comme modèle dans le cadre de ces travaux. Plus précisément, nous avons utilisé deux espèces appartenant au genre *Populus* : l'hybride *Populus tremula x alba* (ou clone INRAE 717-1B4) et le peuplier noir européen (*Populus nigra* L.)

1. Mise en place du microbiote racinaire de l'arbre

De nombreux travaux relatifs au microbiote racinaire du peuplier sont aujourd'hui disponibles dans la littérature scientifique. Il a notamment été démontré que la composition du microbiote rhizosphérique et endosphérique varie à travers les différents gradients environnementaux (Gottel et al., 2011 ; Shakya et al., 2013 ; Cregger et al., 2018) et entre les génotypes ou espèces de peuplier (Bonito et al., 2014 ; Veach et al., 2019). Il a également été mis en évidence que certains isolats microbiens du peuplier telles que les bactéries endophytes des genres *Enterobacter, Burkholderia* et *Pseudomonas* améliorent la santé, la croissance et le développement de l'arbre

hôte (Taghavi et al., 2009 ; Timm et al., 2016). La différentiation entre les communautés microbiennes du sol et celles de l'endosphère semble être due à la sélection d'un consortium microbien ayant la capacité de pénétrer et de coloniser l'environnement de l'arbre hôte (Gottel et al., 2011). Néanmoins, aucune étude n'a été menée concernant la dynamique de colonisation racinaire du peuplier par les communautés fongiques et bactériennes. Dans le cadre de cette thèse (Chapitre II), nous avons choisi d'étudier cette colonisation en utilisant des jeunes plants de peuplier, inialement cultivés en conditions contrôlées et axéniques, mis au contact de sol naturel de peupleraie. L'utilisation de la technique du métabarcoding combinée à la microscopie confocale nous a permis de caractériser les communautés fongiques et bactériennes ayant colonisé les racines à différents stades de développement. Les résultats soulignent trois stades de colonisation successifs pour les champignons : une phase précoce (de 2 à 4 jours de croissance) marquée par la forte abondance relative (environ 50 %) de champignons saprotrophes et endophytes, une phase intermédiaire (de 7 à 15 jours de croissance) marquée par la sélection des partenaires fongiques par l'hôte et, enfin, une phase tardive (de 30 à 50 jours de croissance) marquée par la forte diminution de l'abondance relative des saprotrophes et des endophytes au profit de l'installation des champignons ectomycorhiziens. En parallèle, nous avons observé une sélection très précoce des communautés bactériennes dans les racines des jeunes peupliers suivie par une évolution lente de la composition du microbiote racinaire jusqu'à 50 jours de croissance.

Bien que ces résultats soient originaux compte tenu du manque de connaissance actuelle sur cette question, des observations complémentaires au microscope pourraient être menées afin de permettre la détection des bactéries endophytes en utilisant l'un des marqueurs bactériens utilisés lors des travaux de thèse de Cora Miquel-Gennoc (DAPI ou TOPRO3 ; Miquel-Guennoc et al., 2018). Ce type d'expérimentation nous permettrait d'observer si les bactéries colonisent l'apex, la surface et/ou l'apoplaste des racines et si cette colonisation est dépendante de celle des champignons.
D'autre part, des analyses de co-occurrence des données de metabaroding/amplicons permettraient peut-être de découvrir de potentielles interactions entre micro-organismes qui pourraient par la suite être validées expérimentalement. Toutefois, le nombre de réplicats peu élevé (5) risque d'être un frein à une telle analyse.

2. Effet du type de sol sur le microbiote racinaire de l'arbre

Dans le cadre de mes travaux de thèse, l'effet du type de sol est apparu comme le facteur environnemental explicatif majeur de la structuration taxonomique et fonctionnelle des communautés microbiennes racinaires (Figure 13). Il est aujourd'hui admis que le sol est le principal réservoir de micro-organismes susceptibles de coloniser la rhizosphère et les racines des plantes (Lareen et al., 2016 ; Fierer et al., 2017).

2. A. Origine du sol

Un des objectifs de ma thèse était de mettre en évidence la contribution relative de l'origine du sol sur la composition taxonomique et fonctionelle du microbiote racinaire du peuplier noir (Chapitre III). La matrice de sol ainsi étudiée était les sédiments de deux rivières localisées dans deux régions de France : la Drôme et la Loire. Les résultats générés sont originaux puisque ce type de matrice est peu étudié dans le cadre de projets scientifiques visant à comprendre le rôle des facteurs abiotiques sur la composition et la structuration du microbiote racinaire des arbres. De plus, contrairement à des matrices associées à des habitats purement terrestres tels que le sol forestier, les sédiments sont soumis à d'autres types de contraintes environnementales telle que la dynamique des cours d'eau qu'ils bordent et les phénomènes de crues et de décrues. Nous avons observé de fortes différences entre les sédiments de la Drôme et ceux de la Loire particulièrement en termes de texture et granulométrie et en terme de fertilité. Ces différentes conditions de sol sont connues depuis longtemps par les agriculteurs et les sylviculteurs car elles se traduisent par des différences de rendements, et par les physiologistes végétaux car elles sont à l'origine de changements de la qualité et de la quantité des exsudats racinaires. Dans notre étude, nous avons observé que la croissance aérienne d'une des trois familles de peupliers noirs étudiées était affectée par le changement de sédiments utilisés pour sa culture. Le microbiote de ces deux types de sédiments présentait également de fortes différences de composition avec 40 % des OTUs bactériens et 80 % des OTUs fongiques spécifiques des sédiments de la Loire ou de ceux de la Drôme.

Dans le but d'étudier si ces changements significatifs de la composition du microbiote entre les deux types de sédiments avaient un impact sur la composition taxonomique et fonctionnelles des communautés microbiennes associées aux racines, l'utilisation de mésocosmes s'est révélée être la technique la plus appropriée. En effet, elle nous a permis de nous rapprocher des conditions naturelles des familles de peupliers étudiées en faisant abstraction des phénomènes de crues et de décrues ayant lieu dans la Loire et la Drôme pour se concentrer uniquement sur l'effet du sol et du climat sur la composition et la structure du microbiote racinaire. Grâce à ce dispositif expérimental, nous avons mis en évidence la forte contribution du type de sol qui est le facteur de structuration et de composition le plus important pour la colonisation des racines de peuplier (Bonito et al., 2014). En effet, nous avons observé un changement significatif de la composition taxonomique des communautés de bactéries et de champignons associées à la rhizosphère et à l'endosphère entre les jeunes arbres cultivés dans leurs sédiments d'origine et ceux cultivés dans les sédiments originaires de l'autre rivière. Néanmoins, nous avons observé que les communautés fongiques rhizosphériques et endosphériques étaient plus sensibles au changement de sédiments que les communautés bactériennes. Bien que cette observation ait également été réalisée dans d'autres études, elle semble majoritairement concerner les champignons ectomycorhiziens (Goldmann et al., 2015 ; Sun et al., 2017). Dans notre cas, le changement de sédiments pour la culture des jeunes peupliers noirs a engendré une modification significative de l'abondance relative des champignons mycorrhiziens à arbuscule et des pathogènes. Bien que ces deux guildes fongiques sont plutôt minoritaires dans la rhizosphère et l'endosphère des peupliers noirs, l'effet de l'orgine des sédiments sur la composition fonctionelle du microbiote racinaire du peuplier noir reste à étudier. Se pose ègalament la question de savoir si

les champignons AM sont si minoritaires que cela en réalité. En effet, plusieurs travaux ont mis en évidence que le séquençage haut débit des marqueurs 18S et ITS est difficile pour les champignons AM (Danielsen et al., 2012 ; Bonito et al., 2019) et ne donne pas une bonne image des proportions réelles des champignons AM dans les échantillons de sol ou de racines (Chapitre II de cette thèse). Des analyses supplémentaires avec d'autres amorces pour amplifier l'ADN des champignons AM sont à réaliser (Voriskova et al., 2017).

2. B. Variation du microbiote du sol au cours du temps

Au cours de cette thèse, je me suis également interéssée à l'effet de la variation de la composition du microbiote du sol ayant lieu, par exemple, à la même saison d'une année à l'autre, sur la composition et la structure taxonomique et fonctionnelle du microbiote racinaire de l'arbre (Chapitre IV). Bien qu'il ait été démontré que la dynamique saisonnière influence de manière significative les communautés bactériennes de la rhizosphère et de l'endosphère de *Populus deltoides* (Cregger et al., 2018), aucune étude recensée dans la littérature scientifique ne concerne l'impact des variations inter-annuelles sur les communautés microbiennes associées aux racines du peuplier.

Les principaux résultats de notre étude, publiée en mars 2020 dans le journal *Phytobiomes* mettent en évidence l'effet significatif de la variation de la composition du microbiote du sol d'une année à l'autre sur la composition du microbiote racinaire et sur le métabolome du peuplier. En effet, la diminution des précipitations cumulées au cours de l'hiver et du printemps de la deuxième année de notre expérience a été corrélée avec un changement significatif des communautés microbiennes du sol dont une réduction significative de la diversité bactérienne. Ces observations sont en cohérence avec la littérature puisque des études antérieures ont démontré que les communautés bactériennes sont plus sensibles aux variations environnementales que les communautés fongiques (Barnard et al., 2013 ; Ochoa-Hueso et al., 2018). Comme dans le sol, un changement significatif de la composition et de la structure taxonomique et fonctionnelle des communautés microbiennes a été observée dans les racines de peupliers suggérant que le sol est le principal facteur impliqué dans l'assemblage du microbiote racinaire comme l'ont démontré d'autre travaux (Bonito et al., 2014 ; Veach et al., 2019). Cependant, d'autres études mais, cette fois réalisées sur des plantes herbacées, ont démontré que les racines exercent un recrutement des communautés microbiennes du sol indépendant de la composition de l'inoculum source (Edwards et al., 2015 ; Perez-Jaramillo et al., 2016). Ces informations mettent en évidence l'importance relative des différents facteurs intrinsèques et/ou extrinsèques sur la composition du microbiote racinaire en fonction de l'hôte étudié. En parallèle, l'étude du métabolome racinaire des deux générations de jeunes peupliers cultivés en serre lors des deux années de l'expérience nous a permis d'observer une altération des métabolismes primaire et secondaire liée à la modification du microbiote racinaire.

Aborder des questions d'écologie microbienne d'un point de vue fonctionnel dans un environnement si dynamique et si hétérogène que le sol est un véritable enjeu compte tenu des différents facteurs qui modulent de

façon directe ou indirecte les interactions entre les différents acteurs. Dans l'étude ci-dessus, l'objectif était de comprendre, d'une part, le lien entre le climat et les micro-organismes du sol et, d'autre part, le lien entre microbiote et métabolisme racinaire. La première question a été abordée en prélevant du sol naturel de peupleraie. Cette dernière est un modèle de choix puisque, bien qu'elle soit dominée par d'autres espèces de peuplier que le clone INRAE 717-1B4, elle abrite un cortège de micro-organismes caractéristiques de la rhizosphère du peuplier tout en étant soumise aux variations environnementales naturelles des aires d'occupation de cette essence. Cependant, on peut se demander si le cortège microbien régit par les caractéristiques propres du site telles que l'âge des arbres, le nombre d'arbres ou encore la présence de racines de plantes adventives est adapté pour observer les variations du microbiote racinaire de jeunes boutures de peupliers. Nous pourrions réaliser un échantillonnage des racines des arbres présents sur le site pour caractériser la composition du microbiote racinaire de ces arbres et ainsi la comparer avec nos résultats.

3. Effet du climat sur le microbiote racinaire de l'arbre

En plus des caractères physico-chimiques et de l'origine du sol, le climat est un facteur important de régulation du microbiote racinaire des arbres. Il peut avoir un impact direct mais également un impact indirect sur le microbiote racinaire des arbres, d'une part en modifiant la composition du microbiote du sol, principal réservoir de micro-organismes pour les racines et, d'autre part, en altérant la physiologie et le métabolisme de l'arbre hôte (Figure 13).

L'effet du climat (température et luminosité) sur le microbiote racinaire de l'arbre a été étudié grâce à une expérience en mésocosmes. Ce système nous a permis d'observer l'impact des températures de deux régions climatiquement contrastées (une différence moyenne de 2,6 °C a été enregistrée entre les deux sites sur la période de notre expérience) sur les communautés de micro-organismes colonisant la matrice de sol et celles colonisant la rhizosphère et l'endosphère de l'arbre (Chapitre III). Pour ce faire, nous nous sommes focalisés sur les communautés de bactéries et de champignons associées à la rhizosphère et aux racines du peuplier noir, espèce ripisylve menacée par les effets du changement climatique. Des différences significatives ont été observées concernant la croissance aérienne des jeunes peupliers noirs cultivés sous le climat opposé par rapport à celle des jeunes arbres cultivés sous leur climat d'origine. Par exemple, les individus appartenant à la progénie D15 poussait beaucoup moins bien dans la Loire (- 75%) que dans la Drôme suggérant que la diminution de la température moyenne et la diminution de la fertilité de la matrice sol sont responsables d'une altération significative de la croissance aérienne des jeunes arbres. Cette hypothèse est en cohérence avec d'autres études menées sur les arbres à feuilles caduques montrant que l'augmentation de température ainsi que la fertilité du sol favorisent la croissance du hêtre, du chêne et du frêne (Levesque et al., 2016). De plus, nous avons observé un changement significatif de la composition taxonomique des communautés bactériennes et fongiques associées à la rhizosphère et l'endosphère entre les peupliers noirs cultivés sous leur climat d'origine et ceux cultivés sous le climat opposé. D'un point de vue trophique, une différence significative a été observé

concernant la distribution relative des guildes fongiques associés uniquement au compartiment rhizophérique entre les peupliers noirs cultivés sous leur climat d'origine et ceux cultivés sous le climat opposé. En effet, les champignons saprotrophes et ectomycorhiziens étaient les champignons les plus impactés en terme d'abondance relative par le changement de condition climatique dans la rhizosphère alors que l'abondance relative des champignons mycorhiziens à arbuscule et les pathogènes était significativement différente dans l'endosphère entre les arbres cultivés sous leur climat d'origine et ceux cultivés sous le climat opposé.

Cette expérimentation en mésocosmes nous a permis de remédier aux problèmes d'interprétation induits par les conditions naturelles variables dans le temps et l'espace. De plus, cette approche était la plus appropriée pour transporter facilement les jeunes arbres dans les sites d'intérêt afin d'observer l'effet du climat d'une région sur les peupliers originaires de l'autre région. Néanmoins, les conditions régnants au sein des mésocosmes peuvent être très différentes des conditions naturelles. Les études en micro ou mésocosmes soustraient de l'équation de nombreuses variables et de nombreux types d'interactions entre les plantes, les micro-organismes et les paramètres variables de l'environnement, tels que les flux d'eau. En effet, bien que la dynamique du cours d'eau n'ait pas été prise en compte dans notre étude, il est facile d'imaginer que ce paramètre soit très important pour la germination des graines puis le développement des semis de peupliers noirs. Les habitats contenus dans chaque mésocosme n'ont pas cette dynamique, ce qui les éloignent beaucoup des conditions que l'on peut retrouver en milieu naturel. Ainsi, l'utilisation de mésocosmes permet de faciliter la compréhension de processus naturels en se concentrant uniquement sur quelques paramètres d'intérêts mais il est difficile de transposer ces résultats directement sur un écosystème naturel. Une des perspectives à ce projet serait de réaliser un échantillonnage des sédiments et des racines de peupliers noirs directement sur les deux sites d'intérêt. Cela nous permettrait de connaître l'effet des flux d'eau sur la composition et la structure du microbiote des sédiments et des racines en comparant ces résultats avec ceux précédemment récoltés suite à notre expérimentation en mésocosmes.

4. Effet de la plante sur le microbiote racinaire de l'arbre

Enfin, les derniers paramètres étudiés lors de cette thèse sont ceux liés directement à l'arbre hôte (Figure 13). Chez le peuplier, le génotype est connu pour être un facteur important dans la structuration et la composition du microbiote racinaire mais minoritaire par rapport aux facteurs extrinsèques comme le sol (Bonito et al., 2014 ; Veach et al., 2019 ; Bonito et al., 2019). De plus, s'il est aujourd'hui admis que les arbres ont la capacité de sélectionner, pour une part, les micro-organismes avec lesquels ils s'associent via la rhizosphère (Calvaruso et al., 2010) et l'endosphère, les mécanismes impliqués dans cette sélection sont mal connus. Nous avons émis l'hypothèse que les voies de défense, en particulier, la voie de signalisation et de biosynthèse de l'acide jasmonique (AJ) employée contre les pathogènes jouent un rôle dans cette sélection.

4. A. Effet « génotype »

L'impact du génotype sur la structure et la composition taxonomique du microbiote du peuplier a été testée dans le cadre du projet POPMICROCLIM (Chapitre III). En effet, nous souhaitions observer si les différentes progénies du peuplier noir (*Populus nigra*) originaires de la Drôme et de la Loire présentaient des différences significatives en termes de structure et de composition des communautés de micro-organismes associées à la rhizosphère et à l'endosphère.

En conditions natives de culture, nous avons observé une différence de croissance des parties aériennes des jeunes plants de peupliers noirs appartenant aux trois progénies originaires de la Drôme (D11, D13 et D15) et la Loire (L04, L06, L08). De plus, l'étude des communautés bactériennes et fongiques présentes dans la rhizosphère et l'endosphère de ces jeunes arbres nous a permis de mettre en évidence certaines différences significatives concernant la composition des communautés microbiennes de la rhizosphère entre les trois progénies originaires du même site (Drôme ou Loire). Ces différences détectées au niveau du phylum et du genre pour les bactéries et uniquement au niveau du genre pour les champignons concernaient les communautés microbiennes les plus abondantes détéctées dans la rhizosphère (Chloroflexi ≅ 20%, *Niastella* ≅ 1.5% et le champignon EcM *Geopora* ≅ 10% dans la rhizosphère des progénies de la Drôme, Actinobacteria ≅ 15% et *Geopora* ≅ 15% dans la rhizosphère des progénies de la Loire). A ce stade, deux questions se sont posées : les différences observées au niveau de la composition des microbiotes racinaires entre les différentes progénies sont-elles essentiellement liées au taux de croissance de chaque progénie ? La présence de certaines communautés microbiennes est-elle responsable de la différence de croissance aérienne constatée entre les trois progénies ? Pour répondre à ces questions, nous pouvons envisager une expérimentation visant à mettre en contact de façon individuelle une des souches identifiées (*Niastella* et *Geopora*) et chacune des progénies de peupliers noirs précédemment citées afin de comprendre si ces souches d'intérêt sont réellement reponsables de l'augmentation de croissance aérienne des jeunes arbres.

En conditions de culture transplantée (sédiments et climat non-natifs), nous avons observé une diminution de la croissance aérienne des progénies de la Drôme et une augmentation de la croissance aérienne des progénies de la Loire. De plus, nous avons observé une colonisation significativement plus abondante de la rhizosphère des progénies de la Drôme par le champignon ECM *Geopora*. Bien que les champignons EcM dont *Geopora* soient connus pour promouvoir la croissance des arbres (Müller et al., 2013 ; Van der Heijden et al., 2015 ; Gehring et al., 2017), l'amélioration de la croissance n'a été constatée que dans l'une des deux conditions de transplantation testées pour les progénies de la Drôme. Néanmoins, nos observations et celles réalisées sur d'autres génotypes de peupliers (Bonito et al., 2014 ; Veach et al., 2019) suggèrent que les conditions de culture c'est-à-dire les facteurs environnementaux sont les paramètres les plus influants de la croissance et de la composition du microbiote racinaire des arbres.

4. B. Rôle des hormones de défense

Dans le chapitre V, nous nous sommes interessés à l'effet de la voie de signalisation de l'AJ sur la composition et la structure du microbiote racinaire du Peuplier. Pour ce faire, nous avons utilisé des boutures de peuplier (*Populus tremula x alba*) génétiquement modifié pour exprimer de façon constitutive le gène fongique MiSSP7. L'effecteur fongique MiSSP7 est connu pour être sécrété spécifiquement par le champignon ectomycorhizien *Laccaria bicolor* dans les cellules racinaires du peuplier afin de bloquer la voie de signalisation de l'AJ via son interaction avec le répresseur JAZ6 et, ainsi, coloniser les racines de l'arbre et permettre la mise en place de la symbiose ectomycorhizienne (Plett et al., 2014). Quatre lignées de peupliers MiSSP7 ont été générées par le Dr Jonathan Plett (Plett et al., 2011). Après une période d'acclimatation dans du sol naturel, ces quatre lignées issues de la même transformation génétique ont été maintenues en serre de confinement OGM depuis leur génération en 2011.

Bien que nous ne connaissons pas précisément l'effet de l'expression de MiSSP7 chez le peuplier sur la voie de signalisation de l'AJ, notamment sur les gènes régulés par l'interaction LbMiSPP7 / PtJAZ6 (Thèse de Veronica Basso 2016-2019), nous avons fait l'hypothèse que cette expression constitutive module la voie de signalisation de la phytohormone. Cette modulation se ferait en empêchant l'AJ d'être actif via une compétition au niveau du site de liaison de l'AJ à la protéine PtJAZ6.

Une première expérimentation a été débutée au printemps 2016 (Année 1) en serre afin de comparer le microbiote racinaire de ces boutures de « peupliers MiSSP7 » et celui de peupliers non génétiquement modifiés (*Populus tremula x alba* sauvages). Le témoin « plasmide vide » ayant été perdu lors de l'acclimatation des plants in vitro dans du sol en serre en 2011, il nous a été impossible de connaître l'effet direct de la transfection du matèriel génétique sur la physiologie et le phénotype des peupliers testés.

Les résultats de l'année 1 montrent un effet significatif de l'expression ectopique de MiSSP7 sur les communautés fongiques, et, dans une moindre mesure, sur les communautés bactériennes de la rhizosphère et de l'endosphère. Nous avons observé une modification de la balance de colonisation racinaire entre les champignons ectomycorhiziens et les endophytes. Plus précisément, les racines de peupliers MiSSP7 sont significativement plus colonisées par des champignons ectomycorhiziens alors que les racines des peupliers sauvages sont significativement plus colonisées par des endophytes. De plus, ces résultats ont été confirmés par l'analyse métabolomique des racines de peupliers. En effet, une accumulation de sucres marqueurs des associations symbiotiques (trehalose, glucose et fructose) a été observée dans les racines des peupliers MiSSP7 par rapport aux racines de peupliers sauvages, suggérant une augmentation significative de la colonisation racinaire par des champignons.

Suite à l'obtention de ces résultats encourageants, nous avons choisi de répéter cette expérimentation l'année suivante (en 2017, Année 2) afin de confirmer nos premières observations. En plus des approches déjà réalisées durant l'expérimentation de l'année 1 (Séquençage haut débit d'amplicons 16S rRNA et ITS rDNA et analyse du

métabolome racinaire), nous avons choisi d'étudier l'effet de l'expression de MiSSP7 sur la physiologie, le transcriptome du peuplier et le métatrancriptome eucaryote rhizosphérique.

En comparant le microbiote racinaire des boutures de peupliers MiSSP7 et celui des boutures de peupliers non génétiquement modifiés (*Populus tremula x alba* sauvages) cultivées l'année 2, nous avons observé un effet constrasté de l'expression de MiSSP7 sur les communautés de micro-organismes (champignons et bactéries) de la rhizosphère et de l'endosphère par rapport à l'année 1. Si l'abondance relative des endophytes fongiques est significativement plus élevée dans les racines des peupliers sauvages, nous n'avons pas observé d'augmentation significative des champignons ectomycorhiziens dans les racines des peupliers MISSP7 l'année 2. De plus, les métabolites significativement régulés par l'expression de MiSSP7 l'année 2 ne sont pas les mêmes que ceux identifiés l'année 1.

Trois hypothèses peuvent alors être émises pour expliquer la différence de résultats obtenus à la suite des expérimentations de l'année 1 et l'année 2 :

- Nous avons constaté un effet significatif de l'expression de MiSSP7 chez le peuplier sur la communauté de champignons ectomycorhiziens dominée l'année 1 par *Paxillus involutus*. Aucune OTU assignée à cette espèce n'a été détectée dans les jeux de données de l'année 2 suggérant l'absence totale de ce champignon. Bien que MiSSP7 soit une protéine spécifiquement sécrétée par le champignon *Laccaria bicolor*, nous savons que *Paxillus involutus* possède également la capacité de sécréter ce genre de protéines pour coloniser les racines des arbres hôtes (Kohler et al., 2015). Cette capacité n'est pas encore connue chez les champignons ectomycorhiziens appartenant aux *Thelephoraceae*, famille fongique dominante dans les racines des peupliers cultivés l'année 2. Nous pensons donc que l'effet significatif de l'expression de MiSSP7 observé l'année 1 sur les communautés fongiques rhizosphérique et endosphérique peut être lié à (i) l'action simultanée des MiSSP sécrétée par *Paxillus involutus* ou (ii) que l'action des MiSSP de *Paxillus involutus* sur la voie de signalisation utilisée par ce champignon pour la mise en place de la symbiose est la même que celle utilisée par *Laccaria*. La symbiose ectomycorhizienne étant apparue à plusieurs reprises dans l'évolution (Kohler et al., 2015), il est envisageable que plusieurs champignons utilisent la même voie (*Paxillus* / *Laccaria*) mais aussi que d'autres utilisent des voies alternatives (*Thelephora*) et donc ne bénéficient pas de l'effet de MiSSP7.

Des expériences complémentaires pourront être menées afin de comprendre les différences existantes entre les mécanismes de colonisation de *Laccaria*, *Paxillus* et *Thelephora*. Bien que le génôme de *Thelephora terrestris* vient tout juste d'être séquencé (Mycocosm, UH-Tt-Lm1 v1.0), d'autres expériences devront être menées pour découvrir l'existence potentielle d'effecteurs fongiques chez ce champignon. En effet, l'ADN utilisé pour réaliser le séquençage de ce génôme a été extrait à partir du mycélium du champignon. De ce fait, des expériences de mycorhization contrôlée en boîtes de Pétri (entre *Populus tremula x alba* et *Thelephora terrestris*) pourront être réalisées pour confirmer ou réfuter notre hypothèse.

- Nous avons observé une légère diminution du taux d'expression de MiSSP7 dans les boutures de peupliers génétiquement modifiés cultivées l'année 2 par rapport à celles cultivées l'année 1. De plus, un certain nombre de boutures récoltées pour l'expérience de l'année 2 n'exprimaient plus le gène fongique. Une perte de transgène a déjà été décrit chez plusieurs arbres génétiquement modifiés dont le peuplier (Hawkins et al., 2003 ; Li et al., 2009). Cette observation suggère que certaines parties du plant mère ont perdu ou inactivé le vecteur de transformation induisant ainsi une perte d'efficacité de l'altération de la voie de signalisation de l'AJ. Bien que ces boutures ait été retirées de nos analyses, nous ne pouvons pas totalement exclure que les boutures utilisées présentent une altération réelle de leur voie de signalisation de la phytohormone d'intérêt. Pour répondre à cette question, nous pourrions essayer de récupérer des exsudats racinaires à partir des boutures de peupliers sauvages et MiSSP7 et d'analyser leur composition afin d'observer si l'expression de MiSSP7 est corrélée avec une modification de la concentration en AJ (Michalet et al., 2013 ; Carvalhais et al., 2015).
La classification choisie pour tester l'impact du taux d'expression de MiSSP7 sur la composition et la structure du microbiote racinaire du peuplier pourrait être à l'origine de biais concernant les observations réalisées l'année 2. En effet, la différence de niveau d'expression entre les boutures classifiées comme faiblement exprimantes (MiSSP7_Low expression) et celles classifiées comme fortement exprimentantes (MiSSP7_High expression) est faible, par exemple 0.02. Des analyses complémentaires devront être effectuées afin de s'assurer que cette classification ne fausse pas les résultats. Par exemple, une catégorie intermédiaire pourrait être ajoutée pour classifier les boutures avec une expression modérée du gène d'intérêt (MiSSP7_Medium expression).

- Enfin, un problème technique pourrait également expliquer les différences observées entre les deux années de manipulations. En effet, bien que nous ayons pris soin de suivre exactement le même protocole d'échantillonnage l'année 1 et l'année 2, il est possible que la multiplication des approches expérimentales utilisées l'année 2 ait développé un biais lié à l'utilisation de différentes parties du système racinaire utilisées, par exemple, pour l'extraction d'ADN.

En résumé, aucune conclusion ne peut actuellement être faite concernant l'impact de la voie de signalisation de l'AJ sur la composition et la structure taxonomique du microbiote racinaire du peuplier. Les observations réalisées lors de la première expérimentation restent à confirmer. Une des perspectives envisagées pour confirmer nos observations est la répétition de cette expérimentation en comparant, cette fois-ci, la structure et la composition du microbiote rhizosphèrique et endosphérique de boutures de peupliers ARNi (ARN à interférence) PtJAZ6 mis au point par Félix Fracchia (thèse 2019-2022) avec celles de boutures de peupliers sauvages. L'interaction entre LbMiSSP7 et PtJAZ6 permet la stabilisation du complexe et, ainsi, la répression des gènes de la voie de signalisation induite par l'AJ (Plett et al., 2014). Ainsi en l'absence du représseur PtJAZ6, les gènes de la voie de signalisation induite par l'AJ devraient être constamment exprimés dans les boutures de peupliers mutants. Par conséquent, si l'hypothèse émise lors de notre première expérimentation est correcte, nous

devrions observer une diminution significative de la colonisation des racines de peupliers ARNi PtJAZ6 par les champignons EcM en comparaison avec les peupliers sauvages et les peupliers MiSSP7.

5. Le « core microbiome » du Peuplier

Le concept de « core microbiome » ou « microbiome central » en français est initialement apparu dans le domaine médical. Il a été défini comme les communautés microbiennes essentielles au bon fonctionnement de l'écosystème. Plus particulièrement, il s'agit de la partie du microbiome conservée entre individus d'une même espèce jouant un rôle essentiel dans la santé et la nutrition de l'hôte. De plus, il est aujourd'hui admis que le microbiome central d'une plante est constitué de taxons microbiens essentiels pour la fitness de la plante hôte, établis par des mécanismes évolutifs de sélection et d'enrichissement de taxons microbiens contenant des gènes importants pour la fitness de l'holobionte (Compant et al., 2019).

Chez le peuplier, les travaux de Shakya et al. (2013) ont mis en évidence un microbiome central de la rhizosphère de *Populus deltoides* comprenant seulement 35 OTUs bactériens, appartenant principalement aux ordres des *Burkholderiales* et *Rhizobiales*, et 4 OTUs fongiques principalement assignés au genre *Mortierella*, *Neonectria* et *Exophiala*. Parallèlement, les travaux de Timm et al. (2018) ont permis la détection de 97 OTUs bactériens dont l'abondance relative est significativement régulée lors de stress environnementaux tels que la sécheresse, le manque de luminosité et la présence d'une grande concentration de métaux dans le sol.
Dans le cadre de ma thèse, le microbiome central du peuplier gris (*Populus tremula x alba*) et celui du peuplier noir (*Populus nigra*) a pu être établi en tenant compte des OTUs fongiques et bactériens associées à l'endosphère de l'ensemble des arbres échantillonnés dans nos études (sans prendre en compte le type se sol, le climat, le temps de colonisation ou l'année d'échantillonnage).
Le microbiome central du peuplier gris comprend 20 OTUs fongiques appartenant majoritairement aux genres *Meliniomyces* (champignon ectendomycorhizien) et *Cadophora* (endophyte) et 52 OTUs bactériens appartenant majoritairement aux ordres *Burkholderiales* et *Sphingobacteriales* alors que le microbiome central du peuplier noir est constitué de seulement 2 OTUs fongiques attribué au genre *Tritirachium* (saprotrophes) et 45 OTUs bactériens majoritairement assignés aux *Burkholderiales.* Si le microbiome central bactérien du genre *Populus* semble conservé, le microbiome central fongique semble varier entre les génotypes d'un point de vue structural. On peut alors se demander quel est le rôle du microbiome central endosphérique. Ces micro-organismes conservés sont-ils réellement sélectionnés par l'arbre hôte ou correspondent-ils à des micro-organismes ubiquistes adaptés à toutes les conditions ?

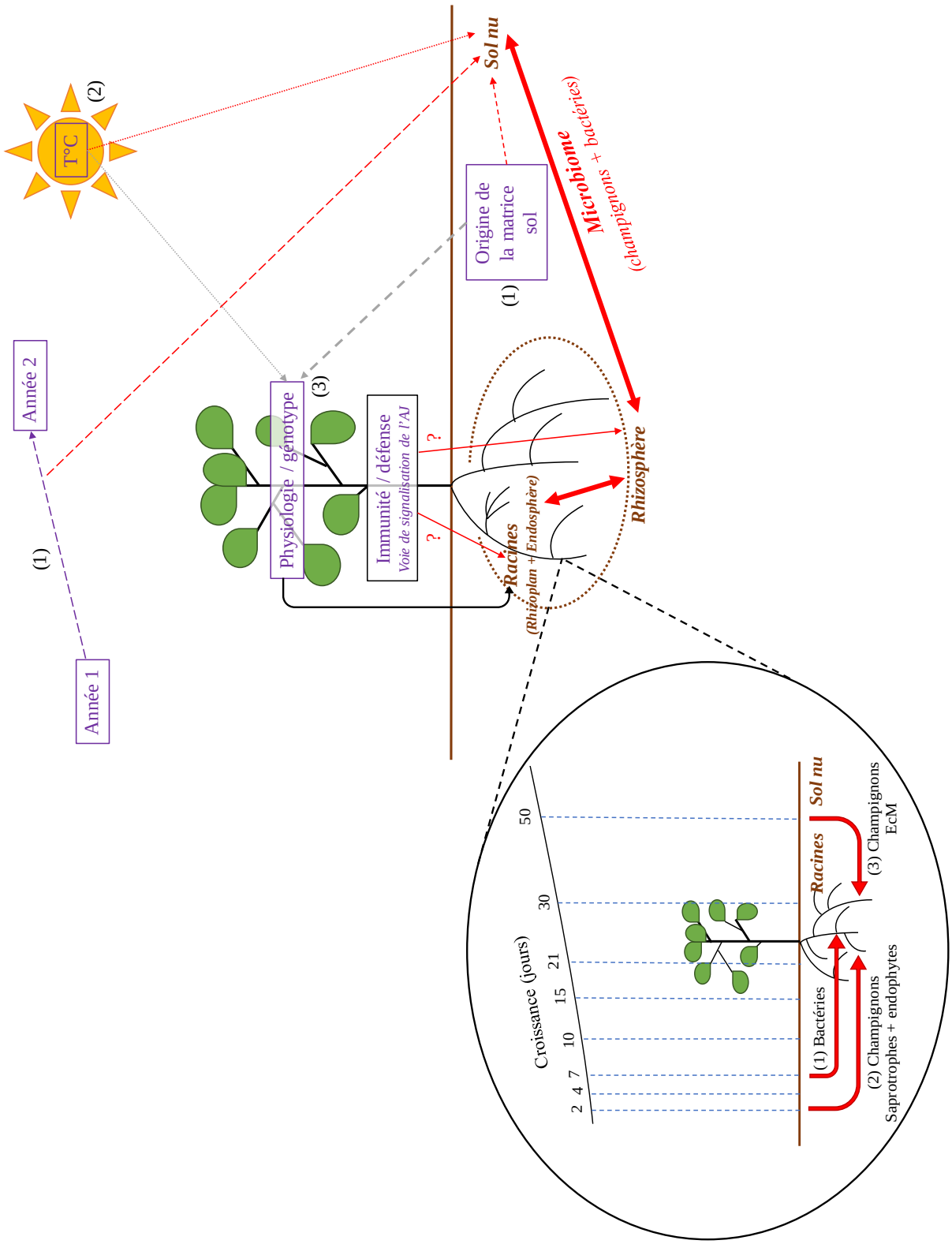


Figure 46 - Modèle de synthèse de la régulation du microbiote du peuplier réalisé à partir des résultats obtenus au cours de cette thèse.

II. Perspectives

Les résultats de ma thèse ont permis de faire avancer les connaissances dans le domaine des interactions arbres / micro-organismes malgré les biais techniques ou biologiques expliqués dans la section précédente. De nouvelles interrogations sont apparues et pourront trouver réponse dans les expérimentations déjà réalisées, en cours ou à venir. Par exemple, il serait intéressant de procéder à des échantllionnages directement sur le terrain afin d'étudier l'effet de la densité d'arbres ou encore l'effet de l'âge de l'arbre hôte sur la composition et la structure taxonomique et fonctionnelle des communautés de micro-organismes associées à la rhizosphère et aux racines de l'arbre (Shakya et al., 2013 ; Bonito et al., 2014 ; Beckers et al., 2017 ; Cregger et al., 2018).

1. Effet du stress hydrique sur le microbiote racinaire du peuplier

Dans le Chapitre IV de cette thèse, nous avons étudié l'impact d'une variation de la composition du microbiote du sol d'une peupleraie sur la composition et la structuration taxonomique et fonctionnelle du microbiote racinaire. Les résultats de cette étude suggeraient que la réduction du niveau de précipitations cumulées au cours de l'hiver entraîne un changement de composition du microbiote du sol.

Le peuplier noir est une espèce ripisylve particulièrement résistante au stress hydrique notamment lors des phénomènes de décrues (Villar & Forestier, 2009). Afin de comprendre le lien entre le génotype, le microbiote racinaire et la résistance au stress hydrique du peuplier noir, une expérience en rhizotrons a été mise en place et menée à la pépinière ONF (Office National des Forêts) de Guémené-Penfao au printemps 2018. Dans le but d'étudier les mécanismes génétiques impliqués dans la résistance des jeunes plants de peupliers noirs au stress hydrique, des graines provenant d'arbres mères originaires de la Drôme et de la Loire (utilisées lors de l'expérimentation détaillée dans le Chapitre III) ont été mises à germer puis transférées dans des rhizotrons contenant les sédiments de la Loire ou de la Drôme. Des triplicats de chaque rhizotron ont été réalisés afin de comparer le microbiote racinaire des plants arrosés et de ceux soumis à un stress hydrique. L'arrosage a été stoppé de manière différentielle en fonction de la matrice sol. L'accroissement des tiges et des racines a été surveillées durant toute la durée de l'expérience, puis les racines et le sol ont été récoltés en septembre 2018 pour déterminer la composition et la structuration taxonomique et fonctionnelle du microbiote racinaire des différents génotypes. L'ADN des échantillons de sol et de racines ont été extraits et les amplifications PCR des marqueurs taxonomiques bactériens (16S rRNA) et fongiques (région ITS) réalisées. Les produits amplifiés ont été envoyés pour marquage et séquençage MiSeq Illumina Next Generation à la plateforme de séquençage GeT PlaGe INRAE de Toulouse, France.

2. Approche SIP (Stable Isotope Probing) : déterminer les communautés microbiennes actives du microbiote racinaire de l'arbre

Le marquage à l'aide d'isotope stable (SIP ou Stable Isotope Probing en anglais) est une technique utilisée notamment en écologie microbienne pour tracer les flux de nutriments entre l'organisme hôte et son cortège microbien. Pour ce faire, un substrat est enrichi d'un isotope stable plus lourd qui est consommé par les micro-organismes à étudier. L'utilisation de cette technique dans le cadre de mes travaux de thèse nous permettrait de connaître précisemment quelles sont les communautés microbiennes actives du microbiote racinaire de l'arbre. Cette information a déjà été obtenue dans le cadre de ma thèse (Chapitre V) via les analyses métatranscriptomiques réalisées sur les racines de peupliers génétiquement modifiés et celles des peupliers sauvages. Nous avons mis en évidence que les champignons EcM sont les micro-organismes les plus actifs de la communauté fongique associée aux racines des boutures. Néanmoins, aucune conclusion n'a pu être faite concernant les communautés bactériennes.

Bien que le marquage à l'aide d'isotope stable ait été utilisé dans des études concernant le microbiote de plantes herbacées (Guyonnet et al., 2018) ou de plantes de grandes cultures telles que le maïs, le blé (Haichar et al., 2008) et le colza (Gkarmiri et al., 2017), aucun travail n'est disponible dans la littérature sur la détection par SIP de communautés actives de micro-organismes associées aux racines d'arbres.

Lors de la culture des boutures de peupliers dans des pots contenant le sol naturel de peupleraie en serre, un marquage au carbone 13 ($^{13}CO_2$) a été réalisé pendant 24 heures sur un lot de peupliers sauvages (non transformés) avec l'aide du Docteur Caroline Plain (UMR 1137, Silva, INRAE Nancy). Les feuilles, les tiges, les racines, le sol rhizosphérique et le sol nu ont été récoltés pour chaque bouture marquée et non marquée. Ces échantillons ont été directement plongés dans l'azote liquide puis conservés à -20°C avant d'être broyés en fine poudre pour de prochaines analyses.

Les premiers résultats de cette expérimentation ont mis en évidence un radiomarquage effectif des différents tissus des jeunes arbres et du sol ainsi qu'une consommation du CO_2 marqué par les micro-organismes au bout de 24 heures.

3. Effet des exsudats racinaires sur le microbiote du sol

Suite à l'obtention des résultats de métabolomique relatifs aux expériences détaillées dans les chapitres IV et V, une expérience a été menée dans le but de tester l'effet de chaque métabolite d'intérêt sur les communautés de micro-organismes du sol naturel de peupleraie. En supposant qu'ils soient sécrétés dans la rhizosphère dans les mêmes quantités et sous la même forme qui a permis leur détection dans les racines des jeunes arbres, nous souhaitions connaître les rôles de ces métabolites dans la prolifération ou, au contraire, dans l'inhibition de certains taxons microbiens. Les analyses du sol marqué au carbone 13 issues de l'expérimentation précédente (II. 2.), réalisées par GC-MS par le Dr Zahar Haichar (UMR 5557 Ecologie Microbienne, Université de Lyon) confirme la présence de ces métabolites sécrétés par les jeunes peupliers mais également par les communautés

de micro-organismes. En effet, les résultats préliminaires de ces analyses mettent en évidence la présence de tréhalose, mannitol, saccharose, acide benzoïque, glucosamine, galactose et glucose dans la rhizosphère.

Pour se faire, du sol de peupleraie (utilisé dans les expériences des Chapitres IV et V) tamisé, séché et humidifié jusqu'à un taux de 80 %, a été réparti dans des boîtes de Pétri de 4,5 cm de diamètre (4 boîtes par composé à tester). Dix composés ont été testés : le saccharose, le glucose, le mannitol, l'arbutine, l'hydroquinone, l'acide salycilique, le methyl-jasmonate (MeJA), l'acide jasmonique et une solution d'exsudats racinaires récoltés à partir de racines de boutures de peuplier (*Populus tremula x alba*). Chaque jour, 1 mL de solution contenant le métabolite d'intérêt a été ajouté dans les bôites de Pétri ainsi que 1 mL d'une solution stérile de NH_4NO_3 afin de garantir le ratio carbone/azote typique d'un sol de peupleraie (environ 10). Au bout de 24h, 48h et 8 jours, environ 250 mg de sol a été prélevé dans chaque boîte et conservé à -20 °C jusqu'à l'extraction d'ADN puis l'amplification par PCR des marqueurs taxonomiques bactérien (16S rRNA) et fongiques (région ITS). Ces amplicons ont ensuité été envoyés à la plateforme de séquençage GeT-PlaGe (INRAE Occitanie-Toulouse) pour ajout des codes-barres puis séquençage haut-débit (Illumina MiSeq).

L'analyse des premiers résultats met en évidence une stabilité de la composition et de la structure des communautés bactériennes du sol collecté après 48h et 8 jours entre chaque métabolite testé, et ce, à chaque rang taxonomique. Une étude réalisée sur *Pinus radiata* a notamment démontré que l'ajout de métabolites d'intérêt (sucres et acides organiques) dans le sol provoquait un changement significatif de la composition et de la structure des communautés bactériennes du sol récolté après 15 jours (Shi et al., 2011).

Concernant les communautés de champignons, le MeJA semble favoriser la prolifération des genres *Penicillium* et *Talaromyces* au détriment des champignons appartenant au genre *Cryptococcus* au bout de 8 jours. S'agissant de moisissures, les observations réalisées restent discutables. En effet, le développement de ces moisissures semble être lié à l'accumulation de la solution testée dans la boîte de Pétri au cours du temps. L'expérience sera répétée en utilisant des contenants plus grands et plus profonds afin d'améliorer l'absortion complète des solutions de métabolites apportées aux échantillons de sol.

4. Effet de l'acide salycilique, de l'éthylène et de l'acide gibbérilique sur le microbiote racinaire du peuplier

Le microbiote racinaire des plantes et des arbres est à l'origine de l'amélioration de la croissance et du développement de l'hôte mais également de l'amélioration de ses défenses face aux stress biotiques et abiotiques. L'acide gibbérélique (AG), l'acide jasmonique (AJ), l'acide salycilique (AJ) et l'éthylène (ET) sont les quatre phytohormones majeures impliquées dans l'équilibre croissance / immunité et sont induites lors d'attaques de la plante hôte par des micro-organismes pathogènes ou des parasites (Jones & Dangl, 2006 ; Badri & Vivanco, 2009).

Un des principaux objectifs de ma thèse était de comprendre le rôle de la voie de signalisation de l'acide jasmonique sur la composition et la structuration taxonomique et fonctionnelle du microbiote racinaire du peuplier. Nos premiers résulats ont montré que l'altération de la voie de signalisation de l'AJ via l'expression de

l'effecteur fongique MiSSP7 chez le peuplier modifie la composition des communautés fongiques associées aux racines des arbres transformés génétiquement par rapport aux arbres non transformés (Chapitre V). De ce fait, il serait interessant de procéder au même type d'expérience en comparant, cette fois-ci, le microbote racinaire de peupliers sauvages et de peupliers modifiés génétiquement dans les voie de signalisation et/ou de biosynthèse des trois autres hormones de défense. Deux types de lignées de peupliers transgéniques, surexpresseurs et sous-expresseurs, ont été générés à partir du clone 717-1B4 *Populus tremula x alba* (Félix Fracchia, Master 2). Le premier type de lignée surexprime des gènes impliqués dans les voies de biosynthèse et de signalisation de l'AG, et de l'AS tandis que le second type de lignée réduit l'expression de ces mêmes gènes par extinction via interférence ARN (Félix Fracchia, Master 2). A l'heure actuelle, ces différentes lignées sont maintenues en chambre phytotronique en conditions contrôlées et stériles. Le passage de ce mode de culture à un mode de culture dans du sol et en serre est une étape délicate et particulièrement longue. Pour exemple, la mise au point des lignées de peupliers exprimant le gène fongique MiSSP7 par le Dr Jonathan Plett (Plett et al., 2011) et utilisées dans le Chapitre V, a nécessité plus d'une année de la transformation à l'acclimatation en serre. L'option choisi dans le Chapitre II de cette thèse visant à transférer des plants stériles dans des pots contenant du sol naturel et à les maintenir en chambre phytotronique semble être une stratégie efficace pour raccourcir ce délai et pouvoir étudier l'impact des phytohormones sur les communautés de micro-organismes qui s'associent aux racines du peuplier, au moins durant les premiers mois de sa vie.

5. Applications possibles des résultats

En France, le peuplier est la deuxième essence feuillue récoltée après le chêne avec près de 230 000 hectares cultivés et une récolte annuelle de 1 500 000 m^3 de bois. La France est donc le premier pays producteur en Europe et le deuxième producteur mondial après la Chine (https://www.peupliersdefrance.org).

Il serait interessant de tester certaines souches bactériennes et fongiques comme, par exemple, *Burkholderia* et *Cadophora* mis en évidence dans cette thèse et dans d'autres études pour leur capacité de résistance face à différents stress environnementaux ou leurs capacités d'amélioration de la nutrition et de la croissance des plantes (Compant et al., 2008 ; Paungfoo-Lonhienne et al., 2016 ; Kia et al., 2017) et des arbres hôtes (Timm et al., 2016 ; Berthelot et al., 2016 ; Kandel et al., 2017). En effet, ayant une vision globale de l'impact des paramètres testés sur le microbiote racinaire du peuplier, il serait maintenant judicieux de sélectionner des communautés d'intérêt pour observer leur évolution face à différents paramètres intrinsèques et extrinsèques et leurs effets sur l'arbre hôte. En fonction des résultats obtenus, la mise au point d'inoculum microbien à ajouter au sol naturel des peupleraies pourrait améliorer la croissance et le dévelopement des arbres et leur résistance face aux parasites et aux pathogènes et, par conséquent, les rendements de bois.

Références bibliographiques

Abarca-Grau, A. M., Penyalver, R., López, M. M. & Marco-Noales, E. (2011). Pathogenic and non-pathogenic *Agrobacterium tumefaciens*, *A. rhizogenes* and *A. vitis* strains form biofilms on abiotic as well as on root surfaces. *Plant Pathology*, *60*(3), 416–425. https://doi.org/10.1111/j.1365-3059.2010.02385.x

Adam, E., Groenenboom, A. E., Kurm, V., Rajewska, M., Schmidt, R., Tyc, O., ... Salles, J. F. (2016). Controlling the microbiome: Microhabitat adjustments for successful biocontrol strategies in soil and human gut. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01079

Afgan, E., Baker, D., Van den Beek, M., Blankenberg, D., Bouvier, D., Čech, M., ... Goecks, J. (2016). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Research*, *44*(1), 3–10. https://doi.org/10.1093/nar/gkw343

Aguirre-Von-Wobeser, E., Rocha-Estrada, J., Shapiro, L. R., & De La Torre, M. (2018). Enrichment of Verrucomicrobia, Actinobacteria and *Burkholderiales* drives selection of bacterial community from soil by maize roots in a traditional milpa agroecosystem. *PLoS ONE*, *13*(12), 1–18.

Ahemad, M. & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University - Science*, *26*(1), 1–20. https://doi.org/10.1016/j.jksus.2013.05.001

Aitken, S. N., Yeaman, S., Holliday, J. A., Wang, T., Curtis-McLane, S. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* 1: 95-111

Akroume, E., Maillard, F., Bach, C., Hossann, C., Brechet, C., Angeli, N., Zeller, B., Saint-André, L. & Buée, M. (2019). First evidences that the ectomycorrhizal fungus *Paxillus involutus* mobilizes nitrogen and carbon from saprotrophic fungus necromass. *Environmental Microbiology*, 21: 197-208. doi:10.1111/1462-2920.14440

Albrectsen, B. R., Varad, A., Wedin, M. & Jansson, S. (2010). Endophytic fungi in European aspen (*Populus tremula*) leaves – Diversity, detection, and a suggested correlation with herbivory resistance. ISSN 1560-2745, Volume 41, Number 1. https://doi.org/10.1007/s13225-009-0011-y

Aziz Khan, H., Ziaf, K., Amjad, M. & Iqbal, Q. (2012). Exogenous Application of Polyamines Improves Germination and Early Seedling Growth of Hot Pepper. *Chilean Journal of Agricultural Research*, *72*(3), 429–433. https://doi.org/10.4067/s0718-58392012000300018

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., ... Smith, D. L. (2018). Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Frontiers in Plant Science*, *9*, 1–17. https://doi.org/10.3389/fpls.2018.01473

Badeau, V., Dambrine, E. & Walter, C. (1999). Propriétés des sols forestiers français : Résultats du premier inventaire systématique. *Etud. Gest. Sols, 6*(3) :165-180

Badri, D. V. & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell and Environment*, *32*(6), 666–681. https://doi.org/10.1111/j.1365-3040.2009.01926.x

Badri, D. V., Zolla, G., Bakker, M. G., Manter, D. K. & Vivanco, J. M. (2013). Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytologist*, *198*(1), 264–273. https://doi.org/10.1111/nph.12124

Bahram, M., Põlme, S., Kõljalg, U. & Tedersoo, L. 2011. A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiology Ecology*, 75: 313-320. doi:10.1111/j.1574-6941.2010.01000.x

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S. & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*, 233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Baldrian, P. (2017). Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiology Reviews*, *41*(2), 109–130. https://doi.org/10.1093/femsre/fuw040

Balint, M., Bartha, L., O'Hara, R. B., Olson, M. S., Otte, J., Pfenninger, M., Robertson, A. L., Tiffin, P. & Schmitt, I. (2014). Relocation, high - latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology*, *24*(1). https://doi.org/10.1111/mec.13018

Balint, M., Tiffin, P., Hallström, B., O'Hara, R. B., Olson, M. S., Fankhauser, J. D., Piepenbring, M. & Schmitt, I. (2013). Host Genotype Shapes the Foliar Fungal Microbiome of Balsam Poplar (*Populus balsamifera*). *PLoS ONE*, *8*(1): e53987. doi:10.1371/journal.pone.0053987

Ban, Y., Tang, M., Chen, H., Xu, Z., Zhang, H. & Yang, Y. (2012). The Response of Dark Septate Endophytes (DSE) to Heavy Metals in Pure Culture. *PLoS ONE*, *7*(10). https://doi.org/10.1371/journal.pone.0047968

Barbi, F., Prudent, E., Vallon, L., Buée, M., Dubost, A., Legout, A., ... Luis, P. (2016). Tree species select diverse soil fungal communities expressing different sets of lignocellulolytic enzyme-encoding genes. *Soil Biology and Biochemistry*, *100*, 149–159. https://doi.org/https://doi.org/10.1016/j.soilbio.2016.06.008

Bardgett, R. D., De Deyn, G. B. & Ostle, N. J. (2009). Editorial: Plant-soil interactions and the carbon cycle. *Journal of Ecology*, *97*(5), 838–839. https://doi.org/10.1111/j.1365-2745.2009.01545.x

Barrat-Segretain, M. H. (1996). Strategies of reproduction, dispersion and competition in river plant: A review. *Vegetatio*, 123:13-37.

Basso, V., De Freitas Pereira, M., Maillard, F., Mallerman, J., Mangeot-Peter, L., Zhang, F. & Bonnot, C. (2018). Facing global change: the millennium challenge for plant scientists. *New Phytologist,* 220: 25– 29.

Bastien, C., Chenault, N., Dowkiw, A., Villar, M., Klein, E. & Frey, P. (2009). Interactions entre populations naturelles et cultivées. L'exemple du Peuplier. *Biofutur*, 305: 31-34.

Baum, C., Hrynkiewicz, K., Szymanska, S., Vitow, N., Hoeber, S., Fransson, P. M. A. & Weih, M. (2018). Mixture of *Salix* Genotypes promotes root colonization with dark septate endophytes and changes P cycling in the mycorrhizosphere. *Frontiers in Microbiology*, *9*, 1–10. https://doi.org/10.3389/fmicb.2018.01012

Baum, C. & Makeschin, F. (2000). Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula x tremuloides*). *Journal of Plant Nutrition and Soil Sciences*, 163, 491-497.

Beauchamp, V. B., Stromberg, J. C. & Stutz, J. C. (2006). Arbuscular mycorrhizal fungi associated with *Populus-Salix* in a semiarid riparian ecosystem. *New Phytologist*, *170*(2). https://doi.org/10.1111/j.1469-8137.2006.01668.x

Beck, A., Persoh, D. & Rambold, G. (2014). First evidence for seasonal fluctuations in lichen- and bark-colonising fungal communities. *Folia Microbiol,* 59:155–7. https://doi.org/10.1007/s12223-013-0278-y

Beckers, B., Op De Beeck, M., Weyens, N., Boerjan, W. & Vangronsveld, J. (2017). Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome*, *5*(1), 1–17. https://doi.org/10.1186/s40168-017-0241-2

Beckers, B., Op De Beeck, M., Weyens, N., Van Acker, R., Van Montagu, Boerjan, W. & Vangronsveld, J. (2016). Lignin engineering in field-grown poplar trees affects the endosphere bacterial microbiome. *PNAS*, *113*(8). https://doi.org/10.1073/pnas.1523264113

Beneduzi, A., Ambrosini, A. & Passaglia, L. M. P. (2012). Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology*, *35*(4), 1044–1051. https://doi.org/10.1590/S1415-47572012000600020

Berendsen, R. L., Pieterse, C. M. J. & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, *17*(8), 478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berg, G. & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, *68*(1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Berthelot, C., Leyval, C., Foulon, J., Chalot, M. & Blaudez, D. (2016). Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal-polluted poplar phytomanagement sites. *FEMS Microbiology Ecology*, *92*(10), 1–14. https://doi.org/10.1093/femsec/fiw144

Beule, L., Grüning, M. M., Karlovsky, P. & Arnold, A. M. (2017). Changes of Scots Pine Phyllosphere and Soil Fungal Communities during Outbreaks of Defoliating Insects. *Forest*, *8*(316) 1–14. https://doi.org/10.3390/f8090316

Blair, P. M., Land, M. L., Piatek, M. J., Jacobson, D. A., Lu, T. Y. S., Doktycz, M. J. & Pelletier, D. A. (2018). Exploration of the biosynthetic potential of the *Populus* microbiome. *mSystems 3*: e00045-18. https://doi.org/10.1128/mSystems .00045-18.

Bisson, M. M., Bleckmann, A., Allekotte, S. & Groth, G. (2009). EIN2, the central regulator of ethylene signalling, is localized at the ER membrane where it interacts with the ethylene receptor ETR1. *Biochemical Journal, 424*(1):1-6.

Bleecker, A. B., Estelle, M. A., Somerville, C. & Kende, H. (1988). Insensitivity to ethylene conferred by a dominant mutation in Arabidopsis thaliana. *Science 241*: 1086–1089

Bockheim, J. G., Gennadiyev, A. N., Hartemink, A. E. & Brevik, E. C. (2014). Soil-forming factors and Soil Taxonomy. *Geoderma 226–227*, 231–237. doi:10.1016/j.geoderma.2014.02.016

Boller, T. & Felix, G. (2009). A Renaissance of Elicitors : Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annual Review of Plant Biology,* 60: 379-406. https://doi.org/10.1146/annurev.arplant.57.032905.10534

Bonito, G., Benucci, G. M. N., Hameed, K., Weighill, D., Jones, P., Chen, K. H., ... Vilgalys, R. (2019). Fungal-Bacterial Networks in the Populus Rhizobiome Are Impacted by Soil Properties and Host Genotype. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00481

Bonito, G., Hameed, K., Toome-heller, M., Healy, R., Reid, C., Liao, H. L., Aime, C., Schadt C. W. & Vilgalys, R. (2017). *Atractiella rhizophila*, sp. No. An endorrhizal fungus isolated from the *Populus* root microbiome. *Mycologia*, *109*(1), 18–26. https://doi.org/10.1080/00275514.2016.1271689

Bonito, G., Hameed, K., Ventura, R., Krishnan, J., Schadt, C. W. & Vilgalys, R. (2016). Isolating a functionally relevant guild of fungi from the root microbiome of Populus. *Fungal Ecology*, *22*, 35–42. https://doi.org/10.1016/j.funeco.2016.04.007

Bonito, G., Reynolds, H., Robeson, M. S., Nelson, J., Hodkinson, B. P., Tuskan, G., ... Vilgalys, R. (2014). Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. *Molecular Ecology*, *23*(13), 3356–3370. https://doi.org/10.1111/mec.12821

Bowles, T. M., Jackson, L. E. & Cavagnaro, T. R. (2018). Mycorrhizal fungi enhance plant nutrient acquisition and modulate nitrogen loss with variable water regimes. *Global Change Biology*, 24, 171-182. https://doi.org/10.1111/gcb.13884

Braatne, J. H., Rood, S. B. & Heilman, P. E. (1996). Life history, ecology, and conservation of riparian cottonwoods in North America. In: *Biology of Populus and its Implications for Management and Conservation*. Stettler, R.F., Bradshaw, H.D., Heilman, P.E., and Hinckley, T.M., eds. NRC Research Press, Ottawa, pp. 57–85.

Bredemeier, M., Busch, G., Hartmann, L., Jansen, M., Richter, F. & Lamersdorf, N. P. (2015). Fast growing plantations for wood production – integration of ecological effects and economic perspectives. *Front. Bioeng. Biotechnol*. https://doi.org/10.3389/fbioe.2015.00072

Brockwell, J., Bottomley, PJ. & Thies JE. (1995). Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. *Plant and Soil* 174: 143-180.

Brodersen, P., Petersen, M., Bjørn, Nielsen., Zhu, S., Newman, MA., Shokat, KM., Rietz, S., Parker, J. & Mundy, J. (2006). Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. *Plant Journal, 47* :532–546.

Brun, A., Chalot, M., Finlay, R. D. & Söderström, B. (1995). Structure and function of the ectomycorrhizal association between *Paxillus involutus* (Batsch) Fr . and *Betula pendula* Roth*. New Phytologist*, 129, 487–493.

Brundrett, M. C., Piché, Y. & Peterson, R. L. (2008). A new method for observing the morphology of vesicular–arbuscular mycorrhizae. *Canadian Journal of Botany*, *62*(10), 2128–2134. https://doi.org/10.1139/b84-290

Buchfink, B., Xie, C. & Huson, D. (2015). Fast and sensitive protein alignment using DIAMOND. *Nat Methods* 12, 59–60. doi:10.1038/nmeth.3176

Bücking, H., Liepold, E. & Ambilwade, P. (2012). The Role of the Mycorrhizal Symbiosis in Nutrient Uptake of Plants and the Regulatory Mechanisms Underlying These Transport Processes. *Plant Science*, Nabin Kumar Dhal and Sudam Charan Sahu, IntechOpen, DOI: 10.5772/52570.

Buée, M., De Boer, W., Martin, F., van Overbeek, L. & Jurkevitch, E. (2009). The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil*, *321*(1–2), 189–212. https://doi.org/10.1007/s11104-009-9991-3

Bueno de Mesquita, C. P., Sartwell, S. A., Ordemann, E. V., Porazinska, D. L., Farrer, E. C., King, A. J., ... Schmidt, S. K. (2018). Patterns of root colonization by arbuscular mycorrhizal fungi and dark septate endophytes across a mostly-unvegetated, high-elevation landscape. *Fungal Ecology*, *36*, 63–74. https://doi.org/10.1016/j.funeco.2018.07.009

Bugg, T. D. H., Ahmad, M., Hardiman, E. M. & Rahmanpour, R. (2011). Pathways for degradation of lignin in bacteria and fungi. *Natural Product Reports*, *28*(12), 1883–1896. https://doi.org/10.1039/c1np00042j

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E. & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, *64*, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver, E., Van Themaat, L., Ahmadinejad, N., ... Schulze-lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, *488*(7409), 91–95. https://doi.org/10.1038/nature11336

Busby, P. E, Peay, K. G. & Newcombe, G. (2015). Common foliar fungi of *Populus trichocarpa* modify *Melampsora* rust disease severity. *New Phytologist*, *209*(4). https://doi.org/10.1111/nph.13742

Busby, P. E, Ridout, M. & Newcombe, G. (2016). Fungal endophytes: modifiers of plant disease. *Plant Molecular Biology*, *90*(6), 645–655. https://doi.org/10.1007/s11103-015-0412-0

Busov, V., Meilan, R., Pearce, D. W., Rood, S. B., Ma, C., Tschaplinski, T. J. & Strauss, S. H. (2006). Transgenic modification of gai or rgl1 causes dwarfing and alters gibberellins, root growth, and metabolite profiles in *Populus*. *Planta*, 224: 288. https://doi.org/10.1007/s00425-005-0213-9

Calvaruso, C., Turpault, M. P., Leclerc, E., Ranger, J., Garbaye, J., Uroz, S. & Frey-Klett, P. (2010). Influence of Forest Trees on the Distribution of Mineral Weathering-Associated Bacterial Communities of the *Scleroderma citrinum* Mycorrhizosphere. *Applied and Environmental Microbiology*, *76*(14), 4780–4787. https://doi.org/10.1128/AEM.03040-09

Canarini, A., Kaiser, C., Merchant, A., Richter, A. & Wanek, W. (2019). Root exudation of primary metabolites: Mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00157

Cao, H., Chen, R., Wang, L., Jiang, L., Yang, F., Zheng, S., ... Lin, X. (2016). Soil pH, total phosphorus, climate and distance are the major factors influencing microbial activity at a regional spatial scale. *Scientific Reports*, *6*, 1–10. https://doi.org/10.1038/srep25815

Cao, H., Glazebrook, J., Clarke, J. D., Volko, S. & Dong, X. (1997). The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell*, *88*(1), 57–63. https://doi.org/10.1016/S0092-8674(00)81858-9

Caroll, G. (1988). Fungal Endophytes in Stems and Leaves: From Latent Pathogen to Mutualistic Symbiont *Ecology*, *69*(1), 2–9. Ecological Society of America Stable. URL: http://www.jstor.org/stable/1943154.

Carvalhais, L. C., Dennis, P. G., Badri, D. V, Kidd, B. N., Vivanco, J. M. & Schenk, P. M. (2015). Linking Jasmonic Acid Signaling, Root Exudates, and Rhizosphere Microbiomes. *Molecular Plant-Microbe Interactions : MPMI*, *28*(9), 1049–1058. https://doi.org/10.1094/MPMI-01-15-0016-R

Carvalhais, L. C., Dennis, P. G., Badri, D. V., Tyson, G. W., Vivanco, J. M. & Schenk, P. M. (2013). Activation of the Jasmonic Acid Plant Defence Pathway Alters the Composition of Rhizosphere Bacterial Communities. *PLOS One, 8*(2), 1–5. https://doi.org/10.1371/journal.pone.0056457

Castaño, C., Dejene, T., Mediavilla, O., Geml, J., Oria-de-Rueda, J. A. & Martín-Pinto, P. (2019). Changes in fungal diversity and composition along a chronosequence of *Eucalyptus grandis* plantations in Ethiopia. *Fungal Ecology*, *39*, 328–335. https://doi.org/https://doi.org/10.1016/j.funeco.2019.02.003

Castro-Rodríguez, V., García-Gutiérrez, A., Canales, J., Cañas, R. A., Kirby, E. G., Avila, C. & Cánovas, F. M. (2016). Poplar trees for phytoremediation of high levels of nitrate and applications in bioenergy. *Plant Biotechnology Journal*, *14*(1), 299–312. https://doi.org/10.1111/pbi.12384

Certini, G. & Ugolini, F.C. (2013). An updated, expanded, universal definition of soil. *Geoderma 192*, 378–379. doi:10.1016/j.geoderma.2012.07.008

Chamaillard, S. (2011). Efficience de l'utilisation de l'eau chez le peuplier noir (*Populus nigra* L.) : variabilité et plasticité en réponse aux variations de l'environnement. PhD thesis of the Université d'Orléans : Orléans, France.

Chao, Q., Rothenberg, M., Solano, R., Roman, G., Terzaghi, W. & Ecker, J. R. (1997). Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell, 89*: 1133–1144

Chaparro, J. M., Sheflin, A. M., Manter, D. K. & Vivanco, J. M. (2012). Manipulating the soil microbiome to increase soil health and plant fertility. *Biology and Fertility of Soils*, *48*(5), 489–499. https://doi.org/10.1007/s00374-012-0691-4

Chilvers, G. A., Lapeyrie, F. F. & Hogan, D. P. (1987). Ectomycorrhizal Vs Endomycorrhizal Fungi Within the Same Root System. *New Phytologist*, *107*(2), 441–448. https://doi.org/10.1111/j.1469-8137.1987.tb00195.x

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J. M., Lorenzo, O., ... Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*, *448*(7154), 666–671. https://doi.org/10.1038/nature06006

Clark, K. L., Larsen, P. B., Wang, X. & Chang, C. (1998). Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. *Proceedings of the National Academy of Sciences of USA*, 95:5401–5406.

Clarke, J. D., Volko, S. M., Ledford, H., Ausubel, F. M. & Dong, X. (2000). Roles of Salicylic Acid, Jasmonic Acid, and Ethylene in. *The Plant Cell*, *12*, 2175–2190.

Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S, Moore, J. A. M., Cregger, M. A., Moorhead, L. C. & Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*, *6*. https://doi.org/10.1890/es15-00217.1

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., ... Lindahl, B. D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, *340*(6127), 1615–1618. https://doi.org/10.1126/science.1231923

Cleveland, C. C., Townsend, A. R., Schimel, D. S., Perakis, S. & Fischer, C. Von. (1999). Global Patterns of Terrestrial Biological Nitrogen (N2) Fixation in Natural Ecosystems. *Global patterns of terrestrial biological nitrogen (Nz)*. *13*(2), 623–645.

Colin, Y., Nicolitch, O., Van Nostrand, J. D., Zhou, J. Z., Turpault, M. P. & Uroz, S. (2017). Taxonomic and functional shifts in the beech rhizosphere microbiome across a natural soil toposequence. *Scientific Reports*, *7*(1), 1–17. https://doi.org/10.1038/s41598-017-07639-1

Compant, S., Nowak, J., Coenye, T., Clément, C. & Ait Barka, E. (2008). Diversity and occurrence of *Burkholderia spp*. in the natural environment. *FEMS Microbiology Reviews*, *32*(4), 607–626. https://doi.org/10.1111/j.1574-6976.2008.00113.x

Compant, S., Kaplan, H., Sessitsch, A., Nowak, J., Ait Barka, E. & Clément, C. (2008). Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: From the rhizosphere to inflorescence tissues. *FEMS Microbiology Ecology*, *63*(1), 84–93. https://doi.org/10.1111/j.1574-6941.2007.00410.x

Compant, S., Van Der Heijden, M. G. A. & Sessitsch, A. (2010). Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiology Ecology*, *73*(2), 197–214. https://doi.org/10.1111/j.1574-6941.2010.00900.x

Compant, S., Samad, A., Faist, H. & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*. https://doi.org/10.1016/j.jare.2019.03.004

Cordier, T., Robin, C., Capdevielle, X., Fabreguettes, O., Desprez-Loustau, D. & Vacher, C. (2012). The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*) varies significantly along an elevation gradient. *New Phytologist*, 196 :510-519. https://doi.org/10.1111/j.1469-8137.2012.04284.x

Corenblit, D., Steiger, J., González, E., Gurnell, A. M., Charrier, G., Darrozes, J., ... Voldoire, O. (2014). The biogeomorphological life cycle of poplars during the fluvial biogeomorphological succession: A special focus on *Populus nigra* L. *Earth Surface Processes and Landforms*, *39*(4), 546–563. https://doi.org/10.1002/esp.3515

Corradi, N. & Bonfante, P. (2012). The Arbuscular Mycorrhizal Symbiosis: Origin and Evolution of a Beneficial Plant Infection. *PLoS Pathogens*, *8*(4), e1002600. https://doi.org/10.1371/journal.ppat.1002600

Cottrell, J. (2004). Conservation of black poplar (*Populus nigra* L.). *Forestry Commission.*

Cregger, M. A., Veach, A. M., Yang, Z. K., Crouch, M. J., Vilgalys, R., Tuskan, G. A. & Schadt, C. W. (2018). The *Populus* holobiont: dissecting the effects of plant niches and genotype on the microbiome. *Microbiome*, 1–14. https://doi.org/10.1186/s40168-018-0413-8

Crombie, A. T., Larke-Mejia, N. L., Emery, H., Dawson, R., Pratscher, J., Murphy, G. P., McGenoty, T. J. & Murell, C. J. (2018). Poplar phyllosphere harbors disparate isoprene-degrading bacteria. *PNAS*. *15*(51) 13081-13086; DOI:10.1073/pnas.1812668115

Denman, S., Doonan, J., Ransom-Jones, E., Broberg, M., Plummer, S., Kirk, S., Scarlett, K., Griffiths, A. R., Kacsmarek, M., Forster, J., Peace, A., Golyshin, P. N., Hassard, F., Brown, N., Kenny, J. G. & Mcdonald, J. E. (2017). Microbiome and infectivity studies reveal complex polyspecies tree disease in Acute Oak Decline. *ISME Journal*, *12*(2), 386–399. https://doi.org/10.1038/ismej.2017.170

Danielsen, L., Thürmer, A., Meinicke, P., Buée, M., Morin, E., Martin, F., ... Reich, M. (2012). Fungal soil communities in a young transgenic poplar plantation form a rich reservoir for fungal root communities. *Ecology and Evolution*, *2*(8), 1935–1948. https://doi.org/10.1002/ece3.305

Daguerre, Y., Plett, J. M. & Veneault-Fourrey, C. (2016). Signaling pathways driving the development of ectomycorrhizal symbiosis. *Molecular Mycorrhizal Symbiosis*. 141–157. https://doi.org/doi:10.1002/9781118951446.ch9

Deveau, A., Kohler, A., Frey-Klett, P. & Martin, F. (2008). The major pathways of carbohydrate metabolism in the ectomycorrhizal basidio- mycete *Laccaria bicolor* S238N. *New Phytoogistl*. 180:379-390.

Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., Hacquard, S., Hervé, V., Labbé, J., Lastovetsky, O. A., Mieszkin, S., Millet, L. J., Vajna, B., Junier, P., Bonfante, P., Krom, B.P., Olsson, S., van Elsas, J. D. & Wick, L. Y. (2018). Bacterial–fungal interactions: ecology, mechanisms and challenges, *FEMS Microbiology Reviews*, *42*(3), 335–352, https://doi.org/10.1093/femsre/fuy008

De Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., ... Bardgett, R. D. (2018). Soil bacterial networks are less stable under drought than fungal networks. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-05516-7

Devadas, S. K., Enyedi, A. & Raina, R. (2002). The Arabadopsis Hr11 Mutation Reveals Novel Overlapping Roles for Salicyclic Acid, Jasmonic Acid, and Ethylene Signaling in Cell Death and Defence Against Pathogens. *The Plant Journal: For Cell and Molecular Biology*, *30*(4), 467–480.

De Boer, W., Folman, L. B., Summerbell, R. C. & Boddy, L. (2005). Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews*, *29*(4), 795–811. https://doi.org/10.1016/j.femsre.2004.11.005

De Vos, M., Van Zaanen, W., Koornneef, A., Korzelius, JP., Dicke, M., Van Loon, LC. & Pieterse, CMJ. (2006). Herbivore-induced resistance against microbial pathogens in Arabidopsis. *Plant Physiology, 142*: 352–363

Dilkes, N. B., Jones, D. L. & Farrar, J. (2004). Temporal Dynamics of Carbon Partitioning and Rhizodeposition in Wheat. *Plant Physiology*, 134, 706–715. https://doi.org/10.1104/pp.103.032045.cesses

Dodds, P. N. & Rathjen, J. P. (2010). Plant immunity: Towards an integrated view of plant pathogen interactions. *Nature Reviews Genetics*, *11*(8), 539–548. https://doi.org/10.1038/nrg2812

Doornbos, R. F., Geraats, B. P. J., Kuramae, E. E., Van Loon, L. C. & Bakker, P. A. H. M. (2010). Effects of Jasmonic Acid, Ethylene, and Salicylic Acid Signaling on the Rhizosphere Bacterial Community of *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*, *24*(4), 395–407. https://doi.org/10.1094/mpmi-05-10-0115

Doornbos, R. F., Van Loon, L. C. & Bakker, P. A. H. M. (2012). Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable Development*, *32*(1), 227–243. https://doi.org/10.1007/s13593-011-0028-y

Doty, S. L., Sher, A. W., Fleck, N. D., Khorasani, M., Bumgarner, R. E., Khan, Z., Ko, A. W. K., Kim, S. H. & DeLuca, T. H. (2016). Variable Nitrogen Fixation in Wild Populus*. PLoS ONE* 11(5): e0155979. doi:10.1371/journal.pone.0155979

Dupont, P. Y., Eaton, C. J., Wargent, J. J., Fechtner, S., Solomon, P., Schmid, J., ... Cox, M. P. (2015). Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. *New Phytologist*, *208*(4), 1227–1240. https://doi.org/10.1111/nph.13614

Durand, A., Maillard, F., Alvarez-Lopez, V., Guinchard, S., Bertheau, C., Valot, B., Blaudez, D. & Chalot, M. (2018). Science of the Total Environment Bacterial diversity associated with poplar trees grown on a Hg-contaminated site: Community characterization and isolation of Hg-resistant plant growth-promoting bacteria. *Science of the Total Environment*, *622–623*, 1165–1177. https://doi.org/10.1016/j.scitotenv.2017.12.069

Durand, A., Maillard, F., Foulon, J., Gweon, H. S., Valot, B. & Chalot, M. (2017). Environmental Metabarcoding Reveals Contrasting Belowground and Aboveground Fungal Communities from Poplar at a Hg Phytomanagement Site. *Microbial Ecology*, *74*(4), 795-809. doi: 10.1007/s00248-017-0984-0.

Durrant, W. E. & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology, 42* :185–209.

Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., ... Pascal, G. (2018). FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics*, *34*(8), 1287–1294. https://doi.org/10.1093/bioinformatics/btx791

Eckenwalder, J. E. (1996). Systematics and evolution of *Populus*. Biology of *Populus*. Stettler RF, Bradshaw HD, Heilman PE, et Hinckley TM. (Eds) NRC Research Press: 7-32.

Ecker, J. R. (1995). The ethylene signal transduction pathway in plants. *Science, 268* :667–675.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., ... Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences*, *112*(8), 911–920. https://doi.org/10.1073/pnas.1414592112

Ekberg, A., Buchmann, N. & Gleixner G. (2007). Rhizospheric influence on soil respiration and decomposition in a temperate Norway spruce stand. *Soil Biology & Biochemistry, 39* :2103–2110.

Escobar, M. A. & Dandekar, A. M. (2003). *Agrobacterium tumefaciens* as an agent of disease. *Trends in Plant Science, 8*(8), 380-386. ISSN 1360-1385,https://doi.org/10.1016/S1360-1385(03)00162-6.

Faivre-Rampant, P., Zaina, G., Jorge, V., Giacomello, S., Segura, V., Scalabrin, S., Guérin, V., De Paoli, E., Aluome, C., Viger, M., Cattonaro, F., Payne, A., PaulStephenRaj, P., Le Paslier, M. C., Berard, A., Allwright, M. R., Villar, M., Taylor, G., Bastien, C. & Morgante, M. (2016). New resources for genetic studies in *Populus nigra*: genome-wide SNP discovery and development of a 12k Infinium array. *Mol Ecol Resour*, 16: 1023-1036. doi:10.1111/1755-0998.12513

Farrar, K., Bryant, D. & Cope-Selby, N. (2014). Understanding and engineering beneficial plant-microbe interactions: Plant growth promotion in energy crops. *Plant Biotechnology Journal*, *12*(9), 1193–1206. https://doi.org/10.1111/pbi.12279

Felix, G. & Boller, T. (2003). Molecular sensing of bacteria in plants. The highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. *J. Biol. Chem*, 278, 6201–6208.

Felsmann, K., Baudis, M., Gimbel, K., Kayler, Z. E., Ellerbrock, R., Bruehlheide, H., ... Welk, E. (2015). Responses to Precipitation Reduction and Forest Management in Forest Ecosystems across Germany. *PLoS ONE*, *10*(4), 1–24. https://doi.org/10.1371/journal.pone.0122539

Felten, J., Kohler, A., Morin, E., Bhalerao, R. P., Palme, K., Martin, F., ... Legué, V. (2009). The ectomycorrhizal fungus laccaria bicolor stimulates lateral root formation in poplar and arabidopsis through auxin transport and signaling. *Plant Physiology*, *151*(4), 1991–2005. https://doi.org/10.1104/pp.109.147231

Fernandez, O., Theocharis, A., Bordiec, S., Feil, R., Jacquens, L., Clément, C., Fontaine, F. & Barka, E. A. (2012). *Burkholderia phytofirmans* PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. *Mol. Plant-Microbe Interaction*. 25, 496–504. doi: 10.1094/MPMI-09-11-0245

Fierer, N. & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(3), 626–631. https://doi.org/10.1073/pnas.0507535103

Fierer, N., Bradford, M. A. & Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology*, *88*(6), 1354–1364. https://doi.org/10.1007/s00209-018-2115-0

Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., ... Caporaso, J. G. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences*, *109*(52), 21390–21395. https://doi.org/10.1073/pnas.1215210110

Fierer, N., Ladau, J., Clemente, J. C., Leff, J. W., Owens, S. M., Pollard, K. S., ... McCulley, R. L. (2013). Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science*, *342*(6158), 621–624. https://doi.org/10.1126/science.1243768

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*(10), 579–590. https://doi.org/10.1038/nrmicro.2017.87

Fillatti, J. J., Sellmer, J., McCown, B., Haissig, B. & Comai, L. (1987). *Agrobacterium* mediated transformation and regeneration of *Populus*. *Mol Gen Genet, 206* :192. https://doi.org/10.1007/BF00333574

Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M. & Johnson, M. T. J. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences*, *115*(6), E1157–E1165. https://doi.org/10.1073/pnas.1717617115

Fleming, L. V. (1985). Experimental study of sequences of ectomycorrhizal fungi on 170 birch (*Betula sp.*) seedling root systems. *Soil Biology & Biochemistry*, 17, 591-600.

Foulon, J., Zappelini, C., Durand, A., Valot, B., Blaudez, D. & Chalot, M. (2016). Impact of poplar-based phytomanagement on soil properties and microbial communities in a metal-contaminated site. *FEMS Microbiology Ecology*, *92*(10), 1–13. https://doi.org/10.1093/femsec/fiw163

Frank, A. C., Saldierna Guzmán, J. P. & Shay, J. E. (2017). Transmission of Bacterial Endophytes. *Microorganisms*, *5*, 70.

Frey-Klett, P., Garbaye, J. & Tarkka, M. (2007). The mycorrhiza helper bacteria revisited. *New Phytologist,* 176, 22–36. doi: 10.1111/j.1469-8137.2007.02191.x

Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M. & Sarniguet, A. (2011). Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews: MMBR*, *75*(4), 583–609. https://doi.org/10.1128/MMBR.00020-11

Gaiero, J. R., McCall, C. A., Thompson, K. A., Day, N. J., Best, A. S. & Dunfield, K. E. (2013). Inside the root microbiome: Bacterial root endophytes and plant growth promotion. *American Journal of Botany*, *100*(9), 1738–1750. https://doi.org/10.3732/ajb.1200572

Gallart, M., Adair, K. L., Love, J., Meason, D. F., Clinton, P. W., Xue, J. & Turnbull, M. H. (2018). Host Genotype and Nitrogen Form Shape the Root Microbiome of *Pinus radiata*. *Microbial Ecology*, 75 :419–433. https://doi.org/10.1007/s00248-017-1055-2

Garbaye, J. (1994). Helper bacteria: a new dimension to the mycorrhizal symbiosis (Tansley Review, 76). *New Phytologist* 128: 197–210.

Gehring, C. A., Mueller, R. C. & Whitham, T. G. (2006). Environmental and genetic effects on the formation of ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. *Oecologia*, *149*(1), 158–164. https://doi.org/10.1007/s00442-006-0437-9

Gehring, C. A., Sthultz, C. M., Flores-Rentería, L., Whipple, A. V. & Whitham, T. G. (2017). Tree genetics defines fungal partner communities that may confer drought tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(42), 11169–11174. https://doi.org/10.1073/pnas.1704022114

Germaine, K., Keogh, E., Garcia-Cabellos, G., Borremans, B., Van der Lelie, D., Barac, T., ..., Dowling, D. N. (2004). Colonisation of poplar trees by *gfp* expressing bacterial endophytes, *FEMS Microbiology Ecology*, *48*(1), 109 118. https://doi.org/10.1016/j.femsec.2003.12.009

Germida, J. J., Siciliano, S. D., Renato de Freitas, J. & Seib, A.M. (1998) Diversity of root-associated bacteria associated with field- grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). *FEMS Microb Ecol* 26: 43–50.

Ghodsalavi, B., Svenningsen, N. B., Hao, X., Olsson, S., Nicolaisen, M. H., Al-Soud, W. A., Sorensen, S. J. & Nybroe, O. (2017). A novel baiting microcosm approach used to identify the bacterial community associated with *Penicillium bilaii* hyphae in soil. *PLoS One*, 12. DOI: 10.1371/journal.pone.0187116.

Gibert, A., Magda, D. & Hazard, L. (2015). Interplay between endophyte prevalence, effects and transmission: Insights from a natural grass population. *PLoS ONE*, *10*(10), 1–17. https://doi.org/10.1371/journal.pone.0139919

Gimenez-Ibanez, S. & Solano, R. (2013). Nuclear jasmonate and salicylate signaling and crosstalk in defense against pathogens. *Frontiers in Plant Science*, *4*, 1–11. https://doi.org/10.3389/fpls.2013.00072

Gkarmiri, K., Mahmood, S., Ekblad, A., Alström, S., Högberg, N. & Finlay, R. (2017). Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape. *Applied and Environmental Microbiology*, *83*(22), 1–14. https://doi.org/10.1128/AEM.01938-17

Goh, C., Vallejos, D. F. V., Nicotra, A. B. & Mathesius, U. (2013). The Impact of Beneficial Plant-Associated Microbes on Plant Phenotypic Plasticity. *J Chem Ecol,* 39: 826–839. https://doi.org/10.1007/s10886-013-0326-8

Goldmann, K., Schröter, K., Pena, R., Schöning, I., Schrumpf, M., Buscot, F., Polle, A. & Wubet, T. (2016). Divergent habitat filtering of root and soil fungal communities in temperate beech forests. *Scientific Report*, 1–10. https://doi.org/10.1038/srep31439

Goldmann, K., Schöning, I., Buscot, F. & Wubet, T. (2015). Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. *Frontiers in Microbiology*, *6*, 1–11. https://doi.org/10.3389/fmicb.2015.01300

Gomes, E. A., Lana, U. G. P., Quensen, J. F., de Sousa, S. M., Oliveira, C. A., Guo, J., ... Tiedje, J. M. (2018). Root-Associated Microbiome of Maize Genotypes with Contrasting Phosphorus Use Efficiency. *Phytobiomes Journal*, *2*(3), 129–137. https://doi.org/10.1094/pbiomes-03-18-0012-r

González-Chávez, Torres-Cruz, T. J., Sánchez, S. A., Carrillo-González, R., Carrillo-López, L. M. & Porras-Alfaro, A. (2018). Microscopic characterization of orchid mycorrhizal fungi: Scleroderma as a putative novel orchid mycorrhizal fungus of Vanilla in different crop systems. *Mycorrhiza*, *28*(2), 147–157. https://doi.org/10.1007/s00572-017-0808-6

Gottel, N. R., Castro, H. F., Kerley, M., Yang, Z., Pelletier, D. A., Podar, M., ... Schadt, C. W. (2011). Distinct Microbial Communities within the Endosphere and Rhizosphere of *Populus deltoides* Roots across Contrasting Soil Types. *Applied and Environmental Microbiology*, *77*(17), 5934–5944. https://doi.org/10.1128/aem.05255-11

Grayston, S. J., Wang, S., Campbell, C. D. & Edwards, A. C. (1998) Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol Biochem,* 30: 369–378.

Guevara, G., Bonito, G., Trappe, J. M., Cázares, E., Williams, G., Healy, R. A., Schadt, C. W & Vilgalys, R. (2013). New North American truffles (*Tuber* spp.) and their ectomycorrhizal associations, *Mycologia*, 105:1, 194-209, DOI: 10.3852/12-087

Gundale, M. J., Kardol, P., Nilsson, M. C., Nilsson, U., Lucas, R. W., & Wardle, D. A. (2014). Interactions with soil biota shift from negative to positive when a tree species is moved outside its native range. *New Phytologist*, *202*(2), 415–421. https://doi.org/10.1111/nph.12699

Guyonnet, J. P., Guillemet, M., Dubost, A., Simon, L., Ortet, P., Barakat, M., ... Haichar, F. el Z. (2018). Plant nutrient resource use strategies shape active rhizosphere microbiota through root exudation. *Frontiers in Plant Science*, *871*, 1–13. https://doi.org/10.3389/fpls.2018.01662

Haas, J. C., Street, N. R., Sjödin, A., Lee, N. M., Högberg, M. N., Näsholm, T. & Hurry, V. (2018). Microbial community response to growing season and plant nutrient optimisation in a boreal Norway spruce forest. *Soil Biology and Biochemistry*, *125*, 197–209. https://doi.org/10.1016/j.soilbio.2018.07.005

Hacquard, S. & Schadt, C. W. (2015). Towards a holistic understanding of the beneficial interactions across the Populus microbiome. *New Phytologist*, *205*(4), 1424–1430. https://doi.org/10.1111/nph.13133

Hacquard, S., Spaepen, S., Garrido-Oter, R., & Schulze-Lefert, P. (2017). Interplay Between Innate Immunity and the Plant Microbiota. *Annual Review of Phytopathology*, *55*(1), 565–589. https://doi.org/10.1146/annurev-phyto-080516-035623

Haichar, F. E. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., ... Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME Journal*, *2*(12), 1221–1230. https://doi.org/10.1038/ismej.2008.80

Hardoim P. R., Van Overbeek, L. S. & Van Elsas, J. D. (2008). Properties of bacterial endophytes and their proposed role in plant growth. *Plant Cell*, 463–471. https://doi.org/10.1016/j.tim.2008.07.008

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M. & Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev*. Doi:10.1128/MMBR.00050-14.

Hari, P. & Nöjd, P. (2009). The effect of temperature and PAR on the annual photosynthetic production of scots pine in northern Finland during 1906-2002. *Boreal Environment Research*, *14*, 5–18.

Harley, J. L. & Smith, S. E. (1983). Mycorrhizal symbiosis. New York: Academic Press.

Hart, N.M, Townsend, P.A, Chowyuk, A. & Gustafson, R. (2018). Stakeholder Assessment of the Feasibility of Poplar as a Biomass Feedstock and Ecosystem Services Provider in Southwestern Washington, USA. *Forests*, *9*(10), 655; doi:10.3390/f9100655

Hartmann, A., Schmid, M., Van Tuinen, D. & Berg, G. (2009). Plant-driven selection of microbes. *Plant Soil,* 321, 235–257. doi: 10.1007/s11104-008-9814-y

Hartmann, A., Rothballer, M. & Schmid, M. (2008). Lorenz Hiltner, pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant Soil*, 312: 7. https://doi.org/10.1007/s11104-007-9514-z

Hassani, M. A., Durán, P. & Hacquard, S. (2018). Microbial interactions within the plant holobiont. *Microbiome, 6*(58), 1–17. https://doi.org/10.1186/s40168-018-0445-0

Hättenschwiler, S., Tiunov, A. V., & Scheu, S. (2005). Biodiversity and Litter Decomposition in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

Hayden, H. L., Mele, P. M., Bougoure, D. S., Allan, C. Y., Norng, S., Piceno, Y. M., Brodie, E. L., Desantis, T. Z., Andersen, G. L., Williams, A. L. & Hovenden, M. J. (2012). Changes in the microbial community structure of bacteria, archaea and fungi in response to elevated CO2 and warming in an Australian native grassland soil. *Environ Microbiol 14*, 3081–3096.

Heller, R., Esnault, R. & Lance, C. (1993). Physiologie végétale. 1.Nutrition. (5e Ed.). Masson, Paris.

Helm, D. J., Allen, E. B. & Trappe, J. M. (1994). Mycorrhizal chronosequence near Exit Glacier, Alaska, (Cdzares 1992). *Canadian Journal of Botany*.

Hiiesalu, I., Pärtel, M., Davison, J., Gerhold, P., Metsis, M., Moora, M., Öpik, M., Vasar, M., Zobel, M. & Wilson, S. D. (2014). Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. *New Phytol*. 203, 233–244. doi: 10.1111/nph.12765

Hiltner, L. (1904). Uber neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderden berucksichtigung und Brache. Arb. Dtsch. *Landwirtsch*. Gesellschaft 98, 59–78.

Hoagland, D. C. & Arnon, D. I. (1950). The water culture method for growing plant without soil. California Agricultural Experiment. *Circular 337. The College of Agriculture University of California. Berkeley.*

Hodgson, S., de Cates, C., Hodgson, J., Morley, N. J., Sutton, B. C. & Gange, A. C. (2014). Vertical transmission of fungal endophytes is widespread in forbs. *Ecology and Evolution*, *4*(8), 1199–1208. https://doi.org/10.1002/ece3.953

Högberg, M. N. & Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, 791–795.

Horan, D. P., Chilvers, G. A. & Lapeyrie, F. F. (1988). Time sequence of the infection process eucalypt ectomycorrhizas. *New Phytologist*, *109*(4), 451–458. https://doi.org/10.1111/j.1469-8137.1988.tb03720.x

Hu, L., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., ... Erb, M. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications*, *9*(1), 1–13. https://doi.org/10.1038/s41467-018-05122-7

Hua, J., Chang, C., Sun, Q. & Meyerowitz, E. M. (1995). Ethylene insensitivity conferred by Arabidopsis EPS gene. *Science*, *269*(5231), 1712–1714. https://doi.org/10.1126/science.7569898

Huang, X., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q. & Vivanco. J. M. (2014). Rhizosphere interactions: root exudates, microbes, and microbial communities. *Botany*, 92: 267–275.

Imbert, E. & Lefèvre, F. (2003). Dispersal and gene flow of *Populus nigra* (*Salicaceae*) along a dynamic river system. *Journal of Ecology, 91*, 447-456.

Iniguez, A. L., Dong, Y., Carter, H. D., Ahmer, B. M. M., Stone, J. M. & Triplett, E. W. (2005). Regulation of Enteric Endophytic Bacterial Colonization by Plant Defenses. *Molecular Plant-Microbe Interactions*, *18*(2), 169–178. https://doi.org/10.1094/mpmi-18-0169

Izuno, A., Kanzaki, M., Artchawakom, T., Wachrinrat, C. & Isagi, Y. (2016). Vertical Structure of Phyllosphere Fungal Communities in a Tropical Forest in Thailand Uncovered by High-Throughput Sequencing. *PLoS ONE*, 11(11): e0166669. doi:10.1371/journal.pone.0166669

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A. & Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Frontiers in Plant Science*, *8*(1). https://doi.org/10.3389/fpls.2017.01617

Jones, J. & Dangl, J. (2006). The plant immune system. *Nature*, *444*(7117), 323–329. https://doi.org/10.1038/nature05286

Ju, C. & Chang, C. (2015). Mechanistic Insights in Ethylene Perception and Signal Transduction. *Plant Physiology*, *169*(1), 85–95. https://doi.org/10.1104/pp.15.00845

Jumpponen, A. & Trappe, JM. (1998). Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist*, 140, 295-310.

Kaiser, C., Kilburn, M. R., Clode, P. L., Fuchslueger, L., Koranda, M., Cliff, J. B., ... Kaiser, C. (2015). Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. *New Phytologist.* 205, 1537–1551.

Kaiser, C., Fuchslueger, L., Koranda, M., Gorfer, M., Stange, C.F., Kitzler, B., Rasche, F., Strauss, J., Sessitsch, A., Zechmeister-Boltenstern, S. & Richter, A. (2011). Plants control the seasonal dynamic of microbial N cycling in a beech forest soil by belowground C allocation. *Ecology, 92*:1036–1051.

Kamoun, S. (2006). A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology, 44* :41-60.

Kandel, S. L., Firrincieli, A., Joubert, P. M., Okubara, P. A., Leston, N. D., McGeorge, K. M., ... Doty, S. L. (2017). An in vitro study of bio-control and plant growth promotion potential of salicaceae endophytes. *Frontiers in Microbiology*, *8*(MAR). https://doi.org/10.3389/fmicb.2017.00386

Karliński, L., Rudawska, M., Kieliszewska-Rokicka, B. & Leski, T. (2010). Relationship between genotype and soil environment during colonization of poplar roots by mycorrhizal and endophytic fungi. *Mycorrhiza*, *20*(5), 315–324. https://doi.org/10.1007/s00572-009-0284-8

Karst, J., Gaster, J., Wiley, E. & Landhäusser, S. M. (2017). Stress differentially causes roots of tree seedlings to exude carbon. *Tree Physiology*, *37*(2), 154–164. https://doi.org/10.1093/treephys/tpw090

Kennedy, P. G., Peay, K. G. & Bruns, T. D. (2009). Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? *Ecology 90*(8): 2098‑107.

Khan, Z., Rho, H., Firrincieli, A., Hung, S. H., Luna, V., Masciarelli, O., Kim, S. H. & Doty S. L. (2016). Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. *Current Plant Biology*, *6*, 38–47. https://doi.org/10.1016/j.cpb.2016.08.001

Khatoon, H., Solanki, P., Narayan, M., Tewari, L., Rai, J. & Hina Khatoon, C. (2017). Role of microbes in organic carbon decomposition and maintenance of soil ecosystem. *International Journal of Chemical Studies*, *5*(6), 1648–1656. Retrieved from http://www.chemijournal.com/archives/2017/vol5issue6/PartW/5-6-133-734.pdf

Kia, S. H., Jurkechova, M., Glynou, K., Piepenbring, M. & Maciá-Vicente, J. G. (2018). The effects of fungal root endophytes on plant growth are stable along gradients of abiotic habitat conditions. *FEMS Microbiology Ecology*, *94*(2), 1–10. https://doi.org/10.1093/femsec/fix162

Kim, D., Langmead, B. & Salzberg, S. (2015). HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* 12, 357–360. doi:10.1038/nmeth.3317

Kipfer, T., Wohlgemuth, T., van der Heijden, M. G. A., Ghazoul, J., & Egli, S. (2012). Growth response of drought-stressed pinus sylvestris seedlings to single- and multi-species inoculation with ectomycorrhizal fungi. *PLoS ONE*, *7*(4). https://doi.org/10.1371/journal.pone.0035275

Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., ... Martin, F. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, *47*(4), 410–415. https://doi.org/10.1038/ng.3223

Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F. C., Van Loon, L. C. & Pieterse, C. M. J. (2008). Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. *Plant Physiology*, *147*(3), 1358–1368. https://doi.org/10.1104/pp.108.121392

Koranda, M., Kaiser, C., Fuchslueger, L., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S. & Richter, A. (2013). Seasonal variation in functional properties of microbial communities in beech forest soil. *Soil Biology and Biochemistry*, *60*, 95–104. https://doi.org/10.1016/j.soilbio.2013.01.025

Kurth, F., Mailänder, S., Bönn, M., Feldhahn, L., Herrman, S., Große, I., Buscot, F., Schrey, S. D. & Tarkka, M. T. (2014). Streptomyces -Induced Resistance Against Oak Powdery Mildew Involves Host Plant Responses in Defense, Photosynthesis, and Secondary Metabolism Pathways. *MPMI*, *27*(9), 891–900.

Labbé, J. L., Weston, D. J., Dunkirk, N., Pelletier, D. A. & Tuskan, G. A. (2014). Newly identified helper bacteria stimulate ectomycorrhizal formation in *Populus*. *Frontiers in Plant Science*, *5*, 1–10. https://doi.org/10.3389/fpls.2014.00579

Labbé, J., Jorge, V., Kohler, A., Vion, P., Marcais, B. T., Bastien, C., et al. (2011). Identification of quantitative trait loci affecting ectomycorrhizal symbiosis in an interspecific F1 poplar cross and differential expression of genes in ectomycorrhizas of the two parents: *Populus deltoides* and *Populus trichocarpa*. *Tree Genetics & Genomes* 7 617–627. 10.1007/s11295-010-0361-3

Laforest-Lapointe, I., Messier, C. & Kembel, S. W. (2016), Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species. *PeerJ*, 4: e2367; DOI 10.7717/peerj.2367

Lakshmanan, V., Selvaraj, G. & Bais, H. P. (2014). Functional Soil Microbiome: Belowground Solutions to an Aboveground Proble. *Plant Physiology*, 166 :689-700.

Lambais, M. R., Crowley, D. E., Cury, J. C., Bu, R. C. & Rodrigues, R. R. (2006). Bacterial Diversity in Tree Canopies of the Atlantic Forest. *Science*, *312*(5782), 1917. https://doi.org/10.1126/science.1124696

Lamit, L.J., Lau, M. K., Sthultz, C. M., Sthultz, C. M., Wooley, S. C., Whitham, T. G. & Gehring, C. A. (2014). Tree genotype and genetically based growth traits structure twig endophyte communities. *American Journal of Botany*, *101*(3). https://doi.org/10.3732/ajb.1400034

Lamit, L. J., Holeski, L. M., Flores-Rentería, L., Whitham, T. G. & Gehring, C. A. (2016). Tree genotype influences ectomycorrhizal fungal community structure: Ecological and evolutionary implications. *Fungal Ecology*, *24*, 124–134. https://doi.org/https://doi.org/10.1016/j.funeco.2016.05.013

Lang, C., Seven, J. & Polle, A. (2011). Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed central european forest. *Mycorrhiza* 21, 297–308. doi: 10.1007/s00572- 010-0338-y

Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology*, *10*(3). https://doi.org/10.1186/gb-2009-10-3-r25

Lapeyrie, F. & Chilvers, G. (1985). An Endomycorrhiza-Ectomycorrhiza Succession Associated with Enhanced Growth of *Eucalyptus dumosa* Seedlings Planted in a Calcareous Soil. *The New Phytologist, 100*(1), 93-104.

Lareen, A., Burton, F. & Schäfer, P. (2016). Plant root-microbe communication in shaping root microbiomes. *Plant Molecular Biology*, *90*(6), 575–587. https://doi.org/10.1007/s11103-015-0417-8

Lau, J. A. & Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences*, *109*(35), 14058–14062. https://doi.org/10.1073/pnas.1202319109

Lau, J. A., Lennon, J. T. & Heath, K. D. (2017). Trees harness the power of microbes to survive climate change. *Proceedings of the National Academy of Sciences*, *114*(42), 11009–11011. https://doi.org/10.1073/pnas.1715417114

Law, B. E. (2015). Regional analysis of drought and heat impacts on forests: current and future science directions. *Global Change Biology*, 20, 3595–3599. https://doi.org/10.1111/gcb.12651

Lauber, C. L., Strickland, M. S., Bradford, M. A. & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry*, *40*(9), 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021

Laurie-Berry, N., Joardar, V., Street, I. H., & Kunkel, B. N. (2006). The *Arabidopsis thaliana* JASMONATE INSENSITIVE 1 gene is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. *Mol. Plant Microbe Interact. 19*, 789–800.

Lee, H. Y. & Yoon, G. M. (2017). Kinase Assay for CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) in Arabidopsis thaliana. In: Binder B., Eric Schaller G. (eds) Ethylene Signaling. Methods in Molecular Biology, vol 1573. Humana Press, New York, NY

Lebeis, S. L., Herrera Paredes, S., Lundberg, D. S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., Glavina del Rio, T., Jones, C. D., Tringe, S. G. & Dangl, J. L. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*, 349(6250), 860-864. DOI: 10.1126/science.aaa8764

Lefèvre, F., Légionnet, A., de Vries, S. & Turok, J. (2009). Strategies for the conservation of a pioneer tree species, *Populus nigra* L., in Europe. *Genetics Selection Evolution*, *30*, S181. https://doi.org/10.1186/1297-9686-30-s1-s181

Lemoine, M. (1973). Amélioration des peupliers de la section Leuce sur sols hydromorphes [Thesis]: University of Nancy, France

Le Tacon, F. & Bouchard, D. (1986). Effects of different ectomycorrhizal fungi on growth of larch, Douglas fir, Scots pine and Norway spruce seedlings in fumigated nursery soil. *Oecologia. Applic. 7*: 389-407.

Letey, J. (1958). Relationship between Soil Physical Properties and Crop Production. In: Stewart B.A. (eds) Advances in Soil Science. Advances in Soil Science, vol 1. Springer, New York, NY

Leveau, J. H. J., Uroz, S. & de Boer, W. (2010). The bacterial genus *Collimonas*: Mycophagy, weathering and other adaptive solutions to life in oligotrophic soil environments. *Environmental Microbiology*, *12*(2), 281–292. https://doi.org/10.1111/j.1462-2920.2009.02010.x

Lévesque, M., Walthert, L. & Weber, P. (2016). Soil nutrients influence growth response of temperate tree species to drought. *Journal of Ecology*, *104*(2), 377–387. https://doi.org/10.1111/1365-2745.12519

Lewis, D. H. & Harley, J. L. (1965). Carbohydrate physiology of mycorrhizal roots of beech. *New Phytologist*, 64: 256-269. doi:10.1111/j.1469-8137.1965.tb05395.x

Li, D., Liu, C. M., Luo, R., Sadakane, K. & Lam, T. W. (2015). MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*, *31*(10), 1674–1676. https://doi.org/10.1093/bioinformatics/btv033

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li, J., Brunner, A. M., Meilan, R. & Strauss, S. H. (2009). Stability of transgenes in trees: Expression of two reporter genes in poplar over three field seasons. *Tree Physiology*, *29*(2), 299–312. https://doi.org/10.1093/treephys/tpn028

Li, L., Xu, M., Ali, M. E., Zhang, W., Duan, Y. & Li, D. (2018). Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. *PLoS ONE*, *13*(9), 1–18. https://doi.org/10.1371/journal.pone.0203812

Li, N., Han, X., Feng, D., Yuan, D. & Huang, L. J. (2019). Signaling Crosstalk between Salicylic Acid and Ethylene/Jasmonate in Plant Defense: Do We Understand What They Are Whispering? *International Journal of Molecular Sciences*, *20*(3), 671. https://doi.org/10.3390/ijms20030671

Liang, Y., Jiang, Y., Wang, F., Wen, C., Deng, Y., Xue, K., ... Sun, B. (2015). Long-term soil transplant simulating climate change with latitude significantly alters microbial temporal turnover. *ISME Journal*, *9*(12), 2561–2572. https://doi.org/10.1038/ismej.2015.78

Liao, H. L., Bonito, G., Rojas, J. A., Hameed, K., Wu, S., Schadt, C. W., Labbe, J. L., Tuskan, G., Martin, F. M., Grigoriev, I. V. & Vilgalys, R. (2019). Fungal endophytes of *Populus trichocarpa* alter host phenotype, gene expression and rhizobiome composition. *MPMI*., doi: 10.1094/MPMI-05-18-0133-R.

Liao, Y., Smyth, G. K., & Shi, W. (2014). FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923–930. https://doi.org/10.1093/bioinformatics/btt656

Lilleskov, E. A. & Bruns, T. D. (2011). Root colonization dynamics of two ectomycorrhizal fungi of contrasting life history strategies are mediating by addition of organic nutrient patches. *New Phytologist*, *159*, 141–151. https://doi.org/10.1046/j.0028-646x.2003.00794.x

Lindhal, B. D. & Tunlid, A. (2014). Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist*, 1–5. https://doi.org/10.1016/j.envres.2011.05.025

Lingua, G., Franchin, C., Todeschini, V., Castiglione, S., Biondi, S., Burlando, B., Parravicini, V., Torrigiani, P. & Berta, G. (2008). Arbuscular mycorrhizal fungi differentially affect the response to high zinc concentrations of two registered poplar clones. *Environmental Poullution*, *153*, 137–147. https://doi.org/10.1016/j.envpol.2007.07.012

Liu, B., Li, H., Zhu, B., Koide, R. T., Eissenstat, D. M. & Guo, D. (2015). Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species. *New Phytol*. 208, 125–136. doi: 10.1111/nph.13434

Liu, H., Carvalhais, L. C., Schenk, P. M. & Dennis, P. G. (2017). Effects of jasmonic acid signalling on the wheat microbiome differ between body sites. *Scientific Reports*, *7*, 1–8. https://doi.org/10.1038/srep41766

Liu, T., Sheng, M., Wang, C. Y., Chen, H., Li, Z. & Tang, M. (2015). Impact of arbuscular mycorrhizal fungi on the growth, water status, and photosynthesis of hybrid poplar under drought stress and recovery. *Photosynthetica*, *53*(2), 250–258. https://doi.org/10.1007/s11099-015-0100-y

Lladó, S., López-Mondéjar, R. & Baldrian, P. (2017). Forest Soil Bacteria: Diversity, Involvement in Ecosystem Processes, and Response to Global Change. *Microbiology and Molecular Biology Reviews: MMBR*, *81*(2), 1–27. https://doi.org/10.1128/MMBR.00063-16

Lodge, D. J. (1989). The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in *Populus* and *Salix*. *Plant and Soil*, 117: 243–253.

Long, D., Liu, J., Han, Q., Wang, X. & Huang, J. (2016). Ectomycorrhizal fungal communities associated with *Populus simonii* and *Pinus tabuliformis* in the hilly-gully region of the Loess Plateau, China. *Scientific Reports*, *6*, 1–10. https://doi.org/10.1038/srep24336

Lorenzo, O. & Solano, R. (2005). Molecular players regulating the jasmonate signalling network. *Curr. Opin. Plant Biol*. 8, 532–540.

Lou, Y., Clay, S. A., Davis, A. S., Dille, A., Felix, J., Ramirez, A. H. M., ... Yannarell, A. C. (2014). An Affinity-Effect Relationship for Microbial Communities in Plant-Soil Feedback Loops. *Microbial Ecology*, *67*(4), 866–876. https://doi.org/10.1007/s00248-013-0349-2

Love, M. I., Huber, W. & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 1–21. https://doi.org/10.1186/s13059-014-0550-8

Lugtenberg, B. J. J., Caradus, J. R. & Johnson, L. J. (2018). Fungal endophytes for sustainable crop production. *FEMS Microbiology Ecology*, 92: 1–17. https://doi.org/10.1093/femsec/fiw194

Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D. & Dangl, J. L. (2013). Practical innovations for high-throughput amplicon sequencing. *Nature Methods*, *10*(10), 999–1002. https://doi.org/10.1038/nmeth.2634

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., ... Ley, R. E. (2012). Defining the core Arabidopsis thaliana root microbiome. *Nature*, *488*(7409), 86–90. https://doi.org/10.1038/nature11237

Luo, Z. B., Li, K., Jiang, X. & Polle, A. (2009). Ectomycorrhizal fungus (*Paxillus involutus*) and hydrogels affect performance of *Populus euphratica* exposed to drought stress. *Ann. For. Sci*, 66. DOI: 10.1051/forest:2008073

Mader, M., Le Paslier, M.C., Bounon, R., Berard, A., Faivre Rampant, P., Fladung, M., Leple, J-C. & Kersten, B. (2016). Whole-genome draft assembly of *Populus tremula x Populus alba* clone INRA 717-1B4. *Silvae Genetica, 65*(2): 74-79

Mangeot-Peter, L., Tschaplinski, T. J., Engle, N. L., Veneault-Fourrey, C., Martin, F. & Deveau, A. (2020). Impacts of soil microbiome variations on root colonization by fungi and bacteria and on the metabolome of *Populus tremula x alba*. *Phytobiomes Journal*, 1–14. https://doi.org/10.1094/pbiomes-08-19-0042-r

Martin, F., Aerts, A., Ahrén, D., Brun, A., Danchin, E. G. J., Duchaussoy, F., ... Grigoriev, I. V. (2008). The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature*, *452*(7183), 88–92. https://doi.org/10.1038/nature06556

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C. & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, *14*, 760. https://doi.org/10.1038/nrmicro.2016.149

Marupakula, S., Mahmood, S., Jernberg, J., Nallanchakravarthula, S., Fahad, Z.A. & Finlay, R.D. (2017). Bacterial microbiomes of individual ectomycorrhizal *Pinus sylvestris* roots are shaped by soil horizon and differentially sensitive to nitrogen addition. *Environ Microbiol*, 19: 4736-4753. doi:10.1111/1462-2920.13939

Marupakula, S., Mahmood, S. & Finlay, R. D. (2016). Analysis of single root tip microbiomes suggests that distinctive bacterial communities are selected by *Pinus sylvestris* roots colonized by different ectomycorrhizal fungi. *Environnmental Microbiology*, *18*(5):1470–1483. https://doi.org/10.1111/1462-2920.13102

Massicotte, H. B., Melville, L. H., Molina, R. & Peterson, R. L. (1993). Structure and histochemistry of mycorrhizae synthesized between *Arbutus menziesii* (*Ericaceae*) and two basidiomycetes, *Pisolithus tinctorius* (*Pisolithaceae*) and *Piloderma bicolor* (*Corticiaceae*). *Mycorrhiza, 3*: 1-11.

Massicotte, H. B., Melville, L. H. & Peterson, R. L. (2005). Structural features of mycorrhizal associations in two members of the *Monotropoideae*, *Monotropa uniflora* and *Pterospora andromedea*. *Mycorrhiza*, *15*(2), 101–110. https://doi.org/10.1007/s00572-004-0305-6

Mcdowell, J. M. & Dangl, J. L. (2000). Signal transduction in the plant immune response. *Trends in Biochemical Sciences. 25*(2), 79–82.

McMurdie, P. J. & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Meaden, S., Metcalf, C. J. E. & Koskella, B. (2016). The effects of host age and spatial location on bacterial community composition in the English Oak tree. *Environmental Microbiology Reports*, *8*(5). https://doi.org/10.1111/1758-2229.12418

Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A., ... Minhas, P. S. (2017). Abiotic Stress Responses and Microbe-Mediated Mitigation in Plants: The Omics Strategies. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.00172

Meisner, A., Jacquiod, S., Snoek, B. L., Ten Hooven, F. C., & Van der Putten, W. H. (2018). Drought legacy effects on the composition of soil fungal and prokaryote communities. *Frontiers in Microbiology*, *9*(3), 1–12. https://doi.org/10.3389/fmicb.2018.00294

Mela, F., Fritsche, K., de Boer, W., van den Berg, M., van Veen, J. A., Maharaj, N. N., & Leveau, J. H. J. (2012). Comparative genomics of bacteria from the genus Collimonas: linking (dis)similarities in gene content to phenotypic variation and conservation. *Environmental Microbiology Reports*, *4*(4), 424–432. https://doi.org/10.1111/j.1758-2229.2012.00336.x

Mendes, R., Garbeva, P., Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms, *FEMS Microbiology Reviews*, *37*(5), 634–663. https://doi.org/10.1111/1574-6976.12028

Mercado-Blanco, J., Abrantes, I., Barra Caracciolo, A., Bevivino, A., Ciancio, A., Grenni, P., Hrynkiewicz, K., Kredics, L. & Proença, D. N. (2018). Belowground Microbiota and the Health of Tree Crops. *Front. Microbiol*. 9:1006. doi: 10.3389/fmicb.2018.01006

Mesanza, N., Crawford, B. D., Coulson, T. J. D., Iturritxa, E. & Patten, C. L. (2019). Colonization of *Pinus radiata* D. Don seedling roots by biocontrol bacteria *Erwinia billingiae and Bacillus simplex*. *Forests*, *10*(7), 1–13. https://doi.org/10.3390/f10070552

Mestre, M. C., Rosa, C. A., Safar, S. V. B., Libkind, D. & Fontenla, S. B. (2011). Yeast communities associated with the bulk-soil, rhizosphere and ectomycorrhizosphere of a Nothofagus pumilio forest in northwestern Patagonia, Argentina. *FEMS Microbiology Ecology*, *78*(3), 531–541. https://doi.org/10.1111/j.1574-6941.2011.01183.x

Michalet, S., Rohr, J., Warshan, D., Bardon, C., Roggy, J.-C., Domenach, A.-M., ... Poly, F. (2013). Phytochemical analysis of mature tree root exudates in situ and their role in shaping soil microbial communities in relation to tree N-acquisition strategy. *Plant Physiology and Biochemistry*, *72*, 169–177. https://doi.org/https://doi.org/10.1016/j.plaphy.2013.05.003

Miquel-Guennoc, C., Rose, C., Labbé, J. & Deveau, A. (2018). Bacterial biofilm formation on the hyphae of ectomycorrhizal fungi: A widespread ability under controls? *FEMS Microbiology Ecology*, *94*(7), 1–14. https://doi.org/10.1093/femsec/fiy093

Müller, A., Volmer, K., Mishra-Knyrim, M. & Polle, A. (2013). Growing poplars for research with and without mycorrhizas. *Frontiers in Plant Science*, *4*, 1–11. https://doi.org/10.3389/fpls.2013.00332

Naik, D., Smith, E. & Cumming, J. R. (2009). Rhizosphere carbon deposition, oxidative stress and nutritional changes in two poplar species exposed to aluminum. *Tree Physiology*, 29, (3), 423–436. https://doi.org/10.1093/treephys/tpn035

Nascimento, F. X., Rossi, M. J. & Glick, B. R. (2018). Ethylene and 1-aminocyclopropane-1-carboxylate (ACC) in plant–bacterial interactions. *Frontiers in Plant Science*, *9*(2), 1–17. https://doi.org/10.3389/fpls.2018.00114

Naylor, D. & Coleman-Derr, D. (2018). Drought Stress and Root-Associated Bacterial Communities. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.02223

Nehls, U. (2008). Mastering ectomycorrhizal symbiosis: The impact of carbohydrates. *Journal of Experimental Botany*, *59*(5), 1097–1108. https://doi.org/10.1093/jxb/erm334

Nehls, U. (2004). Carbohydrates and nitrogen: nutrients and signals in ectomycorrhizas. In: Varma A, Abbott L, Werner D, Hampp R, eds. *Plant surface microbiology*. Berlin: Springer Verlag, 373–392.

Newman, E. I. (1985). The rhizosphere: carbon sources and microbial populations. In Ecological Interactions in Soil (A.H. Fitter, D. Atkinson. D. J. Read and M. B. Usher, Eds), pp. 107-121. Blackwell Scientific Publications, Oxford.

Newman, M. A., Sundelin, T., Nielsen, J. T. & Erbs, G. (2013). MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Frontiers in Plant Science*, *4*, 1–14. https://doi.org/10.3389/fpls.2013.00139

Nicolitch, O., Feucherolles, M., Churin, J. L., Fauchery, L., Turpault, M. P. & Uroz, S. (2019). A microcosm approach highlights the response of soil mineral weathering bacterial communities to an increase of K and Mg availability. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-50730-y

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U.,... Van Kleunen, M. (2010). Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, *15*(12), 684–692.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., ... Kennedy, P. G. (2016). FUNGuild : An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Noirot-Gros, M. F., Shinde, S., Larsen, P. E., Zerbs, S., Korajczyk, P. J., Kemner, K. M. & Noirot, P. H. (2018). Dynamics of aspen roots colonization by Pseudomonads reveals strain-specific and mycorrhizal-specific patterns of biofilm formation. *Frontiers in Microbiology*, *9*, 1–16. https://doi.org/10.3389/fmicb.2018.00853

Nonaka, S., Sugawara, M., Minamisawa, K., Yuhashi, K. I. & Ezura, H. (2008). 1-Aminocyclopropane-1-carboxylate deaminase enhances *Agrobacterium tumefaciens*-mediated gene transfer into plant cells. *Applied and Environmental Microbiology*, *74*(8), 2526–2528. https://doi.org/10.1128/AEM.02253-07

Nunes da Rocha, U., Van Elsas, J. D. & Van Overbeek, L. S. (2011). Verrucomicrobia subdivision 1 strains display a difference in the colonization of the leek (*Allium porrum*) rhizosphere. *FEMS Microbiol Ecol*, 78:297–305.

Nylund, J. E. & Wallander, H. (1989). Effects of ectomycorrhiza on host growth and carbon balance in a semi-hydroponic cultivation system. New Phytologist 112: 389–398.

Oburger, E. & Jones, D. L. (2018). Sampling root exudates – mission impossible? *Rhizosphere*, 6, 116–133. DOI: 10.1016/j.rhisph.2018.06.004

Oksanen, J. (2015). Multivariate Analysis of Ecological Communities in R : vegan tutorial. https://doi.org/10.1016/0169-5347(88)90124-3

Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E. & Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME Journal*, *9*(4), 980–989. https://doi.org/10.1038/ismej.2014.196

Pant, M., Negi, GCS. & Kumar, P. (2017). Macrofauna contributes to organic matter decomposition and soil quality in Himalayan agroecosystems, India. *Appl. Soil Ecol. 120*, 20– 29.

Parsons, T. J., Sinkar, V. P., Stettler, R. F., Nester, E. W. & Gordon, M. P. (1986). Transformation of Poplar by *Agrobacterium tumefaciens*. *Bio/Technology*, *4*(6), 533–536. https://doi.org/10.1038/nbt0686-533

Pauwels, L. & Goossens, A. (2011). The JAZ Proteins: A Crucial Interface in the Jasmonate Signaling Cascade. *The Plant Cell*, *23*(9), 3089–3100. https://doi.org/10.1105/tpc.111.089300

Pena, R. & Polle, A. (2014). Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. *ISME Journal*, *8*(2), 321–330. https://doi.org/10.1038/ismej.2013.158

Pascazio, S., Crecchio, C., Ricciuti, P., Palese, A. M., Xiloyannis, C. & Sofo, A. (2015). Phyllosphere and carposhere bacterial communities in olive plants subjected to different cultural practices. *International Journal of Plant Biology*, 15–19. https://doi.org/10.4081/pb.2015.6011

Patle, P. N., Navnage, N. P. & Ramteke, P. R. (2018). Endophytes in plant system: Roles in growth promotion, mechanism and their potentiality in achieving agriculture sustainability. *International Journal of Chemical Studies*, *6*(1), 270–274.

Paungfoo-Lonhienne, C., Lonhienne, T. G. A., Yeoh, Y. K., Donose, B. C., Webb, R. I., Parsons, J., ... Ragan, M. A. (2016). Crosstalk between sugarcane and a plant-growth promoting Burkholderia species. *Scientific Reports*, *6*. https://doi.org/10.1038/srep37389

Pellegrin, C., Daguerre, Y., Ruytinx, J., Guinet, F., Kemppainen, M., Frey, N.F.d., Puech-Pagès, V., Hecker, A., Pardo, A.G., Martin, F.M. and Veneault-Fourrey, C. (2019). *Laccaria bicolor* MiSSP8 is a small-secreted protein decisive for the establishment of the ectomycorrhizal symbiosis. *Environ Microbiol*, 21: 3765-3779. doi:10.1111/1462-2920.14727

Penninckx, I. A. M. A., Thomma, B. P. H. J., Buchala, A., Metraux, J.-P. & Broekaert, W. F. (2007). Concomitant Activation of Jasmonate and Ethylene Response Pathways Is Required for Induction of a Plant Defensin Gene in Arabidopsis. *The Plant Cell*, *10*(12), 2103. https://doi.org/10.2307/3870787

Pérez, J., Muñoz-Dorado, J., De La Rubia, T. & Martínez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. *International Microbiology*, *5*(2), 53–63. https://doi.org/10.1007/s10123-002-0062-3

Pérez-Izquierdo, L., Zabal-Aguirre, M., Flores-Rentería, D., González-Martínez, S. C., Buée, M. & Rincón, A. (2017). Functional outcomes of fungal community shifts driven by tree genotype and spatial-temporal factors in Mediterranean pine forests. *Environmental Microbiology*, *19*(4), 1639–1652. https://doi.org/10.1111/1462-2920.13690

Perotto, S. & Bonfante, P. (1998). Genetic and functional diversity of ericoid mycorrhizal fungi. *Symbiosis*, *25*(1–3), 19–27.

Peterson, E. B. & Peterson, N. M. (1992). Ecology, management, and use of aspen and balsam poplar in the prairie provinces. *Forestry Canada, Special Report 1*, 1–252.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., ... Mundy, J. (2000). Arabidopsis map kinase 4 negatively regulates systemic acquired resistance. *Cell, 103*(7):1111–20. DOI:https://doi.org/10.1016/S0092-8674(00)00213-0

Philippot, L., Raaijmakers, J. M., Lemanceau, P. & Van der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology*, *11*, 789. https://doi.org/10.1038/nrmicro3109

Pieterse, C. M. J. & Van Loon, L. C. (2004). NPR1: the spider in the web of induced resistance signaling pathways. *Current Opinion in Plant Biology, 7* :456–464.

Pii, Y., Mimmo, T., Terzano, R., Cesco, S. & Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant growth- promoting rhizobacteria on nutrient acquisition process. A review. *Biology and Fertility of Soils*. 51(4), 403–415. https://doi.org/10.1007/s00374-015-0996-1

Pineda, A. & Dicke, M. (2013). Beneficial microbes in a changing environment : are they always helping plants to deal with insects ? *Functional Ecology*, 27, 574–586. https://doi.org/10.1111/1365-2435.12050

Pilate, G., Dejardin, A. & Leple, J. C. (2012). Chapter 1 - Field trials with lignin-modi ed transgenic trees. In: Lise J, Catherine L (eds) Advances in Botanical Research. Volume 61 (Lignins). London, Oxford, Boston, New York, San Diego: Aca- demic Press, pp 1-36. ISBN 0124160239

Pilipovic, A., Orlovic, S., Nikolic, N. & Galic, Z. (2006). Investigating potential of some poplar (*Populus sp.*) clones for phytoremediation of nitrates through biomass production. *Environmental Applications of Poplar and Willow Working Party, Northern Ireland*, 18–20.

Phillips, D. A., Fox, T. C., King, M. D., Bhuvaneswari, T. V. & Teuber, L. R. (2004). Microbial products trigger amino acid exudation from plant roots. *Plant Physiol*. 136, 2887–2894. doi: 10.1104/pp.104.044222

Plett, J. M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, S. J., ... Martin, F. (2014). Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences*, *111*(22), 8299–8304. https://doi.org/10.1073/pnas.1322671111

Plett, J. M., Kemppainen, M., Kale, S. D., Kohler, A., Legué, V., Brun, A., ... Martin, F. (2011). A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Current Biology*, *21*(14), 1197–1203. https://doi.org/10.1016/j.cub.2011.05.033

Pré, M., Atallah, M., Champion, A., De Vos, M., Pieterse, C. M. J. & Memelink, J. (2008). The AP2/ERF Domain Transcription Factor ORA59 Integrates Jasmonic Acid and Ethylene Signals in Plant Defense. *Plant Physiology*, *147*(3), 1347–1357. https://doi.org/10.1104/pp.108.117523

Pritchard, S. G. (2011). Soil organisms and global climate change. *Plant Pathology*, *60*(1), 82–99. https://doi.org/10.1111/j.1365-3059.2010.02405.x

Proença, D. N., Francisco, R., Kublik, S., Schöler, A., Schloter, M. & Morais, P. V. (2017). The Microbiome of Endophytic, Wood Colonizing Bacteria from Pine Trees as Affected by Pine Wilt Disease. *Scientific Reports*, 1–9. https://doi.org/10.1038/s41598-017-04141-6

Pusztahelyi, T., Holb, I. J & Pocsi, I. (2015). Secondary metabolites in fungus-plant interactions. *Frontiers in Plant Science*, *6*, 1–23. https://doi.org/10.3389/fpls.2015.00573

Qin, R., Hirano, Y. & Brunner, I. (2007). Exudation of organic acid anions from poplar roots after exposure to Al, Cu and Zn. *Tree Physiology*, *27*(2), 313–320. https://doi.org/10.1093/treephys/27.2.313

Quan, M. & Liang, J. (2017). The influences of four types of soil on the growth, physiological and biochemical characteristics of *Lycoris aurea* (L' Her.) Herb. *Scientific Reports*, *7*, 1–9. https://doi.org/10.1038/srep43284

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*, 590–596. https://doi.org/10.1093/nar/gks1219

Quoreshi, A. M. (2008). The Use of Mycorrhizal Biotechnology in Restoration of Disturbed Ecosystem. In: Siddiqui Z.A., Akhtar M.S., Futai K. (eds) Mycorrhizae: Sustainable Agriculture and Forestry. Springer, Dordrecht

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C. & Moënne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, *321*(1–2), 341–361. https://doi.org/10.1007/s11104-008-9568-6

Raaijmakers, J. M. & Mazzola, M. (2012). Diversity and Natural Functions of Antibiotics Produced by Beneficial and Plant Pathogenic Bacteria. *Annual Review of Phytopathology*, *50*(1), 403–424. https://doi.org/10.1146/annurev-phyto-081211-172908

Rae, A. M., Robinson, K. M., Street, N. R. & Taylor, G. (2004). Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. *Canadian Journal of Forest Research*, 34 (7), 1488-1498. (doi:10.1139/x04-033).

Rascovan, N., Carbonetto, B., Perrig, D., Díaz, M., Canciani, W., Abalo, M., … Vazquez, M. P. (2016). Integrated analysis of root microbiomes of soybean and wheat from agricultural fields. *Scientific Reports*, *6*(1150), 1–12. https://doi.org/10.1038/srep28084

R Core Team. (2016). R : A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rey, T. & Schornack, S. (2013). Interactions of benefi cial and detrimental rootcolonizing fi lamentous microbes with plant hosts. *Genome Biology*, *14*(6), 4–9. https://doi.org/10.1186/gb-2013-14-6-121

Richard, F., Roy, M., Shahin, O., Sthultz, C., Duchemin, M., Joffre, R. & Selosse, M. A. (2011). Ectomycorrhizal communities in a Mediterranean forest ecosystem dominated by *Quercus ilex*: Seasonal dynamics and response to drought in the surface organic horizon. *Annals of Forest Science*, *68*(1), 57–68. https://doi.org/10.1007/s13595-010-0007-5

Riedlinger, J., Schrey, S. D., Tarkka, M. T., Hampp, R., Kapur, M. & Fiedler, H. P. (2006). Auxofuran, a novel metabolite that stimulates the growth of fly agaric, is produced by the mycorrhiza helper bacterium *Streptomyces* strain AcH 505. *Applied and Environmental Microbiology*, *72*(5), 3550–3557. https://doi.org/10.1128/AEM.72.5.3550-3557.2006

Rincón, A., Ruiz-Díez, B., García-Fraile, S., García, J. A. L., Fernández-Pascual, M., Pueyo, J. J. & De Felipe, M. R. (2005). Colonisation of *Pinus halepensis* roots by *Pseudomonas fluorescens* and interaction with the ectomycorrhizal fungus *Suillus granulatus*. *FEMS Microbiology Ecology*, *51*(3), 303–311. https://doi.org/10.1016/j.femsec.2004.09.006

Rincon-Florez, V. A., Carvalhais, L. C., Dang, Y. & Schenk, P. (2015). Soil microbial communities under tillage systems in Australia. *Plant, Soil and Microbes*, *1*, 93-102.

Rodriguez, R. J., White, J. F., Arnold, A. E. & Redman, R. S. (2009). Fungal endophytes: diversity and functional roles. *New Phytologist, 182*, 314–330. doi: 10.1111/j.1469-8137.2009.02773.x

Rood, S. B. & Mahoney J. M. (1990). Collapse of riparian poplar forests downstream from dams in western prairies: Probable causes and prospects for mitigation. *Environmental Management*, 14: 451. https://doi.org/10.1007/BF02394134

Ruffinoni, C., Trémolières, M. & Sanchez-Perez, J. M. (2003). Végétation alluviale et flux de nutriments : des liens interactifs. In : Les forêts riveraines des cours d'eau. Écologie, fonctions et gestion / H. Piégay, G. Pautou, C. Ruffinoni, éditeurs. Paris: IDF: 134-154.

Ryan, M. G. (2010). Editorial: Temperature and tree growth. *Tree Physiology*, *30*(6), 667–668. https://doi.org/10.1093/treephys/tpq033

Sarabia, M., Jakobsen, I., Grønlund, M., Carreon-Abud, Y. & Larsen, J. (2018). Rhizosphere yeasts improve P uptake of a maize arbuscular mycorrhizal association. *Applied Soil Ecology*, *125*, 18–25. https://doi.org/10.1016/j.apsoil.2017.12.012

Sasse, J., Martinoia, E. & Northen, T. (2018). Feed your friends: do plant exudates shape the root microbiome? *Trends Plant Sci.* 23, 25–41. doi: 10.1016/j.tplants.2017.09.003

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis, *Nature methods*, *9*(7): 671-675, PMID 22930834

Schreiter, S., Ding, G. C., Heuer, H., Neumann, G., Sandmann, M., Grosch, R., … Smalla, K. (2014). Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. *Frontiers in Microbiology*, *5*, 1–13. https://doi.org/10.3389/fmicb.2014.00144

Schweiger, P., Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Rasche, F., … Richter, A. (2010). Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. *New Phytologist*, *187*(3), 843–858. https://doi.org/10.1111/j.1469-8137.2010.03321.x

Selosse, M. A. & Le Tacon, F. (1998). The land flora: a phototroph-fungus partnership? *Tree, 13*(1).

Selosse, M. A., Bouchard, D., Martin, F. & Tacon, F. Le. (2011). Effect of *Laccaria bicolor* strains inoculated on Douglas-fir (*Pseudotsuga menziesii*) several years after nursery inoculation. *Canadian Journal of Forest Research*, *30*(3), 360–371. https://doi.org/10.1139/x99-221

Setaro, S., Weiß, M., Oberwinkler, F. & Kottke, I. (2006). *Sebacinales* form ectendomycorrhizas with *Cavendishia nobilis*, a member of the Andean clade of Ericaceae, in the mountain rain forest of southern Ecuador. *New Phytologist*, *169*(2), 355–365. https://doi.org/10.1111/j.1469-8137.2005.01583.x

Seyfferth, C. & Tsuda, K. (2014). Salicylic acid signal transduction: the initiation of biosynthesis, perception and transcriptional reprogramming. *Frontiers in Plant Science*, *5*, 1–10. https://doi.org/10.3389/fpls.2014.00697

Shakya, M., Gottel, N., Castro, H., Yang, Z. K., Gunter, L., Labbé, J., … Schadt, C. W. (2013). A Multifactor Analysis of Fungal and Bacterial Community Structure in the Root Microbiome of Mature *Populus deltoides* Trees. *PLoS ONE*, *8*(10). https://doi.org/10.1371/journal.pone.0076382

Schmidt, H., Eickhorst, T. & Tippkotter, R. (2011). Monitoring of root growth and redox conditions in paddy soil rhizotrons by redox electrodes and image analysis. *Plant Soil 341*, 221–232. doi: 10.1007/s11104-010- 0637-2

Schortemeyer, M., Hartwig, U. A., Hendrey, G. R. & Sadowsky, M. J. (1996). Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). *Soil Biology and Biochemistry, 28*, 1717–1724.

Schulz-Bohm, K., Tyc, O., de Boer, W., Peereboom, N., Debets, F., Zaagman, N., Janssens, T. K. S., Garbeva, P. (2016). Fungus-associated bacteriome in charge of their host behavior. *Fungal Genet Biol*. DOI: 10.1016/j.fgb.2016.07.011.

Shi, S., Condron, L., Larsen, S., Richardson, A. E., Jones, E., Jiao, J., … Stewart, A. (2011). In situ sampling of low molecular weight organic anions from rhizosphere of radiata pine (*Pinus radiata*) grown in a rhizotron system. *Environmental and Experimental Botany*, *70*(2), 131–142. https://doi.org/https://doi.org/10.1016/j.envexpbot.2010.08.010

Sieber, T. (2007). Endophytic fungi in forest trees: are they mutualists? *Fungal Biology Review*, 21(2-3), 75-89. DOI : https://doi.org/10.1016/j.fbr.2007.05.004

Slot, M. & Winter, K. (2017). In situ temperature response of photosynthesis of 42 tree and liana species in the canopy of two Panamanian lowland tropical forests with contrasting rainfall regimes. *New Phytologist*, *214*(3), 1103–1117. https://doi.org/10.1111/nph.14469

Smith, S. E. & Read, D. (2008). Mycorrhizal symbiosis, 3rd edn. Academic Press, London

Song, Y., Chan, Q., Ci, D., Shao, X. & Zhang, D. (2014). Effects of high temperature on photosynthesis and related gene expression in poplar. *BMC Plant Biology*, *14*(111), 1–20. https://doi.org/10.3329/bjb.v44i3.38553

Sørensen, J. (1997). The rhizosphere as a habitat for soil microorganisms, p. 21-45. In J. D. Van Elsas, J. T. Trevors, and E. M. H. Wellington (ed.), Modern soil microbiology. Marcel Dekker, Inc. New York, N.Y.

Sperber, J. I. & Rovira, A. D. (1995). A study of the bacteria associated with the roots of subterranean clover and Wimmera ryegrass. *Journal of Applied Bacteriology*. 22, 85-95.

Spring, S., Scheuner, C., Göker, M. & Klenk, H. P. (2015). A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. *Frontiers in Microbiology*, *6*, 1–17. https://doi.org/10.3389/fmicb.2015.00281

Stopnisek, N., Zühlke, D., Carlier, A., Barberan, A., Fierer, N., Becher, D., Riedel, K., Eberl, L. & Weisskopf, L. (2016). Molecular mechanisms underlying the close association between soil Burkholderia and fungi. *ISME J*, 10:253–64.

Strullu-Derrien, C., Selosse, M. A., Kenrick, P. & Martin, F. M. (2018). The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist*, *220*(4), 1012–1030. https://doi.org/10.1111/nph.15076

Sun, S., Li, S., Avera, B.N., Strahm, B. D. & Badgley, B. D. (2017). Soil Bacterial and Fungal Communities Show Distinct Recovery Patterns during Forest Ecosystem Restoration. *Applied and Environmental Microbiology*, *83*(14) e00966-17. DOI: 10.1128/AEM.00966-17

Taffner, J., Erlacher, A., Bragina, A., Berg, C., Moissl-Eichinger, C. & Berg, G. (2018). What Is the Role of Archaea in Plants? New Insights from the Vegetation of Alpine Bogs. *MSphere*, *3*(3), 1–14. https://doi.org/10.1128/msphere.00122-18

Taghavi, S., Garafola, C., Newman, L., Hoffman, A., Weyens, N., Barac, T., Vangronsveld, J. & Van der Lelie, D. (2009). Genome Survey and Characterization of Endophytic Bacteria Exhibiting a Beneficial Effect on Growth and Development of Poplar Trees. *Applied and Environmental Microbiology*, *75*(3), 748–757. https://doi.org/10.1128/AEM.02239-08

Takacs, T., Radimzsky, L. & Nemeth, T. (2005). The Arbuscular Mycorrhizal Status of Poplar Clones Selected for Phytoremediation of Soils Contaminated with Heavy Metals. *Zeitschrift fur Naturforschung*, *60*(3-4): 357-361.

Tedersoo, L., Bahram, M., Cajthaml, T., Polme, S., Hiiesalu, I., Anslan, S., Harend, H., Buegger, F., Pritsch, K., Koricheva, J. & Abarenkov, K. (2016). Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. *ISME J*; *10*(2):346-62.

Terhonen, E., Blumenstein, K., Kovalchuk, A. & Asiegbu, F. O. (2019). Forest tree microbiomes and associated fungal endophytes: Functional roles and impact on forest health. *Forests*, *10*(1), 1–32. https://doi.org/10.3390/f10010042

Terrat, S., Horrigue, W., Dequietd, S., Saby, N. P. A., Lelièvre, M., Nowak, V., … Ranjard, L. (2017). Mapping and predictive variations of soil bacterial richness across France. *PLoS ONE*, *12*(10), 5–8. https://doi.org/10.1371/journal.pone.0186766

Tesar, M., Reichenauer, T. G. & Sessitsch, A. (2002). Bacterial rhizosphere populations of black poplar and herbal plants to be used for phytoremediation of diesel fuel. *Soil Biology & Biochemistry,* 34: 1883– 1892.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., … Browse, J. (2007). JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. *Nature*, *448*(7154), 661–665. https://doi.org/10.1038/nature05960

Thoen, E., Aas, A. B., Vik, U., Brysting, A. K., Skrede, I., Carlsen, T. & Kauserud, H. (2019). A single ectomycorrhizal plant root system includes a diverse and spatially structured fungal community. *Mycorrhiza*, 29: 167. https://doi.org/10.1007/s00572-019-00889-z

Tian, J., Pourcher, AM., Bouchez, T., Gelhaye, E. & Peu, P. (2014). Occurrence of lignin degradation genotypes and phenotypes among prokaryotes. *Appl Microbiol Biotechnol*, 98: 9527. https://doi.org/10.1007/s00253-014-6142-4

Tian, J., Wang, J., Dippold, M., Gao, Y., Blagodatskaya, E. & Kuzyakov, Y. (2016). Biochar affects soil organic matter cycling and microbial functions but does not alter microbial community structure in a paddy soil. *Science of the Total Environment*, *556*, 89–97. https://doi.org/10.1016/j.scitotenv.2016.03.010

Timm, C. M., Carter, K. R., Carrell, A. A., Jun, S., Jawdy, S. S., Vélez, J. M., Gunter, L. E., Yang, Z., Nookaew, I., Engle, N. L., Lu, T. Y. S., Schadt, C. W., Tschaplinski, T. J., Doktycz, M. J., Tuskan, G. A., Pelletier, D.A. & Weston, D. J. (2018). Abiotic Stresses Shift Belowground Populus –Associated bacteria toward a core stress microbiome. *mSystems*, *3*(1), 1–17.

Timm, C. M., Pelletier, D. A., Jawdy, S. S., Gunter, L. E., Henning, J. A., Engle, N., Aufrecht, J., Gee, E., Nookaew, I., Yang, Z., Lu, T. Y., Tschaplinski, T. J., Doktycz, M. J., Tuskan, G. A. & Weston, D. J. (2016). Two poplar-associated bacterial isolates induce additive favorable responses in a constructed plant-microbiome system. *Front Plant Sci* 7:497. https://doi.org/10.3389/fpls.2016.00497.

Timm, C. M., Campbell, A. G., Utturkar, S. M., Jun, S. R., Parales, R. E., Tan, W. A., … Pelletier, D. A. (2015). Metabolic functions of *Pseudomonas fluorescens* strains from *Populus deltoides* depend on rhizosphere or endosphere isolation compartment. *Frontiers in Microbiology*, *6*, 1–13. https://doi.org/10.3389/fmicb.2015.01118

Thomma, B. P. H. J., Tierens, K. F. M., Penninckx, I. A. M. A., Mauch-Mani, B., Broekaert, W. F. & Cammue, B. P. A. (2001). Different micro-organisms differentially induce Arabidopsis disease response pathways. *Plant Physiology and Biochemistry. 39*(7-8), 673-680.

Toju, H., Tanabe, A. S. & Sato, H. (2018). Network hubs in root-associated fungal metacommunities. *Microbiome*, 1–16. https://doi.org/10.1186/s40168-018-0497-1

Tschaplinski, T. J., Plett, J. M., Engle, N. L., Deveau, A., Cushman, K. C., Martin, M. Z., Doktycz, M. J., Tuskan, G. A., Brun, A., Kohler, A. & Martin, F. (2014). *Populus trichocarpa* and *Populus deltoides* exhibit different metabolomic responses to colonization by the symbiotic fungus *Laccaria bicolor*. *Mol Plant Microbe Interact*. *27*(6):546-56. doi: 10.1094/MPMI-09-13-0286-R.

Turner, J. G., Ellis, C. & Devoto, A. (2002). The Jasmonate Signal Pathway. *The Plant Cell*, *14*, S153–S164. https://doi.org/10.1105/tpc.000679

Turner, T. R., James, E. K., Poole, P. S., Gilbert, J., Meyer, F., Jansson, J., … Jones, J. (2013). The plant microbiome. *Genome Biology*, *14*(6), 209. https://doi.org/10.1186/gb-2013-14-6-209

Tuskan, G. A., Di Fazio, S., S, J., J. Bohlmann, Grigoriev, I., Hellsten, U., … Rokhsar, D. (2006). The Genome of Black Cottonwood, *Science*, *313*(September), 1596–1604. https://doi.org/10.1126/science.1128691

Uehling, J., Deveau, A. & Paoletti, M. (2017). Do fungi have an innate immune response? An NLR-based comparison to plant and animal immune systems. *PLOS Pathogens*, *13*(10), e1006578. Retrieved from https://doi.org/10.1371/journal.ppat.1006578

Ulrich, A. & Ewald, D. (2008). Diversity of endophytic bacterial communities in poplar grown under field conditions. *FEMS*, 63(2), 169-180. https://doi.org/10.1111/j.1574-6941.2007.00419.x

Uren, N. C. (2000). Types, amounts and possible functions of com- pounds released into the rhizosphere by soil-grown plants. In The Rhizosphere, Biochemistry and Organic Substances at the Soil–Plant Interface (eds R. Pinton, Z. Varanini & P. Nannipieri), pp. 19–40. Marcel Dekker, New York, NY, USA.

Uroz, S., Buée, M., Deveau, A., Mieszkin, S. & Martin, F. (2016). Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. *SBB*, 103, 471-488. https://doi.org/10.1016/j.soilbio.2016.09.006

Uroz, S., Oger, P., Tisserand, E., Cébron, A., Turpault, M. P., Bueé, M., ... Frey-Klett, P. (2016). Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. *Scientific Reports*, *6*(May), 1–11. https://doi.org/10.1038/srep27756

Uroz, S., Calvaruso, C., Turpault, M. P., Sarniguet, A., de Boer, W., Leveau, J. H. J. & Frey-Klett, P. (2010). Efficient mineral weathering is a distinctive functional trait of the bacterial genus *Collimonas*. *Soil Biology and Biochemistry*, *41*(10), 2178–2186. https://doi.org/10.1016/j.soilbio.2009.07.031

Uroz, S., Calvaruso, C., Turpault, M. P., Pierrat, J. C., Mustin, C. & Frey-Klett, P. (2007). Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. *Applied and Environmental Microbiology*, *73*(9), 3019–3027. https://doi.org/10.1128/AEM.00121-07

Uselman, S. M., Qualls, R. G. & Thomas, R. B. (2000). Effects of increased atmospheric CO_2, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). *Plant and Soil*, 222: 191. https://doi.org/10.1023/A:1004705416108

Van Der Heijden, M. G. A., Bardgett, R. D. & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, *11*(6), 651. https://doi.org/10.1111/j.1461-0248.2008.01199.x

Van der Heijden, M. G. A., Martin, F. M., Selosse, M. A. & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, *205*(4), 1406–1423. https://doi.org/10.1111/nph.13288

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Duhamel, M. & Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist*, *206*(4), 1196–1206. https://doi.org/10.1111/nph.13312

Varma, A., Verma, S., Sudha, Sahay, N., Bütehorn, B. & Franken, P. (1999). *Piriformospora indica*, a Cultivable Plant-Growth-Promoting Root Endophyte. *Applied and Environmental Microbiology*, *65*(6), 2741–2744.

Vayssier-Taussat, M., Albina, E., Citti, C., Cosson, J. F., Jacques M. A., Lebrun, M. H., Le Loir, Y., Ogliastro, M., Petit, M. A., Roumagnac, P. & Candresse, T. (2014). Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta-omics. *Front Cell Infect Microbiol*, 4:29. doi:10.3389/fcimb.2014.00029

Vayssières, A., Pěnčík, A., Felten, J., Kohler, A., Ljung, K., Martin, F. & Legué, V. (2015). Development of the poplar-*Laccaria bicolor* ectomycorrhiza modifies root auxin metabolism, signaling, and response. *Plant Physiology*, *169*(1), 890–902. https://doi.org/10.1104/pp.114.255620

Veach, A. M, Yip, D., Engle, N. L., Yang, Z. K., Bible, A. & Schadt, C. W. (2018). Modification of plant cell wall chemistry impacts metabolome and microbiome composition in *Populus* PdKOR1 RNAi plants. *Plant Soil*, 429: 349–361.

Veach, A. M., Morris, R., Yip, D. Z., Yang, D. K., Engle, N. L., Cregger, M. A., Tschaplinski, T. J. & Schadt, C. W. (2019). Rhizosphere microbiomes diverge among Populus trichocarpa plant-host genotypes and chemotypes, but, it depends on soil origin. *Microbiome, 7*(76). https://doi.org/10.1186/s40168-019-0668-8.

Vick, B. A. & Zimmerman, D. C. (1984). Biosynthesis of Jasmonic Acid by Several Plant Species. *Plant Physiology*, *75*(2), 458–461. https://doi.org/10.1104/pp.75.2.458

Vierheilig, H., Schweiger, P. & Brundrett, M. (2005). An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum*, *125*(4), 393–404. https://doi.org/10.1111/j.1399-3054.2005.00564.x

Villar, M. & Forestier, O. (2009). Le Peuplier noir en France : pourquoi conserver ses ressources génétiques et comment les valoriser ? *Revue forestière française*, vol. LXI, n° 5, pp. 457-466.

Villeneuve, N., Le Tacon, F. & Bouchard, D. (1991). Survival of inoculated Laccaria bicolor in competition with native ectomycorrhizal fungi and effects on the growth of outplanted Douglas fir seedlings. *Plant Soil, 135*: 95. https://doi.org/10.1007/BF00014782

Visioli, G., Sanangelantoni, A. M., Vamerali, T., Dal Cortivo, C. & Blandino, M. (2018). 16S rDNA profiling to reveal the influence of seed-applied biostimulants on the rhizosphere of young maize plants. *Molecules*, *23*(6), 1–18. https://doi.org/10.3390/molecules23061461

Voříšková, A., Jansa, J., Püschel, D., Krüger, M., Cajthaml, T., Vosátka, M. & Janoušková, M. (2017). Real-time PCR quantification of arbuscular mycorrhizal fungi: does the use of nuclear or mitochondrial markers make a difference? *Mycorrhiza*, *27*(6), 577–585. https://doi.org/10.1007/s00572-017-0777-9

Voříšková, J. & Baldrian, P. (2013). Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME J.* 7, 477–486. doi: 10.1038/ismej.2012.116

Wagner, M. R., Lundberg, D. S., Del Rio, T. G., Tringe, S. G., Dangl, J. L. & Mitchell-Olds, T. (2016). Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nat. Commun.* 7:12151. doi: 10.1038/ncomms12151

Walker, C. (2005). A simple blue staining technique for arbuscular mycorrhizal and other root-inhabiting fungi. *Inoculum* 56: 68–69.

Wallenstein, M. D. & Hall, E. K. (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry*, *109*(1–3), 35–47. https://doi.org/10.1007/s10533-011-9641-8

Walter, C., Fladung, M. & Boerjan, W. (2010). The 20-year environmental safety record of GM trees. *Nat Biotechnol*, *28*(7): 656-658. http://dx.doi.org/10.1038/ nbt0710-656

Wang, M., Gao, L., Dong, S., Sun, Y., Shen, Q. & Guo, S. (2017). Role of Silicon on Plant–Pathogen Interactions. *Frontiers in Plant Science*, *8*, 1–14. https://doi.org/10.3389/fpls.2017.00701

Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, Y., Zhang, W., Ding, C., Zhang, B., Huang, Q., Huang, R. & Su, X. (2019). Endophytic communities of transgenic poplar were determined by the environment and niche rather than by transgenic events. *Frontiers in Microbiology*, *10*, 1–18. https://doi.org/10.3389/fmicb.2019.00588

Warmink J. A, Nazir R. & Van Elsas J. D. (2009). Universal and species-specific bacterial “fungiphiles” in the mycospheres of different basidiomycetous fungi. *Environ Microbiol*., 11:300–12.

Wawra, S., Fesel, P., Widmer, H., Timm, M., Seibel, J., Leson, L., ... Zuccaro, A. (2016). The fungal-specific β-glucan-binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms13188

Whipps, J. M., Hand, P., Pink, D. & Bending, G. D. (2008). Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology*, *105*(6), 1744–1755. https://doi.org/10.1111/j.1365-2672.2008.03906.x

Wu, G., Liu, H., Hua, L., Luo, Q., Lin, Y., He, P., ... Ye, Q. (2018). Differential responses of stomata and photosynthesis to elevated temperature in two co-occurring subtropical forest tree species. *Frontiers in Plant Science*, *9*(April), 1–8. https://doi.org/10.3389/fpls.2018.00467

Yadav, R., Arora, P., Kumar, S. & Chaudhury, A. (2010). Perspectives for genetic engineering of poplars for enhanced phytoremediation abilities. *Ecotoxicology*, 19, 1754–1588.

Yip, D. Z., Veach, A. M., Yang, Z. K., Cregger, M. A. & Schadt, C. W. (2018). Rapid report Methanogenic Archaea dominate mature heartwood habitats of Eastern Cottonwood (*Populus deltoides*). *New Phytologist*. https://doi.org/10.1111/nph.15346

Yu, K., Pieterse, C. M. J., Bakker, P. A. H. M. & Berendsen, R. L. (2019). Beneficial microbes going underground of root immunity. *Plant Cell Environ*, 1–11. https://doi.org/10.1111/pce.13632

Yu, T. E., Egger, K. N. & Peterson, L. R. (2001). Ectendomycorrhizal associations - Characteristics and functions. *Mycorrhiza*, *11*(4), 167–177. https://doi.org/10.1007/s005720100110

Yurkov, A. M. (2018). Yeasts of the soil – obscure but precious. *Yeast*, *35*(5), 369–378. https://doi.org/10.1002/yea.3310

Zamioudis, C. & Pieterse, C. M. J. (2011). Modulation of Host Immunity by Beneficial Microbes. *Molecular Plant-Microbe Interactions*, *25*(2), 139–150. https://doi.org/10.1094/mpmi-06-11-0179

Zelicourt, A., Al-Yousif, M. & Hirt, H. (2013). Rhizosphere Microbes as Essential Partners for Plant Stress Tolerance. *Molecular Plant Advance*, 1–4. https://doi.org/10.1093/mp/sst028

Zhang, K., Shi, Y., Cui, X., Yue, P., Li, K., Liu, X., ... Chu, H. (2019). Salinity Is a Key Determinant for Soil Microbial Communities in a Desert Ecosystem. *MSystems*, *4*(1), 1–11. https://doi.org/10.1128/msystems.00225-18

Ziska, L.H. & Bunce, J.A. (2006). Plant responses to rising atmospheric carbon dioxide. In: Plant growth and climate change (Ed. By Morison, J.I.L & Morecroft, M.D.). Blackwell Publishing, Oxford.
Zilber-Rosenberg, I. & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS*, 32 : 723-735. https://doi.org/10.1111/j.1574-6976.2008.00123.x
Zuñiga, C., Zaramela, L. & Zengler, K. (2017). Elucidation of complexity and prediction of interactions in microbial communities. *Microbial Biotechnology*, *10*(6), 1500–1522. https://doi.org/10.1111/1751-7915.12855

Annexes

Chapitre II : Dynamique de colonisation des racines du peuplier par les communautés de micro-organisme

SUPPLEMENTAL INFORMATIONS

Table S1 (1/4) - Relative abundance of members of bacterial communities at each taxonomic rank (>1% relative abundance in at least one time point) detected in the bulk soil and/or roots samples collecting from T0 to T50. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant differences between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05). The asterisks denote significant difference in relative abudance of fungal communities between bulk soil samples and root samples collected from T2 (One-way ANOVA, factor=sampling time, P<0.05).

Bacterial phylum	**Bulk Soil**	**T2**	**T4**	**T7**	**T10**	**T15**	**T21**	**T30**	**T50**
Armatimonadetes	0.31 ± 0.01 *	0.04 ± 0.01 a	0.13 ± 0.05 a	0.21 ± 0.05 ab	0.22 ± 0.06 ab	0.76 +/- 0.23 abc	0.52 ± 0.07 ab	1.0 +/- 0.3 bc	1.4 +/- 0.3 c
Gemmatimonadetes	1.2 +/- 0.1 *	0.16 ± 0.04 a	0.11 ± 0.02 a	0.10 ± 0.01 a	0.10 ± 0.06 a	0.05 ± 0.02 a	0.19 ± 0.04 a	0.22 ± 0.06 a	0.15 ± 0.01 a
Other	2.9 +/- 0.1 *	0.37 +/- 0.10 ab	0.21 ± 0.05 a	0.33 +/- 0.12 ab	0.47 +/- 0.25 ab	0.21 ± 0.03 a	0.61 ± 0.08 ab	0.95 +/- 0.26 b	0.95 +/- 0.15 ab
Chloroflexi	3.8 +/- 0.2 *	0.43 +/- 0.14 a	0.27 ± 0.01 a	0.24 ± 0.05 a	0.25 ± 0.04 a	0.21 ± 0.09 a	0.99 +/- 0.30 a	2.2 +/- 1.3 ab	3.8 +/- 0.4 b
Unidentified phyla	2.2 +/- 0.1 *	0.45 ± 0.08 ab	0.60 +/- 0.12 abc	0.42 ± 0.04 ab	0.29 ± 0.08 a	0.44 ± 0.02 ab	0.67 ± 0.08 abc	1.0 +/- 0.2 c	0.99 +/- 0.14 bc
Firmicutes	0.06 ± 0.01	0.66 +/- 0.17 a	10.5 +/- 8.5 a	0.23 +/- 0.10 a	1.1 +/- 0.9 a	0.39 +/- 0.25 a	0.10 ± 0.05 a	0.89 +/- 0.80 a	0.08 ± 0.04 a
Planctomycetes	7.6 +/- 0.3 *	2.7 +/- 0.7 ab	1.2 +/- 0.1 b	1.3 +/- 0.4 b	1.3 +/- 0.5 ab	1.7 +/- 0.5 ab	2.7 +/- 0.7 ab	3.7 +/- 0.7 a	2.6 +/- 0.2 ab
Verrucomicrobia	22.6 +/- 1.2 *	3.4 +/- 0.7 a	3.1 +/- 1.1 a	3.3 +/- 0.9 a	3.1 +/- 0.6 a	5.7 +/- 1.6 ab	7.8 +/- 1.4 ab	9.2 +/- 0.7 b	10.7 +/- 0.4 b
Acidobacteria	25.9 +/- 0.1 *	3.9 +/- 1.0 abcd	2.5 +/- 0.4 abc	2.2 +/- 0.5 ab	1.7 +/- 0.4 ab	1.6 +/- 0.5 a	5.3 +/- 0.9 bcd	5.9 +/- 1.4 cd	7.6 +/- 0.6 d
Bacteroidetes	3.4 +/- 0.1	4.1 +/- 0.9 c	9.7 +/- 3.5 abc	16.0 +/- 2.5 abc	21.6 +/- 7.0 ab	23.3 +/- 3.9 a	15.5 +/- 3.2 abc	15.0 +/- 1.8 abc	16.9 +/- 0.8 abc
Actinobacteria	4.1 +/- 0.4	5.2 +/- 0.9 ab	7.9 +/- 1.8 b	5.4 +/- 0.6 ab	2.3 +/- 0.9 a	2.6 +/- 0.5 a	3.2 +/- 0.7 ab	4.2 +/- 1.3 ab	3.8 +/- 1.0 ab
Proteobacteria	26.1 +/- 0.4	78.6 +/- 3.4 * a	63.7 +/- 4.7 ab	70.2 +/- 2.8 ab	67.5 +/- 6.0 ab	63.0 +/- 3.2 ab	62.4 +/- 5.9 ab	55.7 +/- 3.1 b	51.0 +/- 1.7 b
Bacterial class	**Bulk Soil**	**T2**	**T4**	**T7**	**T10**	**T15**	**T21**	**T30**	**T50**
Clostridia	0.0 ± 0.0	0.0 ± 0.0 a	4.5 +/- 4.2 a	0.05 ± 0.04 a	0.11 +/- 0.11 a	0.27 +/- 0.24 a	0.01 ± 0.00 a	0.63 +/- 0.62 a	0.04 ± 0.03 a
Anaerolineae	0.67 ± 0.08 *	0.06 ± 0.01 a	0.06 ± 0.02 a	0.06 ± 0.01 a	0.04 ± 0.01 a	0.05 ± 0.02 a	0.27 ± 0.09 b	0.22 ± 0.05 b	1.2 +/- 0.3 c
Blastocatellia (Subgroup 4)	1.2 +/- 0.1 *	0.11 ± 0.02 a	0.07 ± 0.01 a	0.07 ± 0.01 a	0.06 ± 0.01 a	0.08 ± 0.01 a	0.22 ± 0.09 b	0.19 ± 0.05 b	0.25 ± 0.02 b
AD3	1.4 +/- 0.1 *	0.14 ± 0.05 a	0.04 ± 0.02 a	0.02 ± 0.00 a	0.02 ± 0.01 a	0.01 ± 0.00 a	0.04 ± 0.01 a	0.08 ± 0.04 a	0.04 ± 0.00 a
Ktedonobacteria	1.3 +/- 0.1 *	0.14 ± 0.06 a	0.10 ± 0.02 a	0.11 ± 0.02 a	0.10 ± 0.03 a	0.10 ± 0.04 a	0.55 +/- 0.13 ab	1.8 +/- 1.2 ab	2.5 +/- 0.4 b
Gemmatimonadetes	1.2 +/- 0.1 *	0.16 ± 0.05 b	0.10 ± 0.02 a	0.10 ± 0.01 a	0.09 ± 0.05 ac	0.04 ± 0.01 c	0.18 ± 0.04 b	0.21 ± 0.06 b	0.15 ± 0.01 b
Phycisphaerae	1.9 +/- 0.1 *	0.20 ± 0.02 a	0.16 ± 0.05 a	0.31 +/- 0.11 ab	0.36 +/- 0.12 b	0.54 +/- 0.17 b	0.63 +/- 0.12 b	1.2 +/- 0.1 c	0.68 ± 0.09 b
Holophagae	1.5 +/- 0.1 *	0.20 ± 0.06 ab	0.13 ± 0.04 a	0.11 ± 0.03 a	0.11 ± 0.03 a	0.11 ± 0.04 a	0.32 ± 0.06 b	0.30 ± 0.08 b	0.50 ± 0.09 c
Subgroup 6	3.7 +/- 0.2 *	0.53 +/- 0.17 b	0.22 ± 0.02 a	0.18 ± 0.06 a	0.12 ± 0.03 a	0.15 ± 0.07 a	0.46 ± 0.06 b	0.37 +/- 0.11 b	0.52 ± 0.05 b
Deltaproteobacteria	4.4 +/- 0.1 *	0.54 +/- 0.15 a	0.36 ± 0.02 a	0.38 ± 0.08 a	0.44 +/- 0.12 a	0.60 +/- 0.14 a	1.4 +/- 0.3 b	2.5 +/- 0.6 c	4.8 +/- 1.3 d
Unidentified classes	4.0 ± 0.0 *	0.61 +/- 0.15 ab	0.67 +/- 0.13 ab	0.53 ± 0.09 a	0.36 ± 0.09 a	0.57 ± 0.03 a	0.87 ± 0.08 ab	1.3 +/- 0.3 b	1.3 +/- 0.2 b
Bacilli	0.05 ± 0.00	0.64 +/- 0.19 a	6.0 +/- 4.4 b	0.17 ± 0.06 c	0.99 +/- 0.89 a	0.10 ± 0.03 c	0.08 ± 0.05 c	0.08 ± 0.01 c	0.02 ± 0.00 c
Other	4.6 +/- 0.1 *	0.79 +/- 0.24 a	0.57 ± 0.08 a	0.70 +/- 0.19 a	0.92 +/- 0.27 a	1.2 +/- 0.1 b	1.7 +/- 0.3 c	2.6 +/- 0.6 d	2.9 +/- 0.3 d
Planctomycetacia	5.3 +/- 0.4 *	2.5 +/- 0.7 a	1.1 +/- 0.1 b	0.96 +/- 0.31 b	0.97 +/- 0.34 b	1.1 +/- 0.5 b	2.0 +/- 0.5 b	2.4 +/- 0.8 b	1.8 +/- 0.1 b
Acidobacteriia	18.4 +/- 0.2 *	2.9 +/- 0.9 abc	2.0 +/- 0.3 ab	1.8 +/- 0.4 a	1.3 +/- 0.3 a	1.2 +/- 0.4 a	4.1 +/- 0.8 abc	4.9 +/- 1.2 bc	6.0 +/- 0.4 c
Verrucomicrobiae	22.5 +/- 1.2 *	3.4 +/- 0.8 a	3.1 +/- 1.1 a	3.3 +/- 0.9 a	3.1 +/- 0.6 a	5.7 +/- 1.6 b	7.8 +/- 1.4 b	9.2 +/- 0.7 bc	10.6 +/- 0.4 c
Bacteroidia	3.2 +/- 0.1	4.1 +/- 1.0 c	9.6 +/- 3.5 abc	16.0 +/- 2.5 abc	21.5 +/- 7.1 ab	23.2 +/- 3.9 a	15.4 +/- 3.2 abc	14.9 +/- 1.8 abc	16.7 +/- 0.8 abc
Alphaproteobacteria	11.1 +/- 0.4 *	4.3 +/- 0.6 b	6.4 +/- 1.8 bc	8.0 +/- 0.8 abc	8.5 +/- 0.9 abc	13.7 +/- 2.2 a	7.6 +/- 0.8 bc	10.7 +/- 1.4 ac	11.7 +/- 0.8 ac
Actinobacteria	2.9 +/- 0.3	4.9 +/- 1.0 ab	7.8 +/- 1.8 b	5.3 +/- 0.6 ab	2.2 +/- 0.9 a	2.5 +/- 0.5 a	2.9 +/- 0.5 a	3.9 +/- 1.2 ab	3.7 +/- 1.0 ab
Betaproteobacteria	10.5 +/- 0.1	73.7 +/- 4.5 * b	56.8 +/- 4.7 abc	61.7 +/- 3.4 ab	58.5 +/- 6.5 ab	48.5 +/- 5.1 ac	53.3 +/- 6.9 abc	42.4 +/- 3.8 ac	34.4 +/- 2.9 cd

Table S1 (2/4) - Relative abundance of members of bacterial communities at each taxonomic rank (>1% relative abundance in at least one time point) detected in the bulk soil and/or roots samples collecting from T0 to T50. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant differences between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05). The asterisks denote significant difference in relative abudance of fungal communities between bulk soil samples and root samples collected from T2 (One-way ANOVA, factor=sampling time, P<0.05).

Bacterial order	Bulk Soil	T2	T4	T7	T10	T15	T21	T30	T50
Clostridiales	0.01 ± 0.01	0.01 ± 0.00 a	4.5 +/- 4.2 b	0.06 ± 0.04 a	0.11 +/- 0.11 a	0.28 +/- 0.24 ab	0.02 ± 0.00 a	0.64 +/- 0.62 ab	0.05 ± 0.03 a
Methylacidiphilales	0.13 ± 0.02 *	0.03 ± 0.00 a	0.03 ± 0.01 a	0.05 ± 0.01 a	0.03 ± 0.01 a	0.10 ± 0.03 a	0.40 ± 0.09 b	0.82 +/- 0.38 bc	1.3 +/- 0.2 c
WD260	1.1 +/- 0.1 *	0.09 ± 0.02 a	0.09 ± 0.02 a	0.06 ± 0.02 a	0.05 ± 0.01 a	0.05 ± 0.02 a	0.18 ± 0.04 a	0.15 ± 0.04 a	0.55 +/- 0.13 b
Ktedonobacterales	1.2 +/- 0.1 *	0.13 ± 0.05 a	0.10 ± 0.02 a	0.10 ± 0.02 a	0.09 ± 0.02 a	0.10 ± 0.04 a	0.54 +/- 0.13 ab	1.7 +/- 1.2 ab	2.5 +/- 0.4 b
Unknown order of AD3	1.4 +/- 0.1 *	0.14 ± 0.05 a	0.04 ± 0.02 a	0.03 ± 0.01 a	0.02 ± 0.01 a	0.04 ± 0.01 a	0.09 ± 0.04 a	0.05 ± 0.01 a	0.03 ± 0.01 a
Cytophagales	0.37 ± 0.02	0.14 ± 0.05 a	1.2 +/- 1.0 a	0.77 +/- 0.37 b	0.78 +/- 0.29 b	0.65 +/- 0.24 b	2.2 +/- 0.7 c	1.1 +/- 0.4 a	3.6 +/- 0.4 d
Gemmatimonadales	1.2 +/- 0.1 *	0.16 ± 0.04 a	0.11 ± 0.02 a	0.10 ± 0.01 a	0.09 ± 0.05 a	0.05 ± 0.01 a	0.19 ± 0.04 a	0.22 ± 0.06 a	0.15 ± 0.01 a
Micropepsales	1.2 +/- 0.1 *	0.18 ± 0.03 a	0.10 ± 0.03 b	0.12 ± 0.03 ab	0.16 ± 0.03 ab	0.26 ± 0.04 c	0.34 ± 0.07 c	0.56 ± 0.06 d	0.58 ± 0.01 d
Tepidisphaerales	1.9 +/- 0.1 *	0.20 ± 0.02 a	0.16 ± 0.05 a	0.31 +/- 0.11 b	0.37 +/- 0.12 b	0.54 +/- 0.17 bc	0.61 +/- 0.11 c	1.2 +/- 0.1 d	0.66 ± 0.09 c
Myxococcales	1.9 +/- 0.1 *	0.20 ± 0.04 a	0.17 ± 0.03 a	0.17 ± 0.04 a	0.17 ± 0.02 a	0.23 ± 0.07 a	0.85 +/- 0.19 b	1.5 +/- 0.3 c	3.6 +/- 1.2 d
Subgroup 7	1.5 +/- 0.1 *	0.20 ± 0.05 a	0.12 ± 0.03 ab	0.09 ± 0.02 b	0.07 ± 0.01 b	0.09 ± 0.04 b	0.23 ± 0.04 a	0.19 ± 0.05 ab	0.32 ± 0.04 c
Gammaproteobacteria Incertae Sedis	1.5 +/- 0.1 *	0.24 ± 0.04 a	0.33 +/- 0.11 a	0.32 ± 0.08 a	0.42 +/- 0.15 a	0.54 +/- 0.14 a	1.1 +/- 0.3 b	1.4 +/- 0.8 ab	1.4 +/- 0.3 b
Frankiales	1.3 +/- 0.17 *	0.25 ± 0.08 b	0.09 ± 0.02 a	0.08 ± 0.02 a	0.07 ± 0.03 a	0.14 ± 0.08 ab	0.36 +/- 0.24 ab	0.29 +/- 0.12 ab	0.26 ± 0.01 b
RCP2-54	2.0 +/- 0.1 *	0.26 ± 0.07 a	0.11 ± 0.01 b	0.10 ± 0.01 b	0.06 ± 0.02 c	0.07 ± 0.03 c	0.22 ± 0.04 a	0.32 +/- 0.10 a	0.32 ± 0.01 a
Sphingomonadales	0.22 ± 0.02	0.27 ± 0.04 a	0.49 +/- 0.15 a	0.91 +/- 0.18 b	1.5 +/- 0.3 c	3.4 +/- 1.0 d	0.76 +/- 0.17 b	1.34 +/- 0.27 c	0.53 +/- 0.11 b
Solibacterales	2.5 +/- 0.1 *	0.31 +/- 0.10 abc	0.16 ± 0.02 ab	0.15 ± 0.05 ab	0.10 ± 0.01 ab	0.09 ± 0.03 a	0.38 ± 0.07 bc	0.35 ± 0.09 abc	0.55 ± 0.03 c
Pedosphaerales	3.2 +/- 0.2 *	0.35 ± 0.07 ab	0.39 +/- 0.12 a	0.82 +/- 0.40 ab	1.1 +/- 0.4 ab	2.3 +/- 0.8 abc	3.8 +/- 0.8 c	3.5 +/- 0.6 c	5.7 +/- 0.4 d
Elsterales	2.3 +/- 0.1 *	0.52 +/- 0.16 a	0.25 ± 0.05 a	0.17 ± 0.04 a	0.16 ± 0.06 a	0.21 ± 0.06 a	0.54 +/- 0.19 a	0.44 +/- 0.13 a	0.37 ± 0.01 a
Unidentified order of *Subgroup 6*	3.7 +/- 0.2 *	0.54 +/- 0.16 a	0.23 ± 0.03 a	0.13 ± 0.03 b	0.16 ± 0.07 ab	0.47 ± 0.06 c	0.37 +/- 0.11 c	0.52 ± 0.05 d	0.18 ± 0.06 ab
Caulobacterales	0.26 ± 0.03	0.58 +/- 0.29 b	1.9 +/- 0.7 a	2.5 +/- 0.4 a	2.6 +/- 0.4 a	4.6 +/- 1.2 c	1.9 +/- 0.3 a	2.9 +/- 0.7 ac	3.0 +/- 0.2 ac
Unidentified orders	4.0 +/- 0.1 *	0.61 +/- 0.14 a	0.68 +/- 0.13 a	0.36 ± 0.09 a	0.58 ± 0.03 a	0.87 ± 0.08 a	1.3 +/- 0.3 b	1.3 +/- 0.2 b	0.53 ± 0.09 a
Bacillales	0.06 ± 0.1	0.64 +/- 0.17 a	5.9 +/- 4.4 b	0.17 ± 0.06 c	0.99 +/- 0.89 ac	0.10 ± 0.03 c	0.08 ± 0.05 c	0.08 ± 0.01 c	0.02 ± 0.00 c
Chitinophagales	2.0 +/- 0.1 *	0.74 ± 0.08 b	1.4 +/- 0.5 b	5.4 +/- 1.0 a	12.6 +/- 5.8 c	12.4 +/- 1.8 c	4.7 +/- 1.7 ac	8.9 +/- 1.9 ac	4.5 +/- 0.6 ac
Isosphaerales	1.4 +/- 0.2	1.0 +/- 0.2 b	0.48 ± 0.07 a	0.40 +/- 0.14 a	0.39 +/- 0.14 a	0.51 +/- 0.25 a	0.80 +/- 0.10 ab	1.0 +/- 0.3 ab	0.66 ± 0.03 a
Gemmatales	2.7 +/- 0.2 *	1.0 +/- 0.3 b	0.42 ± 0.06 a	0.40 +/- 0.13 a	0.42 +/- 0.15 a	0.42 +/- 0.18 a	0.90 +/- 0.30 b	0.98 +/- 0.33 b	0.74 ± 0.06 ab
Subgroup 2	7.0 +/- 0.1 *	1.1 +/- 0.4 ab	0.59 ± 0.08 a	0.53 +/- 0.12 a	0.35 +/- 0.11 a	0.33 +/- 0.13 a	1.2 +/- 0.3 ab	1.3 +/- 0.4 ab	1.9 +/- 0.2 b
Xanthomonadales	1.1 +/- 0.1	1.4 +/- 0.1 a	2.0 +/- 0.7 a	1.3 +/- 0.1 a	0.57 +/- 0.13 b	1.4 +/- 0.1 a	0.9 +/- 0.2 a	2.0 +/- 0.9 a	1.6 +/- 0.3 a
Acidobacteriales	8.8 +/- 0.3 *	1.5 +/- 0.3 a	1.2 +/- 0.2 a	1.1 +/- 0.2 a	0.84 +/- 0.20 a	0.79 +/- 0.19 a	2.5 +/- 0.4 b	3.2 +/- 0.8 bc	3.6 +/- 0.3 c
Rhizobiales	5.7 +/- 0.3 *	2.4 +/- 0.3 a	3.4 +/- 0.9 ab	4.0 +/- 0.4 abc	3.8 +/- 0.5 abc	4.7 +/- 0.4 abc	3.4 +/- 0.4 ab	4.6 +/- 0.8 abc	6.3 +/- 0.7 c
Chthoniobacterales	18.2 +/- 1.1 *	2.9 +/- 0.6 a	1.8 +/- 0.3 b	2.0 +/- 0.5 ab	1.6 +/- 0.5 b	2.4 +/- 0.6 ab	2.8 +/- 0.4 a	3.9 +/- 0.7 c	2.7 +/- 0.1 a
Pseudomonadales	0.22 ± 0.04	3.0 +/- 0.44 * a	4.1 +/- 3.6 ab	1.0 +/- 0.6 b	1.4 +/- 0.7 b	0.67 +/- 0.14 c	0.41 +/- 0.11 c	0.46 +/- 0.29 c	0.17 ± 0.03 d
Other	12.7 +/- 0.3 *	3.0 +/- 0.6 a	3.0 +/- 1.0 a	3.5 +/- 0.6 a	5.0 +/- 1.1 a	6.5 +/- 1.0 a	7.9 +/- 1.3 ab	10.6 +/- 1.1 b	2.8 +/- 0.5 a
Sphingobacteriales	0.47 +/- 0.11	3.0 +/- 0.8 * a	7.0 +/- 2.6 b	9.8 +/- 1.5 b	7.6 +/- 1.6 b	9.5 +/- 2.1 b	8.5 +/- 2.0 b	5.0 +/- 0.4 b	8.6 +/- 1.6 b
Streptomycetales	0.59 +/- 0.11	4.0 +/- 0.8 * a	7.1 +/- 1.7 b	4.8 +/- 0.6 ab	1.7 +/- 0.7 c	2.1 +/- 0.5 c	1.8 +/- 0.3 c	2.8 +/- 0.8 ac	2.3 +/- 1.0 a
Betaproteobacteriales	6.1 +/- 0.1	68.8 +/- 4.4 * b	50.3 +/- 6.7 a	58.6 +/- 4.0 a	55.8 +/- 6.7 a	45.2 +/- 5.1 a	49.6 +/- 7.2 a	37.6 +/- 3.7 c	29.3 +/- 3.4 c

Table S1 (3/4) - Relative abundance of members of bacterial communities at each taxonomic rank (>1% relative abundance in at least one time point) detected in the bulk soil and/or roots samples collecting from T0 to T50. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant differences between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05). The asterisks denote significant difference in relative abudance of fungal communities between bulk soil samples and root samples collected from T2 (One-way ANOVA, factor=sampling time, P<0.05).

Bacterial family	Bulk Soil	T2	T4	T7	T10	T15	T21	T30	T50
Clostridiaceae 1	0.0 ± 0.0	0.0 ± 0.0 a	4.5 +/- 4.2 b	0.05 ± 0.04 b	0.11 +/- 0.11 ab	0.18 +/- 0.15 b	0.01 ± 0.00 b	0.27 +/- 0.27 ab	0.01 ± 0.01 ab
Methylophilaceae	0.0 ± 0.0	0.0 ± 0.0 a	0.58 +/- 0.51 b	0.79 +/- 0.29 b	0.25 +/- 0.13 b	1.5 +/- 0.7 c	1.5 +/- 0.9 c	0.92 +/- 0.46 bc	0.52 ± 0.08 bc
BIrii41	0.22 ± 0.02 *	0.02 ± 0.00 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.01 ± 0.00 a	0.02 ± 0.02 ab	0.13 ± 0.02 c	0.28 +/- 0.13 c	1.2 +/- 0.6 d
Labraceae	0.01 ± 0.00	0.02 ± 0.00 a	0.52 +/- 0.23 b	0.27 ± 0.07 b	0.19 ± 0.06 b	0.46 +/- 0.15 b	0.14 ± 0.04 b	0.29 ± 0.05 b	1.1 +/- 0.2 c
Methylacidiphilaceae	0.12 ± 0.02 *	0.02 ± 0.00 a	0.03 ± 0.01 a	0.05 ± 0.01 a	0.02 ± 0.01 a	0.09 ± 0.03 b	0.40 ± 0.09 c	0.82 +/- 0.38 cd	1.3 +/- 0.2 d
Polyangiaceae	0.56 ± 0.04 *	0.08 ± 0.03 a	0.08 ± 0.01 a	0.08 ± 0.01 a	0.09 ± 0.01 a	0.08 ± 0.03 a	0.39 +/- 0.10 b	0.56 +/- 0.12 b	1.6 +/- 0.5 c
Unidentified family of *WD260*	1.1 ± 0.05 *	0.09 ± 0.02 a	0.09 ± 0.02 a	0.06 ± 0.02 a	0.05 ± 0.01 a	0.05 ± 0.02 a	0.18 ± 0.04 b	0.15 ± 0.04 b	0.55 +/- 0.13 bc
Unidentified family of *Betaproteobacteriales*	1.4 +/- 0.1 *	0.09 ± 0.04 a	0.09 ± 0.06 a	0.07 ± 0.03 a	0.02 ± 0.01 a	0.07 ± 0.02 a	0.29 +/- 0.12 b	0.42 +/- 0.15 bc	0.66 +/- 0.10 c
Ktedonobacteraceae	1.0 +/- 0.1 *	0.11 ± 0.04 a	0.09 ± 0.02 a	0.09 ± 0.02 a	0.08 ± 0.02 a	0.08 ± 0.03 a	0.50 +/- 0.12 b	1.6 +/- 1.1 bc	2.3 +/- 0.4 c
Nitrosomonadaceae	1.4 ± 0.0 *	0.14 ± 0.02 ab	0.09 ± 0.01 a	0.04 ± 0.01 a	0.04 ± 0.01 a	0.06 ± 0.02 a	0.16 ± 0.03 ab	0.14 ± 0.04 ab	0.27 ± 0.04 b
Microscillaceae	0.33 ± 0.01 *	0.14 ± 0.05 a	1.1 +/- 0.9 a	0.63 +/- 0.37 b	0.52 +/- 0.21 b	0.41 +/- 0.17 ab	1.6 +/- 0.4 c	0.58 +/- 0.31 ab	3.4 +/- 0.4 d
Gemmatimonadaceae	1.2 +/- 0.1 *	0.16 ± 0.04 a	0.10 ± 0.02 a	0.10 ± 0.01 a	0.09 ± 0.05 a	0.04 ± 0.01 b	0.18 ± 0.04 a	0.21 ± 0.06 a	0.15 ± 0.01 a
Micropepsaceae	1.2 ± 0.0 *	0.17 ± 0.03 a	0.09 ± 0.03 b	0.11 ± 0.03 ab	0.15 ± 0.03 ab	0.26 ± 0.04 c	0.33 ± 0.07 c	0.56 ± 0.06 d	0.58 ± 0.01 d
WD2101 soil group	1.7 +/- 0.1 *	0.18 ± 0.02 abc	0.12 ± 0.04 b	0.17 ± 0.03 bc	0.25 ± 0.09 bc	0.27 ± 0.09 abc	0.51 +/- 0.11 abc	0.61 +/- 0.16 a	0.61 ± 0.08 ac
Unidentified family of *Subgroup 7*	1.5 +/- 0.1 *	0.20 ± 0.05 a	0.12 ± 0.04 ab	0.09 ± 0.02 b	0.07 ± 0.02 b	0.09 ± 0.05 b	0.23 ± 0.04 a	0.20 ± 0.05 a	0.32 ± 0.05 c
Acidothermaceae	1.2 +/- 0.2 *	0.20 ± 0.07 b	0.06 ± 0.01 a	0.07 ± 0.02 a	0.06 ± 0.03 a	0.13 ± 0.08 c	0.33 +/- 0.22 bc	0.27 +/- 0.11 bc	0.23 ± 0.01 bc
Unidentified family of *Gammaproteobacteria Incertae Sedis*	1.5 +/- 0.1 *	0.24 ± 0.04 a	0.33 +/- 0.11 a	0.32 ± 0.09 a	0.42 +/- 0.16 a	0.54 +/- 0.14 b	1.1 +/- 0.3 c	1.4 +/- 0.8 bc	1.4 +/- 0.3 c
Sphingomonadaceae	0.21 ± 0.02	0.26 ± 0.04 a	0.49 +/- 0.15 b	0.91 +/- 0.18 c	1.5 +/- 0.3 d	3.4 +/- 1.0 e	0.76 +/- 0.17 c	1.3 +/- 0.3 d	0.53 +/- 0.11 c
Koribacteraceae	1.5 +/- 0.1 *	0.26 ± 0.06 a	0.21 ± 0.03 a	0.17 ± 0.04 a	0.15 ± 0.04 ab	0.08 ± 0.02 b	0.29 ± 0.05 a	0.67 +/- 0.19 c	0.35 ± 0.02 a
Unidentified family of *RCP2-54*	2.0 +/- 0.1 *	0.26 ± 0.07 b	0.11 ± 0.01 a	0.10 ± 0.02 a	0.06 ± 0.02 a	0.07 ± 0.03 a	0.22 ± 0.04 ab	0.32 +/- 0.11 ab	0.32 ± 0.01 ab
Solibacteraceae (Subgroup 3)	2.5 ± 0.0 *	0.31 +/- 0.10 abc	0.15 ± 0.02 ab	0.14 ± 0.05 ab	0.10 ± 0.01 ab	0.08 ± 0.03 a	0.37 ± 0.07 bc	0.34 ± 0.09 abc	0.54 ± 0.03 c
Pedosphaeraceae	3.2 +/- 0.2 *	0.34 ± 0.07 a	0.38 +/- 0.12 a	0.82 +/- 0.40 a	1.1 +/- 0.4 a	2.3 +/- 0.8 b	3.8 +/- 0.8 bc	3.5 +/- 0.5 bc	5.7 +/- 0.4 c
Unidentified family of *Elsterales*	2.2 +/- 0.1 *	0.48 +/- 0.16 b	0.17 ± 0.01 a	0.12 ± 0.04 a	0.11 ± 0.04 a	0.16 ± 0.06 a	0.49 +/- 0.20 b	0.39 +/- 0.12 b	0.30 ± 0.01 b
Caulobacteraceae	0.26 ± 0.03	0.58 +/- 0.29 b	1.9 +/- 0.7 a	2.5 +/- 0.4 b	2.6 +/- 0.4 b	4.6 +/- 1.2 c	1.9 +/- 0.3 b	2.9 +/- 0.7 b	3.0 +/- 0.2 b
Chitinophagaceae	2.0 ± 0.0 *	0.74 ± 0.08 a	1.4 +/- 0.5 b	5.4 +/- 1.0 c	12.5 +/- 5.8 d	12.3 +/- 1.81 d	4.7 +/- 1.7 bc	8.9 +/- 1.9 d	4.5 +/- 0.6 bc
Rhizobiaceae	0.03 ± 0.01	0.77 +/- 0.14 * b	1.2 +/- 0.4 ab	1.8 +/- 0.2 a	1.6 +/- 0.3 a	1.6 +/- 0.3 a	0.89 +/- 0.20 ab	0.95 +/- 0.18 ab	1.2 +/- 0.2 ab
Unidentified family of *Acidobacteriales*	6.3 +/- 0.2 *	0.95 +/- 0.22 a	0.72 +/- 0.14 a	0.68 +/- 0.15 a	0.42 +/- 0.10 b	0.42 +/- 0.14 ab	1.7 +/- 0.3 c	1.8 +/- 0.6 c	2.7 +/- 0.3 d
Isosphaeraceae	1.4 +/- 0.2	1.0 +/- 0.2 b	0.48 ± 0.07 a	0.40 +/- 0.14 a	0.38 +/- 0.14 a	0.51 +/- 0.25 a	0.77 +/- 0.13 ab	1.0 +/- 0.3 bc	0.66 ± 0.03 a
Gemmataceae	2.7 +/- 0.2 *	1.0 +/- 0.3 b	0.42 ± 0.06 a	0.40 +/- 0.13 a	0.42 +/- 0.15 a	0.42 +/- 0.18 a	0.91 +/- 0.34 ab	0.91 +/- 0.30 ab	0.74 ± 0.06 ab
Rhodanobacteraceae	0.59 ± 0.03	1.1 +/- 0.1 * a	1.9 +/- 0.7 a	1.3 +/- 0.1 a	0.47 +/- 0.11 b	1.2 +/- 0.1 a	0.63 +/- 0.12 b	1.7 +/- 0.9 ab	0.78 +/- 0.11 ab
Unidentified family of *Subgroup 2*	7.0 +/- 0.1 *	1.1 +/- 0.4 a	0.60 ± 0.08 a	0.54 +/- 0.13 a	0.35 +/- 0.11 b	0.33 +/- 0.13 b	1.2 +/- 0.3 c	1.3 +/- 0.4 c	1.9 +/- 0.2 c
Xanthobacteraceae	4.8 +/- 0.3 *	1.4 +/- 0.4 a	1.6 +/- 0.2 a	1.8 +/- 0.3 ab	1.7 +/- 0.3 ab	2.6 +/- 0.1 abc	2.1 +/- 0.2 ab	3.1 +/- 0.5 bcd	3.8 +/- 0.5 c
Unidentified	11.5 +/- 0.1 *	1.7 +/- 0.4 a	1.2 +/- 0.2 a	1.0 +/- 0.2 b	0.87 +/- 0.17 b	1.1 +/- 0.1 b	2.7 +/- 0.3 c	2.8 +/- 0.6 c	3.4 +/- 0.4 c
Chthoniobacteraceae	17.4 +/- 1.1 *	2.7 +/- 0.6 b	1.7 +/- 0.2 a	1.8 +/- 0.4 a	1.5 +/- 0.5 a	2.2 +/- 0.6 a	2.4 +/- 0.4 a	3.5 +/- 0.6 c	2.4 +/- 0.1 a
Pseudomonadaceae	0.21 ± 0.04	3.0 +/- 0.4 * a	4.0 +/- 3.6 ab	1.0 +/- 0.6 b	1.3 +/- 0.7 b	0.66 +/- 0.14 bc	0.31 +/- 0.12 c	0.38 +/- 0.29 c	0.01 ± 0.00 d
Sphingobacteriaceae	0.37 ± 0.09	3.0 +/- 0.8 * a	7.0 +/- 2.6 b	9.8 +/- 1.4 c	7.6 +/- 1.6 b	9.5 +/- 2.1 c	8.4 +/- 2.0 c	4.8 +/- 0.4 bc	8.5 +/- 1.6 c
Streptomycetaceae	0.58 +/- 0.10	4.0 +/- 0.7 * a	7.1 +/- 1.7 b	4.8 +/- 0.6 a	1.7 +/- 0.7 c	2.1 +/- 0.5 c	1.8 +/- 0.3 d	2.8 +/- 0.8 cd	2.3 +/- 1.0 cd
Other	16.8 +/- 0.2 *	4.6 +/- 0.8 a	9.9 +/- 3.9 b	4.1 +/- 0.6 a	5.5 +/- 1.5 b	6.6 +/- 1.4 b	8.5 +/- 1.4 bc	11.8 +/- 1.7 c	14.0 +/- 1.2 c
Burkholderiaceae	1.6 ± 0.0	68.4 +/- 4.5 * a	49.3 +/- 6.7 b	57.3 +/- 4.3 b	55.3 +/- 6.7 b	43.3 +/- 5.2 b	47.3 +/- 7.6 bc	35.3 +/- 3.6 bc	26.5 +/- 3.3 c

Table S1 (4/4) - Relative abundance of members of bacterial communities at each taxonomic rank (>1% relative abundance in at least one time point) detected in the bulk soil and/or roots samples collecting from T0 to T50. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant differences between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05). The asterisks denote significant difference in relative abudance of fungal communities between bulk soil samples and root samples collected from T2 (One-way ANOVA, factor=sampling time, P<0.05).

Bacterial genus	Bulk Soil	T2	T4	T7	T10	T15	T21	T30	T50
Unidentified genus of *Methylophilaceae*	0.0 ± 0.0	0.0 ± 0.0 a	0.58 +/- 0.52 b	0.79 +/- 0.29 bc	0.25 +/- 0.14 b	1.5 +/- 0.7 c	1.5 +/- 0.9 c	0.46 +/- 0.29 bc	0.52 ± 0.08 bc
Unidentified genus of *BIrii41*	0.22 ± 0.02 *	0.02 ± 0.01 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.01 ± 0.01 a	0.03 ± 0.02 a	0.13 ± 0.02 b	0.28 +/- 0.13 b	1.2 +/- 0.6 c
Unidentified genus of *Ktedonobacteraceae*	0.22 ± 0.03 *	0.02 ± 0.01 a	0.04 ± 0.01 a	0.04 ± 0.01 a	0.05 ± 0.01 a	0.04 ± 0.01 a	0.31 ± 0.08 b	1.3 +/- 0.9 b	1.7 +/- 0.4 c
Unidentified genus of *Methylacidiphilaceae*	0.13 ± 0.02 *	0.03 ± 0.01 a	0.03 ± 0.01 a	0.05 ± 0.02 a	0.03 ± 0.01 a	0.09 ± 0.04 a	0.40 +/- 0.10 b	0.82 +/- 0.39 bc	1.3 +/- 0.2 bc
Unidentified genus of *Microscillaceae*	0.18 ± 0.01	0.11 ± 0.05 a	1.1 +/- 0.9 ab	0.60 +/- 0.37 ab	0.50 +/- 0.21 ab	0.38 +/- 0.16 ab	1.5 +/- 0.4 b	0.49 +/- 0.24 a	3.0 +/- 0.3 c
Unidentified genus of *Pedosphaeraceae*	1.6 +/- 0.2 *	0.15 ± 0.03 a	0.27 +/- 0.12 a	0.72 +/- 0.37 a	1.1 +/- 0.4 a	2.2 +/- 0.8 a	3.4 +/- 0.7 a	3.2 +/- 0.6 a	5.1 +/- 0.4 b
Unidentified genus of *Micropepsaceae*	1.2 +/- 0.1 *	0.18 ± 0.04 a	0.10 ± 0.03 a	0.12 ± 0.03 a	0.16 ± 0.03 a	0.26 ± 0.04 b	0.34 ± 0.07 b	0.56 ± 0.07 c	0.58 ± 0.01 c
Unidentified genus of *WD2101 soil group*	1.7 +/- 0.1 *	0.19 ± 0.03 a	0.12 ± 0.04 a	0.18 ± 0.03 a	0.26 ± 0.09 a	0.28 ± 0.09 a	0.51 +/- 0.12 a	0.62 +/- 0.16 a	0.61 ± 0.08 a
Unidentified genus of *Chitinophagaceae*	1.6 +/- 0.1 *	0.28 ± 0.02 a	0.58 +/- 0.13 b	1.4 +/- 0.1 c	4.8 +/- 3.8 cd	5.3 +/- 1.9 d	2.4 +/- 0.6 c	4.2 +/- 1.0 d	2.6 +/- 0.2 c
Unidentified genus of *Xanthobacteraceae*	2.7 +/- 0.1 *	0.71 +/- 0.21 a	0.37 ± 0.05 b	0.35 ± 0.09 b	0.39 +/- 0.13 b	0.37 +/- 0.11 b	0.64 +/- 0.14 a	0.82 +/- 0.28 ac	1.2 +/- 0.1 c
Unidentified genus of *Sphingobacteriaceae*	0.14 ± 0.07	0.76 +/- 0.19 a	2.4 +/- 0.9 bc	4.0 +/- 0.9 b	2.0 +/- 0.5 b	3.5 +/- 0.8 c	2.3 +/- 0.6 bc	2.0 +/- 0.3 b	2.4 +/- 0.6 b
Clostridium sensu stricto 9	0.0 ± 0.0	0.0 ± 0.0 a	2.3 +/- 2.2 a	0.04 ± 0.04 a	0.10 +/- 0.10 a	0.12 +/- 0.10 a	0.0 ± 0.0 a	0.05 ± 0.05 a	0.0 ± 0.0 a
Chthoniobacter	0.36 ± 0.03 *	0.02 ± 0.00 a	0.07 ± 0.01 b	0.25 +/- 0.10 c	0.24 ± 0.05 c	1.1 +/- 0.5 d	0.64 +/- 0.29 d	1.2 +/- 0.4 d	0.52 ± 0.07 d
Labrys	0.01 ± 0.00	0.02 ± 0.00 a	0.52 +/- 0.23 b	0.27 ± 0.07 b	0.19 ± 0.06 b	0.46 +/- 0.15 b	0.14 ± 0.04 c	0.29 ± 0.05 b	1.1 +/- 0.2 d
Asticcacaulis	0.01 ± 0.00	0.02 ± 0.01 a	0.70 +/- 0.41 b	0.40 +/- 0.11 b	0.77 +/- 0.22 b	1.5 +/- 0.9 b	0.34 ± 0.05 c	0.41 +/- 0.22 b	0.92 +/- 0.12 b
Rhodoferax	0.01 ± 0.0	0.03 ± 0.01 a	0.0 ± 0.0 a	0.04 ± 0.01 a	0.48 +/- 0.26 a	0.09 ± 0.04 a	0.20 +/- 0.15 a	0.16 +/- 0.12 a	5.8 +/- 3.0 b
Pajaroellobacter	0.52 ± 0.05 *	0.05 ± 0.01 a	0.06 ± 0.02 a	0.06 ± 0.01 a	0.07 ± 0.00 a	0.07 ± 0.02 a	0.30 ± 0.07 b	0.37 +/- 0.11 b	1.2 +/- 0.5 c
Rhizobacter	0.05 ± 0.01	0.06 ± 0.00 a	0.08 ± 0.04 a	0.18 ± 0.06 a	0.28 ± 0.09 b	0.26 +/- 0.15 b	0.49 +/- 0.14 b	0.24 +/- 0.13 b	1.2 +/- 0.3 c
Niastella	0.04 ± 0.00	0.07 ± 0.03 a	0.55 +/- 0.25 b	2.1 +/- 1.3 cd	1.4 +/- 0.4 c	1.4 +/- 0.3 c	1.4 +/- 0.9 c	2.1 +/- 0.2 d	1.5 +/- 0.4 c
Phenylobacterium	0.16 ± 0.02	0.08 ± 0.01 a	0.71 +/- 0.20 b	1.6 +/- 0.3 c	1.3 +/- 0.4 bc	2.5 +/- 0.6 c	0.77 +/- 0.13 b	1.4 +/- 0.5 bc	0.64 ± 0.07 b
Ellin6067	1.0 ± 0.0 *	0.12 ± 0.02 ab	0.08 ± 0.01 ab	0.03 ± 0.01 a	0.03 ± 0.01 a	0.05 ± 0.01 a	0.12 ± 0.03 ab	0.10 ± 0.03 ab	0.18 ± 0.04 b
Bryobacter	1.6 +/- 0.1 *	0.18 ± 0.06 ab	0.07 ± 0.01 a	0.07 ± 0.02 a	0.04 ± 0.00 a	0.04 ± 0.02 a	0.18 ± 0.03 ab	0.18 ± 0.06 ab	0.30 ± 0.01 b
ADurb.Bin063-1	1.4 ± 0.0 *	0.19 ± 0.04 b	0.08 ± 0.01 a	0.08 ± 0.03 a	0.05 ± 0.01 a	0.09 ± 0.03 a	0.30 ± 0.05 c	0.32 ± 0.07 c	0.50 ± 0.05 d
Acidothermus	1.2 +/- 0.2 *	0.20 ± 0.07 b	0.06 ± 0.01 a	0.07 ± 0.02 a	0.06 ± 0.03 a	0.13 ± 0.08 ab	0.33 +/- 0.22 b	0.27 +/- 0.11 b	0.23 ± 0.01 b
Sphingomonas	0.16 ± 0.02	0.21 ± 0.03 a	0.45 +/- 0.14 b	0.78 +/- 0.16 c	1.1 +/- 0.2 c	3.0 +/- 0.9 d	0.69 +/- 0.14 b	1.2 +/- 0.3 c	0.48 +/- 0.11 b
Acidibacter	1.5 +/- 0.1 *	0.23 ± 0.04 ab	0.32 +/- 0.10 abc	0.25 ± 0.05 ab	0.19 ± 0.03 a	0.41 ± 0.08 abc	0.57 +/- 0.10 abc	0.72 +/- 0.15 c	0.70 +/- 0.15 bc
Candidatus Koribacter	1.5 +/- 0.1 *	0.26 ± 0.06 a	0.21 ± 0.03 a	0.17 ± 0.04 a	0.15 ± 0.04 a	0.08 ± 0.02 b	0.29 ± 0.05 a	0.67 +/- 0.19 c	0.35 ± 0.02 a
Dyella	0.0 ± 0.0	0.28 ± 0.06 * a	1.1 +/- 0.8 a	0.38 +/- 0.11 a	0.11 ± 0.06 a	0.36 +/- 0.18 a	0.05 ± 0.02 a	0.75 +/- 0.57 a	0.0 ± 0.0 b
Undibacterium	0.01 ± 0.00	0.30 ± 0.06 a	0.25 +/- 0.17 ab	0.54 +/- 0.23 ab	1.3 +/- 0.6 b	1.5 +/- 0.7 b	0.67 +/- 0.36 ab	0.56 +/- 0.42 ab	0.19 +/- 0.10 a
Chitinophaga	0.02 ± 0.00	0.33 +/- 0.10 * b	0.22 +/- 0.13 b	1.8 +/- 0.6 ab	6.2 +/- 2.7 c	5.5 +/- 0.8 c	0.84 +/- 0.48 a	2.4 +/- 1.2 ab	0.23 +/- 0.17 b
Paenibacillus	0.03 ± 0.00	0.59 +/- 0.16 a	2.4 +/- 1.3 b	0.16 ± 0.06 a	0.97 +/- 0.88 b	0.07 ± 0.01 c	0.06 ± 0.03 c	0.05 ± 0.01 c	0.01 ± 0.00 c
Bradyrhizobium	1.9 +/- 0.1 *	0.66 +/- 0.13 a	1.2 +/- 0.2 ab	1.4 +/- 0.2 b	1.3 +/- 0.2 b	2.0 +/- 0.3 c	1.4 +/- 0.1 b	2.2 +/- 0.3 c	2.5 +/- 0.4 c
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.03 ± 0.01	0.77 +/- 0.14 * a	1.2 +/- 0.4 ab	1.8 +/- 0.2 b	1.5 +/- 0.3 ab	1.4 +/- 0.4 ab	0.84 +/- 0.20 ab	0.90 +/- 0.17 ab	1.1 +/- 0.2 ab
Pseudoduganella	0.01 ± 0.00	0.84 +/- 0.52 a	0.06 ± 0.06 b	1.4 +/- 0.8 a	0.27 +/- 0.17 ab	0.09 ± 0.06 b	0.23 +/- 0.21 b	0.01 ± 0.00 b	0.0 ± 0.0 b
Collimonas	0.17 ± 0.05	0.89 ± 0.09 * a	2.1 +/- 1.2 a	11.5 +/- 3.6 b	20.6 +/- 11.8 b	7.1 +/- 4.2 ab	25.4 ± 10.0 c	11.4 +/- 4.8 bc	3.5 +/- 0.5 a
Unidentified genus of *Gemmataceae*	2.5 +/- 0.2 *	1.0 +/- 0.3 a	0.41 ± 0.06 b	0.37 +/- 0.13 b	0.38 +/- 0.14 b	0.38 +/- 0.17 b	0.84 +/- 0.31 ab	0.82 +/- 0.28 ab	0.64 ± 0.05 ab
Streptomyces	0.16 ± 0.03	1.3 +/- 0.3 a	0.93 +/- 0.17 a	1.3 +/- 0.3 a	0.40 ± 0.09 b	0.58 +/- 0.14 b	0.31 +/- 0.10 b	0.28 ± 0.07 b	0.27 +/- 0.15 b
Duganella	0.09 ± 0.03	12.7 +/- 5.0 * a	10.8 +/- 5.2 a	11.8 +/- 2.3 a	10.5 +/- 4.0 a	5.8 +/- 0.7 a	4.1 +/- 1.9 a	1.5 +/- 0.6 a	0.44 +/- 0.10 b
Unidentified genus of *Streptomycetaceae*	0.43 ± 0.07	2.6 +/- 0.4 * a	6.1 +/- 1.6 b	3.5 +/- 0.5 a	1.3 +/- 0.6 a	1.5 +/- 0.3 a	1.4 +/- 0.2 a	2.5 +/- 0.8 a	2.0 +/- 0.8 a
Mucilaginibacter	0.24 ± 0.07	2.1 +/- 0.6 * a	4.6 +/- 1.6 b	5.7 +/- 0.8 b	5.4 +/- 1.7 b	5.8 +/- 1.3 b	6.1 +/- 1.7 b	2.8 +/- 0.4 ab	6.1 +/- 1.2 b
Candidatus Udaeobacter	17.1 +/- 1.1 *	2.6 +/- 0.6 a	1.2 +/- 0.1 b	1.1 +/- 0.3 b	0.79 +/- 0.22 b	0.96 +/- 0.38 b	1.6 +/- 0.3 b	1.9 +/- 0.7 b	1.6 +/- 0.1 b
Pseudomonas	0.22 ± 0.04	3.0 +/- 0.4 * a	4.0 +/- 3.6 ab	1.0 +/- 0.6 b	1.3 +/- 0.7 b	0.66 +/- 0.14 b	0.31 +/- 0.12 b	0.38 +/- 0.29 b	0.01 ± 0.00 c
Cupriavidus	0.01 ± 0.00	3.7 +/- 1.1 * b	0.78 +/- 0.23 a	0.58 +/- 0.17 a	0.26 ± 0.09 c	0.61 +/- 0.28 a	0.15 ± 0.05 c	0.19 ± 0.07 c	0.01 ± 0.00 d
Burkholderia-Caballeronia-Paraburkholderia	0.75 ± 0.06	36.4 +/- 5.3 * a	12.9 +/- 3.8 b	16.2 +/- 1.8 b	4.4 +/- 1.3 c	13.1 +/- 1.6 b	2.9 +/- 0.5 c	6.3 +/- 0.8 bc	1.2 +/- 0.2 c
Unidentified genus of *Burkholderiaceae*	0.48 ± 0.03	5.1 +/- 1.2 * a	9.5 +/- 2.9 b	11.8 +/- 1.7 b	9.5 +/- 2.9 b	10.2 +/- 0.7 b	11.3 +/- 1.1 b	13.2 +/- 2.1 b	13.8 +/- 1.2 b
Unidentified genus	36.7 +/- 0.2 *	5.6 +/- 1.5 a	7.2 +/- 3.1 a	3.4 +/- 0.8 b	2.7 +/- 0.7 b	3.1 +/- 0.6 b	8.6 +/- 1.3 a	9.7 +/- 2.4 a	13.3 +/- 1.5 c
Other	19.8 +/- 0.5 *	6.8 +/- 0.9 a	8.8 +/- 1.6 a	6.8 +/- 1.0 a	7.7 +/- 1.3 a	10.5 +/- 2.1 a	11.7 +/- 1.6 a	15.9 +/- 1.7 ab	17.1 +/- 1.2 b
Massilia	0.03 ± 0.01	7.9 +/- 2.4 * a	12.2 +/- 6.2 a	2.5 +/- 0.9 b	6.9 +/- 4.6 ab	3.4 +/- 0.8 ab	0.92 +/- 0.50 c	1.1 +/- 0.9 c	0.07 ± 0.03 d

Table S2 (1/3) - Relative abundance of members of fungal communities at each taxonomic rank (>1% relative abundance in at least one time point) detected in the bulk soil and/or roots samples collected from T0 to T50. Genera highlighted in red correspond to EcM fungi, in grey to saprotrophic fungi and in yellow to endophytes. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant differences between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05). The asterisks denote significant difference in relative abudance of fungal communities between bulk soil samples and root samples collected from T2 (One-way ANOVA, factor=sampling time, P<0.05).

Fungal phylum	**Bulk Soil**	**T2**	**T4**	**T7**	**T10**	**T15**	**T21**	**T30**	**T50**
Glomeromycota	0.09 ± 0.05	0.0 ± 0.0 a	0.0 ± 0.0 a	0.06 ± 0.04 a	0.49 ± 0.44 a	0.0 ± 0.0 a	0.04 ± 0.04 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Chytridiomycota	0.13 ± 0.03	0.03 ± 0.03 a	0.0 ± 0.0 a	0.15 ± 0.10 a	0.23 ± 0.14 a	0.04 ± 0.04 a	0.02 ± 0.02 a	0.01 ± 0.01 a	0.04 ± 0.04 a
Rozellomycota	1.2 ± 0.0 *	0.03 ± 0.03 a	0.03 ± 0.02 a	0.06 ± 0.02 a	0.07 ± 0.02 a	0.17 ± 0.11 a	0.03 ± 0.02 a	0.01 ± 0.01 a	0.0 ± 0.0 a
Unidentified phyla	2.5 ± 0.1	1.1 ± 0.4 ab	0.62 ± 0.28 ab	0.5 ± 0.2 ab	2.0 ± 0.8 ab	2.8 ± 1.4 a	0.50 ± 0.21 ab	0.30 ± 0.16 ab	0.12 ± 0.07 b
Basidiomycota	51.6 ± 2.9 *	23.9 ± 6.9 a	15.4 ± 0.9 a	24.4 ± 13.6 a	7.3 ± 3.5 a	30.7 ± 12.8 ab	71.3 ± 6.4 bc	35.6 ± 14.2 ab	87.2 ± 3.1 c
Ascomycota	20.1 ± 2.3	31.5 ± 7.5 bc	56.0 ± 7.9 ab	51.8 ± 10.8 abc	74.6 ± 2.7 a	63.1 ± 13.5 ab	24.7 ± 5.5 bc	63.8 ± 14.1 ab	12.7 ± 3.1 c
Zygomycota	24.4 ± 1.3	43.4 ± 10.9 * bc	27.9 ± 7.2 bc	23.0 ± 7.7 abc	15.4 ± 5.6 ab	3.2 ± 1.1 ab	3.4 ± 1.3 ab	0.32 ± 0.07 a	0.03 ± 0.02 a
Fungal class	**Bulk Soil**	**T2**	**T4**	**T7**	**T10**	**T15**	**T21**	**T30**	**T50**
Eurotiomycetes	1.3 ± 0.1	0.16 ± 0.08 a	0.09 ± 0.05 a	0.18 ± 0.11 a	1.5 ± 1.1 a	0.19 ± 0.06 a	0.15 ± 0.06 a	0.01 ± 0.01 a	0.06 ± 0.02 a
Wallemiomycetes	1.8 ± 0.2 *	0.39 ± 0.22 b	0.13 ± 0.12 a	0.02 ± 0.02 a	0.0 ± 0.0 a	0.26 ± 0.21 a	0.04 ± 0.02 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Other	2.6 ± 0.3 *	0.40 ± 0.20 b	0.63 ± 0.33 b	0.33 ± 0.17 b	0.32 ± 0.01 b	0.54 ± 0.33 b	0.16 ± 0.07 b	0.07 ± 0.03 c	0.02 ± 0.01 c
Pezizomycetes	3.9 ± 0.4 *	0.74 ± 0.25 a	1.5 ± 0.7 a	15.8 ± 7.3 b	54.7 ± 13.5 c	19.1 ± 8.9 b	15.5 ± 5.1 b	48.2 ± 13.2 c	11.3 ± 3.5 b
Unidentified classes	2.5 ± 0.1 *	1.1 ± 0.5 b	0.61 ± 0.28 b	0.52 ± 0.18 b	2.0 ± 0.8 b	2.8 ± 1.4 a	0.50 ± 0.23 b	0.30 ± 0.18 b	0.11 ± 0.08 b
Microbotryomycetes	0.27 ± 0.09	1.1 ± 0.8 a	0.27 ± 0.15 a	0.13 ± 0.05 a	0.01 ± 0.0 a	0.04 ± 0.02 a	0.06 ± 0.03 a	0.02 ± 0.02 a	0.03 ± 0.01 a
Leotiomycetes	4.8 ± 2.2	1.2 ± 0.4 a	0.90 ± 0.38 a	6.2 ± 5.2 a	1.4 ± 1.1 a	5.8 ± 2.8 b	2.8 ± 1.1 a	2.6 ± 1.4 ab	1.0 ± 0.4 a
Sordariomycetes	4.1 ± 1.0	1.7 ± 0.5 a	11.9 ± 4.2 b	10.0 ± 2.8 b	11.9 ± 6.1 b	24.9 ± 12.5 b	3.4 ± 1.2 a	12.6 ± 10.1 a	0.15 ± 0.05 c
Tremellomycetes	8.4 ± 0.8	12.2 ± 4.1 b	4.9 ± 2.5 ab	1.9 ± 1.0 a	0.35 ± 0.12 c	2.0 ± 0.8 a	0.40 ± 0.10 c	0.07 ± 0.03 c	0.02 ± 0.01 c
Mucoromycotina_cls_Incertae_sedis	12.3 ± 0.9	14.0 ± 5.0 b	16.8 ± 8.0 b	2.7 ± 1.5 a	0.43 ± 0.26 c	0.48 ± 0.23 c	1.2 ± 1.0 c	0.04 ± 0.01 c	0.0 ± 0.0 c
Unidentified classes of Ascomycota	2.2 ± 0.1 *	21.1 ± 9.2 b	32.8 ± 7.8 b	15.2 ± 5.0 b	4.0 ± 1.6 a	9.7 ± 7.2 ab	1.4 ± 0.6 ac	0.17 ± 0.06 c	0.03 ± 0.02 c
Mortierellomycotina_cls_Incertae_sedis	12.0 ± 1.1	29.4 ± 8.8 * b	11.1 ± 5.0 a	20.2 ± 7.9 ab	14.9 ± 5.8 ab	2.8 ± 1.1 a	2.2 ± 1.2 a	0.27 ± 0.07 c	0.03 ± 0.01 c
Dothideomycetes	2.9 ± 1.2	6.3 ± 1.6 a	8.5 ± 6.0 a	4.3 ± 1.3 a	0.88 ± 0.24 b	3.2 ± 1.1 a	1.4 ± 0.6 a	0.04 ± 0.02 b	0.05 ± 0.03 b
Agaricomycetes	40.7 ± 3.3 *	9.9 ± 3.7 a	9.3 ± 2.7 a	22.3 ± 12.9 a	6.8 ± 3.4 a	28.3 ± 11.9 a	70.8 ± 13.8 b	35.4 ± 14.2 ab	87.1 ± 15.8 b
Fungal order	**Bulk Soil**	**T2**	**T4**	**T7**	**T10**	**T15**	**T21**	**T30**	**T50**
Microascales	0.05 ± 0.02	0.10 ± 0.09 a	0.06 ± 0.03 a	5.3 ± 2.7 b	5.7 ± 0.7 b	5.9 ± 3.8 b	0.64 ± 0.42 a	0.05 ± 0.05 a	0.0 ± 0.0 a
Chaetothyriales	1.1 ± 0.0 *	0.16 ± 0.08 a	0.03 ± 0.01 a	0.17 ± 0.11 a	1.5 ± 1.1 b	0.10 ± 0.03 a	0.10 ± 0.06 a	0.01 ± 0.01 a	0.06 ± 0.03 a
Thelephorales	6.3 ± 2.1 *	0.17 ± 0.14 a	0.07 ± 0.03 a	16.0 ± 11.0 b	1.5 ± 1.3 a	1.1 ± 0.9 a	25.5 ± 8.3 b	12.8 ± 10.0 ab	34.4 ± 18.4 b
Hypocreales	2.5 ± 0.4 *	0.31 ± 0.21 a	0.30 ± 0.24 a	2.6 ± 0.9 b	5.6 ± 3.0 b	14.4 ± 8.9 b	2.4 ± 1.1 b	12.5 ± 10.1 b	0.08 ± 0.04 a
Chaetosphaeriales	0.50 ± 0.12	0.31 ± 0.26 a	1.5 ± 1.3 a	0.41 ± 0.25 a	0.0 ± 0.0 b	0.10 ± 0.08 a	0.04 ± 0.02 a	0.01 ± 0.01 ab	0.0 ± 0.0 b
Geminibasidiales	1.8 ± 0.2 *	0.39 ± 0.22 a	0.14 ± 0.12 a	0.03 ± 0.02 a	0.0 ± 0.0 b	0.27 ± 0.22 a	0.04 ± 0.02 a	0.01 ± 0.01 ab	0.0 ± 0.0 b
Pezizales	3.9 ± 0.4 *	0.75 ± 0.25 a	1.5 ± 0.7 a	15.9 ± 7.3 b	54.7 ± 13.5 c	19.2 ± 9.0 b	15.6 ± 5.1 b	48.2 ± 13.3 c	11.3 ± 3.5 b
Sordariomycetidae_ord_Incertae_sedis	0.24 ± 0.05	0.77 ± 0.40 a	10.0 ± 3.6 b	1.8 ± 0.4 ab	0.73 ± 0.35 a	4.4 ± 3.0 ab	0.23 ± 0.07 a	0.13 ± 0.07 a	0.08 ± 0.02 a
Trechisporales	1.0 ± 0.7	0.0 ± 0.0 a	0.0 ± 0.0 a	0.76 ± 0.69 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Trichosporonales	0.12 ± 0.02	0.0 ± 0.0 a	1.3 ± 1.0 b	0.02 ± 0.02 a	0.0 ± 0.0 a	0.30 ± 0.27 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Unidentified orders of *Dothideomycetes*	0.24 ± 0.12	0.06 ± 0.03 a	0.38 ± 0.26 b	1.6 ± 0.6 c	0.47 ± 0.17 b	1.5 ± 0.9 c	0.10 ± 0.05 b	0.02 ± 0.01 a	0.0 ± 0.0 a
Other	0.75 ± 0.52	0.17 ± 0.09 a	0.11 ± 0.06 a	0.09 ± 0.05 a	0.07 ± 0.05 a	0.09 ± 0.03 a	0.54 ± 0.15 b	0.08 ± 0.05 a	0.04 ± 0.02 a
Helotiales	4.6 ± 2.2 *	1.1 ± 0.4 a	0.87 ± 0.36 a	6.1 ± 5.2 a	1.4 ± 1.1 a	5.7 ± 2.8 ab	2.2 ± 0.9 ab	2.5 ± 1.4 ab	0.97 ± 0.33 a
Pleosporales	1.8 ± 1.1	1.6 ± 0.6 a	0.99 ± 0.80 a	0.70 ± 0.30 a	0.12 ± 0.04 b	1.1 ± 0.6 a	1.1 ± 0.6 a	0.0 ± 0.0 c	0.03 ± 0.03 ac
Tremellales	8.3 ± 0.8	12.2 ± 4.1 b	3.6 ± 1.6 a	1.9 ± 1.0 a	0.35 ± 0.13 c	1.7 ± 0.7 a	0.35 ± 0.13 c	0.08 ± 0.03 c	0.03 ± 0.01 c
Mucorales	12.4 ± 0.9	14.0 ± 5.0 a	16.8 ± 8.0 a	2.7 ± 1.5 b	0.43 ± 0.27 c	0.49 ± 0.23 c	1.2 ± 1.0 bc	0.04 ± 0.01 d	0.0 ± 0.0 d
Unidentified orders	6.1 ± 0.2	22.5 ± 8.4 * b	33.5 ± 7.8 b	15.9 ± 4.9 ab	6.0 ± 2.1 a	12.6 ± 8.4 ab	1.9 ± 0.7 c	0.49 ± 0.18 d	0.15 ± 0.08 d
Mortierellales	12.1 ± 1.1	29.4 ± 8.8 * b	11.1 ± 5.0 a	20.2 ± 7.9 ab	14.9 ± 5.8 ab	2.8 ± 1.1 c	2.2 ± 1.2 c	0.28 ± 0.08 d	0.03 ± 0.02 d
Sebacinales	21.2 ± 1.2 *	3.0 ± 1.4 a	5.8 ± 1.9 a	3.6 ± 2.2 a	4.5 ± 2.2 a	20.2 ± 11.0 ab	37.3 ± 8.2 b	20.1 ± 7.0 ab	47.6 ± 17.6 b
Capnodiales	0.18 ± 0.04	3.3 ± 1.1 * a	6.5 ± 5.0 a	2.0 ± 1.3 a	0.20 ± 0.05 b	0.30 ± 0.15 b	0.15 ± 0.07 b	0.01 ± 0.01 c	0.0 ± 0.0 c
Agaricales	12.2 ± 2.8 *	4.5 ± 2.8 a	2.3 ± 0.9 a	2.0 ± 1.1 a	0.89 ± 0.53 b	7.0 ± 2.5 a	8.0 ± 2.4 a	2.6 ± 1.6 a	5.1 ± 3.4 a

Table S2 (2/3) - Relative abundance of members of fungal communities at each taxonomic rank (>1% relative abundance in at least one time point) detected in the bulk soil and/or roots samples collected from T0 to T50. Genera highlighted in red correspond to EcM fungi, in grey to saprotrophic fungi and in yellow to endophytes. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant differences between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05). The asterisks denote significant difference in relative abudance of fungal communities between bulk soil samples and root samples collected from T2 (One-way ANOVA, factor=sampling time, P<0.05).

Fungal family	Bulk Soil	T2	T4	T7	T10	T15	T21	T30	T50
Microascales_fam_Incertae_sedis	0.03 ± 0.0	0.0 ± 0.0 a	0.05 ± 0.03 a	5.3 ± 2.6 b	5.7 ± 3.7 b	5.9 ± 3.8 b	0.63 ± 0.42 c	0.05 ± 0.04 a	0.0 ± 0.0 a
Trichosporonaceae	0.12 ± 0.02	0.0 ± 0.0 a	1.3 ± 0.9 b	0.02 ± 0.02 a	0.0 ± 0.0 a	0.29 ± 0.26 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Unidentified families of *Mortierellales*	0.05 ± 0.03	0.0 ± 0.0 a	0.15 ± 0.08 b	0.15 ± 0.15 ab	5.4 ± 4.8 c	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Unidentified families of *Sebacinales*	0.73 ± 0.05 *	0.0 ± 0.0 a	1.6 ± 0.9 b	2.7 ± 2.3 b	2.3 ± 1.2 b	12.6 ± 9.8 b	16.4 ± 8.4 b	11.2 ± 3.4 b	17.6 ± 7.5 b
Mycenaceae	2.2 ± 2.2	0.0 ± 0.0 a	0.0 ± 0 0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Amylostereaceae	0.0 ± 0.0	0.01 ± 0.01 a	1.1 ± 0.9 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Unidentified families of *Pezizales*	0.01 ± 0.01	0.01 ± 0.01 a	0.0 ± 0.0 a	4.5 ± 4.5 ab	3.3 ± 2.0 b	0.0 ± 0.0 a	0.01 ± 0.01 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Vibrisseaceae	0.11 ± 0.02	0.02 ± 0.01 a	0.13 ± 0.11 a	1.6 ± 1.2 b	1.3 ± 1.1 b	3.0 ± 2.4 b	0.45 ± 0.20 ab	0.65 ± 0.43 ab	0.16 ± 0.09 a
Hypocreales_fam_Incertae_sedis	0.09 ± 0.0 *	0.04 ± 0.03 a	0.0 ± 0.0 a	1.0 ± 0.7 b	5.5 ± 3.0 c	10.3 ± 5.9 c	1.9 ± 0.9 b	3.0 ± 1.8 bc	0.0 ± 0.0 a
Unidentified families of *Hypocreales*	0.56 ± 0.32 *	0.06 ± 0.04 a	0.06 ± 0.04 a	0.02 ± 0.01 a	0.04 ± 0.03 a	3.8 ± 3.4 b	0.21 ± 0.12 a	9.4 ± 9.4 ab	0.01 ± 0.01 a
Strophariaceae	5.5 ± 3.7	0.11 ± 0.04 a	0.14 ± 0.10 a	0.18 ± 0.10 a	0.0 ± 0.0 b	0.60 ± 0.24 a	1.1 ± 0.4 ac	0.47 ± 0.25 a	4.1 ± 3.5 c
Discinaceae	2.1 ± 0.2 *	0.12 ± 0.06 a	0.12 ± 0.10 a	1.3 ± 0.6 b	0.01 ± 0.0 a	2.4 ± 0.9 b	2.0 ± 1.5 b	0.33 ± 0.17 a	0.59 ± 0.38 a
Herpotrichiellaceae	1.1 ± 0.0 *	0.16 ± 0.08 a	0.03 ± 0.01 b	0.16 ± 0.10 a	1.5 ± 1.1 a	0.08 ± 0.03 a	0.10 ± 0.05 a	0.01 ± 0.01 b	0.06 ± 0.02 ab
Thelephoraceae	6.3 ± 2.1 *	0.17 ± 0.14 a	0.06 ± 0.03 a	16.0 ± 11.0 b	1.5 ± 1.3 a	1.1 ± 0.9 a	25.4 ± 8.3 b	12.8 ± 10.0 ab	34.4 ± 18.4 b
Hypocreaceae	1.7 ± 0.2 *	0.17 ± 0.16 a	0.23 ± 0.20 a	1.1 ± 0.5 b	0.03 ± 0.02 a	0.10 ± 0.05 a	0.14 ± 0.08 a	0.05 ± 0.03 a	0.05 ± 0.04 a
Chaetosphaeriaceae	0.49 ± 0.12	0.31 ± 0.26 a	1.5 ± 1.3 a	0.40 ± 0.24 a	0.0 ± 0.0 b	0.14 ± 0.07 a	0.03 ± 0.01 a	0.0 ± 0.0 b	0.0 ± 0.0 b
Geminibasidiaceae	1.8 ± 0.2 *	0.39 ± 0.22 a	0.13 ± 0.12 ab	0.02 ± 0.02 ab	0.0 ± 0.0 b	0.3 ± 0.2 a	0.04 ± 0.02 a	0.0 ± 0.0 b	0.0 ± 0.0 b
Helotiales_fam_Incertae_sedis	0.42 ± 0.09	0.54 ± 0.29 a	0.21 ± 0.14 a	4.4 ± 4.0 ab	0.02 ± 0.01 c	1.8 ± 0.9 b	1.2 ± 0.6 ab	1.7 ± 1.3 ab	0.38 ± 0.12 a
Pyronemataceae	0.54 ± 0.09	0.61 ± 0.21 a	0.90 ± 0.76 a	8.5 ± 4.1 b	51.3 ± 13.2 c	16.4 ± 9.3 b	12.8 ± 4.6 b	47.7 ± 13.2 c	10.4 ± 3.3 b
Annulatascaceae	0.05 ± 0.04	0.76 ± 0.40 a	10.0 ± 3.6 b	1.8 ± 0.4 a	0.59 ± 0.27 a	4.4 ± 3.0 ab	0.20 ± 0.06 a	0.12 ± 0.07 ac	0.07 ± 0.02 c
Unidentified families of *Agaricales*	2.9 ± 0.01 *	1.0 ± 0.4 ab	1.9 ± 0.7 ab	1.5 ± 1.0 ab	0.20 ± 0.10 b	5.2 ± 2.3 a	6.2 ± 1.9 a	2.1 ± 1.6 ab	1.0 ± 0.5 ab
Sporidiobolales_fam_Incertae_sedis	0.02 ± 0.01	1.1 ± 0.8 a	0.10 ± 0.08 b	0.12 ± 0.06 ab	0.01 ± 0.00 b	0.03 ± 0.02 b	0.03 ± 0.01 b	0.02 ± 0.02 b	0.03 ± 0.01 b
Unidentified families of *Pleosporales*	1.4 ± 1.0	1.5 ± 0.6 a	0.80 ± 0.68 a	0.56 ± 0.34 a	0.07 ± 0.03 b	0.34 ± 0.14 ab	0.50 ± 0.28 ab	0.0 ± 0.0 c	0.03 ± 0.03 c
Tremellales_fam_Incertae_sedis	8.3 ± 0.8	12.1 ± 4.1 b	3.6 ± 1.6 a	1.9 ± 1.0 a	0.35 ± 0.12 c	1.7 ± 0.7 a	0.35 ± 0.13 c	0.07 ± 0.03 d	0.02 ± 0.01 d
Umbelopsidaceae	12.2 ± 0.8	13.9 ± 5.0 a	16.4 ± 8.2 a	2.7 ± 1.5 b	0.43 ± 0.26 c	0.46 ± 0.21 c	0.96 ± 0.81 c	0.04 ± 0.01 d	0.0 ± 0.0 d
Polyporaceae	0.0 ± 0.0	2.2 ± 2.0 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Other	10.2 ± 1.5 *	2.8 ± 1.0 a	3.6 ± 1.1 a	3.7 ± 1.6 a	1.9 ± 0.6 a	3.0 ± 0.9 a	2.9 ± 0.9 a	0.35 ± 0.14 b	0.71 ± 0.21 b
Unidentified families	7.1 ± 0.6	22.7 ± 8.3 * a	34.0 ± 2.6 a	17.6 ± 5.1 a	6.6 ± 1.9 ac	14.2 ± 10.0 a	2.5 ± 0.7 c	0.59 ± 0.17 b	0.19 ± 0.12 b
Mortierellaceae	12.0 ± 1.1 *	29.4 ± 8.8 b	10.9 ± 5.0 a	20.0 ± 7.9 ab	9.5 ± 5.5 ab	2.8 ± 1.1 c	2.2 ± 1.2 c	0.27 ± 0.07 d	0.03 ± 0.01 d
Sebacinaceae	20.4 ± 1.2 *	3.0 ± 1.4 a	4.2 ± 1.9 a	0.90 ± 0.30 b	2.2 ± 1.0 a	7.6 ± 4.2 a	20.9 ± 5.1 c	8.9 ± 5.9 a	29.9 ± 11.5 c
Cortinariaceae	1.3 ± 0.3	3.2 ± 2.7 a	0.17 ± 0.14 b	0.20 ± 0.13 b	0.03 ± 0.03 bc	1.1 ± 0.9 a	0.40 ± 0.34 b	0.0 ± 0.0 c	0.0 ± 0.0 c
Mycosphaerellaceae	0.18 ± 0.04	3.3 ± 1.1 * a	6.5 ± 5.0 a	1.6 ± 1.0 a	0.18 ± 0.04 b	0.31 ± 0.14 b	0.13 ± 0.06 b	0.01 ± 0.01 c	0.0 ± 0.0 c

Table S2 (3/3) - Relative abundance of members of fungal communities at each taxonomic rank (>1% relative abundance in at least one time point) detected in the bulk soil and/or roots samples collected from T0 to T50. Genera highlighted in red correspond to EcM fungi, in grey to saprotrophic fungi and in yellow to endophytes. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant differences between each sampling time from T2 to T50 (One-way ANOVA, factor=sampling time, P<0.05). The asterisks denote significant difference in relative abudance of fungal communities between bulk soil samples and root samples collected from T2 (One-way ANOVA, factor=sampling time, P<0.05).

Fungal genus	Bulk Soil	T2	T4	T7	T10	T15	T21	T30	T50
Sphaeronaemella	0.03 ± 0.01 a	0.0 ± 0.0 a	0.06 ± 0.04 b	5.3 ± 2.7 c	5.7 ± 4.0 c	5.9 ± 4.1 c	0.64 ± 0.46 d	0.05 ± 0.05 ab	0.0 ± 0.0 a
Trichosporon	0.12 ± 0.02 a	0.0 ± 0.0 a	1.3 ± 1.1 b	0.02 ± 0.02 a	0.0 ± 0.0 a	0.30 ± 0.30 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Unidentified genera of *Thelephoraceae*	3.5 ± 2.4 *	0.0 ± 0.0 a	0.05 ± 0.04 a	15.3 ± 11.9 b	1.5 ± 1.4 c	0.80 ± 0.80 ac	25.2 ± 7.9 b	12.8 ± 10.9 b	34.4 ± 19.4 b
Exophiala	0.39 ± 0.10 a	0.02 ± 0.01 a	0.03 ± 0.02 a	0.05 ± 0.03 a	1.0 ± 1.0 a	0.07 ± 0.03 a	0.06 ± 0.05 a	0.0 ± 0.0 a	0.01 ± 0.01 a
Phialocephala	0.11 ± 0.02 a	0.02 ± 0.01 a	0.14 ± 0.12 a	1.6 ± 1.3 b	1.3 ± 1.2 ab	3.0 ± 2.7 b	0.46 ± 0.21 c	0.66 ± 0.46 c	0.17 ± 0.11 c
Amylostereum	0.0 ± 0.0 a	0.02 ± 0.02 a	1.1 ± 1.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.01 ± 0.01 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Hebeloma	0.09 ± 0.03 a	0.02 ± 0.02 a	0.09 ± 0.08 a	0.01 ± 0.01 a	0.0 ± 0.0 a	0.25 ± 0.16 b	0.50 ± 0.14 b	0.17 ± 0.09 ab	4.0 ± 3.8 ab
Cadophora	0.04 ± 0.01 a	0.04 ± 0.03 a	0.11 ± 0.06 a	0.06 ± 0.05 a	0.03 ± 0.02 a	0.15 ± 0.08 a	0.81 ± 0.34 b	1.7 ± 1.4 c	0.28 ± 0.09 a
Geopora	0.0 ± 0.0 a	0.05 ± 0.03 a	0.31 ± 0.28 a	1.4 ± 1.3 a	14.0 ± 4.7 b	0.07 ± 0.05 a	0.01 ± 0.01 a	0.05 ± 0.02 a	0.05 ± 0.05 a
Ilyonectria	0.09 ± 0.01 a	0.05 ± 0.03 a	0.0 ± 0.0 a	1.0 ± 0.8 b	5.5 ± 3.1 c	10.4 ± 6.3 c	1.9 ± 1.0 bc	3.0 ± 1.9 bc	0.08 ± 0.05 a
Unidentified genera of *Sebacinaceae*	1.4 ± 0.4 *	0.05 ± 0.03 a	0.02 ± 0.02 a	0.08 ± 0.05 a	1.1 ± 0.5 b	2.8 ± 2.2 b	11.2 ± 2.4 c	4.6 ± 3.5 bc	16.3 ± 6.2 b
Mycena	2.2 ± 2.2 a	0.08 ± 0.08 a	0.0 ± 0.0 a	0.08 ± 0.08 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Hymenogaster	5.4 ± 3.7 b	0.09 ± 0.04 a	0.06 ± 0.04 a	0.17 ± 0.10 a	0.05 ± 0.05 a	0.35 ± 0.18 b	0.61 ± 0.23 b	0.31 ± 0.18 b	0.05 ± 0.03 a
Hydnotrya	2.0 ± 0.2 a	0.12 ± 0.07 a	0.12 ± 0.12 a	1.3 ± 0.7 b	0.01 ± 0.01 a	2.4 ± 1.0 b	2.0 ± 1.6 b	0.33 ± 0.18 a	0.60 ± 0.41 ab
Tomentella	1.9 ± 1.0 b	0.14 ± 0.13 ab	0.0 ± 0.0 a	0.73 ± 0.73 ab	0.05 ± 0.05 a	0.06 ± 0.06 ab	0.16 ± 0.09 b	0.0 ± 0.0 a	0.04 ± 0.04 a
Trichoderma	1.5 ± 0.2 b	0.18 ± 0.18 a	0.23 ± 0.23 a	1.0 ± 0.5 b	0.03 ± 0.02 a	0.10 ± 0.05 a	0.09 ± 0.04 a	0.05 ± 0.03 a	0.06 ± 0.05 a
Leptodontidium	0.19 ± 0.07 a	0.29 ± 0.29 a	0.11 ± 0.11 a	4.3 ± 4.3 ab	0.0 ± 0.0 a	1.6 ± 1.0 b	0.43 ± 0.30 a	0.05 ± 0.03 a	0.10 ± 0.05 a
Chaetosphaeria	0.50 ± 0.12 a	0.31 ± 0.28 a	1.5 ± 1.4 a	0.41 ± 0.27 a	0.0 ± 0.0 a	0.14 ± 0.09 a	0.04 ± 0.02 a	0.08 ± 0.08 a	0.0 ± 0.0 a
Geminibasidium	1.8 ± 0.2 b	0.39 ± 0.24 b	0.14 ± 0.14 ab	0.03 ± 0.02 a	0.0 ± 0.0 a	0.27 ± 0.24 ab	0.04 ± 0.02 a	0.08 ± 0.08 ab	0.0 ± 0.0 a
Unidentified genera of *Pyronemataceae*	0.54 ± 0.09	0.56 ± 0.23 a	0.58 ± 0.58 a	7.1 ± 3.7 b	37.3 ± 6.9 c	13.2 ± 9.8 b	12.8 ± 4.4 b	47.7 ± 11.3 c	10.4 ± 3.1 b
Conlarium	0.05 ± 0.04 a	0.77 ± 0.43 a	10.0 ± 3.3 b	1.8 ± 0.3 a	0.90 ± 0.03 a	4.4 ± 3.3 ab	0.21 ± 0.06 a	0.13 ± 0.08 a	0.08 ± 0.02 a
Daedaleopsis	0.0 ± 0.0 a	2.2 ± 2.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Sebacina	19.1 ± 0.8 c	3.0 ± 1.4 a	4.2 ± 1.9 b	0.83 ± 0.26 c	1.1 ± 0.5 ac	4.1 ± 2.3 a	9.8 ± 1.4 d	4.3 ± 2.8 b	13.7 ± 5.2 d
Cortinarius	1.2 ± 0.3 a	3.0 ± 3.0 a	0.02 ± 0.01 a	0.14 ± 0.08 a	0.03 ± 0.03 a	1.1 ± 1.0 a	0.39 ± 0.38 a	0.04 ± 0.04 a	0.0 ± 0.0 a
Mycosphaerella	0.18 ± 0.05 a	3.3 ± 1.0 d	6.5 ± 5.6 c	1.0 ± 0.6 c	0.18 ± 0.02 b	0.31 ± 0.15 b	0.12 ± 0.07 b	0.01 ± 0.01 a	0.0 ± 0.0 a
Other	7.2 ± 2.3 *	4.2 ± 1.7 a	3.4 ± 0.9 a	3.8 ± 1.0 a	2.4 ± 0.8 a	13.6 ± 1.5 b	2.5 ± 0.7 a	0.27 ± 0.09 c	0.41 ± 0.13 c
Cryptococcus	8.3 ± 0.8 ab	12.1 ± 3.9 b	3.6 ± 1.6 a	1.9 ± 1.0 a	0.35 ± 0.12 c	1.7 ± 0.7 a	0.35 ± 0.13 c	0.08 ± 0.03 d	0.03 ± 0.02 d
Umbelopsis	12.3 ± 0.8 ab	13.9 ± 4.9 a	16.5 ± 8.4 a	2.7 ± 1.5 b	0.44 ± 0.29 c	0.46 ± 0.22 c	1.0 ± 0.9 bc	0.04 ± 0.01 d	0.0 ± 0.0 d
Unidentified genera	17.7 ± 1.9	25.8 ± 8.8 a	38.9 ± 2.8 b	27.9 ± 4.0 a	18.0 ± 4.5 c	29.7 ± 11.7 a	26.5 ± 9.2 a	23.4 ± 7.5 a	19.3 ± 8.2 ac
Mortierella	12.0 ± 1.1 ab	29.4 ± 7.9 a	11.0 ± 5.0 b	20.1 ± 7.8 ab	9.5 ± 5.8 b	2.8 ± 1.1 c	2.2 ± 1.2 c	0.28 ± 0.06 d	0.03 ± 0.02 d

Table S3 - Distribution of the relative abundance of fungal guilds detected in the bulk soil samples and in the roots samples collected from T2 to T50. Each given value is the average value of 3, 4 or 5 replicates +/- SE. Different letters denote significant difference between each sampling time from T2 to T50 (One way ANOVA, factor = sampling time, P<0.05). The asterisks denote significant difference between bulk soil samples root samples collected from T2 (One-way ANOVA, factor = sampling time, P<0.05).

Fungal guild	Bulk Soil	T2	T4	T7	T10
Arbuscular mycorrhizal fungi	0.10 ± 0.05 *	0.0 ± 0.0 a	0.0 ± 0.0 a	0.04 ± 0.02 ab	0.08 ± 0.03 b
Plant pathogen	0.57 ± 0.16	3.3 ± 1.0 a	7.2 ± 6.2 a	1.2 ± 0.6 a	0.87 ± 0.55 a
Ectomycorrhizal fungi	34.9 ± 13.1 *	6.4 ± 3.9 a	4.6 ± 2.0 a	19.3 ± 11.5 ab	2.6 ± 1.9 a
Undefined Saprotroph	16.7 ± 1.8	18.1 ± 5.0 bc	26.9 ± 7.5 c	9.9 ± 3.4 abc	6.9 ± 3.8 ab
Endophyte	13.0 ± 1.2	29.9 ± 8.1 * b	11.3 ± 5.3 ab	26.9 ± 7.7 b	10.9 ± 5.3 ab
Unclassified and other	34.7 ± 1.5	42.3 ± 9.0 a	49.9 ± 4.2 a	42.8 ± 6.6 a	78.7 ± 6.4 a
Fungal guild	**T15**	**T21**	**T30**	**T50**	
Arbuscular mycorrhizal fungi	0.0 ± 0.0 a	0.01 ± 0.01 a	0.0 ± 0.0 a	0.0 ± 0.0 a	
Plant pathogen	0.50 ± 0.18 a	1.1 ± 0.5 a	0.02 ± 0.01 a	0.01 ± 0.01 a	
Ectomycorrhizal fungi	9.5 ± 3.2 a	39.0 ± 8.4 ab	18.0 ± 11.3 ab	52.8 ± 13.1 b	
Undefined Saprotroph	11.3 ± 4.3 abc	2.0 ± 0.3 ab	0.27 ± 0.08 a	0.01 ± 0.01 a	
Endophyte	7.6 ± 2.8 ab	3.9 ± 1.8 a	2.7 ± 1.4 a	0.58 ± 0.21 a	
Unclassified and other	71.2 ± 2.5 a	53.9 ± 7.1 a	79.0 ± 11.7 a	46.5 ± 13.0 a	

Chapitre III : Effet du type de sol, du génotype et du climat sur le microbiote racinaire du Peuplier noir (*Populus nigra*)

TABLES

Table 1 – Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation of compartment for fungal and bacterial communities at the OTU level. R and E samples of the seedlings of the Loire and Drôme progenies cultivated in their native conditions of sediments and climate were included in this analysis (D = Drôme, L = Loire). Statistical significance (p-value) computed based on sequential sums of square from 999 permutations (*** = P-value < 0.01).

Fungi											
E Compartment			**F**	**R2**	**P-value**	**R Compartment**			**F**	**R2**	**P-value**
Seedlings_ origin	Sediment	Climate				Seedlings_ origin	Sediment	Climate			
D	**D**	**D**	3.81	0.124	***	**D**	**D**	**D**	7.82	0.238	***
L	**L**	**L**				**L**	**L**	**L**			
Bacteria											
E Compartment			**F**	**R2**	**P-value**	**R Compartment**			**F**	**R2**	**P-value**
Seedlings_ origin	Sediment	Climate				Seedlings_ origin	Sediment	Climate			
D	**D**	**D**	25.64	0.477	***	**D**	**D**	**D**	21.57	0.444	***
L	**L**	**L**				**L**	**L**	**L**			

Table 2 – Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation of compartment for fungal and bacterial communities at the OTU level. BS, R and E samples of the seedlings of the Loire and Drôme progenies cultivated in the different conditions of sediments and climate were included in this analysis (D = Drôme, L = Loire). Statistical significance (p-value) computed based on sequential sums of square from 999 permutations (*** = P-value < 0.01, ** = P-value < 0.03).

		Fungi											
		Comparison BS vs R						**Comparison R vs E**					
		Seedlings_origin	Sediment	Climate	**F**	**R2**	**P-value**	Seedlings_origin	Sediment	Climate	**F**	**R2**	**P-value**
Drôme seedlings		**D**	**D**	**D**	1.39	0.08	0.21	**D**	**D**	**D**	26.53	0.49	***
		D	L	D	1.75	0.09	0.17	D	L	D	14.47	0.32	***
		D	D	L	1.04	0.07	0.39	D	D	L	11.72	0.37	***
		D	L	L	2.13	0.11	0.07	D	L	L	19.05	0.38	***
Loire seedlings		**L**	**L**	**L**	1.71	0.12	0.15	**L**	**L**	**L**	6.59	0.22	***
		L	D	L	1.40	0.08	0.22	L	D	L	21.97	0.43	***
		L	L	D	2.78	0.17	0.09	L	L	D	13.60	0.36	***
		L	D	D	2,00	0.10	0.08	L	D	D	17.57	0.35	***
		Bacteria											
		Comparison BS vs R						**Comparison R vs E**					
		Seedlings_origin	Sediment	Climate	**F**	**R2**	**P-value**	Seedlings_origin	Sediment	Climate	**F**	**R2**	**P-value**
Drôme seedlings		**D**	**D**	**D**	4.93	0.24	***	**D**	**D**	**D**	22.48	0.45	***
		D	L	D	3.39	0.16	***	D	L	D	18.09	0.38	***
		D	D	L	6.40	0.30	***	D	D	L	56.12	0.70	***
		D	L	L	5.69	0.24	***	D	L	L	30.05	0.49	***
Loire seedlings		**L**	**L**	**L**	5.11	0.25	***	**L**	**L**	**L**	17.69	0.40	***
		L	D	L	6.02	0.26	***	L	D	L	31.97	0.54	***
		L	L	D	1.14	0.07	0.246	L	L	D	10.13	0.27	***
		L	D	D	4.79	0.17	**	L	D	D	23.97	0.41	***

Table 3 – Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation of treatment (Sediments, Climate or Sediments & Climate) for fungal and bacterial communities at the OTU level. R and E samples of the seedlings of the Loire and Drôme cultivated in the different conditions of sediments and climate were included in this analysis (D = Drôme, L = Loire). Statistical significance (p-value) computed based on sequential sums of square from 999 permutations (*** = P-value < 0.01, ** = P-value < 0.03, * = P-value < 0.05).

Fungi			**PermMANOVA**		
Comparision in the R compartment		**Source of variation**	**F**	**R2**	**p-value**
DLD	DDD	Sediments	17.11	0.36	***
LDL	LLL	Sediments	4.83	0.15	**
DDL	DDD	Climate	2.39	0.08	**
LLD	LLL	Climate	3.29	0.12	*
DLL	DDD	Sediments & Climate	7.97	0.22	***
LDD	LLL	Sediments & Climate	4.97	0.18	***
Comparision in the E compartment		**Source of variation**	**F**	**R2**	**p-value**
DLD	DDD	Sediments	6.87	0.19	***
LDL	LLL	Sediments	3.23	0.11	*
DDL	DDD	Climate	3.49	0.14	0.052
LLD	LLL	Climate	1.08	0.04	0.377
DLL	DDD	Sediments & Climate	3.17	0.10	*
LDD	LLL	Sediments & Climate	2.43	0.09	*
Bacteria			**PermMANOVA**		
Comparision in the R compartment		**Source of variation**	**F**	**R2**	**p-value**
DLD	DDD	Sediments	23.32	0.43	***
LDL	LLL	Sediments	16.62	0.41	***
DDL	DDD	Climate	4.22	0.14	***
LLD	LLL	Climate	4.47	0.15	***
DLL	DDD	Sediments & Climate	27.0	0.47	***
LDD	LLL	Sediments & Climate	23.9	0.38	***
Comparision in the E compartment		**Source of variation**	**F**	**R2**	**p-value**
DLD	DDD	Sediments	21.83	0.44	***
LDL	LLL	Sediments	34.77	0.54	***
DDL	DDD	Climate	9.99	0.29	***
LLD	LLL	Climate	6.97	0.20	***
DLL	DDD	Sediments & Climate	31.2	0.51	***
LDD	LLL	Sediments & Climate	27.8	0.42	***

Table 4 – Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation in this study (compartment, progeny and interaction between compartment and progeny (C x P)) for fungal and bacterial communities at the OTU level. BS, R and E samples of the seedlings of the Loire and Drôme progenies cultivated in the different conditions of sediments and climate were included in this analysis (D = Drôme, L = Loire). Statistical significance (p-value) computed based on sequential sums of square from 999 permutations (*** = P-value < 0.01, ** = P-value < 0.03, * = P-value < 0.05).

Fungi		PermMANOVA			Fungi		PermMANOVA		
Comparison BS vs R	**Source of variation**	**F**	**R2**	**p-value**	**Comparison R vs E**	**Source of variation**	**F**	**R2**	**p-value**
DDD	Compartment (C)	14.73	0.34	***	DDD	Compartment (C)	2.69	0.12	*
	Progeny (P)	1.28	0.06	0.199		Progeny (P)	0.31	0.02	0.986
	C*P	0.47	0.02	0.970		C*P	0.19	0.01	1.000
LLL	Compartment (C)	6.49	0.22	***	LLL	Compartment (C)	2.75	0.14	*
	Progeny (P)	0.91	0.06	0.562		Progeny (P)	0.25	0.02	0.968
	C*P	0.77	0.05	0.693		C*P	0.19	0.02	0.997
DLD	Compartment (C)	13.76	0.30	***	DLD	Compartment (C)	3.47	0.14	*
	Progeny (P)	0.81	0.03	0.528		Progeny (P)	0.67	0.05	0.625
	C*P	1.51	0.06	0.178		C*P	0.42	0.03	0.829
LDL	Compartment (C)	24.40	0.43	***	LDL	Compartment (C)	3.28	0.12	**
	Progeny (P)	1.31	0.04	0.245		Progeny (P)	0.77	0.06	0.738
	C*P	0.56	0.02	0.732		C*P	0.42	0.03	0.995
DDL	Compartment (C)	13.42	0.35	***	DDL	Compartment (C)	2.53	0.12	**
	Progeny (P)	1.79	0.09	0.080		Progeny (P)	0.70	0.06	0.861
	C*P	0.64	0.03	0.751		C*P	0.57	0.05	0.935
LLD	Compartment (C)	15.10	0.36	***	LLD	Compartment (C)	6.23	0.23	**
	Progeny (P)	1.99	0.09	0.091		Progeny (P)	1.19	0.09	0.324
	C*P	0.95	0.04	0.415		C*P	0.94	0.07	0.451
DLL	Compartment (C)	21.40	0.39	***	DLL	Compartment (C)	3.89	0.15	**
	Progeny (P)	1.02	0.03	0.367		Progeny (P)	0.34	0.05	0.996
	C*P	1.05	0.03	0.361		C*P	0.17	0.13	0.995
LDD	Compartment (C)	17.13	0.33	***	LDD	Compartment (C)	2.493	0.09	*
	Progeny (P)	1.43	0.05	0.178		Progeny (P)	0.60	0.04	0.860
	C*P	0.93	0.03	0.456		C*P	0.28	0.02	1.000
Bacteria		**PermMANOVA**			**Bacteria**		**PermMANOVA**		
Comparison BS vs R	**Source of variation**	**F**	**R2**	**p-value**	**Comparison R vs E**	**Source of variation**	**F**	**R2**	**p-value**
DDD	Compartment (C)	22.72	0.44	***	DDD	Compartment (C)	21.03	0.52	**
	Progeny (P)	1.18	0.04	0.266		Progeny (P)	0.62	0.03	0.680
	C*P	0.96	0.03	0.431		C*P	0.39	0.02	0.930
LLL	Compartment (C)	18.80	0.41	***	LLL	Compartment (C)	15.70	0.42	**
	Progeny (P)	1.10	0.04	0.325		Progeny (P)	0.91	0.04	0.459
	C*P	0.92	0.04	0.454		C*P	0.54	0.02	0.870
DLD	Compartment (C)	18.33	0.37	***	DLD	Compartment (C)	8.56	0.29	**
	Progeny (P)	1.31	0.05	0.212		Progeny (P)	0.27	0.02	0.968
	C*P	0.88	0.03	0.535		C*P	0.14	0.01	1.000
LDL	Compartment (C)	32.84	0.54	***	LDL	Compartment (C)	16.63	0.44	**
	Progeny (P)	1.48	0.04	0.188		Progeny (P)	0.49	0.02	0.937
	C*P	0.88	0.02	0.479		C*P	0.28	0.01	0.999
DDL	Compartment (C)	56.15	0.70	***	DDL	Compartment (C)	15.27	0.44	**
	Progeny (P)	1.02	0.02	0.370		Progeny (P)	0.56	0.06	0;941
	C*P	0.98	0.02	0.381		C*P	0.14	0.01	1.000
LLD	Compartment (C)	9.76	0.27	***	LLD	Compartment (C)	4.27	0.18	**
	Progeny (P)	0.81	0.04	0.682		Progeny (P)	0.50	0.08	0.983
	C*P	0.69	0.03	0.845		C*P	0.15	0.01	1.000
DLL	Compartment (C)	29.43	0.49	***	DLL	Compartment (C)	22.20	0.50	**
	Progeny (P)	0.95	0.03	0.407		Progeny (P)	0.68	0.03	0.679
	C*P	0.72	0.02	0.600		C*P	0.36	0.01	0.992
LDD	Compartment (C)	27.48	0.54	***	LDD	Compartment (C)	24.42	0.54	**
	Progeny (P)	1.04	0.03	0.345		Progeny (P)	0.58	0.02	0.685
	C*P	0.91	0.04	0.499		C*P	0.51	0.01	0.994

Chapitre III : Effet du type de sol, du génotype et du climat sur le microbiote racinaire du Peuplier noir (*Populus nigra*)

SUPPLEMENTAL INFORMATIONS

Table S1 – Number of biological replicates used for each treatment in this study (D = Drôme, L = Loire).

Treatment	Progeny	Number of seedlings	Sediment	Climate
LLL	L04	5	Loire	Loire
	L06	5		
	L08	5		
LDL	L04	6	Drôme	Loire
	L06	6		
	L08	6		
LLD	L04	5	Loire	Drôme
	L06	5		
	L08	5		
LDD	L04	6	Drôme	Drôme
	L06	6		
	L08	6		
DDD	D11	5	Loire	Loire
	D13	5		
	D15	5		
DLD	D11	5	Drôme	Loire
	D13	6		
	D15	6		
DDL	D11	5	Loire	Drôme
	D13	5		
	D15	4		
DLL	D11	6	Drôme	Drôme
	D13	6		
	D15	5		

Table S2 – Sequences of primers and PNA probes PCR blockers used in this study.

Primer name	Sequence 5’ → 3’
515F_Universal	GTGYCAGCMGCCGCGGTAA
515F_Chloroflexi	GTGCCAGCMGCWGCGGTAA
515F_TM7	GTGCCAGCMGCCGCGGTCA
515F_Nano	GTGGCAGYCGCCRCGGKAA
806R_Universal	GGACTACNVGGGTWTCTAAT
806R_Nano	GGAMTACHGGGGTCTCTAAT
ITS3NGS1	CATCGATGAAGAACGCAG
ITS3NGS2	CAACGATGAAGAACGCAG
ITS3NGS3	CACCGATGAAGAACGCAG
ITS3NGS4	CATCGATGAAGAACGTAG
ITS3NGS5	CATCGATGAAGAACGTGG
ITS3NGS10	CATCGATGAAGAACGCTG
ITS4NGS	TCCTSCGCTTATTGATATGC
pPNA_717-1B4	GGCTCAACCCTGGACAG
mtPNA _717-1B4	GGCAAGTCTTCTTCGGA
ITSspacePNA_717-1B4	CGAGGGCACGTCTGCCTGG

Table S3 – Physico-chemical properties of Drôme and Loire sediments.

		Units	Drome sediments	Loire sediments
Texture	Clay (< 2 µm)	g/kg	101	11
	Fine slit (2/20 µm)	g/kg	105	1
	Coarse silt (20/50 µm)	g/kg	54	3
	Fine sand (50/200 µm)	g/kg	230	9
	Coarse sand (200/2000 µm)	g/kg	510	976
	Total limestone (CaCO3)	g/kg	684	<1
	Phosphorus (P2O5)	g/kg	<0,005	<0,005
Fertility	Organic matter	g/kg	4,71	0,277
	C/N	-	10,1	8
	pH	-	9,05	8,01
	CEC	cmol+/kg	4,05	1,42
Elements	Calcium (Ca)	cmol+/kg	5,84	0,97
	Magnesium (Mg)	cmol+/kg	0,33	0,14
	Sodium (Na)	cmol+/kg	0,01	0,007
	Potassium (K)	cmol+/kg	0,098	0,044
	Iron (Fe)	cmol+/kg	<0,005	<0,005
	Manganese (Mn)	cmol+/kg	<0,005	0,0071
	Aluminium (Al)	cmol+/kg	<0,02	<0,02

Table S4 – Permutational multivariate ANOVA results with Bray-Curtis distance matrices implemented to partition sources of variation in this study (sediments, climate, time, interaction between time and sediment (T x G) and between sediment and climate (S x C)) for fungal and bacterial communities at the OTU level. BS samples of Loire and Drôme sediments collected at T0 (pre-conditioning stage) and after 5 months in mesocosms (T1) were included in this analysis. Statistical significance (p-value) computed based on sequential sums of square from 999 permutations (*** = P-value < 0.01, ** = P-value < 0.03, * = P-value < 0.05).

Fungi			**PermMANOVA**		
BS origin	**Time**	**Source of variation**	**F**	**R2**	**p-value**
Loire and Drôme	T0 and T1	Time (T)	5.42	0.16	***
		Sediments (S)	4.99	0.15	***
		Climate (C)	1.32	0.04	0.10
		T*S	1.89	0.05	*
		S*C	0.85	0.02	0.63
Drôme	T0 andT1	Time	3.37	0.25	**
		Mesocosm	0.63	0.34	0.99
Loire	T0 and T1	Time	3.94	0.28	**
		Mesocosm	0.80	0.40	0.82
Loire and Drôme	T1	Sediments (S)	3.56	0.25	**
		Climate (C)	1.48	0.10	0.13
		S*C	1.03	0.07	0.35
Drôme	T1	Climate	0.97	0.19	0.70
Loire	T1	Climate	1.50	0.27	0.30
Bacteria			**PermMANOVA**		
BS origin	**Time**	**Source of variation**	**F**	**R2**	**p-value**
Loire and Drôme	T0 and T1	Time (T)	13.95	0.24	***
		Sediments (S)	17.83	0.31	***
		Climate (C)	2.28	2.28	0.053
		T*S	3.73	0.06	**
		S*C	1.17	0.02	0.24
Drôme	T0 andT1	Time	6.25	0.34	**
		Mesocosm	0.50	0.29	0.97
Loire	T0 and T1	Time	11.46	0.53	**
		Mesocosm	0.40	0.25	0.94
Loire and Drôme	T1	Sediments (S)	9.52	0.48	***
		Climate (C)	1.10	0.05	0.28
		S*C	1.10	0.05	0.29
Drôme	T1	Climate	0.84	0.17	0.90
Loire	T1	Climate	1.31	0.24	0.10

Table S5 – Relative abundance of the most abundant bacterial and fungal phyla and genera (> 1 % relative abundance in at least one of the two tested conditions) observed in the rhizosphere (R) and in the endosphere (E) of the Populus nigra seedlings of the Drôme and the Loire progenies cultivated in native conditions. The asterisks denote bacterial and fungal genera significantly different in relative abundance between the two studied progenies (ANOVA, P<0.05).

Bacterial genus	**R_Progenies from Loire**	**R_Progenies from Drôme**	**E_Progenies from Loire**	**E_Progenies from Drôme**
Acidibacter	0.4 ± 0.2	1.1 ± 0.3 *	0.8 ± 0.4	2.1 ± 0.6 *
Actinoplanes			11.1 ± 7.1	8.1 ± 4.6
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium			0.8 ± 0.5	1.3 ± 0.9
Azohydromonas	0.3 ± 0.2	1.1 ± 0.4 *	0.3 ± 0.2	1.3 ± 0.6 *
Bacillus	1.4 ± 0.7 *	0.1 ± 0.1		
Bradyrhizobium			0.8 ± 0.3	1.0 ± 0.3
Cellvibrio			1.4 ± 1.5 *	0.1 ± 0.1
Cytophaga			1.3 ± 0.9 *	0.2 ± 0.1
Gaiella	1.4 ± 0.6	1.1 ± 0.5		
Haliangium			2.6 ± 0.9	1.8 ± 0.9
Hyphomicrobium			0.5 ± 0.2	1.2 ± 0.5
Lechevalieria			4.9 ± 3.8 *	0.7 ± 0.5
Niastella	1.2 ± 0.7	0.8 ± 0.3	5.8 ± 3.3	5.5 ± 2.2
Ohtaekwangia	1.8 ± 0.9 *	0.9 ± 0.3	4.6 ± 1.2 *	2.0 ± 0.8
Phytohabitans			0.1 ± 0.1	1.3 ± 0.6 *
Rhodomicrobium			0.1 ± 0.1	1.5 ± 0.8 *
Sphingomonas				
Steroidobacter	0.2 ± 0.1	1.1 ± 0.4 *	0.7 ± 0.4	4.1 ± 1.1 *
Streptomyces			5.6 ± 3.2	4.9 ± 1.7
Fungal genus	**R_Progenies from Loire**	**R_Progenies from Drôme**	**E_Progenies from Loire**	**E_Progenies from Drôme**
Alternaria	0.8 ± 0.2	1.4 ± 0.4		
Ciliophora	0.8 ± 0.5	2.3 ± 0.6		
Cladosporium	6.2 ± 1.2	3.3 ± 0.8		
Corallomycetella	0.7 ± 0.6	4.8 ± 0.9 *		
Geopora	12.5 ± 4.6	9.9 ± 2.1	35.6 ± 6.8	25.2 ± 5.6
Mortierella	0.5 ± 0.1	1.5 ± 0.2 *		
Olpidiaster	2.1 ± 0.9 *	0.02 ± 0.02		
Pulvinula	0.3 ± 0.2	1.4 ± 0.4 *		
Pustularia	0.2 ± 0.1	1.0 ± 0.4		
Tetracladium	0.2 ± 0.2	2.2 ± 0.7 *	0.06 ± 0.05	1.7 ± 0.8 *
Tomentella	0.1 ± 0.06	2.4 ± 0.8 *	0.02 ± 0.01	1.2 ± 0.5 *

Table S6 (1/4) – Relative abundance of the most abundant bacterial genera (> 1 % relative abundance in at least one of the two tested conditions) detected in the bulk sediment (BS) of Loire and Drôme under native or non-native climate and in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and the Loire progenies cultivated in native conditions or in transplant conditions of sediments and/or climate. The asterisks denote bacterial genera significantly different in relative abundance between BS and R compartments or between R and E compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

Bacterial genus	**BS_Native Conditions (Loire)**	**R_Native Conditions (Loire)**	**E_Native Conditions (Loire)**
Actinoplanes	0.22 ± 0.08	* 0.48 ± 0.08	x 11.3 ± 2.0
Bacillus	3.6 ± 0.45 *	1.4 ± 0.2 x	0.01 ± 0.00
Cellvibrio	0.41 ± 0.13	* 0.93 ± 0.19	x 1.4 ± 0.4
Cytophaga	0.04 ± 0.02	* 0.33 ± 0.04	x 1.4 ± 0.3
Gaiella	2.7 ± 0.2 *	1.4 ± 0.2 x	0.03 ± 0.01
Haliangium	0.47 ± 0.13	0.56 ± 0.08	x 2.5 ± 0.2
Lechevalieria	0.02 ± 0.01	* 0.20 ± 0.03	x 4.9 ± 1.1
Niastella	0.15 ± 0.06	* 1.2 ± 0.2	x 6.1 ± 0.8
Ohtaekwangia	0.38 ± 0.09	* 1.8 ± 0.3	x 4.7 ± 0.3
Streptomyces	0.52 ± 0.11	0.38 ± 0.04	x 5.9 ± 0.8
Bacterial genus	**BS_Drôme sediment under Loire climate**	**R_Progenies from Loire in Drôme sediments under Loire climate**	**E_Progenies from Loire in Drôme sediments under Loire climate**
Acidibacter	2.4 ± 0.3 *	1.6 ± 0.1	x 3.7 ± 0.1
Actinocorallia	0.29 ± 0.10 *	0.10 ± 0.01	x 2.1 ± 0.3
Actinoplanes	1.3 ± 0.1 *	0.79 ± 0.11	x 8.8 ± 1.2
Bradyrhizobium	0.78 ± 0.06	0.95 ± 0.09	1.1 ± 0.1
Gaiella	1.2 ± 0.06	1.2 ± 0.1 x	0.02 ± 0.01
Haliangium	0.63 ± 0.11	0.67 ± 0.05	x 2.1 ± 0.2
Niastella	0.82 ± 0.07	* 1.5 ± 0.3	x 6.6 ± 0.5
Ohtaekwangia	1.5 ± 0.2 *	0.92 ± 0.08	x 3.1 ± 0.3
Steroidobacter	0.53 ± 0.13	* 1.2 ± 0.3	x 4.4 ± 0.2
Streptomyces	0.88 ± 0.26	0.96 ± 0.18	x 5.9 ± 0.5
Terrimonas	1.3 ± 0.1 *	0.54 ± 0.04	0.45 ± 0.02

Table S6 (2/4) – Relative abundance of the most abundant bacterial genera (> 1 % relative abundance in at least one of the two tested conditions) detected in the bulk sediment (BS) of Loire and Drôme under native or non-native climate and in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and the Loire progenies cultivated in native conditions or in transplant conditions of sediments and/or climate. The asterisks denote bacterial genera significantly different in relative abundance between BS and R compartments or between R and E compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

Bacterial genus	**BS_Loire sediments under Drôme climate**	**R_Progenies from Loire in Loire sediments under Drôme climate**	**E_Progenies from Loire in Loire sediments under Drôme climate**
Actinoplanes	0.35 ± 0.10	0.60 ± 0.33	x 6.1 ± 1.1
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.81 ± 0.11	* 1.8 ± 0.4	2.6 ± 0.5
Bacillus	1.7 ± 0.6	1.0 ± 0.2 x	0.01 ± 0.00
Blastococcus	0.35 ± 0.09	1.2 ± 1.0 x	0.00 ± 0.00
Bradyrhizobium	0.52 ± 0.11	0.60 ± 0.07	x 1.4 ± 0.1
Cellvibrio	0.26 ± 0.10	* 0.73 ± 0.12	x 2.2 ± 0.4
Cytophaga	0.31 ± 0.05 *	0.14 ± 0.03	x 1.1 ± 0.2
Gaiella	2.3 ± 0.3	1.8 ± 0.2 x	0.06 ± 0.01
Haliangium	0.86 ± 0.04	0.72 ± 0.12	x 4.1 ± 0.5
Hyphomicrobium	1.1 ± 0.1 *	0.65 ± 0.07	x 1.0 ± 0.1
Lechevalieria	0.26 ± 0.12	0.37 ± 0.06	x 6.3 ± 0.8
Leptolyngbya Es-Yyy1000	0.20 ± 0.16	* 1.0 ± 0.4 x	0.00 ± 0.00
Niastella	0.42 ± 0.15	0.60 ± 0.08	x 6.9 ± 0.8
Ohtaekwangia	2.1 ± 0.3 *	1.1 ± 0.2	x 4.6 ± 1.2
Streptomyces	0.44 ± 0.05	0.41 ± 0.05	x 5.3 ± 0.5
Terrimonas	1.5 ± 0.1 *	0.56 ± 0.06	x 0.84 ± 0.08
Bacterial genus	**BS_Native Conditions (Drôme)**	**R_Progenies from Loire in Drôme sediments under Drôme climate**	**E_Progenies from Loire in Drôme sediments under Drôme climate**
Acidibacter	2,1 ± 0.3 *	1,0 ± 0.1	x 2,5 ± 0.1
Actinoplanes	1,1 ± 0.1 *	0,62 ± 0.10	x 7,5 ± 0.9
Azohydromonas	0,16 ± 0.04	* 1.0 ± 0.1	1,4 ± 0.2
Gaiella	1,4 ± 0.1 *	1.1 ± 0.1 x	0,03 ± 0.01
Haliangium	0,74 ± 0.1	0,59 ± 0.05	x 1,6 ± 0.1
Hyphomicrobium	1.0 ± 0.1 *	0,39 ± 0.04	x 1,0 ± 0.1
Niastella	0,80 ± 0.06	1,0 ± 0.2	x 5,5 ± 0.4
Ohtaekwangia	1,9 ± 0.4 *	0,91 ± 0.10	x 2,7 ± 0.2
Rhodomicrobium	0,32 ± 0.08 *	0,15 ± 0.02	x 1,2 ± 0.1
Steroidobacter	0,57 ± 0.14	* 1,0 ± 0.1	x 4,1 ± 0.2
Streptomyces	0,52 ± 0.11	0,55 ± 0.10	x 4,1 ± 0.4
Terrimonas	1,2 ± 0.3 *	0,51 ± 0.04	0,65 ± 0.07

Table S6 (3/4) – Relative abundance of the most abundant bacterial genera (> 1 % relative abundance in at least one of the two tested conditions) detected in the bulk sediment (BS) of Loire and Drôme under native or non-native climate and in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and the Loire progenies cultivated in native conditions or in transplant conditions of sediments and/or climate. The asterisks denote bacterial genera significantly different in relative abundance between BS and R compartments or between R and E compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

Bacterial genus	**BS_Native Conditions (Drôme)**	**R_Native Conditions (Drôme)**	**E_Native Conditions (Drôme)**
Acidibacter	2,2 ± 0.6 *	1,1 ± 0.1	x 2,2 ± 0.2
Actinoplanes	0,23 ± 0.04	* 0,63 ± 0.15	x 8,6 ± 1.2
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0,16 ± 0.03	* 0,52 ± 0.10	x 1,1 ± 0.2
Azohydromonas	0,14 ± 0.05	* 1,1 ± 0.1	1,3 ± 0.2
Gaiella	1,9 ± 0.2 *	1,1 ± 0.1 x	0,05 ± 0.01
Haliangium	0,46 ± 0.05	* 0,66 ± 0.05	x 1,8 ± 0.3
Hyphomicrobium	0,62 ± 0.13	0,48 ± 0.05	x 1,2 ± 0.2
Niastella	0,26 ± 0.08	* 0,86 ±0.09	x 5,6 ± 0.6
Ohtaekwangia	0,40 ± 0.09	* 0,85 ± 0.07	x 1,9 ± 0.2
Phytohabitans	0,02 ± 0.01	0,08 ± 0.01	x 1,4 ± 0.2
Rhodomicrobium	0,19 ± 0.12	0,22 ± 0.05	x 1,5 ± 0.2
Steroidobacter	0,36 ± 0.09	* 1,1 ± 0.1	x 3,9 ± 0.3
Streptomyces	0,18 ± 0.01	* 0,44 ± 0.06	x 4,8 ± 0.5
Bacterial genus	**BS_Loire sediment under Drôme climate**	**R_Progenies from Drôme in Loire sediments under Drôme climate**	**E_Progenies from Drôme in Loire sediments under Drôme climate**
Actinoplanes	0.35 ± 0.10	0.49 ± 0.07	x 6.1 ± 1.5
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.81 ± 0.11	* 1.4 ± 0.2	x 2.3 ± 0.3
Bacillus	1.6 ± 0.6	1.0 ± 0.1 x	0.01 ± 0.00
Cellvibrio	0.26 ± 0.10	* 1.4 ± 0.3	x 2.8 ± 0.7
Cytophaga	0.31 ± 0.05 *	0.17 ± 0.03	x 1.6 ± 0.2
Gaiella	2.3 ± 0.3 *	1.7 ± 0.1 x	0.03 ± 0.01
Haliangium	0.86 ± 0.04	0.86 ± 0.14	x 5.1 ± 0.9
Hyphomicrobium	1.1 ± 0.0 *	0.72 ± 0.05	0.82 ± 0.11
Lechevalieria	0.26 ± 0.12	0.38 ± 0.07	x 5.1 ± 0.6
Niastella	0.42 ± 0.15	* 0.78 ± 0.10	x 5.8 ± 0.5
Ohtaekwangia	2.2 ± 0.3	1.7 ± 0.4	x 5.0 ± 0.6
Steroidobacter	0.35 ± 0.21	0.11 ± 0.01	x 1.3 ± 0.3
Streptomyces	0.44 ± 0.05	0.49 ± 0.07	x 5.8 ± 0.7
Terrimonas	1.5 ± 0.1 *	0.81 ± 0.09	0.83 ± 0.11

Table S6 (4/4) – Relative abundance of the most abundant bacterial genera (> 1 % relative abundance in at least one of the two tested conditions) detected in the bulk sediment (BS) of Loire and Drôme under native or non-native climate and in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and the Loire progenies cultivated in native conditions or in transplant conditions of sediments and/or climate. The asterisks denote bacterial genera significantly different in relative abundance between BS and R compartments or between R and E compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

Bacterial genus	**BS_Drôme sediments under Loire climate**	**R_Progenies from Drôme in Drôme sediments under Loire climate**	**E_Progenies from Drôme in Drôme sediments under Loire climate**
Acidibacter	2.4 ± 0.3	1.8 ± 0.2	x 4.0 ± 0.2
Actinocorallia	0.29 ± 0.10	0.22 ± 0.04	x 3.7 ± 0.5
Actinoplanes	1.3 ± 0.1 *	0.57 ± 0.11	x 6.3 ± 0.8
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.29 ± 0.05	* 0.53 ± 0.07	x 1.1 ± 0.1
Gaiella	1.2 ± 0.1	1.1 ± 0.2 x	0.02 ± 0.01
Haliangium	0.60 ± 0.11	0.71 ± 0.06	x 2.5 ± 0.3
Niastella	0.82 ± 0.07	* 1.4 ± 0.2	x 6.7 ± 0.2
Ohtaekwangia	1.5 ± 0.2 *	0.89 ± 0.08	x 3.8 ± 0.5
Steroidobacter	0.53 ± 0.13	* 1.0 ± 0.1	x 4.1 ± 0.2
Streptomyces	0.88 ± 0.26	0.82 ± 0.15	x 9.0 ± 0.8
Terrimonas	1.3 ± 0.1 *	0.41 ± 0.04	0.41 ± 0.03
Bacterial genus	**BS_Native Conditions (Loire)**	**R_Progenies from Drôme in Loire sediments under Loire climate**	**E_Progenies from Drôme in Loire sediments under Loire climate**
Acidibacter	1,1 ± 0.2 *	0,28 ± 0.03	x 0,61 ± 0.1
Actinoplanes	0,51 ± 0.04	0,41 ± 0.09	x 9,9 ± 1.7
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0,45 ± 0.11	* 1,2 ± 0.2	0,84 ± 0.1
Bacillus	1,9 ± 0.2	1,4 ± 0.3 x	0,00 ± 0.00
Cellvibrio	0,40 ± 0.09	* 1,2 ± 0.2	x 2,3 ± 0.6
Cytophaga	0,24 ± 0.07	* 0,49 ± 0.10	x 3,4 ± 0.5
Gaiella	2,3 ± 0.2 *	1,0 ± 0.1 x	0,01 ± 0.00
Haliangium	1,2 ± 0.2 *	0,53 ± 0.08	x 3,2 ± 0.4
Lechevalieria	0,10 ± 0.03	* 0,31 ± 0.10	x 4,1 ± 0.5
Niastella	0,29 ± 0.07	* 1,0 ± 0.12	x 7,4 ± 0.7
Nodosilinea PCC-7104	0,04 ± 0.01	* 2,6 ± 0.4 x	0,00 ± 0.00
Ohtaekwangia	1,9 ± 0.2	1,9 ± 0.5	x 5.0 ± 0.6
Pirellula	0,52 ± 0.08	* 1,1 ± 0.1 x	0,17 ± 0.02
Sphingomonas	0,74 ± 0.07	1,0 ± 0.1 x	0,04 ± 0.01
Streptomyces	0,41 ± 0.01	0,41 ± 0.04	x 8,4 ± 1.1
Terrimonas	1,6 ± 0.4 *	0,45 ± 0.04	0,31 ± 0.04

Table S7 (1/2) – Relative abundance of the most abundant fungal genera (> 1 % relative abundance in at least one of the two tested conditions) detected in the bulk sediment (BS) of Loire and Drôme under native or non-native climate and in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and the Loire progenies cultivated in native conditions or in transplant conditions of sediments and/or climate. The asterisks denote bacterial genera significantly different in relative abundance between BS and R compartments or between R and E compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

Fungal genus	**BS_Native Conditions (Loire)**	**R_Native Conditions (Loire)**	**E_Native Conditions (Loire)**
Geopora	25.7 ± 18.8	8.1 ± 1.9	x 35.6 ± 6.8
Mortierella	12.1 ± 11.2	0.44 ± 0.13 x	0.00 ± 0.00
Curvularia	1.4 ± 0.7 *	0.03 ± 0.01	0.01 ± 0.0
Aspergillus	1.1 ± 0.5	0.55 ± 0.11 x	0.01 ± 0.0
Cladosporium	1.1 ± 0.4	* 6.6 ± 1.1 x	0.09 ± 0.05
Olpidiaster	0.10 ± 0.04	* 1.3 ± 0.2 x	0.29 ± 0.08
Ciliophora	0.09 ± 0.09	* 1.1 ± 0.5 x	0.00 ± 0.00
Fungal genus	**BS_Drôme sediment under Loire climate**	**R_Progenies from Loire in Drôme sediments under Loire climate**	**E_Progenies from Loire in Drôme sediments under Loire climate**
Geopora	3.9 ± 0.9	* 8.7 ± 1.5	x 31.0 ± 3.5
Peziza	1.0 ± 0.3	1.5 ± 1.0	2.2 ± 1.8
Tetracladium	0.16 ± 0.04	0.91 ± 0.26	1.2 ± 0.2
Ilyonectria	1.0 ± 0.8	0.33 ± 0.08	0.50 ± 0.10
Phaeonectriella	3.8 ± 1.8 *	0.53 ± 0.16	0.36 ± 0.11
Corallomycetella	0.40 ± 0.32	* 2.9 ± 0.8 x	0.28 ± 0.12
Cladosporium	0.22 ± 0.05	* 5.1 ± 1.1 x	0.02 ± 0.01
Anguillospora	2.3 ± 2.2	0.08 ± 0.04	0.01 ± 0.01
Alternaria	0.11 ± 0.02	* 1.2 ± 0.5 x	0.01 ± 0.00
Mortierella	4.3 ± 1.9 *	1.3 ± 0.3 x	0.00 ± 0.00
Ciliophora	4.9 ± 4.4	1.3 ± 0.5 x	0.00 ± 0.00
Hygrocybe	1.7 ± 1.6	0.02 ± 0.01	0.00 ± 0.00
Gloioxanthomyces	1.4 ± 1.4	0.00 ± 0.00	0.00 ± 0.00
Fungal genus	**BS_Loire sediments under Drôme climate**	**R_Progenies from Loire in Loire sediments under Drôme climate**	**E_Progenies from Loire in Loire sediments under Drôme climate**
Geopora	12.1 ± 5.5 *	1.5 ± 0.5	x 15.2 ± 4.7
Pulvinula	0.30 ± 0.30	* 1.8 ± 1.0	1.2 ± 0.5
Cladosporium	0.11 ± 0.01	* 10.9 ± 3.0 x	0.06 ± 0.02
Alternaria	0.04 ± 0.03	* 2.0 ± 0.6 x	0.01 ± 0.01
Ciliophora	4.3 ± 3.2	0.62 ± 0.40 x	0.01 ± 0.01
Talaromyces	1.3 ± 0.3 *	0.40 ± 0.09 x	0.00 ± 0.00
Spizellomyces	1.1 ± 1.1	0.10 ± 0.07	0.00 ± 0.00
Fungal genus	**BS_Native Conditions (Drôme)**	**R_Progenies from Loire in Drôme sediments under Drôme climate**	**E_Progenies from Loire in Drôme sediments under Drôme climate**
Geopora	4.5 ± 1.8	* 13.2 ± 1.9	x 30.1 ± 3.6
Tomentella	1.0 ± 0.5	1.7 ± 0.6	2.0 ± 0.5
Tetracladium	0.29 ± 0.16	* 1.5 ± 0.6	1.2 ± 0.4
Corallomycetella	1.1 ± 0.9	* 5.1 ± 1.0 x	0.82 ± 0.26
Pustularia	1.9 ± 0.6	0.95 ± 0.3	0.67 ± 0.17
Pulvinula	0.19 ± 0.07	* 1.2 ± 0.3 x	0.22 ± 0.10
Cladosporium	0.11 ± 0.06	* 2.1 ± 0.4 x	0.01 ± 0.01
Mortierella	2.9 ± 1.1	1.6 ± 0.3 x	0.01 ± 0.00
Ciliophora	1.2 ± 0.8	1.6 ± 0.4 x	0.00 ± 0.00
Pseudaleuria	3.2 ± 3.2	0.50 ± 0.32 x	0.00 ± 0.00

Table S7 (2/2) – Relative abundance of the most abundant fungal genera (> 1 % relative abundance in at least one of the two tested conditions) detected in the bulk sediment (BS) of Loire and Drôme under native or non-native climate and in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and the Loire progenies cultivated in native conditions or in transplant conditions of sediments and/or climate. The asterisks denote bacterial genera significantly different in relative abundance between BS and R compartments or between R and E compartments (ANOVA, P<0.05). The crosses denote significant difference in relative abundance of bacterial phyla between R and E compartments (ANOVA, P<0.05).

Fungal genus	**BS_Native Conditions (Drôme)**	**R_Native Conditions (Drôme)**	**E_Native Conditions (Drôme)**
Geopora	4.5 ± 1.8	* 9.9 ± 2.1	x 25.0 ± 5.2
Pseudaleuria	3.2 ± 3.2	0.59 ± 0.43 x	0.00 ± 0.00
Mortierella	2.9 ± 1.1	1.5 ± 0.2 x	0.01 ± 0.0
Pustularia	1.9 ± 0.6	1.0 ± 0.4 x	0.15 ± 0.08
Ciliophora	1.2 ± 0.8	2.3 ± 0.6 x	0.00 ± 0.00
Corallomycetella	1.1 ± 0.9	* 4.8 ± 0.9 x	0.40 ± 0.15
Tomentella	0.99 ± 0.48	2.4 ± 0.8	1.3 ± 0.5
Alternaria	0.80 ± 0.66	1.4 ± 0.4 x	0.03 ± 0.02
Tetracladium	0.29 ± 0.16	* 2.2 ± 0.7	1.9 ± 0.8
Pulvinula	0.19 ± 0.07	* 1.4 ± 0.4 x	0.15 ± 0.06
Cladosporium	0.11 ± 0.06	* 3.3 ± 0.8 x	0.06 ± 0.02
Fungal Genus	**BS_Loire sediment under Drôme climate**	**R_Progenies from Drôme in Loire sediments under Drôme climate**	**E_Progenies from Drôme in Loire sediments under Drôme climate**
Geopora	12.1 ± 5.5	8.6 ± 2.1	x 23.9 ± 4.3
Rhizophagus	0.08 ± 0.08	0.10 ± 0.03	x 1.2 ± 0.6
Dactylella	0.00 ± 0.00	0.26 ± 0.18	1.1 ± 1.1
Cladosporium	0.11 ± 0.01	* 6.6 ± 1.9 x	0.05 ± 0.04
Alternaria	0.04 ± 0.03	* 1.3 ± 0.5 x	0.02 ± 0.01
Talaromyces	1.25 ± 0.34	0.63 ± 0.12 x	0.00 ± 0.00
Ciliophora	4.3 ± 3.2 *	0.48 ± 0.14 x	0.00 ± 0.00
Spizellomyces	1.1 ± 1.1	0.15 ± 0.12	0.00 ± 0.00
Fungal genus	**BS_Drôme sediments under Loire climate**	**R_Progenies from Drôme in Drôme sediments under Loire climate**	**E_Progenies from Drôme in Drôme sediments under Loire climate**
Geopora	3.9 ± 0.9	* 14.4 ± 3.1	x 38.3 ± 5.6
Clohesyomyces	0.03 ± 0.03	0.42 ± 0.40	2.3 ± 2.2
Tetracladium	0.16 ± 0.04	* 1.3 ± 0.4	1.3 ± 0.4
Psathyrella	0.00 ± 0.00	* 0.36 ± 0.21	1.1 ± 0.7
Ilyonectria	1.0 ± 0.8	0.57 ± 0.17	0.52 ± 0.13
Phaeonectriella	3.8 ± 1.8 *	0.34 ± 0.08	0.23 ± 0.11
Peziza	1.0 ± 0.3 *	0.20 ± 0.05 x	0.05 ± 0.03
Anguillospora	2.3 ± 2.2	2.3 ± 2.2 x	0.04 ± 0.04
Cladosporium	0.22 ± 0.05	* 4.0 ± 1.0	0.02 ± 0.01
Alternaria	0.11 ± 0.02	* 1.8 ± 0.8	0.00 ± 0.00
Ciliophora	4.9 ± 4.4	1.3 ± 0.4 x	0.00 ± 0.00
Mortierella	4.3 ± 1.9	2.3 ± 0.4 x	0.00 ± 0.00
Hygrocybe	1.7 ± 1.6	0.01 ± 0.01	0.00 ± 0.00
Gloioxanthomyces	1.4 ± 1.4	0.00 ± 0.00	0.00 ± 0.00
Lectera	0.00 ± 0.00	1.3 ± 1.2 x	0.00 ± 0.00
Fungal genus	**BS_Native Conditions (Loire)**	**R_Progenies from Drôme in Loire sediments under Loire climate**	**E_Progenies from Drôme in Loire sediments under Loire climate**
Geopora	25.7 ± 18.8	8.5 ± 1.8	x 22.1 ± 3.9
Cladosporium	1.1 ± 0.4	* 4.4 ± 1.1	1.4 ± 1.4
Alternaria	0.14 ± 0.14	* 1.9 ± 1.0 x	0.54 ± 0.52
Ciliophora	0.09 ± 0.09	* 1.1 ± 0.5 x	0.02 ± 0.01
Verrucocladosporium	0.00 ± 0.00	* 2.0 ± 0.8 x	0.01 ± 0.01
Mortierella	12.1 ± 11.2	1.3 ± 0.5 x	0.01 ± 0.00
Aspergillus	1.1 ± 0.5 *	0.28 ± 0.07 x	0.01 ± 0.00
Curvularia	1.4 ± 0.7 *	0.07 ± 0.03 x	0.00 ± 0.00
Peziza	0.09 ± 0.09	2.0 ± 2.0	0.00 ± 0.00

Table S8 (1/3) – Relative abundance of the most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated in their native sediments or in the other type of sediments. Most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated under their native climate or under the opposite climate. Most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated in their native conditions of soil and climate or in the transplant conditions of soil and cliamte. The asterisks denote bacterial genera significantly different in relative abundance between the two studied conditions (ANOVA, P<0.05).

Bacterial genus	R_Loire progenies in Drôme sediments	R_Loire progenies in Loire sediments (Native conditions)	E_Loire progenies in Drôme sediments	E_Loire progenies in Loire sediments (Native conditions)
Acidibacter	1.7 ± 0.1 *	0.4 ± 0.1	3.7 ± 0.1 *	0.8 ± 0.1
Actinocorallia			2.1 ± 0.3 *	0.01 ± 0.01
Actinoplanes			8.8 ± 1.2	11.3 ± 2.0
Bacillus	0.05 ± 0.01	1.4 ± 0.2 *		
Bradyrhizobium			1.1 ± 0.1 *	0.8 ± 0.1
Cellvibrio			0.2 ± 0.1	1.4 ± 0.4 *
Cytophaga			0.5 ± 0.1	1.4 ± 0.3 *
Haliangium			2.1 ± 0.2	2.5 ± 0.2
Lechevalieria			0.2 ± 0.1	4.9 ± 1.1 *
Gaiella	1.2 ± 0.1	1.41 ± 0.17		
Niastella	1.5 ± 0.3	1.22 ± 0.18	6.6 ± 0.5	6.1 ± 0.8
Ohtaekwangia	0.9 ± 0.1	1.8 ± 0.3 *	3.1 ± 0.3	4.7 ± 0.3 *
Steroidobacter	1.2 ± 0.3 *	0.2 ± 0.1	4.4 ± 0.2 *	0.7 ± 0.1
Streptomyces			5.9 ± 0.5	5.9 ± 0.8
Bacterial genus	**R_Drôme progenies in Loire sediments**	**R_Drôme progenies in Drôme sediments (Native conditions)**	**E_Drôme progenies in Loire sediments**	**E_Drôme progenies in Drôme sediments (Native conditions)**
Acidibacter	0.2 ± 0.1	1.1 ± 0.1 *	0.9 ± 0.3	2.1 ± 0.2 *
Actinoplanes			6.1 ± 1.5	8.1 ± 1.2
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	1.4 ± 0.2 *	0.5 ± 0.1	2.4 ± 0.3 *	1.3 ± 0.2
Azohydromonas	0.3 ± 0.1	1.1 ± 0.1 *	0.7 ± 0.1	1.3 ± 0.2 *
Bacillus	1.0 ± 0.1 *	0.03 ± 0.01	1.0 ± 0.1	1.0 ± 0.1
Bradyrhizobium				
Cellvibrio	1.4 ± 0.3 *	0.1 ± 0.1	2.8 ± 0.7 *	0.1 ± 0.1
Cytophaga			1.6 ± 0.2 *	0.4 ± 0.1
Haliangium			5.1 ± 0.9 *	1.8 ± 0.2
Hyphomicrobium			0.8 ± 0.1	1.2 ± 0.1 *
Lechevalieria			5.1 ± 0.6 *	0.7 ± 0.1
Niastella			5.8 ± 0.5	5.5 ± 0.6
Gaiella	1.7 ± 0.1 *	1. ± 0.1 *		
Ohtaekwangia	1.7 ± 0.4 *	0.9 ± 0.1	5.0 ± 0.6 *	2.0 ± 0.2
Phytohabitans			0.03 ± 0.01	1.3 ± 0.2 *
Rhodomicrobium			0.2 ± 0.0	1.5 ± 0.2 *
Steroidobacter	0.1 ± 0.1	1.0 ± 0.1 *	1.3 ± 0.3	4.0 ± 0.3 *
Streptomyces			5.8 ± 0.7	4.9 ± 0.4
Bacterial genus	**R_Loire progenies under Drôme climate**	**R_Loire progenies under Loire climate (Native conditions)**	**E_Loire progenies under Drôme climate**	**E_Loire progenies under Loire climate (Native conditions)**
Actinoplanes			6.1 ± 1.1	11.1 ± 1.8 *
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	1.8 ± 0.4 *	0.7 ± 0.1	2.6 ± 0.5 *	0.8 ± 0.1
Bacillus	1.0 ± 0.1	1.5 ± 0.2		
Bradyrhizobium			1.4 ± 0.1 *	0.8 ± 0.1
Blastococcus	1.2 ± 1.0	0.10 ± 0.01		
Cellvibrio			2.2 ± 0.4	1.4 ± 0.4
Cytophaga			1.1 ± 0.2	1.3 ± 0.2
Haliangium			4.1 ± 0.5 *	2.6 ± 0.2
Hyphomicrobium			1.0 ± 0.1 *	0.5 ± 0.1
Lechevalieria			6.3 ± 0.8	4.9 ± 1.0
Gaiella	1.8 ± 0.2	1.4 ± 0.2		
Leptolyngbya Es-Yyy1000	1.0 ± 0.4 *	0.10 ± 0.04		
Niastella	0.6 ± 0.1	1.2 ± 0.2 *	6.9 ± 0.8	5.8 ± 0.8
Ohtaekwangia	1.1 ± 0.2	1.76 ± 0.25	4.6 ± 1.2	4.6 ± 0.3

Table S8 (2/3) – Relative abundance of the most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated in their native sediments or in the other type of sediments. Most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated under their native climate or under the opposite climate. Most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated in their native conditions of soil and climate or in the transplant conditions of soil and cliamte. The asterisks denote bacterial genera significantly different in relative abundance between the two studied conditions (ANOVA, P<0.05).

Bacterial genus	R_Drôme progenies under Loire climate	R_Drôme progenies under Drôme climate (Native conditions)	E_Drôme progenies under Loire climate	E_Drôme progenies under Drôme climate (Native conditions)
Acidibacter	1,8 ± 0,2 *	1,1 ± 0,1	4.1 ± 0.2 *	2.1 ± 0.2
Actinocorallia			3.9 ± 0.6 *	0.20 ± 0.02
Actinoplanes			6.1 ± 0.8	8.1 ± 1.2
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium			1.1 ± 0.1	1.3 ± 0.2
Azohydromonas	0,5 ± 0,1	1,1 ± 0,1 *	0.5 ± 0.1	1.3 ± 0.2 *
Bradyrhizobium			0.9 ± 0.1	1.0 ± 0.1
Gaiella	1,1 ± 0,2	1,1 ± 0,1		
Haliangium			2.4 ± 0.3	1.8 ± 0.2
Hyphomicrobium			0.8 ± 0.1	1.2 ± 0.1 *
Niastella	1,4 ± 0,2 *	0,8 ± 0,1	6.7 ± 0.3	5.5 ± 0.6
Ohtaekwangia			3.8 ± 0.5 *	2.0 ± 0.2
Phytohabitans			0.4 ± 0.1	1.3 ± 0.2 *
Rhodomicrobium			0.6 ± 0.1	1.5 ± 0.2 *
Steroidobacter	1,0 ± 0,1	1,0 ± 0,1	4.0 ± 0.2	3.9 ± 0.3
Streptomyces			9.0 ± 0.8 *	4.9 ± 0.4
Bacterial genus	**R_Loire progenies in Drôme sediments and under Drôme climate**	**R_Loire progenies in Loire sediments under Loire climate (Native conditions)**	**E_Loire progenies in Drôme sediments and under Drôme climate**	**E_Loire progenies in Loire sediments under Loire climate (Native conditions)**
Acidibacter	1.0 ± 0.1 *	0.62 ± 0.10	2.5 ± 0.1 *	0.79 ± 0.09
Actinoplanes			7.5 ± 0.9	11.1 ± 1.8
Azohydromonas			1.4 ± 0.2 *	0.28 ± 0.05
Bacillus	0.05 ± 0.01	1.5 ± 0.2 *		
Cellvibrio			0.13 ± 0.03	1.4 ± 0.4 *
Cytophaga			0.57 ± 0.07	1.3 ± 0.2 *
Gaiella	1.1 ± 0.1	1.4 ± 0.2		
Haliangium			1.6 ± 0.1	2.6 ± 0.2 *
Hyphomicrobium			1.0 ± 0.1 *	0.53 ± 0.05
Lechevalieria			0.39 ± 0.07	4.9 ± 1.0 *
Niastella	1.0 ± 0.2	1.2 ± 0.2	5.5 ± 0.4	5.8 ± 0.9
Ohtaekwangia	0.91 ± 0.10	1.8 ± 0.3 *	2.7 ± 0.2	4.6 ± 0.3 *
Rhodomicrobium			1.2 ± 0.1 *	0.10 ± 0.01
Steroidobacter	1.0 ± 0.1 *	0.19 ± 0.04	4.1 ± 0.2 *	0.70 ± 0.10
Streptomyces			4.1 ± 0.4	5.6 ± 0.8

Table S8 (3/3) – Relative abundance of the most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated in their native sediments or in the other type of sediments. Most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated under their native climate or under the opposite climate. Most abundant bacterial genera (> 1 % relative abundance) detected in the rhizosphere (R) and the endosphere (E) of the Populus nigra seedlings of the Drôme and Loire progenies cultivated in their native conditions of soil and climate or in the transplant conditions of soil and cliamte. The asterisks denote bacterial genera significantly different in relative abundance between the two studied conditions (ANOVA, P<0.05).

Bacterial genus	R_Drôme progenies in Loire sediments and under Loire climate	R_Drôme progenies in Drôme sediments under Drôme climate (Native conditions)	E_Drôme progenies in Loire sediments and under Loire climate	E_Drôme progenies in Drôme sediments under Drôme climate (Native conditions)
Acidibacter	0.28 ± 0.03	1.1 ± 0.1 *	0.61 ± 0.07	2.1 ± 0.2 *
Actinoplanes			9.9 ± 1.7	8.1 ± 1.2
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	1.2 ± 0.2 *	0.55 ± 0.10	0.84 ± 0.14	1.3 ± 0.2
Azohydromonas	0.23 ± 0.02	1.1 ± 0.1 *	0.33 ± 0.05	1.3 ± 0.2 *
Bacillus	1.4 ± 0.3 *	0.04 ± 0.01		
Bradyrhizobium			0.66 ± 0.07	1.0 ± 0.1 *
Cellvibrio	1.2 ± 0.2 *	0.12 ± 0.02	2.3 ± 0.6 *	0.12 ± 0.03
Cytophaga			3.4 ± 0.6 *	0.35 ± 0.05
Gaiella	1.1 ± 0.1	1.1 ± 0.1		
Haliangium			3.2 ± 0.5	1.8 ± 0.2 *
Hyphomicrobium			0.40 ± 0.04	1.2 ± 0.1 *
Lechevalieria			4.1 ± 0.5 *	0.70 ± 0.15
Niastella	1.1 ± 0.1	0.84 ± 0.08	7.4 ± 0.7	5.5 ± 0.6
Nodosilinea PCC-7104	2.6 ± 0.4 *	0.02 ± 0.01		
Ohtaekwangia	1.9 ± 0.5	0.86 ± 0.07	5.0 ± 0.6 *	2.0 ± 0.2
Phytohabitans			0.08 ± 0.02	1.3 ± 0.2 *
Pirellula	1.1 ± 0.1 *	0.80 ± 0.03		
Rhodomicrobium			0.11 ± 0.02	1.5 ± 0.2 *
Sphingomonas	1.0 ± 0.1 *	0.57 ± 0.06		
Steroidobacter	0.12 ± 0.02	1.1 ± 0.1 *	0.75 ± 0.07	4.0 ± 0.3 *
Streptomyces			8.4 ± 1.1 *	4.9 ± 0.5

Chapitre IV : Impact des variations de la composition et de la structure du microbiote du sol sur la colonisation racinaire et le métabolome du Peuplier (*Populus tremula x alba*)

SUPPLEMENTAL INFORMATIONS

Table S1– Sequences of primers and of PNA PCR blockers used in this study.

Primer name	Sequence 5' → 3'
515F_Universal	GTGYCAGCMGCCGCGGTAA
515F_Chloroflexi	GTGCCAGCMGCWGCGGTAA
515F_TM7	GTGCCAGCMGCCGCGGTCA
515F_Nano	GTGGCAGYCGCCRCGGKAA
806R_Universal	GGACTACNVGGGTWTCTAAT
806R_Nano	GGAMTACHGGGGTCTCTAAT
ITS3NGS1	CATCGATGAAGAACGCAG
ITS3NGS2	CAACGATGAAGAACGCAG
ITS3NGS3	CACCGATGAAGAACGCAG
ITS3NGS4	CATCGATGAAGAACGTAG
ITS3NGS5	CATCGATGAAGAACGTGG
ITS3NGS10	CATCGATGAAGAACGCTG
ITS4NGS	TCCTSCGCTTATTGATATGC
pPNA_717-1B4	GGCTCAACCCTGGACAG
mtPNA 717-1B4	GGCAAGTCTTCTTCGGA
ITSspacePNA_717-1B4	CGAGGGCACGTCTGCCTGG

Table S2 – Physico-chemical properties of bulk soil 1 T0 and bulk soil 2 T0.

	Soil 1	Soil 2
Carbon total (g/kg)	20.7	27.7
Nitrogen total (g/kg)	1.48	1.78
Phosphorus (g/kg)	0.015	0.02
C/N	14.0	15.6
N/P	98.7	89,0
CEC Metson (cmol+/kg)	10.2	12.5
Calcium (cmol+/kg)	2.65	3.21
Aluminium (cmol+/kg)	1.98	2.3
Magnesium (cmol+/kg)	0.693	0.859
Potassium (cmol+/kg)	0.406	0.376
Manganese (cmol+/kg)	0.39	0.626
Sodium (cmol+/kg)	0.04	0.115
Iron (cmol+/kg)	0.0082	0.0124
pH	5.09	4.9

Table S3 –Relative abundance of fungal and bacterial phyla (%) detected in the bulk soil T0. Numbers in bold denote the highest relative abundance of fungal and bacterial phyla detected between Soil 1 and Soil 2. The asterisks denote significant difference in the relative abundance of fungal and bacterial phyla between Soil 1 and Soil 2 (Student *t*-test, p<0,05, means +/- SE).

Fungal phylum	Soil 1	Soil 2
Acomycota	**42.1 +/- 4.8**	32.40 +/- 4.23
Basidiomycota	36.7 +/- 13.3	**48.5 +/- 6.2**
Zygomycota	**18.1 +/- 8.2**	15.9 +/- 1.4
Unidentified	**2.40 +/- 0.32**	2.01 +/- 0.50
Glomeromycota	**0.36 +/- 0.12**	0.28 +/- 0.06
Chytridiomycota	**0.23 +/- 0.04**	0.19 +/- 0.21
Rozellomycota	0.13 +/- 0.07	**0.70 +/- 0.10 ***
Bacterial phylum	**Soil 1**	**Soil 2**
Acidobacteria	32.8 +/- 2.6	**36.7 +/- 0.4**
Proteobacteria	27.3 +/- 1.5	**32.3 +/- 0.6**
Verrucomicrobia	**11.4 +/- 0.4 ***	5.6 +/- 0.3
Bacteroidetes	**7.4 +/- 0.2**	5.3 +/- 0.76
Chloroflexi	**6.9 +/- 0.3 ***	3.3 +/- 0.3
Planctomycetes	**4.0 +/- 0.2 ***	2.6 +/- 0.1
Actinobacteria	3.3 +/- 0.6	**8.0 +/- 0.4 ***
Gemmatimonadetes	1.7 +/- 0.2	**2.1 +/- 0.1**
Unidentified	1.3 +/- 0.2	**1.4 +/- 0.1**
WPS-2	**1.1 +/- 0.1**	0.90 +/- 0.07
Elusimicrobia	**0.62 +/- 0.04**	0.44 +/- 0.04
Nitrospirae	**0.58 +/- 0.09**	0.24 +/- 0.07
Armatimonadetes	**0.54 +/- 0.18**	0.46 +/- 0.00
Patescibacteria	**0.48 +/- 0.04**	0.17 +/- 0.03
Thaumarchaeota	**0.20 +/- 0.04**	0.12 +/- 0.02
Dependentiae	**0.13 +/- 0.02**	0.07 +/- 0.03
Firmicutes	**0.10 +/- 0.02**	0.04 +/- 0.01
FCPU426	**0.10 +/- 0.01 ***	0.01 +/- 0.01
Chlamydiae	0.06 +/- 0.01	**0.12 +/- 0.01**
Latescibacteria	**0.04 +/- 0.01**	0.00
Cyanobacteria	0.03 +/- 0.02	0.03 +/- 0.02
Fibrobacteres	0.03 +/- 0.02	0.00
Crenarchaeota	0.02 +/- 0.02	0.00
Euryarchaeota	0.01 +/- 0.01	0.00
GAL15	0.01 +/- 0.01	0.00
Rokubacteria	0.01 +/- 0.01	0.02 +/- 0.01
Spirochaetes	0.01 +/- 0.01	0.00
FBP	0.00	0.01 +/- 0.01

Table S4 - Relative abundance of fungal OTUs specifically detected in one of the two bulk soils T0. The fungal OTUs specific to each of the two soils have been grouped according to the fungal genus with which they are affiliated. The last line TOTAL corresponds to the relative abundance of fungal genera and the sum of fungal OTUs found to be specific of Soil 1 and Soil 2. UG = Unidentified genus.

Fungal genus Soil 1 specific	Relative abundance (%)	Number of OTUs	Fungal genus Soil 2 specific	Relative abundance (%)	Number of OTUs
Hymenogaster	4.3	2	*Tuber*	1.7	2
Podospora	0.9	1	*Tomentella*	0.9	2
UG of *Coniochaetaceae*	0.6	1	*Rugosomyces*	0.3	2
Entorrhiza	0.2	3	*Hymenoscyphus*	0.2	3
Peziza	0.2	1	*Hydnotrya*	0.2	1
UG of *Herpotrichiellaceae*	0.2	2	*Penicillium*	0.2	8
Pseudeurotium	0.1	2	*Pezizella*	0.1	1
Cunninghamella	0.1	1	*Phragmocephala*	0.01	1
UG of *Glomeraceae*	0.1	5	UG of *Trichocomaceae*	0.09	1
UG of *Helvellaceae*	0.1	1	*Clavulina*	0.08	1
UG of *Sordariaceae*	0.09	1	*Calicium*	0.05	1
Drechslera	0.08	1	*Hypomyces*	0.04	3
Plectania	0.08	1	*Psilocybe*	0.04	1
Coprinellus	0.07	1	*Rigidoporus*	0.04	1
Ophiosphaerella	0.06	1	*Spirosphaera*	0.03	1
Cenococcum	0.06	1	*Endogone*	0.03	2
Rhizophagus	0.06	2	*Tremella*	0.03	3
Fusarium	0.05	3	UG of *Dipodascaceae*	0.02	1
Byssonectria	0.04	1	UG of *Mycosphaerellaceae*	0.02	1
Colletotrichum	0.04	1	*Inocybe*	0.02	2
Ceratostomella	0.04	2	*Phialophora*	0.02	1
Entoloma	0.04	3	*Pseudoidriella*	0.02	1
UG of *Pyronemataceae*	0.04	1	*Sporothrix*	0.02	1
Conlarium	0.03	2	UG of *Venturiaceae*	0.02	3
Pyrenochaetopsis	0.03	1	*Capnobotryella*	0.01	1
Clavaria	0.03	2	*Ilyonectria*	0.01	1
Hypholoma	0.03	2	UG of *Onygenaceae*	0.01	1
Russula	0.03	1	UG of *Sebacinales*	0.01	2
Betamyces	0.02	2	*Adisciso*	0.01	1
Hyalodendriella	0.02	1	*Athelopsis*	0.01	1
Nectria	0.02	1	*Ceratobasidium*	0.01	1

Uleiella	0.02	1	*Chalastospora*	0.01	1
UG of *Elaphomycetaceae*	0.02	1	*Chloridium*	0.01	1
Volutella	0.02	1	*Clathrosphaerina*	0.01	1
Bensingtonia	0.02	1	*Dactylella*	0.01	1
Geminibasidium	0.02	1	*Dendryphion*	0.01	1
Lacrymaria	0.02	1	*Lyophyllum*	0.01	1
Maasoglossum	0.02	1	*Metarhizium*	0.01	1
Monacrosporium	0.02	1	*Miniancora*	0.01	1
Rhizophlyctis	0.02	2	*Pleurotheciella*	0.01	1
Gyoerffyella	0.01	1	UG of *Clavariaceae*	0.01	2
Pseudogymnoascus	0.01	1	UG of *Sebacinaceae*	0.01	1
Rhizoscyphus	0.01	1	UG of *Xylariaceae*	0.01	1
Tylospora	0.01	1	*Absidia*	0.005	1
Unguicularia	0.01	1	*Auricularia*	0.005	1
UG of *Phaeosphaeriaceae*	0.01	2	*Calocera*	0.005	1
Archaeorhizomyces	0.01	1	*Capronia*	0.005	1
Basidiobolus	0.01	1	*Ciboria*	0.005	1
Gongronella	0.01	2	*Collophora*	0.005	1
Powellomyces	0.01	1	*Cyberlindnera*	0.005	1
Rhexocercosporidium	0.01	1	*Cystofilobasidium*	0.005	1
UG of Archaeosporaceae	0.01	1	*Didymosphaeria*	0.005	1
UG of Nectriaceae	0.01	1	*Eutypella*	0.005	1
Acremonium	0.005	1	*Exobasidium*	0.005	1
Bjerkandera	0.005	1	*Fulvoflamma*	0.005	1
Botrytis	0.005	1	*Gymnostellatospora*	0.005	1
Clitocybe	0.005	1	*Helicoma*	0.005	1
Clonostachys	0.005	1	*Hemibeltrania*	0.005	1
Coniochaeta	0.005	1	*Hymenopellis*	0.005	1
Cylindrotrichum	0.005	1	*Kappamyces*	0.005	1
Dictyosporium	0.005	1	*Leptospora*	0.005	1
Dimorphospora	0.005	1	*Letendraea*	0.005	1
Gaeumannomyces	0.005	1	*Malassezia*	0.005	1
Geoglossum	0.005	1	*Metacordyceps*	0.005	1
Glomus	0.005	1	*Pluteus*	0.005	1
Haptocillium	0.005	1	*Pochonia*	0.005	1
Hypochnicium	0.005	1	*Polytolypa*	0.005	1
Hypoxylon	0.005	1	*Pseudoclitocybe*	0.005	1
Lipomyces	0.005	1	*Rhizopogon*	0.005	1
Menispora	0.005	1	*Spiromyces*	0.005	1
Microstroma	0.005	1	*Wilcoxina*	0.005	1

Mollisia	0.005	1	**TOTAL**	**4.6**	**93**
Monographella	0.005	1			
Ophiognomonia	0.005	1			
Paecilomyces	0.005	1			
Phaeococcomyces	0.005	1			
Phialea	0.005	1			
Pluteus	0.005	1			
Sordaria	0.005	1			
UG of Entolomataceae	0.005	1			
UG of Helotiaceae	0.005	1			
UG of Sarcosomataceae	0.005	1			
UG of Sporormiaceae	0.005	1			
TOTAL	**8.4**	**105**			

Table S5 – Relative abundance of bacterial OTUs specifically detected one of the two bulk soils T0. The bacterial OTUs specific to each of the two soils have been grouped according to the bacterial genus with which they are affiliated. The last line TOTAL corresponds to the relative abundance of fungal genera and the sum of fungal OTUs found to be specific of Soil 1 and Soil 2.UG = Unidentified genus.

Bacterial genus Soil 1 specific	Relative abundance (%)	Number of OTUs	Bacterial genus Soil 2 specific	Realtive abundance (%)	Number of OTUs
Bdellovibrio	0.1	5	*Terrimonas*	0.1	1
Acidicapsa	0.09	2	UG of *Holophagales*	0.06	1
Aquisphaera	0.07	3	*Dongia*	0.05	3
Achromobacter	0.06	1	*Sphingobium*	0.03	2
Gemmata	0.06	4	*Cloacibacterium*	0.02	1
Arenimonas	0.04	2	*Niastella*	0.02	1
Armatimonas	0.04	2	*1174-901-12*	0.02	1
Bauldia	0.04	2	*Occallatibacter*	0.02	4
JGI 0001001-H03	0.04	4	*Pseudacidovorax*	0.02	1
Candidatus Nostocoida	0.04	2	*Actinoallomurus*	0.01	1
Acidisoma	0.04	1	*Dokdonella*	0.01	2
Acidocella	0.04	1	*Hyphomicrobium*	0.01	1
Reyranella	0.04	5	*Pseudoflavitalea*	0.01	2
Herbaspirillum	0.03	3	*Undibacterium*	0.01	2
Holophaga	0.03	3	UG of *Nitrosotaleales*	0.01	1
Luedemannella	0.03	3	*Dactylosporangium*	0.01	1
Actinocorallia	0.03	1	*Nitrospira*	0.01	2
Actinoplanes	0.03	1	*Psychrobacillus*	0.01	1
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.02	1	*Roseococcus*	0.01	1
Altererythrobacter	0.02	1	*Terriglobus*	0.01	1
Amnibacterium	0.02	1	UG of *Candidatus Zambryskibacteria*	0.01	1
Amycolatopsis	0.02	1	UG of *Chloroflexales*	0.01	1
Azohydromonas	0.02	1	UG of *SAR324 clade (Marine group B)*	0.01	1
Georgfuchsia	0.02	2	*Arthrobacter*	0.007	1
LD29	0.02	2	*Diplorickettsia*	0.007	1

Pseudonocardia	0.02	3	*FukuN18 freshwater group*	0.007	1
Steroidobacter	0.02	3	*Hydrogenispora*	0.007	1
UG of *Sphingomonadales*	0.02	5	*Luteolibacter*	0.007	1
Candidatus Amoebophilus	0.02	1	*Ralstonia*	0.007	2
Candidatus Paracaedibacter	0.02	1	*Tahibacter*	0.007	1
UG of *Anaerolineales*	0.02	4	UG of *Verrucomicrobiales*	0.007	1
UG of *JG36-TzT-191*	0.02	4	*AAP99*	0.003	1
Chujaibacter	0.01	1	*Aetherobacter*	0.003	1
Clostridium sensu stricto 12	0.01	1	*Aquicella*	0.003	1
Collimonas	0.01	1	*Bosea*	0.003	1
Cupriavidus	0.01	1	*Candidatus Ovatusbacter*	0.003	1
Curvibacter	0.01	1	*Chitinimonas*	0.003	1
Desulfitobacterium	0.01	1	*Chitinophaga*	0.003	1
Desulfosporosinus	0.01	1	*Chryseobacterium*	0.003	1
Devosia	0.01	1	*CL500-29 marine group*	0.003	1
Edaphobacter	0.01	1	*Clostridium sensu stricto 13*	0.003	1
Gaiella	0.01	1	*Crossiella*	0.003	1
Pseudolabrys	0.01	2	*Heliimonas*	0.003	1
Rhodoblastus	0.01	2	*Nitrosospira*	0.003	1
Rhodovastum	0.01	2	*Phaselicystis*	0.003	1
Spirochaeta 2	0.01	2	*Rhizomicrobium*	0.003	1
UG of *Armatimonadales*	0.01	3	*Rhodomicrobium*	0.003	1
UG of *Phycisphaerales*	0.01	4	*SM1A02*	0.003	1
Mycoavidus	0.01	1	*Sphingopyxis*	0.003	1
Geothrix	0.01	1	*Tumebacillus*	0.003	1
IS-44	0.01	1	UG of *Clostridiales*	0.003	1
Legionella	0.01	1	UG of *R7C24*	0.003	1
OM27 clade	0.01	1	*Vibrionimonas*	0.003	1
UG of *Frankiales*	0.01	3	**TOTAL**	**0.7**	**64**
UG of *S085*	0.01	2			
Pseudochrobactrum	0.007	1			
Rhodopila	0.007	1			

Rhodoplanes	0.007	1
Sideroxydans	0.007	1
Spirosoma	0.007	1
Terracidiphilus	0.007	1
Terrimicrobium	0.007	1
UG of *Selenomonadales*	0.007	1
UG of *KF-JG30-C25*	0.007	2
Zavarzinella	0.007	2
UG of *Chthonomonadales*	0.003	1
UG of *Desulfarculales*	0.003	1
UG of *Lineage IV*	0.003	1
UG of *Rhodobacterales*	0.003	1
UG of *Salinisphaerales*	0.003	1
UG of *Subgroup 12*	0.003	1
UTCFX1	0.003	1
Variovorax	0.003	1
Xylella	0.003	1
TOTAL	**1.5**	**131**

Table S6 –Relative abundance of fungal genera and of bacterial orders and genera (>1% relative abundance in at least one soil) detected in the bulk soil T0 which showed significant differences between Soil 1 and Soil 2 (Student *t*-test, p<0,05, means +/- SE). Numbers in bold denote the highest relative abundance of fungal and bacterial phyla detected between Soil 1 and Soil 2.

Fungal genus	**Soil 1**	**Soil 2**
Unidentified genus of *Pezizaceae*	0.01 +/- 0.01	**2.0 +/- 0.2**
Bacterial order	**Soil 1**	**Soil 2**
Chthoniobacterales	**6.4 +/- 0.2**	2.1 +/- 0.2
Solibacterales	5.6 +/- 0.5	**7.4 +/- 0.2**
Rhizobiales	3.7 +/- 0.1	**5.2 +/- 0.4**
Unidentified order of AD3	**3.31 +/- 0.49**	0.85 +/- 0.20
Micropepsales	**2.3 +/- 0.1**	1.63 +/- 0.17
Cytophagales	**1.9 +/- 0.2**	0.89 +/- 0.14
Subgroup 7	**1.4 +/- 0.1**	0.77 +/- 0.08
WD260	1.2 +/- 0.1	**1.8 +/- 0.1**
Frankiales	1.0 +/- 0.1	**3.3 +/- 0.2**
Gammaproteobacteria Incertae Sedis	0.67 +/- 0.04	**2.4 +/- 0.2**
Caulobacterales	0.56 +/- 0.05	**1.1 +/- 0.1**
Gaiellales	0.55 +/- 0.05	**1.2 +/- 0.1**
Bacterial genus	**Soil 1**	**Soil 2**
Candidatus Udaeobacter	**5.4 +/- 0.1**	1.6 +/- 0.2
Bryobacter	2.7 +/- 0.2	**4.1 +/- 0.2**
Candidatus Solibacter	2.2 +/- 0.2	**2.9 +/- 0.1**
Bradyrhizobium	1.4 +/- 0.1	**2.0 +/- 0.1**
Acidothermus	0.92 +/- 0.07	**3.1 +/- 0.2**
Acidibacter	0.67 +/- 0.04	**2.4 +/- 0.2**

Table S7 – Relative abundance of fungal OTUs specifically detected in *Populus tremula x alba* roots cultivated in either Soil 1 or Soil 2 after 10 days (T1) and 6.5 weeks (T2) of growth. The fungal OTUs specific to roots grown in one of the two soils have been grouped according to the fungal genus with which they are affiliated. The last line "TOTAL" corresponds to the relative abundance of fungal genera and the sum of fungal OTUs found to be specific of roots grown in Soil 1 or Soil 2. UG = Unidentified genus.

T1 : Fungal genus Soil 1 root specific	Relative abundance (%)	Number of OTUs	T1 : Fungal genus Soil 2 root specific	Relative abundance (%)	Number of OTUs
Paxillus	6.9	1	*Tuber*	7.3	2
Fusarium	1.6	3	*Colletotrichum*	1.2	2
Mortierella	1.0	12	UG of *Pyronemataceae*	1.1	3
Delicatula	0.8	1	UG of *Sebacinales_Group_B*	0.4	1
Cunninghamella	0.8	1	*Penicillium*	0.2	5
Cortinarius	0.6	2	*Hydnotrya*	0.1	1
Entorrhiza	0.4	3	UG of *Hydnodontaceae*	0.1	1
Penicillium	0.4	1	UG of *Thelephoraceae*	0.1	2
Cystobasidium	0.3	3	*Cadophora*	0.1	6
Neosetophoma	0.3	1	*Bipolaris*	0.09	1
Hymenogaster	0.3	1	*Hymenoscyphus*	0.09	1
Cryptococcus	0.2	4	*Wilcoxina*	0.08	1
Coprinellus	0.2	2	*Hygrophorus*	0.06	1
Menispora	0.2	1	*Pyrenochaetopsis*	0.06	1
Peziza	0.2	1	UG of *Sebacinaceae*	0.05	1
UG of *Helotiaceae*	0.2	2	*Endogone*	0.05	4
Exophiala	0.1	3	*Sebacina*	0.05	1
Geomyces	0.1	3	*Sphaeronaemella*	0.05	1
Parastagonospora	0.1	1	*Clavulina*	0.04	1
Tremella	0.09	1	*Meliniomyces*	0.04	2
Trimmatostroma	0.08	1	*Gyoerffyella*	0.03	1
UG of *Glomeraceae*	0.08	7	*Tomentella*	0.03	1
UG of *Lasiosphaeriaceae*	0.08	1	UG of *Herpotrichiellaceae*	0.03	1
Dactylella	0.07	1	*Galerina*	0.02	1
Cladophialophora	0.07	5	*Ilyonectria*	0.02	1
Collophora	0.06	1	*Mortierella*	0.02	4
Drechslera	0.06	1	UG of *Clavariaceae*	0.02	1
Calicium	0.05	1	UG of *Entomophthoraceae*	0.02	1
Rhizophagus	0.05	1	*Atractospora*	0.02	1

Trichoderma	0.05	2	*Chaetosphaeria*	0.02	1
UG of *Ceratobasidiaceae*	0.05	2	*Helicoma*	0.02	1
Alternaria	0.04	1	*Leptodontidium*	0.02	1
Betamyces	0.04	2	*Mycoarthris*	0.02	1
Chaetosphaeria	0.04	1	*Oidiodendron*	0.02	2
Hyalopeziza	0.04	1	*Spirosphaera*	0.02	1
Mycoleptodiscus	0.04	1	UG of *Pezizaceae*	0.02	2
Stagonosporopsis	0.04	1	UG of *Trichocomaceae*	0.02	2
Podospora	0.04	2	*Acremonium*	0.01	1
Cryptosporiopsis	0.03	1	*Cladophialophora*	0.01	1
Ceratocystis	0.03	3	*Clonostachys*	0.01	1
Lactarius	0.03	1	*Rhodotorula*	0.01	1
Mucor	0.03	4	UG of *Chaetomiaceae*	0.01	1
Phialea	0.03	1	*Inocybe*	0.01	2
Trechispora	0.03	1	*Calcarisporium*	0.005	1
Cenococcum	0.03	1	*Claroideoglomus*	0.005	1
Umbelopsis	0.03	2	*Cryptococcus*	0.005	1
Erythrobasidium	0.02	1	*Hypoxylon*	0.005	1
Gongronella	0.02	2	*Leptosphaeria*	0.005	1
Leptodontidium	0.02	1	*Lophiostoma*	0.005	1
UG of *Elsinoaceae*	0.02	1	*Mycena*	0.005	1
UG of *Thelephoraceae*	0.02	1	*Pezizella*	0.005	1
Cadophora	0.02	1	*Prosthemium*	0.005	1
Conlarium	0.02	1	*Rickenella*	0.005	1
Hypholoma	0.02	1	*Talaromyces*	0.005	1
Neobulgaria	0.02	1	*Trechispora*	0.005	1
Sphaeronaemella	0.02	1	*Trichoderma*	0.005	1
Sphaerulina	0.02	1	*Trichosporon*	0.005	1
UG of Amphisphaeriaceae	0.02	1	*Umbelopsis*	0.005	1
Capnobotryella	0.01	1	UG of *Coniochaetaceae*	0.005	1
Circinaria	0.01	1	UG of *Glomeraceae*	0.005	1
Clavulinopsis	0.01	1	UG of *Lasiosphaeriaceae*	0.005	1
Diaporthe	0.01	1	UG of *Onygenaceae*	0.005	1
Entoloma	0.01	1	UG of *Venturiaceae*	0.005	1
Glomus	0.01	1	UG of *Xylariaceae*	0.005	1
Oidiodendron	0.01	1	**TOTAL**	**11.7**	**89**

Ophiosphaerella	0.01	1
Pseudoclitocybe	0.01	1
Setophaeosphaeria	0.01	1
Basidiobolus	0.005	1
Chalara	0.005	1
Chloridium	0.005	1
Cladosporium	0.005	1
Devriesia	0.005	1
Farysia	0.005	1
Ilyonectria	0.005	1
Pseudeurotium	0.005	1
Pyrenophora	0.005	1
Stemphylium	0.005	1
Talaromyces	0.005	1
UG of *Cortinariaceae*	0.005	1
TOTAL	**16.5**	**128**

T2 : Fungal genus Soil 1 root specific	**Relative abundance (%)**	**Number of OTUs**
Paxillus	5.057	1
UG of *Ceratobasidiaceae*	2.023	1
Penicillium	0.884	8
Cunninghamella	0.656	1
UG of *Entolomataceae*	0.575	3
Chalara	0.423	1
Rhexocercosporidium	0.346	1
Gibberella	0.317	2
Coprinellus	0.173	2
Fusarium	0.173	4
Oidiodendron	0.129	4
UG of *Lasiosphaeriaceae*	0.125	4
Clathrosphaerina	0.114	2
Mucor	0.11	4
Clonostachys	0.081	1
UG of *Ophiocordycipitaceae*	0.077	2

T2 : Fungal genus Soil 2 root specific	**Relative abundance (%)**	**Number of OTUs**
Sphaerobolus	0.7	1
UG of *Pezizaceae*	0.6	1
Endogone	0.	7
UG of *Hydnodontaceae*	0.03	1
Athelopsis	0.02	1
Alternaria	0.007	1
Galerina	0.007	1
Hydnotrya	0.007	1
Hygrophorus	0.005	1
Peziza	0.005	1
Sporobolomyces	0.005	1
Cladophialophora	0.002	1
Leptodontidium	0.002	1
Menispora	0.002	1
Pluteus	0.002	1
Wilcoxina	0.002	1
TOTAL	**1.5**	**22**

Trichoderma	0.07	4
Trechispora	0.07	2
Geminibasidium	0.06	1
UG of *Chaetomiaceae*	0.06	1
Lactarius	0.06	2
Drechslera	0.05	1
Cryptococcus	0.04	2
Entorrhiza	0.04	3
Ilyonectria	0.04	2
UG of *Hyaloscyphaceae*	0.04	1
Ceratocystis	0.03	2
Exophiala	0.03	1
Mollisia	0.03	1
Sphaeronaemella	0.03	1
Neobulgaria	0.03	2
Umbelopsis	0.03	3
Lachnum	0.03	1
Beauveria	0.02	1
Hymenula	0.02	1
Podospora	0.02	3
UG of *Dermateaceae*	0.02	1
Bipolaris	0.02	1
Helicoma	0.02	2
Mycoarthris	0.02	1
Choiromyces	0.02	1
Geoglossum	0.02	1
Gongronella	0.02	1
Gyoerffyella	0.02	1
Collophora	0.01	1
Humicola	0.01	2
Ophiosphaerella	0.01	1
UG of *Coniochaetaceae*	0.01	1
UG of *Phaeosphaeriaceae*	0.01	2
UG of *Venturiaceae*	0.01	1
Clathrus	0.007	1
Cortinarius	0.007	1

Cyberlindnera	0.007	1
Hannaella	0.007	1
Hymenoscyphus	0.007	1
Lipomyces	0.007	1
Maasoglossum	0.007	1
Pochonia	0.007	1
Ramicandelaber	0.007	1
Talaromyces	0.007	1
Unguicularia	0.007	1
UG of *Clavariaceae*	0.007	1
UG of *Sarcosomataceae*	0.007	1
Aureobasidium	0.004	1
Cantharellus	0.004	1
Chaetomium	0.004	1
Chaetosphaeria	0.004	1
Chalastospora	0.004	1
Chloridium	0.004	1
Cryptosporiopsis	0.004	1
Cylindrotrichum	0.004	1
Cystobasidium	0.004	1
Dictyosporium	0.004	1
Lambertella	0.004	1
Leohumicola	0.004	1
Rhizophagus	0.004	1
Rhizophydium	0.004	1
Rickenella	0.004	1
Trematosphaeria	0.004	1
UG of *Helotiaceae*	0.004	1
TOTAL	**12.4**	**123**

Table S8 – Relative abundance of bacterial OTUs specifically detected in *Populus tremula x alba* roots cultivated in either Soil 1 or Soil 2 after 10 days (T1) and 6.5 weeks (T2) of growth. The bacterial OTUs specific to roots grown in one of the two soils have been grouped according to the bacterial genus with which they are affiliated. UG = Unidentified genus.

T1 : Bacterial genus Soil 1 root specific	Relative abundance (%)	Number of OTUs	T1 : Bacterial genus Soil 2 root specific	Relative abundance (%)	Number of OTUs
Methylophilus	0.4	1	*Candidatus Ovatusbacter*	0.9	7
Erwinia	0.2	1	*Cytophaga*	0.4	7
Amycolatopsis	0.2	3	*Silvanigrella*	0.4	1
Flavobacterium	0.2	1	*Ralstonia*	0.2	5
Chthoniobacter	0.1	8	*Novosphingobium*	0.2	11
Singulisphaera	0.1	12	*Vibrionimonas*	0.2	2
UG of *A4b*	0.1	7	*Niastella*	0.2	5
Paenarthrobacter	0.1	1	*Chthonobacter*	0.2	1
Nocardia	0.1	5	UG of *SM2D12*	0.2	11
1921-2	0.1	4	*Methylotenera*	0.1	1
Granulicella	0.1	7	*LD29*	0.1	2
Pedobacter	0.1	2	*Phenylobacterium*	0.1	10
UG of *Micromonosporaceae*	0.1	6	*Chryseobacterium*	0.1	1
Candidatus Xiphinematobacter	0.1	6	*Asticcacaulis*	0.06	7
Hyphomicrobium	0.1	2	*Rhodanobacter*	0.05	4
Luteolibacter	0.1	1	*Bradyrhizobium*	0.05	5
Tumebacillus	0.05	2	*Granulicella*	0.05	3
Candidatus Nostocoida	0.04	3	*Armatimonas*	0.03	3
IS-44	0.04	2	*Cavicella*	0.03	1
Roseococcus	0.04	3	*Deinococcus*	0.03	2
Tardiphaga	0.04	1	*Edaphobacter*	0.03	1
Chryseobacterium	0.04	1	*Herbaspirillum*	0.02	2
Nitrospira	0.04	1	UG of *Methylophilaceae*	0.02	3
UG of *Solibacteraceae (Subgroup 3)*	0.04	4	*Azohydromonas*	0.01	2
UG of *Phycisphaeraceae*	0.03	5	*Duganella*	0.01	1
Altererythrobacter	0.03	1	*Frateuria*	0.01	1
Pantoea	0.03	1	UG of *Pleomorphomonadaceae*	0.01	1
Parafilimonas	0.03	1	UG of *Spirosomaceae*	0.01	1

Phenylobacterium	0.03	4	*Luteibacter*	0.01	1
Reyranella	0.03	4	*Minicystis*	0.01	1
Solirubrobacter	0.03	1	*Cupriavidus*	0.009	2
UG of *Roseiflexaceae*	0.03	1	*Rhodococcus*	0.009	1
UG of *Tepidisphaeraceae*	0.03	1	*Sporocytophaga*	0.009	2
MND1	0.03	3	UG of *Verrucomicrobiaceae*	0.009	3
UG of *Saccharimonadaceae*	0.03	2	*Rudaea*	0.009	2
RB41	0.03	4	UG of *Blastocatellaceae*	0.009	3
UG of *Microtrichaceae*	0.03	1	*Acinetobacter*	0.007	2
JGI 0001001-H03	0.02	3	*Candidatus Amoebophilus*	0.007	1
Zavarzinella	0.02	3	*Candidatus Jidaibacter*	0.007	1
Anaeromyxobacter	0.02	1	*Curvibacter*	0.007	1
Dokdonella	0.02	2	*Acidicapsa*	0.005	2
Halomonas	0.02	1	*Chitinimonas*	0.005	1
Pirellula	0.02	3	*Brevundimonas*	0.003	2
Pseudoflavitalea	0.02	3	*Chthoniobacter*	0.003	3
Stenotrophobacter	0.02	2	*alphaI cluster*	0.003	1
UG of *BIrii41*	0.02	4	*Candidatus Paracaedibacter*	0.003	1
UG of *Blastocatellaceae*	0.02	4	*Crossiella*	0.003	1
UG of *P3OB-42*	0.02	1	*Dinghuibacter*	0.003	1
UG of *Streptomycetaceae*	0.02	3	*Diplorickettsia*	0.003	1
Pir4 lineage	0.02	2	*Heliimonas*	0.003	1
Catenulispora	0.01	4	*Iamia*	0.003	1
Ellin6067	0.01	3	*Marmoricola*	0.003	1
GAS113	0.01	1	*Pedosphaera*	0.003	1
GOUTA6	0.01	1	UG of *Rhizobiales Incertae Sedis*	0.003	1
Sorangium	0.01	1	*[Aquaspirillum] arcticum group*	0.002	1
UG of *Blfdi19*	0.01	1	*Acidocella*	0.002	1
UG of *Desulfarculaceae*	0.01	3	*Adhaeribacter*	0.002	2
UG of *Xanthomonadaceae*	0.01	3	*Alkanindiges*	0.002	1
UTCFX1	0.01	1	*Chujaibacter*	0.002	1
1174-901-12	0.01	2	*Cutibacterium*	0.002	1
Cellvibrio	0.01	1	*Devosia*	0.002	1

Cephaloticoccus	0.01	1	*Dyella*	0.002	1
CL500-29 marine group	0.01	1	*Escherichia-Shigella*	0.002	1
Desmonostoc PCC-6302	0.01	1	*Paracoccus*	0.002	1
Geobacter	0.01	2	*Phyllobacterium*	0.002	1
Rhodovastum	0.01	1	*Psychrobacillus*	0.002	1
Schlesneria	0.01	1	*Rickettsiella*	0.002	1
SH-PL14	0.01	1	*Terrimonas*	0.002	1
UG of *Archangiaceae*	0.01	1	UG of *Beijerinckiaceae*	0.002	1
UG of *Gimesiaceae*	0.01	2	UG of *Blfdi19*	0.002	1
UG of *TRA3-20*	0.01	3	UG of *Geodermatophilaceae*	0.002	1
Aetherobacter	0.007	1	UG of *Parachlamydiaceae*	0.002	1
Candidatus Berkiella	0.007	1	UG of *Rhodocyclaceae*	0.002	1
Collimonas	0.007	1	UG of *Ruminococcaceae*	0.002	1
Edaphobacter	0.007	1	UG of *UBA12409*	0.002	1
Fimbriiglobus	0.007	1	**TOTAL**	**3.8**	**161**
Gaiella	0.007	1	**T2 : Bacterial genus Soil 2 root specific**	**Relative abundance (%)**	**Number of OTUs**
IMCC26207	0.007	1	*Lacunisphaera*	0.1	7
Luedemannella	0.007	1	UG of *Cytophagales*	0.1	7
OLB12	0.007	1	UG of *Chthoniobacterales*	0.1	5
Paludisphaera	0.007	1	*Rhizobacter*	0.07	1
Porphyrobacter	0.007	1	UG of *Lineage IV*	0.06	2
Pseudorhodoplanes	0.007	1	UG of *Methylacidiphilales*	0.06	6
Rhodomicrobium	0.007	2	UG of *Vampirovibrionales*	0.06	1
Roseateles	0.007	2	*Bradyrhizobium*	0.05	5
UG of *Anaerolineaceae*	0.007	1	*Diplorickettsia*	0.05	1
UG of *Holosporaceae*	0.007	1	UG of *Acetobacterales*	0.05	8
UG of *Hyphomicrobiaceae*	0.007	1	*Heliimonas*	0.04	4
Actinoplanes	0.007	2	*Inquilinus*	0.04	4
Aquicella	0.007	2	*Chitinimonas*	0.04	1
Actinoallomurus	0.003	1	*Spirochaeta 2*	0.03	3
Amnibacterium	0.003	1	*Herbaspirillum*	0.03	4
Bauldia	0.003	1	*Pseudonocardia*	0.03	2

Blastococcus	0.003	1	UG of *Verrucomicrobiales*	0.03	4
Candidatus Omnitrophus	0.003	1	*Methylobacterium*	0.03	4
Chitinophaga	0.003	1	*Gemmatirosa*	0.03	2
Cloacibacterium	0.003	1	*Terrabacter*	0.02	1
Clostridium sensu stricto 9	0.003	1	*Roseiarcus*	0.02	4
FCPS473	0.003	1	*Silvanigrella*	0.02	1
Methylocella	0.003	1	UG of *Solibacterales*	0.02	2
MM1	0.003	1	*Azospirillum*	0.02	1
Paludibaculum	0.003	1	*1959-1*	0.02	2
Pedosphaera	0.003	1	*Labrys*	0.02	2
Phaselicystis	0.003	1	*Cytophaga*	0.02	3
Pseudochrobactrum	0.003	1	*Rhodovastum*	0.02	2
Pseudolabrys	0.003	1	*Amnibacterium*	0.01	2
Ramlibacter	0.003	1	*Candidatus Jidaibacter*	0.01	3
Rhizomicrobium	0.003	1	*Phycicoccus*	0.01	1
Rhizorhapis	0.003	1	*Armatimonas*	0.01	2
Roseimicrobium	0.003	1	*Asticcacaulis*	0.01	1
Shewanella	0.003	1	*Candidatus Ovatusbacter*	0.01	2
SM1A02	0.003	1	*Terracidiphilus*	0.01	2
Sphingopyxis	0.003	1	UG of *WD260*	0.01	4
Spirochaeta 2	0.003	1	*Uliginosibacterium*	0.01	1
Stenotrophomonas	0.003	1	*Microbacterium*	0.007	1
Steroidobacter	0.003	1	*Pedosphaera*	0.007	2
Terrabacter	0.003	1	*Streptacidiphilus*	0.007	2
Terracidiphilus	0.003	1	*alphaI cluster*	0.006	1
Terriglobus	0.003	1	*Fimbriiglobus*	0.006	1
Terrimicrobium	0.003	1	*Frateuria*	0.006	1
Thermincola	0.003	1	*Minicystis*	0.006	1
Uliginosibacterium	0.003	1	*Mycoavidus*	0.006	1
Undibacterium	0.003	1	UG of *Holosporales*	0.006	2
UG of *Bacillaceae*	0.003	1	*Vibrionimonas*	0.006	1
UG of *Enterobacteriaceae*	0.003	1	*Aquabacterium*	0.004	2
UG of *KF-JG30-B3*	0.003	1	*Bauldia*	0.004	2
UG of *Rhodanobacteraceae*	0.003	1	*[Aquaspirillum] arcticum group*	0.004	1
UG of *Rhodospirillaceae*	0.003	1	*Acidisoma*	0.004	1

UG of *Veillonellaceae*	0.003	1	*Acinetobacter*	0.004	1
Xylella	0.003	1	*Candidatus Nitrocosmicus*	0.004	1
TOTAL	**3.3**	**243**	*GAS113*	0.004	1
T2 : Bacterial genus Soil 1 root specific	**Relative abundance (%)**	**Number of OTUs**	*MND1*	0.004	1
Pedobacter	0.3	5	*Ralstonia*	0.004	1
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.2	5	*Rickettsia*	0.004	1
UG of *Micromonosporales*	0.1	5	*Rudaea*	0.004	1
Dyella	0.08	4	UG of *Obscuribacterales*	0.004	1
Chitinophaga	0.07	3	*Amycolatopsis*	0.002	1
Nocardia	0.06	7	*Anaeromyxobacter*	0.002	1
Niastella	0.06	2	*Brevinema*	0.002	1
1921-2	0.05	4	*Candidatus Amoebophilus*	0.002	1
Luteolibacter	0.05	2	*CL500-29 marine group*	0.002	1
Asticcacaulis	0.03	4	*Crossiella*	0.002	1
Nakamurella	0.03	2	*Curvibacter*	0.002	1
Catenulispora	0.02	3	*Dactylosporangium*	0.002	1
Actinospica	0.02	3	*Devosia*	0.002	1
Desulfosporosinus	0.02	4	*Empedobacter*	0.002	1
Erwinia	0.02	1	*Escherichia-Shigella*	0.002	1
Flavobacterium	0.02	2	*Fonticella*	0.002	1
Methylophilus	0.02	1	*Geothrix*	0.002	1
Pseudomonas	0.02	3	*Holophaga*	0.002	1
Sphingopyxis	0.02	1	*Methylotenera*	0.002	1
UG of *Subgroup 7*	0.02	5	*Paludisphaera*	0.002	1
Cellulomonas	0.01	1	*PAUC26f*	0.002	1
Nitrospira	0.01	1	*Pir4 lineage*	0.002	1
Parafilimonas	0.01	1	*Sideroxydans*	0.002	1
RB41	0.01	1	*Sporocytophaga*	0.002	1
Marmoricola	0.01	2	*Stenotrophomonas*	0.002	1
Streptosporangium	0.01	1	UG of *C0119*	0.002	1
JG30a-KF-32	0.008	2	UG of *Chloroflexales*	0.002	1
Nocardioides	0.008	2	UG of *Phycisphaerales*	0.002	1
Pseudochrobactrum	0.008	1	UG of *S085*	0.002	1

Silvimonas	0.008	1	**TOTAL**	**1.4**	**159,0**
Spirosoma	0.008	1			
Undibacterium	0.008	1			
1921-3	0.005	1			
Candidatus Nitrosotalea	0.005	1			
Candidatus Nostocoida	0.005	2			
Collimonas	0.005	2			
GOUTA6	0.005	1			
JGI 0001001-H03	0.005	1			
Luteibacter	0.005	1			
Phyllobacterium	0.005	1			
Stenotrophobacter	0.005	1			
Terrimonas	0.005	2			
UG of *RBG-13-54-9*	0.005	1			
UG of *Salinisphaerales*	0.005	1			
UG of *Steroidobacterales*	0.005	2			
Yersinia	0.005	1			
Actinomadura	0.002	1			
Actinoplanes	0.002	1			
Actinorhabdospora	0.002	1			
TOTAL	**1.4**	**102**			

Table S9 – Relative abundance of fungal genera and bacterial orders and genera detected in roots collected at T1 and T2 which showed significant difference between Soil 1 and Soil 2 roots (Student *t*-test, p<0.05, means +/- SE). Numbers in bold denote the highest relative abundance of fungal and bacterial phyla detected between Soil 1 and Soil 2 roots.

Fungal genus	**Roots_T1_ Soil 1**	**Roots_T1_ Soil 2**	**Roots_T2_ Soil 1**	**Roots_T2_ Soil 2**
Mortierella	**6.26 +/- 1.56**	0.40 +/- 0.15	**1.63 +/- 0.38**	0.07 +/- 0.02
Tuber	1.46 +/- 1.10	**10.2 +/- 3.13**		
Paxillus	**6.89 +/- 1.19**	0,0		
UG of *Thelephoraceae*	8.07 +/- 2.75	**22.6 +/- 5.1**	6.45 +/- 4.89	**24.8 +/- 5.8**
Hydnotrya	0.10 +/- 0.06	**8.46 +/- 3.18**		
Phialocephala	**1.99 +/- 0.13**	0.49 +/- 0.08	**6.71 +/- 1.09**	0.27 +/- 0.04
Phaeomollisia			**1.06 +/- 0.11**	0.05 +/- 0.02
Ramicandelaber			**1.43 +/- 0.42**	0.01 +/- 0.01
Bacterial order	**Roots_T1_ Soil 1**	**Roots_T1_ Soil 2**	**Roots_T2_ Soil 1**	**Roots_T2_ Soil 2**
Gammaproteobacteria Incertae Sedis	0.58 +/- 0.05	**2.05 +/- 0.40**		
Acetobacterales	3.11 +/- 1.74	**9.30 +/- 1.28**		
Coxiellales	0.04 +/- 0.03	**1.03 +/- 0.23**		
Ktedonobacterales			0.90 +/- 0.05	**1.93 +/- 0.32**
Chitinophagales			1.24 +/- 0.32	**5.09 +/- 0.97**
Pedosphaerales			0.33 +/- 0.14	**3.43 +/- 0.70**
Saccharimonadales			**18.3 +/- 4.5**	0.73 +/- 0.17
Catenulisporales			**7.93 +/- 1.54**	2.91 +/- 0.92
Rhizobiales			4.18 +/- 1.14	**9.18 +/- 1.02**
Sphingobacteriales			3.73 +/- 0.57	**9.32 +/- 1.44**
Xanthomonadales	**2.70 +/- 0.12**	0.82 +/- 0.09	**3.42 +/- 0.18**	0.62 +/- 0.07
Bacterial genus	**Roots_T1_ Soil 1**	**Roots_T1_ Soil r2**	**Roots_T2_ Soil 1**	**Roots_T2_ Soil 2**
Rhodanobacter	**1.24 +/- 0.07**	0.27 +/- 0.05		
Acidibacter	0.58 +/- 0.05	**1.12 +/- 0.16**		
Coxiella	0.04 +/- 0.03	**1.03 +/- 0.23**		
Phenylobacterium	0.63 +/- 0.10	**1.85 +/- 0.36**		

Table S10– List of metabolites detected in roots of *Populus tremula x alba* harvested after 10 days (T1) and 6.5 weeks (T2) of growth for which a significant difference of concentration has been measured between Soil 1 and Soil 2 roots (* = P<0.05, One-way ANOVA). Values indicate fold changes between Soil 1 and Soil 2 roots. Metabolites highlighted in yellow and green are involved in primary, secondary metabolisms, respectively. The last column indicates the relative abundance of each metabolite in the total root metabolome of *Populus tremula x alba*seedlings.

Metabolite (RT-m/z)	Plant metabolite	Bacterial or fungal metabolite	*Populus* roots collected in Soil 1 vs. Soil 2 roots		% of all root metabolites of *P. tremula x alba* (%)
			10 days of growth (T1)	6.5 weeks of growth (T2)	
sucrose	X	X	0.61	0.40 *	**30.72**
a-salicyloylsalicin	X		0.03	0.76	**14.15**
malic acid	X	X	1.77	0.59 *	**9.74**
glucose	X	X	4.87	3.13 *	**8.11**
salicin	X		0.81	0.50	**6.67**
tremuloidin	X		0.18	1.11	**3.91**
fructose	X	X	3.46	3.40 *	**3.72**
palmitic acid	X	X	0.78	0.30	**3.31**
B-sitosterol	X		1.12	0.95	**2.09**
catechin	X		1.00	0.42 *	**1.68**
7.69 169 101 75 68	?		ND	3.16 *	**1.65**
phosphate	X	X	2.39	0.73	**1.31**
galactose	X	X	2.46	1.59	**1.26**
myo-inositol	X		8.69	2.49	0.97
citric acid	X	X	0.63	0.46	0.89
salireposide	X		1.95	1.71	0.86
shikimic acid	X	X	2.25	1.30	0.80
5-oxo-proline	X	X	2.83	4.04	0.76
mannitol		X	0.08 *	0.79	0.65
catechol	X		3.34	1.06	0.58
salicylic acid	X	X	2.55 *	0.48	0.51

glycerol	X	X	0.13 *	0.46	0.50
linoleic acid	X	X	1.57	0.90	0.48
salicortin	X		1.75	0.45	0.37
4-hydroxybenzoic acid	X	X	1.93	1.23	0.36
19.00 219 171 331	?		1.33	0.70	0.34
threonic acid	X		1.45	0.25 *	0.33
trehalose		X	0.93	0.59	0.33
2,5-dihydroxybenzoic acid-2-O-glucoside	X	X	ND	0.14	0.30
quinic acid	X	X	1.98	0.63	0.23
alanine	X	X	9.15 *	20.07 *	0.21
succinic acid	X	X	0.75	0.84	0.20
salicyl alcohol	X		0.89	0.74	0.18
arbutin	X		0.68	1.13	0.18
lactic acid	X	X	2.79	0.95	0.18
salicyl-salicylic acid-2-O-glucoside	X	X	8.45	2.44	0.17
6.94 225 240 332 278	?		0.74	0.12	0.15
salicyltremuloidin	X		3.49	2.29	0.14
xylitol	X	X	1.67	0.63	0.12
tremulacin	X		3.67	1.11	0.12
fumaric acid	X	X	21.94	1.02	0.12
GABA	X	X	1.83	4.89	0.12
nonanoic acid	X		2.02	0.48	0.11
glyceric acid	X	X	1.62	0.76	0.11
monogalactosylglycerol	X	X	0.98	0.72	0.10
ethyl-phosphate	?		3.21	3.13 *	0.09
2,5-dihydroxybenzoic acid-5-O-glucoside	X		ND	1.61	0.09
oxalomalic acid	X	X	54.78	7.63 *	0.08
19.18 171 coumaroyl glycoside	?		1.41	0.44	0.08
11.29 393 303 257	?		ND	13.87	0.07

17.65 418 179 193 91 glycoside	?		0.62	0.69	0.07
10.68 217 391 411	?		2.15 *	4.11 *	0.06
a-linolenic acid	X	X	2.10	1.31	0.05
19.69 171 caffeoyl glycoside	?		1.69	0.74	0.05
arabitol	X	X	1.29	3.69	0.04
xylono-1,4-lactone	X		2.72	2.09 *	0.04
ribitol	X	X	ND	6.17	0.04
hydroquinone	X		1.59	0.32	0.04
digalactosylglycerol	X	X	ND	0.81	0.04
glycerol-1/3-P	X	X	2.37	1.61	0.033
14.38 254 inositol conj	?		1.67	ND	0.033
maleic acid	X	X	1.09	0.28 *	0.032
gallocatechin	X		0.64	0.68	0.032
14.09 375 292 217	?		0.34 *	ND	0.030
16.11 guaiacyl lignan	X		0.45 *	0.85	0.028
arabinose	X	X	3.25	ND	0.027
threono-1,4-lactone	X	X	3.83	1.18	0.025
glutamic acid	X	X	ND	1.43	0.023
2-hydroxypentanedioic acid	X	X	1.80	0.67	0.023
deltoidin	X		0.61	ND	0.022
phluoroglucinol	X	X	4.84	4.84 *	0.021
15.24 284 glycoside	?		1.04	1.40	0.021
9.98 98 288 390	?		ND	0,00	0.020
1,2,4-benzenetriol	X	X	ND	0.60	0.020
16.04 guaiacyl lignan	X		0.83	0.95	0.019
6-hydroxy-2-cyclohexenone alcohol	X		3.17	0.29	0.019
14.25 331 263 233 258 M+ glycoside	?		1.18	1.29	0.019
15.18 284 glycoside	?		0.78	1.17	0.018
8.34 256 167	?		ND	1.47	0.016

erythronic acid	X		0.94	0.51 *	0.014
6-hydroxy-2-cyclohexenone-1-carboxylic acid	X	X	0.78	0.25 *	0.013
cis-aconitic acid	X	X	0.23	0.41 *	0.012
13.84 183 256 167	?		6.73	ND	0.012
16.37 guaiacyl lignan	X		ND	1.18	0.011
caffeic acid	X		1.87	0.74	0.011
11.22 450 dehydro sugar	?		1.08	0.34 *	0.009
10.90 450 dehydro sugar	?		ND	1.97	0.007
1,2,3-benzenetriol	X	X	ND	0.34	0.005
a-tocopherol	X	X	2.87	1.67	0.004

Chapitre V : Etude de l'expression hétérologue de l'effecteur fongique MiSSP7 sur la structuration et la composition du microbiote racinaire et sur le métabolome du Peuplier

SUPPLEMENTAL INFORMATIONS

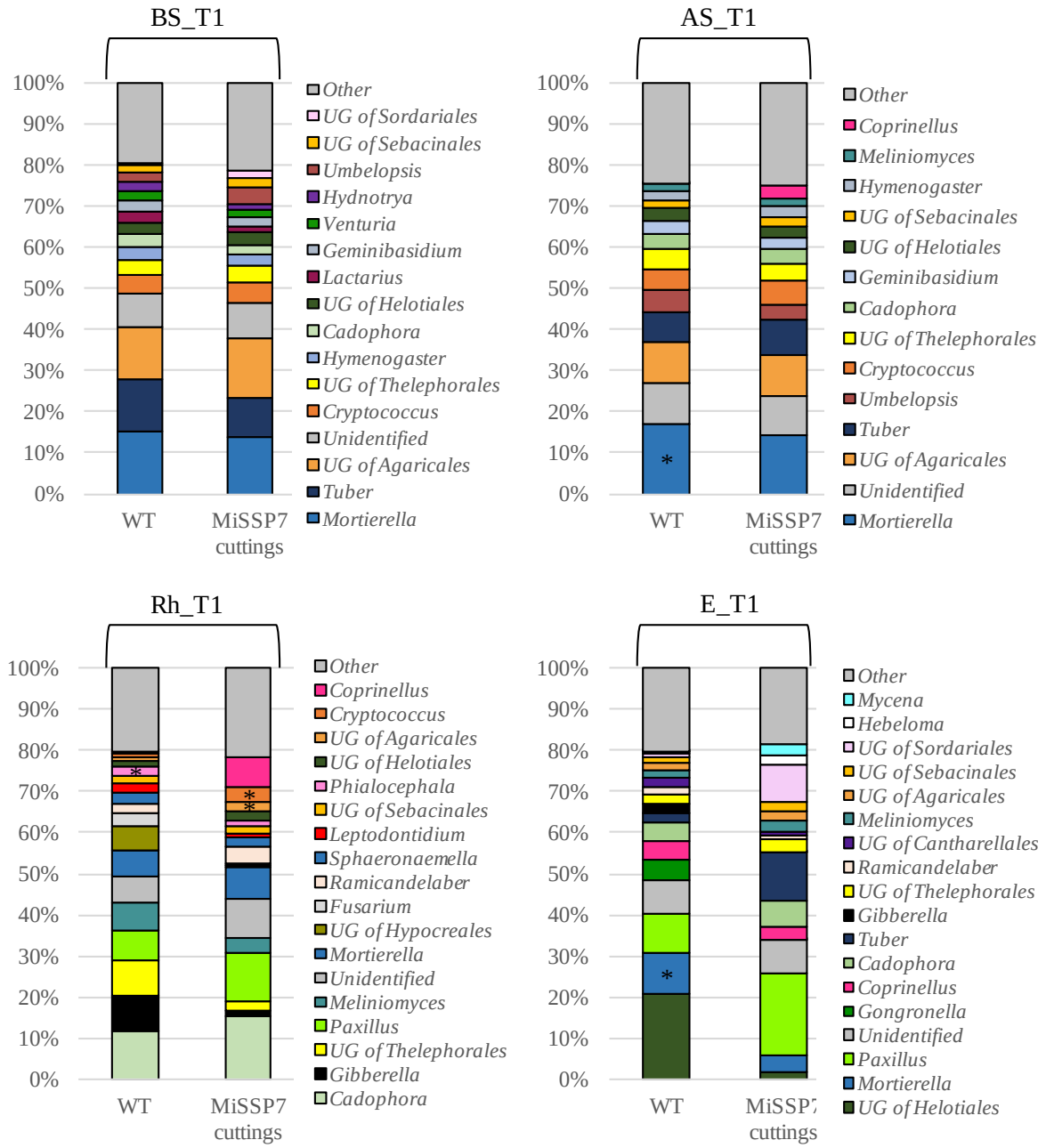


Figure S1 - The distribution of the most dominant fungal genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T1 in Year 1. The asterisks (*) denote significant difference in the relative abundance of fungal genera detected between *Populus* WT and *Populus*-expressing MiSSP7 (P<0.05, ANOVA). Detailed informations are available in Table S2.

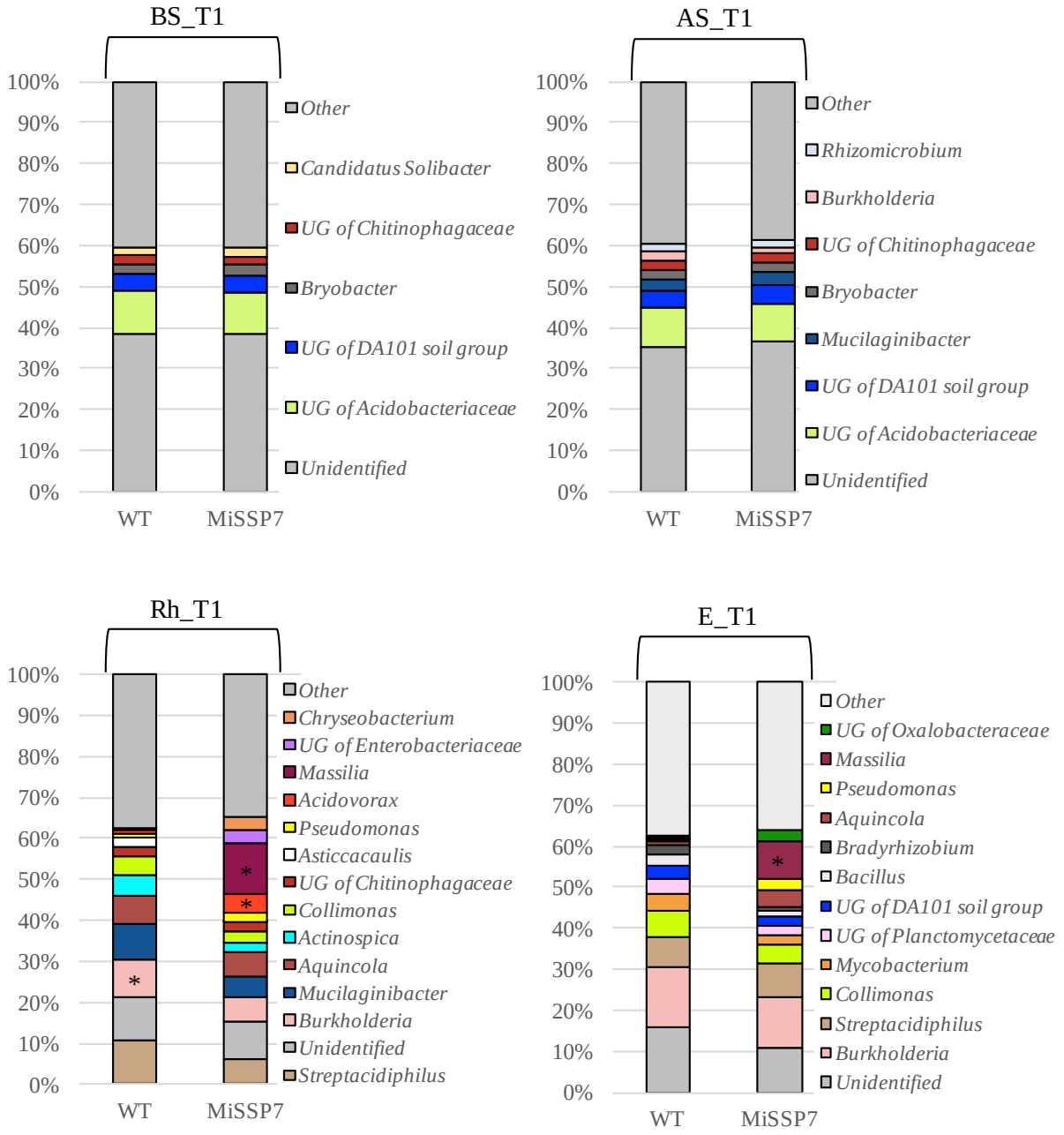


Figure S2 - The distribution of the most dominant bacterial genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T1 in Year 1. The asterisks (*) denote significant difference in the relative abundance of bacterial genera detected between *Populus* WT and *Populus*-expressing MiSSP7 (P<0.05, ANOVA). Detailed informations are available in Table S3.

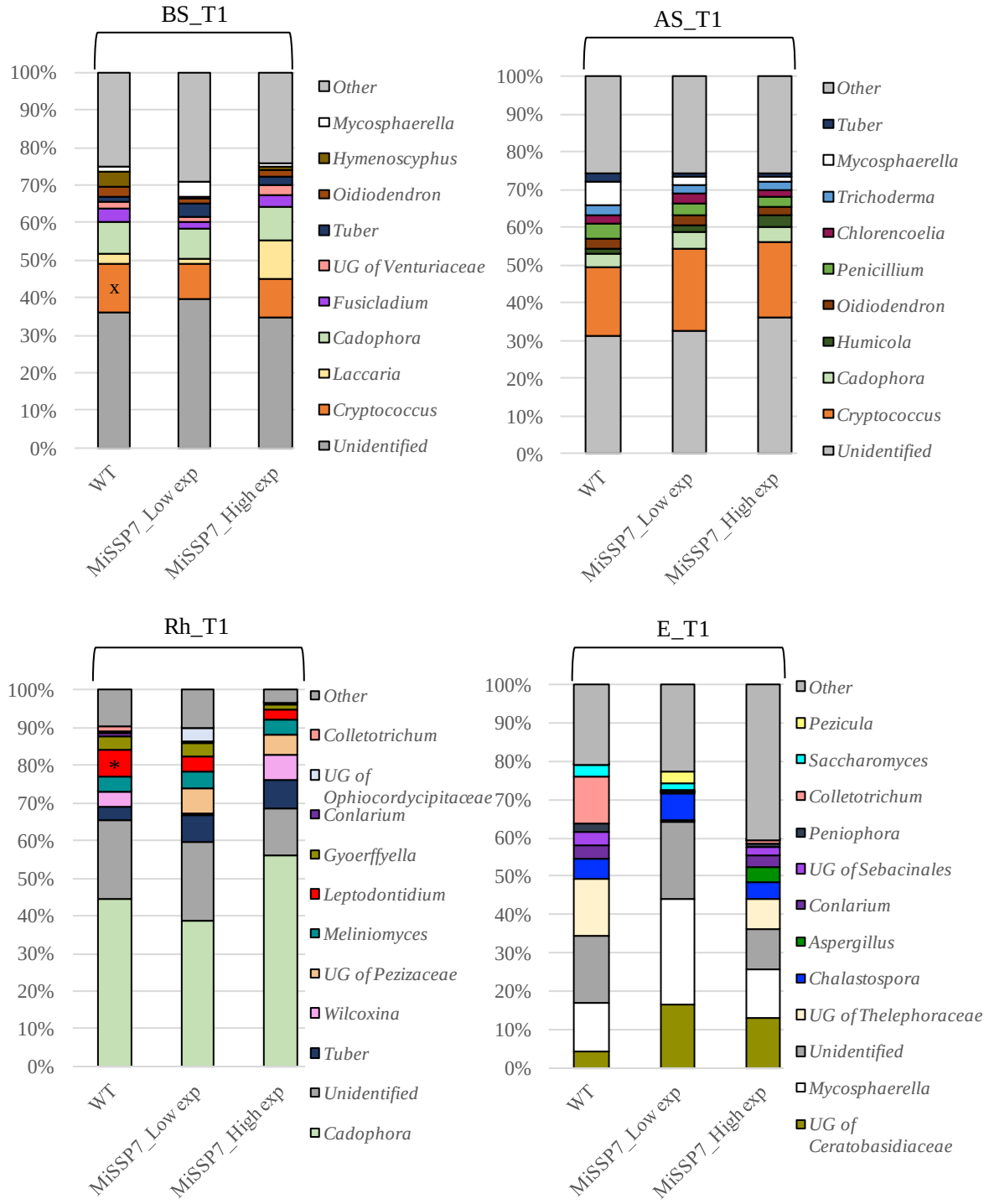


Figure S3 - The distribution of the most dominant fungal genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T1 in Year 2. The crosses (x) denote significant difference in the relative abundance of bacterial genera detected between WT and MiSSP7_Low expression. The asterisks (*) denote significant difference in the relative abundance of fungal genera detected between WT and MiSSP7_High expression (P<0.05, ANOVA). Detailed informations are available in Table S6.

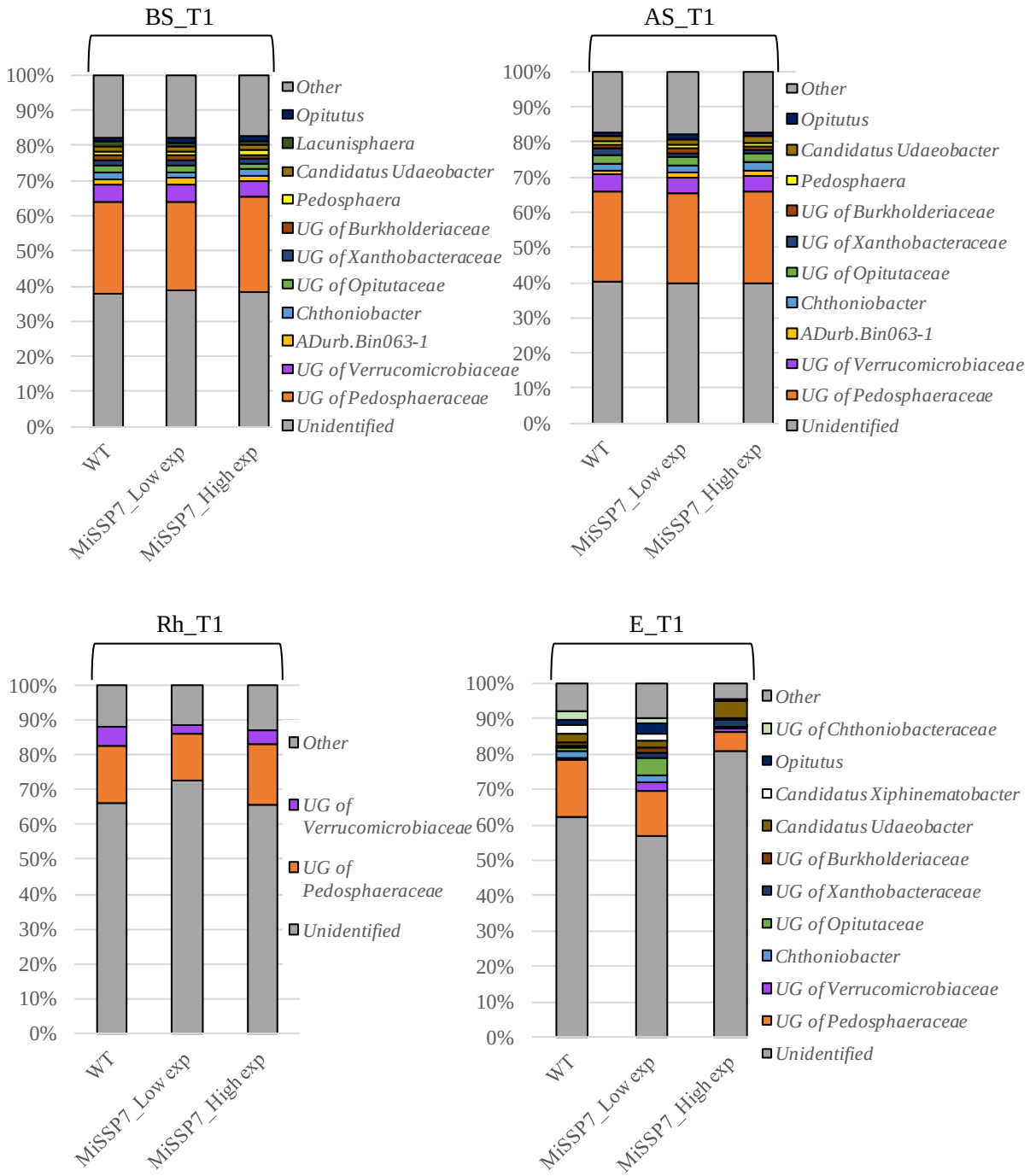


Figure S4 - The distribution of the most dominant bacterial genera (>2 % in relative abundance) detected in BS, AS, Rh and E samples of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T1 in Year 2. Detailed informations are available in Table S7.

Table S1 - Number of cuttings of *Populus* WT and *Populus* MiSSP7 lines harvested at T1 and T2 in Year 1 and Year 2 and used in the analyses performed in this study.

***Populus tremula x alba* cuttings**	**Number of cuttings collected at T1 in Year 1**	**Number of cuttings collected at T1 in Year 1 and used in our analyses**	**Number of cuttings collected at T2 in Year 1**	**Number of cuttings collected at T2 in Year 1 and used in our analyses**
WT	**3**	**3**	**4**	**4**
MiSSP7.1	2	2	3	3
MiSSP7.2	4	3	4	4
MiSSP7.3	2	2	3	3
MiSSP7.6	3	3	4	4
MiSSP7	**11**	**10**	**14**	**14**
***Populus tremula x alba* cuttings**	**Number of cuttings collected at T1 in Year 2**	**Number of cuttings collected at T1 in Year 2 and used in our analyses**	**Number of cuttings collected at T2 in Year 2**	**Number of cuttings collected at T2 in Year 2 and used in our analyses**
WT	**6**	**6**	**6**	**6**
MiSSP7.1	6	0	6	2
MiSSP7.2	6	5	6	5
MiSSP7.3	4	0	4	3
MiSSP7.6	3	3	4	4
MiSSP7	**19**	**8**	**20**	**14**

Table S2 (1/4) - Relative abundance of the most abundant fungal phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_Low exp related samples.

Fungal phylum_Year 1	**WT_BS_T1**	**MiSSP7_BS_T1**	**Fungal phylum_Year 1**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
Ascomycota	37.6 ± 2.0	36.8 ± 2.5	Ascomycota	32.5 ± 2.7	24.2 ± 2.3	29.0 ± 3.3
Basidiomycota	37.2 ± 2.6	38.3 ± 2.1	Basidiomycota	37.3 ± 6.8	48.7 ± 7.5	51.7 ± 6.8
Chytridiomycota	0.48 ± 0.12	0.67 ± 0.19	Chytridiomycota	0.13 ± 0.03	0.37 ± 0.11	0.38 ± 0.12
Glomeromycota	0.55 ± 0.02 *	0.35 ± 0.04	Glomeromycota	0.24 ± 0.06	0.66 ± 0.31	0.23 ± 0.03
Rozellomycota	0.06 ± 0.02	0.07 ± 0.01	Rozellomycota	0.07 ± 0.02	0.05 ± 0.01	0.06 ± 0.02
Unidentified	3.7 ± 0.2	3.4 ± 0.2	Unidentified	5.2 ± 2.0	7.6 ± 2.0	4.1 ± 1.9
Zygomycota	20.2 ± 2.0	20.3 ± 1.4	Zygomycota	24.3 ± 3.4	18.4 ± 3.3	14.4 ± 1.8
Fungal phylum_Year 1	**WT_AS_T1**	**MiSSP7_AS_T1**	**Fungal phylum_Year 1**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
Ascomycota	33.6 ± 3.4	34.2 ± 2.4	Ascomycota	41.3 ± 3.6 x	26.2 ± 2.6	31.9 ± 4.7
Basidiomycota	35.6 ± 0.6	39.5 ± 1.8	Basidiomycota	35.3 ± 2.6	40.1 ± 5.9	44.1 ± 6.6
Chytridiomycota	0.62 ± 0.05	0.62 ± 0.08	Chytridiomycota	0.31 ± 0.04	0.57 ± 0.25	0.20 ± 0.04
Glomeromycota	0.39 ± 0.11	0.37 ± 0.03	Glomeromycota	0.15 ± 0.05	0.21 ± 0.03	0.09 ± 0.01
Rozellomycota	0.11 ± 0.02	0.08 ± 0.01	Rozellomycota	0.08 ± 0.02	0.04 ± 0.00	0.08 ± 0.01
Unidentified	3.9 ± 0.5	3.8 ± 0.2	Unidentified	3.6 ± 0.7	3.8 ± 0.7	3.4 ± 0.4
Zygomycota	25.6 ± 2.4	21.2 ± 1.3	Zygomycota	19.1 ± 2.2	28.9 ± 6.0	20.2 ± 1.7
Fungal phylum_Year 1	**WT_Rh_T1**	**MiSSP7_Rh_T1**	**Fungal phylum_Year 1**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
Ascomycota	60.0 ± 1.6 *	45.5 ± 3.2	Ascomycota	71.5 ± 6.2	45.4 ± 9.0	30.6 ± 12.2
Basidiomycota	27.5 ± 1.9	36.3 ± 4.0	Basidiomycota	21.0 ± 6.0	50.0 ± 9.4	66.3 ± 13.6 *
Chytridiomycota	0.08 ± 0.00	0.06 ± 0.02	Chytridiomycota	0.01 ± 0.00	0.03 ± 0.02	0.0 ± 0.0
Glomeromycota	0.22 ± 0.08	0.61 ± 0.14	Glomeromycota	0.06 ± 0.03	0.31 ± 0.25	0.04 ± 0.01
Rozellomycota	0.01 ± 0.01	0.03 ± 0.01	Rozellomycota	0.01 ± 0.01	0.0 ± 0.0	0.0 ± 0.0
Unidentified	1.6 ± 0.4	3.2 ± 0.5	Unidentified	3.2 ± 1.2	1.3 ± 0.4	0.70 ± 0.31
Zygomycota	10.4 ± 1.2	14.2 ± 1.6	Zygomycota	4.1 ± 0.8	2.8 ± 0.7	2.2 ± 0.8
Fungal phylum_Year 1	**WT_E_T1**	**MiSSP7_E_T1**	**Fungal phylum_Year 1**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
Ascomycota	48.1 ± 9.0	46.9 ± 7.8	Ascomycota	47.7 ± 7.8	31.6 ± 5.2	36.0 ± 4.6
Basidiomycota	27.4 ± 12.2	42.4 ± 7.2	Basidiomycota	38.2 ± 5.1	65.3 ± 5.7 x	58.8 ± 4.6
Chytridiomycota	0.27 ± 0.10	0.08 ± 0.04	Chytridiomycota	0.06 ± 0.04	0.0 ± 0.0	0.08 ± 0.05
Glomeromycota	0.11 ± 0.05	0.20 ± 0.07	Glomeromycota	0.06 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
Rozellomycota	0.13 ± 0.10	0.09 ± 0.07	Rozellomycota	0.01 ± 0.01	0.0 ± 0.0	0.0 ± 0.0
Unidentified	3.7 ± 0.9	3.3 ± 0.6	Unidentified	3.3 ± 1.4	1.0 ± 0.4	1.5 ± 0.2
Zygomycota	20.0 ± 4.9 *	6.9 ± 2.0	Zygomycota	10.5 ± 4.9 x	1.9 ± 0.7	3.3 ± 1.2

Table S2 (2/4) - Relative abundance of the most abundant fungal phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_Low exp related samples.

Fungal genus_Year1	**WT_BS_T1**	**MiSSP7_BS_T1**	**Fungal genus_Year1**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
Mortierella	15.4 ± 1.2	13.8 ± 1.0	*Mortierella*	16.8 ± 2.4	14.5 ± 2.6	10.7 ± 1.5
Tuber	12.6 ± 1.0	9.6 ± 1.1	Unidentified	11.4 ± 2.5	13.1 ± 3.2	9.3 ± 3.3
UG of *Agaricales*	12.4 ± 1.1	14.1 ± 1.1	*Paxillus*	9.6 ± 5.3	12.1 ± 4.8	8.4 ± 2.0
Unidentified	8.3 ± 1.2	8.7 ± 0.5	*Cryptococcus*	7.9 ± 1.9	4.6 ± 0.9	5.9 ± 1.3
Cryptococcus	4.3 ± 0.4	5.1 ± 0.3	UG of *Thelephorales*	7.1 ± 3.8	11.9 ± 5.1	12.4 ± 6.1
UG of *Thelephorales*	3.7 ± 0.3	3.9 ± 0.5	*Cadophora*	4.0 ± 1.6	3.4 ± 1.0	4.1 ± 1.3
Hymenogaster	3.1 ± 0.7	2.8 ± 0.7	*Umbelopsis*	3.4 ± 1.3	1.8 ± 0.3	2.1 ± 0.2
Cadophora	2.9 ± 0.7	2.5 ± 0.3	UG of *Helotiales*	3.1 ± 0.6	2.5 ± 0.6	2.4 ± 0.5
UG of *Helotiales*	2.8 ± 0.3	2.9 ± 0.3	*Geminibasidium*	3.0 ± 0.7	2.6 ± 0.6	1.9 ± 0.5
Lactarius	2.7 ± 2.0	1.3 ± 0.3	UG of *Agaricales*	2.9 ± 0.5	5.0 ± 1.8	3.7 ± 1.3
Geminibasidium	2.7 ± 0.4	2.3 ± 0.2	*Tuber*	2.1 ± 0.2	1.4 ± 0.3	1.2 ± 0.3
Venturia	2.4 ± 1.5	2.1 ± 0.5	*Cunninghamella*	2.0 ± 1.4	0.42 ± 0.11	0.46 ± 0.13
Hydnotrya	2.2 ± 0.2	1.2 ± 0.1	*Meliniomyces*	2.0 ± 0.5	1.5 ± 0.3	1.8 ± 0.2
Umbelopsis	2.2 ± 0.6	3.9 ± 0.7	UG of *Sebacinales*	1.7 ± 0.3	2.0 ± 0.7	5.9 ± 3.7
UG of *Sebacinales*	1.9 ± 0.3	2.3 ± 0.2	*Venturia*	1.7 ± 0.2	1.5 ± 0.2	1.7 ± 0.6
Ramicandelaber	1.6 ± 0.6	1.6 ± 0.3	*Ramicandelaber*	1.5 ± 0.5	0.86 ± 0.17	0.62 ± 0.05
Meliniomyces	1.5 ± 0.4	1.5 ± 0.2	UG of *Chaetothyriales*	1.3 ± 0.7	0.57 ± 0.15	0.60 ± 0.30
Tomentella	1.4 ± 1.2	0.26 ± 0.02	*Pseudeurotium*	1.1 ± 0.9	0.20 ± 0.06	0.16 ± 0.02
Paxillus	1.3 ± 0.2	1.3 ± 0.3	UG of *Sordariales*	0.98 ± 0.26	0.67 ± 0.10	1.3 ± 0.5
UG of *Sordariales*	0.68 ± 0.14	2.0 ± 1.0	*Hebeloma*	0.70 ± 0.54	0.77 ± 0.62	1.2 ± 0.4
Other	13.5 ± 1.6	17.0 ± 2.3	*Lactarius*	0.31 ± 0.17	1.4 ± 0.9	0.41 ± 0.10
Fungal genus_Year1	**WT_AS_T1**	**MiSSP7_AS_T1**	UG of *Cantharellales*	0.25 ± 0.12	0.36 ± 0.24	1.8 ± 1.5
Mortierella	17.1 ± 0.8 *	14.4 ± 0.9	*Coprinellus*	0.05 ± 0.03	2.7 ± 2.7	6.0 ± 4.4
Unidentified	9.9 ± 1.1	9.2 ± 0.3	*Peziza*	0.03 ± 0.01	0.03 ± 0.01	3.3 ± 3.1
UG of *Agaricales*	9.7 ± 1.8	10.1 ± 0.9	Other	14.9 ± 1.8	13.7 ± 2.9	12.3 ± 2.0
Tuber	7.6 ± 2.1	8.4 ± 0.6	**Fungal genus_Year1**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
Umbelopsis	5.4 ± 1.5	3.9 ± 0.4	*Cadophora*	13.4 ± 1.8 x	6.9 ± 1.1	10.6 ± 1.5
Cryptococcus	4.9 ± 0.3	5.6 ± 0.5	*Mortierella*	11.9 ± 0.9	15.0 ± 2.6	12.2 ± 1.2
UG of *Thelephorales*	4.9 ± 1.2	4.2 ± 0.9	UG of *Thelephorales*	10.3 ± 3.4	11.2 ± 4.5	9.2 ± 3.3
Cadophora	3.3 ± 0.2	3.3 ± 0.6	Unidentified	8.1 ± 1.1	7.2 ± 0.9	6.6 ± 0.8
Geminibasidium	3.3 ± 0.1	3.0 ± 0.4	*Meliniomyces*	5.5 ± 0.6	3.7 ± 0.7	4.2 ± 0.3
UG of *Helotiales*	2.9 ± 0.3	2.6 ± 0.2	*Cryptococcus*	4.5 ± 0.5	3.6 ± 0.3	3.2 ± 0.1
UG of *Sebacinales*	2.2 ± 0.1	2.2 ± 0.1	UG of *Sebacinales*	3.7 ± 1.1	2.8 ± 0.9	5.1 ± 2.3

Table S2 (3/4) - Relative abundance of the most abundant fungal phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_Low exp related samples.

Hymenogaster	1.9 ± 0.4	2.6 ± 0.5	*Paxillus*	3.6 ± 1.9	5.3 ± 3.0	8.6 ± 3.2
Meliniomyces	1.8 ± 0.2	2.0 ± 0.5	*Umbelopsis*	3.2 ± 0.2	5.9 ± 2.0	5.1 ± 0.6
Paxillus	1.6 ± 1.0	1.6 ± 0.4	UG of *Helotiales*	2.8 ± 0.3 x*	1.4 ± 0.2	1.5 ± 0.3
Ramicandelaber	1.5 ± 0.2	1.5 ± 0.2	*Geminibasidium*	2.3 ± 0.4	3.2 ± 0.6	2.4 ± 0.1
Venturia	1.4 ± 0.4	1.2 ± 0.2	UG of *Agaricales*	2.2 ± 0.5	2.5 ± 0.5	2.0 ± 0.7
Lactarius	1.1 ± 0.3	1.1 ± 0.2	UG of *Cantharellales*	2.0 ± 0.9	4.6 ± 2.0	1.5 ± 0.5
Pleurotus	0.78 ± 0.36	1.1 ± 0.3	*Mycena*	1.6 ± 1.5	0.03 ± 0.01	0.0 ± 0.0
Coprinellus	0.02 ± 0.01	3.0 ± 1.8	*Chalara*	1.6 ± 1.6	0.20 ± 0.19	0.03 ± 0.02
Other	18.4 ± 1.9	18.8 ± 1.9	*Ramicandelaber*	1.6 ± 0.6	2.5 ± 0.9	0.80 ± 0.10
Fungal genus_Year1	**WT_Rh_T1**	**MiSSP7_Rh_T1**	*Tuber*	1.5 ± 0.1	0.85 ± 0.35	0.80 ± 0.23
Cadophora	11.8 ± 1.4	15.5 ± 2.4	*Mucor*	1.3 ± 1.1	0.68 ± 0.47	0.10 ± 0.02
Gibberella	8.6 ± 4.2	1.1 ± 1.1	*Venturia*	1.1 ± 0.3 x*	0.48 ± 0.08	0.48 ± 0.04
UG of *Thelephorales*	8.4 ± 2.9	2.2 ± 0.8	*Hebeloma*	0.85 ± 0.32	1.0 ± 0.5	1.2 ± 0.4
Paxillus	7.2 ± 1.2	12.0 ± 2.7	*Cunninghamella*	0.78 ± 0.42	4.1 ± 2.1	1.6 ± 0.6
Meliniomyces	6.9 ± 1.1	3.7 ± 0.4	*Sphaeronaemella*	0.35 ± 0.07	2.2 ± 0.7	1.3 ± 0.5
Unidentified	6.4 ± 1.7	9.5 ± 1.3	*Talaromyces*	0.23 ± 0.05	0.28 ± 0.10	1.3 ± 1.0
Mortierella	6.0 ± 1.6	7.3 ± 0.9	*Coprinellus*	0.17 ± 0.10	2.4 ± 2.3	8.6 ± 4.6
UG of *Hypocreales*	5.9 ± 5.9	0.47 ± 0.30	*Peziza*	0.08 ± 0.03	0.28 ± 0.21	2.1 ± 2.1
Fusarium	3.0 ± 1.3	0.66 ± 0.65	Other	15.0 ± 2.3	11.3 ± 1.8	9.4 ± 1.1
Ramicandelaber	2.5 ± 0.6	4.1 ± 1.1	**Fungal genus_Year1**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
Sphaeronaemella	2.4 ± 1.8	2.3 ± 0.6	*Cadophora*	28.7 ± 4.1 *	18.9 ± 4.3	10.7 ± 4.0
Leptodontidium	2.2 ± 1.6	0.54 ± 0.17	*Meliniomyces*	12.1 ± 0.7	7.8 ± 1.6	6.7 ± 3.5
UG of *Sebacinales*	2.2 ± 0.9	2.1 ± 0.6	Unidentified	9.2 ± 0.5	4.0 ± 1.1	2.4 ± 0.9
Phialocephala	2.0 ± 0.1 *	1.1 ± 0.3	*Phialocephala*	6.9 ± 0.8 *	2.9 ± 1.4	0.9 ± 0.3
UG of *Helotiales*	1.6 ± 0.2	2.6 ± 0.7	UG of *Thelephorales*	6.1 ± 4.7	12.2 ± 7.2	17.5 ± 8.8
Tuber	1.3 ± 1.0	1.0 ± 0.3	*Paxillus*	4.9 ± 4.3	13.7 ± 6.1	22.1 ± 10.3 *
Penicillium	1.1 ± 1.1	0.09 ± 0.02	*Oidiodendron*	4.6 ± 2.1	0.96 ± 0.40	2.3 ± 1.3
UG of *Cantharellales*	1.1 ± 1.0	0.33 ± 0.13	UG of *Sebacinales*	3.7 ± 1.4	2.7 ± 0.8	2.9 ± 2.1
Gyoerffyella	1.1 ± 0.2	0.55 ± 0.16	UG of *Cantharellales*	2.7 ± 1.5	4.9 ± 2.1	6.5 ± 5.5
Rhexocercosporidium	1.0 ± 0.9	1.0 ± 0.2	UG of *Helotiales*	1.9 ± 0.6	2.1 ± 0.6	2.5 ± 1.5
UG of *Agaricales*	0.88 ± 0.24	* 2.3 ± 0.4	*Leptodontidium*	1.9 ± 0.7	0.66 ± 0.22	0.61 ± 0.30
Cryptococcus	0.79 ± 0.27	* 3.5 ± 0.9	*Mortierella*	1.6 ± 0.4	1.2 ± 0.3	1.2 ± 0.5
Cunninghamella	0.76 ± 0.34	1.4 ± 0.5	*Ramicandelaber*	1.6 ± 0.4	0.95 ± 0.27	0.37 ± 0.16
Hebeloma	0.66 ± 0.28	1.3 ± 0.5	*Gyoerffyella*	1.4 ± 0.7	0.18 ± 0.06	0.11 ± 0.05
UG of *Sordariales*	0.22 ± 0.08	1.2 ± 0.6	*Phaeomollisia*	1.1 ± 0.1 *	0.51 ± 0.22	0.13 ± 0.04
Coprinellus	0.21 ± 0.17	7.2 ± 4.4	UG of *Agaricales*	0.90 ± 0.39	1.2 ± 0.5	1.5 ± 1.2
Other	13.3 ± 1.9	14.9 ± 2.8	*Tuber*	0.58 ± 0.35	2.0 ± 0.9	0.89 ± 0.31

Table S2 (4/4) - Relative abundance of the most abundant fungal phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_Low exp related samples.

Fungal genus_Year1	**WT_E_T1**	**MiSSP7_E_T1**	UG of *Sordariales*	0.29 ± 0.20	1.2 ± 1.1	0.69 ± 0.32
UG of *Helotiales*	20.7 ± 15.4	1.9 ± 0.6	*Coprinellus*	0.20 ± 0.06	10.0 ± 9.9	11.5 ± 11.3
Mortierella	10.2 ± 0.8 *	4.1 ± 1.3	*Delicatula*	0.17 ± 0.10	2.3 ± 2.0	1.2 ± 1.2
Paxillus	9.2 ± 1.5	19.9 ± 7.3	*Ceratocystis*	0.01 ± 0.01	1.0 ± 1.0	0.0 ± 0.0
Unidentified	8.3 ± 2.9	7.9 ± 1.4	Other	9.4 ± 0.9	8.4 ± 2.4	6.8 ± 1.8
Gongronella	5.1 ± 5.1	0.01 ± 0.01	**Fungal genus_Year1**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
Coprinellus	4.4 ± 4.4	3.1 ± 1.4	UG of *Cantharellales*	14.0 ± 8.5	16.8 ± 8.6	9.8 ± 5.1
Cadophora	4.4 ± 3.2	6.7 ± 1.4	*Cadophora*	8.3 ± 4.0	12.9 ± 2.9	6.4 ± 1.5
Tuber	2.3 ± 0.6	11.6 ± 5.3	Unidentified	7.7 ± 2.0	4.5 ± 2.4	4.4 ± 0.8
Gibberella	2.2 ± 1.9	0.01 ± 0.0	UG of *Thelephorales*	7.0 ± 4.9	13.0 ± 4.9	14.3 ± 12.7
UG of *Thelephorales*	2.2 ± 0.6	2.9 ± 1.3	*Mycena*	5.6 ± 4.6	0.63 ± 0.37	1.24 ± 0.7
Ramicandelaber	2.2 ± 1.2	1.1 ± 0.3	*Phialocephala*	5.6 ± 3.2	1.8 ± 0.70	1.6 ± 0.5
UG of *Cantharellales*	2.1 ± 0.7	0.93 ± 0.46	*Ramicandelaber*	4.8 ± 2.7	0.44 ± 0.16	0.81 ± 0.39
Meliniomyces	1.9 ± 1.1	2.4 ± 0.6	*Meliniomyces*	4.2 ± 2.2	5.9 ± 1.2	3.1 ± 0.7
UG of *Agaricales*	1.7 ± 0.9	2.4 ± 0.8	*Paxillus*	3.9 ± 2.0	13.5 ± 5.4	20.8 ± 5.8
Cryptococcus	1.4 ± 1.3	1.6 ± 0.5	UG of *Helotiales*	3.6 ± 1.3	2.4 ± 0.6	3.2 ± 1.3
Umbelopsis	1.3 ± 0.3	1.1 ± 0.4	*Fusarium*	3.4 ± 3.3	0.0 ± 0.0	0.92 ± 0.90
UG of *Sebacinales*	1.1 ± 0.9	2.1 ± 0.5	*Leptodontidium*	3.0 ± 2.0	0.29 ± 0.11	0.29 ± 0.13
Geminibasidium	1.1 ± 1.1	0.68 ± 0.27	*Mortierella*	2.9 ± 1.5	1.0 ± 0.4	1.3 ± 0.7
UG of *Pleosporales*	1.1 ± 0.4	0.48 ± 0.18	UG of *Pleosporales*	1.9 ± 1.5	0.35 ± 0.27	1.0 ± 0.3
UG of *Sordariales*	1.1 ± 0.1	9.3 ± 8.9	*Menispora*	1.9 ± 1.8	0.10 ± 0.09	0.08 ± 0.05
Phialocephala	0.32 ± 0.23	1.9 ± 1.5	*Sphaeronaemella*	1.8 ± 1.3	0.10 ± 0.04	0.11 ± 0.05
Hebeloma	0.30 ± 0.09	2.3 ± 1.5	*Coprinellus*	1.6 ± 1.0	8.8 ± 8.8	5.7 ± 4.9
Mycena	0.23 ± 0.02	2.5 ± 1.7	*Umbelopsis*	1.6 ± 0.7 x	0.18 ± 0.05	0.49 ± 0.16
Sphaeronaemella	0.08 ± 0.06	1.0 ± 0.37	*Tuber*	1.5 ± 0.6	0.92 ± 0.47	2.4 ± 0.2
Other	14.8 ± 1.2	12.1 ± 3.3	UG of *Sebacinales*	1.3 ± 0.5	3.8 ± 1.1	3.3 ± 1.7
			Cunninghamella	1.0 ± 0.7	0.02 ± 0.01	0.15 ± 0.08
			UG of *Agaricales*	0.72 ± 0.24	3.1 ± 2.2	0.55 ± 0.18
			Hebeloma	0.21 ± 0.08	2.9 ± 1.5	0.31 ± 0.13
			Cladosporium	0.13 ± 0.08	0.01 ± 0.0	7.6 ± 7.2
			Other	12.2 ± 2.3	6.2 ± 2.8	9.9 ± 4.0

Table S3 (1/3) - Relative abundance of the most abundant bacterial phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7 _Low exp related samples.

Bacterial phylum_Year 1	**WT_BS_T1**	**MiSSP7_BS_T1**	**Bacterial phylum_Year 1**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
Actinobacteria	3.9 ± 0.4	3.8 ± 1.2	Actinobacteria	5.9 ± 1.6	3.7 ± 0.1	5.6 ± 1.0
Proteobacteria_*Betaproteobacteria*	7.8 ± 0.4	7.8 ± 1.7	Proteobacteria_*Betaproteobacteria*	6.2 ± 0.3	5.3 ± 0.2	6.5 ± 0.8
Saccharibacteria	1.2 ± 0.1	1.2 ± 0.4	Saccharibacteria	1.8 ± 0.6	2.5 ± 0.3	1.6 ± 0.1
Proteobacteria_*Alphaproteobacteria*	8.4 ± 0.4	8.9 ± 0.8	Proteobacteria_*Alphaproteobacteria*	10.2 ± 0.6	9.4 ± 0.4	10.9 ± 1.2
Bacteroidetes	6.9 ± 0.8	6.1 ± 0.8	Bacteroidetes	5.3 ± 1.1	5.3 ± 0.3	4.5 ± 0.6
Proteobacteria_*Gammaproteobacteria*	6.2 ± 0.2	6.9 ± 0.9	Proteobacteria_*Gammaproteobacteria*	7.4 ± 0.5	6.1 ± 0.3	6.6 ± 1.1
Acidobacteria	32.7 ± 0.9	32.8 ± 3.1	Acidobacteria	30.9 ± 3.3	34.8 ± 0.6	32.2 ± 2.9
Chloroflexi	8.6 ± 0.8	8.4 ± 0.6	Chloroflexi	9.8 ± 0.6	9.0 ± 0.3	8.4 ± 0.8
Verrucomicrobia	9.1 ± 0.5	8.9 ± 0.6	Verrucomicrobia	7.4 ± 0.7	9.1 ± 0.5	8.4 ± 1.4
Planctomycetes	4.3 ± 0.2	4.3 ± 0.3	Planctomycetes	4.8 ± 0.1	4.3 ± 0.2	4.4 ± 0.2
Proteobacteria_*Deltaproteobacteria*	2.7 ± 0.1	2.9 ± 0.1	Proteobacteria_*Deltaproteobacteria*	2.7 ± 0.1	2.8 ± 0.2	2.7 ± 0.1
WD272	1.9 ± 0.1	1.7 ± 0.1	WD272	2.2 ± 0.2	1.9 ± 0.1	1.9 ± 0.1
Gemmatimonadetes	1.7 ± 0.1	1.7 ± 0.2	Gemmatimonadetes	1.4 ± 0.2	1.5 ± 0.1	2.4 ± 0.1 *
Proteobacteria	0.41 ± 0.02	0.40 ± 0.04	Proteobacteria	0.59 ± 0.06	0.53 ± 0.04	0.5 ± 0.1
Firmicutes	0.32 ± 0.05	0.39 ± 0.10	Firmicutes	0.30 ± 0.14	0.19 ± 0.03	0.17 ± 0.02
TM6	0.08 ± 0.01	0.06 ± 0.01	TM6	0.11 ± 0.02	0.10 ± 0.01	0.14 ± 0.02
Other	3.6 ± 0.1	3.5 ± 0.3	Other	2.7 ± 0.2	3.1 ± 0.1	2.6 ± 0.1
Bacterial phylum_Year 1	**WT_AS_T1**	**MiSSP7_AS_T1**	**Bacterial phylum_Year 1**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
Actinobacteria	4.3 ± 0.3	4.2 ± 0.4	Actinobacteria	7.2 ± 0.6	6.3 ± 0.7	6.6 ± 1.1
Proteobacteria_*Betaproteobacteria*	7.6 ± 0.5	8.5 ± 0.5	Proteobacteria_*Betaproteobacteria*	7.0 ± 0.2	7.2 ± 0.6	7.8 ± 0.7
Saccharibacteria	0.88 ± 0.06	0.76 ± 0.09	Saccharibacteria	2.5 ± 0.7	3.1 ± 0.6	2.0 ± 0.1
Proteobacteria_*Alphaproteobacteria*	8.4 ± 0.2	9.0 ± 0.7	Proteobacteria_*Alphaproteobacteria*	9.6 ± 0.4	10.3 ± 0.6	10.3 ± 0.4
Bacteroidetes	8.7 ± 0.7	7.9 ± 0.8	Bacteroidetes	4.9 ± 0.4	7.2 ± 1.0	6.6 ± 1.1
Proteobacteria_*Gammaproteobacteria*	6.9 ± 0.3	7.9 ± 1.1	Proteobacteria_*Gammaproteobacteria*	6.2 ± 0.4	6.5 ± 0.5	7.7 ± 0.8
Acidobacteria	30.3 ± 0.6	30.5 ± 0.7	Acidobacteria	30.8 ± 0.3	27.5 ± 1.3	29.7 ± 2.0
Chloroflexi	7.4 ± 0.4	6.4 ± 0.5	Chloroflexi	9.2 ± 0.6	8.5 ± 0.6	7.4 ± 0.7
Verrucomicrobia	9.7 ± 0.5	9.1 ± 1.2	Verrucomicrobia	8.6 ± 0.9	9.5 ± 0.6	8.1 ± 1.4
Planctomycetes	4.5 ± 0.1	4.8 ± 0.3	Planctomycetes	4.5 ± 0.1	4.6 ± 0.4	3.9 ± 0.3
Proteobacteria_*Deltaproteobacteria*	2.8 ± 0.1	2.8 ± 0.1	Proteobacteria_*Deltaproteobacteria*	2.3 ± 0.1	2.1 ± 0.1	2.1 ± 0.0
WD272	1.4 ± 0.1	1.4 ± 0.1	WD272	1.3 ± 0.1	0.99 ± 0.14	1.3 ± 0.1
Gemmatimonadetes	2.4 ± 0.2	2.0 ± 0.3	Gemmatimonadetes	1.9 ± 0.1	1.9 ± 0.1	2.4 ± 0.2
Proteobacteria	0.52 ± 0.02	0.54 ± 0.01	Proteobacteria	0.55 ± 0.05	0.69 ± 0.04	0.63 ± 0.04
Firmicutes	0.22 ± 0.02	0.25 ± 0.08	Firmicutes	0.22 ± 0.09	0.32 ± 0.07	0.15 ± 0.05
TM6	0.09 ± 0.01	0.09 ± 0.00	TM6	0.07 ± 0.01	0.12 ± 0.01	0.14 ± 0.04
Other	3.6 ± 0.1	3.5 ± 0.2	Other	2.7 ± 0.1	2.9 ± 0.2	2.8 ± 0.1
Bacterial phylum_Year 1	**WT_Rh_T1**	**MiSSP7_Rh_T1**	**Bacterial phylum_Year 1**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
Actinobacteria	14.6 ± 2.1	20.9 ± 7.6	Actinobacteria	29.5 ± 3.4 *	17.3 ± 2.3	17.6 ± 2.7
Proteobacteria_*Betaproteobacteria*	34.1 ± 3.2	26.7 ± 2.9	Proteobacteria_*Betaproteobacteria*	21.6 ± 3.8	25.4 ± 2.3	22.0 ± 3.1
Saccharibacteria	1.2 ± 0.2	1.1 ± 0.5	Saccharibacteria	17.9 ± 4.4	11.3 ± 2.4	7.3 ± 1.3
Proteobacteria_*Alphaproteobacteria*	11.6 ± 1.16	13.4 ± 2.3	Proteobacteria_*Alphaproteobacteria*	11.1 ± 3.5	15.4 ± 2.0	15.9 ± 2.8
Bacteroidetes	13 ± 3.59	12.3 ± 6.2	Bacteroidetes	5.1 ± 0.9	8.4 ± 0.9	5.6 ± 0.2
Proteobacteria_*Gammaproteobacteria*	8.9 ± 3.07	6.0 ± 1.1	Proteobacteria_*Gammaproteobacteria*	4.9 ± 0.1	5.3 ± 0.3	6.0 ± 0.6
Acidobacteria	4.69 ± 0.67	6.9 ± 1.6	Acidobacteria	3.8 ± 0.5	6.6 ± 1.1	11.4 ± 3.3 *
Chloroflexi	1.56 ± 0.3	1.8 ± 0.6	Chloroflexi	1.8 ± 0.2	2.3 ± 0.4	3.1 ± 0.6
Verrucomicrobia	3.79 ± 0.57	4.2 ± 1.7	Verrucomicrobia	1.3 ± 0.5	2.8 ± 0.5	3.8 ± 1.1
Planctomycetes	2.95 ± 0.5	3.5 ± 1.2	Planctomycetes	1.0 ± 0.3	1.9 ± 0.2	2.7 ± 0.5 *
Proteobacteria_*Deltaproteobacteria*	0.69 ± 0.14	0.76 ± 0.21	Proteobacteria_*Deltaproteobacteria*	0.25 ± 0.10	0.52 ± 0.10	0.89 ± 0.25 *
WD272	0.2 ± 0.03	0.29 ± 0.09	WD272	0.18 ± 0.02	0.28 ± 0.07	0.45 ± 0.19
Gemmatimonadetes	0.26 ± 0.05	0.28 ± 0.10	Gemmatimonadetes	0.11 ± 0.03	0.30 ± 0.08	0.69 ± 0.26
Proteobacteria	0.15 ± 0.03	0.33 ± 0.04 *	Proteobacteria	0.09 ± 0.02	0.19 ± 0.04	0.27 ± 0.06
Firmicutes	0.11 ± 0.04	0.10 ± 0.04	Firmicutes	0.07 ± 0.01	0.10 ± 0.03	0.12 ± 0.03
TM6	0.06 ± 0.02	0.04 ± 0.02	TM6	0.05 ± 0.02	0.17 ± 0.06	0.23 ± 0.18
Other	1.87 ± 0.84	1.1 ± 0.4	Other	0.84 ± 0.21	1.4 ± 0.2	1.8 ± 0.1

Table S3 (2/3) - Relative abundance of the most abundant bacterial phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7 _Low exp related samples.

Bacterial phylum_Year 1	**WT_E_T1**	**MiSSP7_E_T1**	**Bacterial phylum_Year 1**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
Actinobacteria	15.0 ± 2.4	17.6 ± 3.9	Actinobacteria	44.4 ± 11.2	27.5 ± 3.6	32.4 ± 5.5
Proteobacteria_*Betaproteobacteria*	37.8 ± 4.4	26.4 ± 7.6	Proteobacteria_*Betaproteobacteria*	11.0 ± 0.6	21.1 ± 2.5	22.4 ± 5.6
Saccharibacteria	0.93 ± 0.18 *	0.23 ± 0.06	Saccharibacteria	11.5 ± 5.1	4.2 ± 0.8	4.5 ± 2.4
Proteobacteria_*Alphaproteobacteria*	9.2 ± 1.3	14.7 ± 2.1	Proteobacteria_*Alphaproteobacteria*	11.0 ± 3.9	14.9 ± 2.0	14.8 ± 7.5
Bacteroidetes	3.9 ± 0.9 *	1.3 ± 0.4	Bacteroidetes	4.7 ± 3.5	6.3 ± 2.2	3.1 ± 2.4
Proteobacteria_*Gammaproteobacteria*	9.0 ± 1.6	7.5 ± 1.1	Proteobacteria_*Gammaproteobacteria*	7.4 ± 1.7	7.7 ± 1.3	9.8 ± 2.7
Acidobacteria	5.2 ± 1.5	3.5 ± 0.4	Acidobacteria	1.5 ± 0.5	4.2 ± 0.8	2.0 ± 0.8
Chloroflexi	1.2 ± 0.3	0.71 ± 0.04	Chloroflexi	1.3 ± 0.7	1.6 ± 0.4	0.97 ± 0.33
Verrucomicrobia	4.4 ± 0.7	5.0 ± 0.9	Verrucomicrobia	1.6 ± 0.4	4.3 ± 0.7	1.5 ± 0.2
Planctomycetes	6.1 ± 1.1	8.7 ± 1.5	Planctomycetes	1.7 ± 0.5	2.8 ± 0.3	2.5 ± 0.3
Proteobacteria_*Deltaproteobacteria*	1.1 ± 0.2	1.9 ± 0.4	Proteobacteria_*Deltaproteobacteria*	0.38 ± 0.18	0.75 ± 0.11	0.49 ± 0.05
WD272	0.29 ± 0.05	0.26 ± 0.06	WD272	0.06 ± 0.01	0.23 ± 0.07	0.11 ± 0.02
Gemmatimonadetes	0.29 ± 0.11	0.25 ± 0.09	Gemmatimonadetes	0.06 ± 0.02	0.18 ± 0.05	0.10 ± 0.02
Proteobacteria	0.16 ± 0.07	1.0 ± 0.5	Proteobacteria	0.08 ± 0.04	0.21 ± 0.04	0.05 ± 0.01
Firmicutes	2.9 ± 1.2	7.2 ± 0.5 *	Firmicutes	1.7 ± 0.8	0.60 ± 0.12	2.3 ± 0.4
TM6	0.55 ± 0.28	2.1 ± 0.7	TM6	0.63 ± 0.31	1.6 ± 0.5	1.8 ± 1.9
Other	1.8 ± 0.9	1.4 ± 0.7	Other	0.58 ± 0.22	1.4 ± 0.2	0.70 ± 0.19
Bacterial genus_Year 1	**WT_BS_T1**	**MiSSP7_BS_T1**	**Bacterial genus_Year 1**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
Unidentified	38.4 ± 0.8	38.4 ± 1.3	Unidentified	39,2 ± 2,0	41,1 ± 0,4	38,4 ± 0,9
UG of *Acidobacteriaceae (Subgroup 1)*	10.2 ± 0.6	10.3 ± 2.2	UG of *Acidobacteriaceae (Subgroup 1)*	9,1 ± 1,3	10,3 ± 0,2	10.0 ± 1,7
UG of *DA101 soil group*	4.0 ± 0.2	4.3 ± 0.4	UG of *DA101 soil group*	3,4 ± 0,4	4,7 ± 0,3	4,8 ± 0,8
Bryobacter	2.6 ± 0.1	2.4 ± 0.1	*Bryobacter*	2,8 ± 0,3	3,1 ± 0,2	2,5 ± 0,2
UG of *Chitinophagaceae*	2.2 ± 0.2	2.0 ± 0.2	*Candidatus Solibacter*	2,4 ± 0,4	2,6 ± 0,1	2,4 ± 0,2
Candidatus Solibacter	2.2 ± 0.1	1.9 ± 0.1	UG of *Chitinophagaceae*	2,7 ± 0,2	2,4 ± 0,1	2,3 ± 0,2
Rhizomicrobium	1.9 ± 0.1	1.9 ± 0.1	*Rhizomicrobium*	2,3 ± 0,2	2,5 ± 0,1	2,2 ± 0,1
UG of *Planctomycetaceae*	1.6 ± 0.1	1.7 ± 0.2	*Acidothermus*	1,7 ± 0,1	1,4 ± 0,1	1,8 ± 0,3
Massilia	0.96 ± 0.26	1.6 ± 0.8	UG of *DA111*	1,5 ± 0,2	1,3 ± 0,2	1,8 ± 0,4
Mucilaginibacter	1.7 ± 0.2	1.6 ± 0.3	UG of *Planctomycetaceae*	1,8 ± 0,1	1,6 ± 0,1	1,7 ± 0,1
UG of *DA111*	1.3 ± 0.2	1.6 ± 0.3	UG of *Xanthomonadaceae*	1,8 ± 0,2	1,4 ± 0,1	1,6 ± 0,1
UG of *Xanthomonadaceae*	1.5 ± 0.1	1.5 ± 0.2	*Burkholderia*	1,4 ± 0,2	0,88 ± 0,07	1,5 ± 0,5
UG of *Cytophagaceae*	1.8 ± 0.2	1.5 ± 0.2	UG of *Gemmatimonadaceae*	1,0 ± 0,1	0,99 ± 0,08	1,5 ± 0,1
Acidothermus	1.3 ± 0.2	1.5 ± 0.5	*Bradyrhizobium*	1,7 ± 0,1	1,4 ± 0,1	1,4 ± 0,1
Bradyrhizobium	1.1 ± 0.1	1.2 ± 0.2	*Mucilaginibacter*	1,4 ± 0,4	1,4 ± 0,1	1,1 ± 0,1
Burkholderia	1.3 ± 0.1	1.2 ± 0.3	*Dyella*	0,72 ± 0,12	0,65 ± 0,09	1,1 ± 0,6
Dyella	0.65 ± 0.08	1.1 ± 0.6	*Holophaga*	0,99 ± 0,18	0,74 ± 0,20	1,0 ± 0,3
Holophaga	1.1 ± 0.2	1.0 ± 0.3	*Streptosporangium*	1,1 ± 1,1	0.0 ± 0.0	0.0 ± 0.0
UG of *Nitrosomonadaceae*	1.0 ± 0.1	0.78 ± 0.19	Other	22,4 ± 1,6	21.0 ± 0,4	22.0 ± 1,1
Other	23.0 ± 0.6	22.2 ± 0.7	**Bacterial genus_Year 1**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
Bacterial genus_Year 1	**WT_AS_T1**	**MiSSP7_AS_T1**	Unidentified	40.6 ± 0.4	37.4 ± 0.9	37.8 ± 0.8
Unidentified	36.7 ± 0.6	35.3 ± 0.6	UG of *Acidobacteriaceae (Subgroup 1)*	7.9 ± 0.3	7.8 ± 0.5	8.7 ± 1.4
UG of *Acidobacteriaceae (Subgroup 1)*	8.9 ± 0.3	9.5 ± 0.8	UG of *DA101 soil group*	3.8 ± 0.5	4.8 ± 0.5	3.8 ± 0.6
UG of *DA101 soil group*	4.6 ± 0.2	4.0 ± 0.6	*Burkholderia*	3.0 ± 0.4	3.6 ± 0.5	3.4 ± 0.6
Mucilaginibacter	3.2 ± 0.3	2.8 ± 0.5	*Bryobacter*	2.7 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
Bryobacter	2.3 ± 0.1	2.6 ± 0.2	*Acidothermus*	2.5 ± 0.3	2.3 ± 0.2	2.4 ± 0.3
UG of *Chitinophagaceae*	2.1 ± 0.1	2.2 ± 0.1	*Rhizomicrobium*	2.3 ± 0.1	1.9 ± 0.2	2.3 ± 0.2
Burkholderia	1.3 ± 0.1	2.1 ± 0.2	UG of *Chitinophagaceae*	2.1 ± 0.1	1.6 ± 0.2	2.1 ± 0.2
Rhizomicrobium	1.9 ± 0.1	2.0 ± 0.2	*Candidatus Solibacter*	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.2
Candidatus Solibacter	1.8 ± 0.1	1.9 ± 0.1	UG of *Planctomycetaceae*	1.7 ± 0.1	1.8 ± 0.2	1.3 ± 0.2
UG of *Planctomycetaceae*	1.6 ± 0.1	1.8 ± 0.2	*Mucilaginibacter*	1.7 ± 0.2	4.4 ± 0.8 x	3.2 ± 0.5
UG of *Cytophagaceae*	2.0 ± 0.2	1.7 ± 0.2	UG of *DA111*	1.6 ± 0.1	2.1 ± 0.3	1.7 ± 0.2
UG of *Xanthomonadaceae*	1.6 ± 0.1	1.7 ± 0.3	UG of *Xanthomonadaceae*	1.6 ± 0.2	1.4 ± 0.2	1.9 ± 0.3
UG of *DA111*	1.4 ± 0.1	1.5 ± 0.1	UG of *Gemmatimonadaceae*	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.18
Acidothermus	1.6 ± 0.1	1.5 ± 0.2	*Bradyrhizobium*	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.1
UG of *Gemmatimonadaceae*	1.4 ± 0.1	1.2 ± 0.1	UG of *HSB OF53-F07*	1.2 ± 0.2	1.2 ± 0.2	0.82 ± 0.11
Bradyrhizobium	0.87 ± 0.02	1.1 ± 0.2	UG of *Anaerolineaceae*	1.1 ± 0.1	0.89 ± 0.09	0.89 ± 0.12
UG of *Anaerolineaceae*	1.2 ± 0.1	1.1 ± 0.1	*Rhodanobacter*	0.88 ± 0.14	0.80 ± 0.12	1.0 ± 0.2
Holophaga	1.4 ± 0.3	1.1 ± 0.3	Other	20.3 ± 0.1	21.1 ± 0.6	21.5 ± 0.5

Table S3 (3/3) - Relative abundance of the most abundant bacterial phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7 _Low exp related samples.

Rhodanobacter	0.84 ± 0.12	1.1 ± 0.2	**Bacterial genus_Year 1**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
Acidibacter	0.80 ± 0.1	1.0 ± 0.3	Unidentified	22.4 ± 3.4	19.8 ± 2.3	21.3 ± 2.5
Other	22.1 ± 0.5	22.6 ± 0.8	*Burkholderia*	17.0 ± 2.8	17.9 ± 2.1	14.8 ± 2.9
Bacterial genus_Year 1	**WT_Rh_T1**	**MiSSP7_Rh_T1**	*Streptacidiphilus*	14.1 ± 2.5 x*	7.3 ± 0.7	5.7 ± 1.5
Streptacidiphilus	6.2 ± 1.2	10.6 ± 4.8	*Actinospica*	7.1 ± 1.3	3.5 ± 1.1	3.3 ± 1.1
Unidentified	9.2 ± 1.2	10.5 ± 2.4	*Asticcacaulis*	3.4 ± 1.5	3.0 ± 0.6	1.1 ± 0.5
Burkholderia	5.9 ± 1.1	9.4 ± 0.9 *	*Mucilaginibacter*	3.4 ± 0.6	5.5 ± 0.7	2.8 ± 0.6
Mucilaginibacter	5.1 ± 1.5	8.7 ± 5.0	*Aquincola*	2.9 ± 1.3	3.5 ± 0.4	3.1 ± 0.8
Aquincola	5.9 ± 1.4	6.5 ± 2.9	*Rhodanobacter*	2.4 ± 0.2	1.5 ± 0.3	1.4 ± 0.3
Actinospica	2.2 ± 0.7	5.0 ± 2.1	*Streptomyces*	2.3 ± 1.2	0.91 ± 0.37	0.94 ± 0.26
Collimonas	3.1 ± 0.5	4.9 ± 0.7	UG of *Streptomycetaceae*	2.2 ± 0.4 x*	0.97 ± 0.26	0.66 ± 0.22
UG of *Chitinophagaceae*	1.9 ± 0.3	2.2 ± 0.7	*Bradyrhizobium*	1.4 ± 0.4	2.3 ± 0.3	2.9 ± 0.5
Asticcacaulis	0.35 ± 0.13	2.1 ± 1.5	*Phenylobacterium*	1.4 ± 0.7	1.3 ± 0.3	1.2 ± 0.4
Bradyrhizobium	1.7 ± 0.3	1.9 ± 0.5	UG of *Acidobacteriaceae (Subgroup 1)*	1.2 ± 0.2	2.1 ± 0.3	3.5 ± 1.0 *
UG of *DA101 soil group*	1.8 ± 0.4	1.7 ± 0.6	*Rhizobium*	1.1 ± 0.5	2.3 ± 0.9	1.7 ± 0.9
UG of *Acidobacteriaceae (Subgroup 1)*	1.3 ± 0.2	1.6 ± 0.3	UG of *Chitinophagaceae*	0.99 ± 0.20	2.2 ± 0.3	1.9 ± 0.3
UG of *Comamonadaceae*	0.49 ± 0.13	1.6 ± 0.8	*Acidothermus*	0.77 ± 0.03	1.0 ± 0.1	1.8 ± 0.3 *
UG of *Planctomycetaceae*	1.3 ± 0.3	1.5 ± 0.6	*Catenulispora*	0.69 ± 0.05	1.1 ± 0.3	0.86 ± 0.23
Rhodanobacter	0.51 ± 0.09	1.3 ± 0.1 *	*Rhizomicrobium*	0.60 ± 0.16	1.0 ± 0.2	1.6 ± 0.5
Acidothermus	1.1 ± 0.3	1.2 ± 0.4	*Acidibacter*	0.60 ± 0.14	0.97 ± 0.14	1.0 ± 0.1
Pseudomonas	2.1 ± 1.0	1.2 ± 1.0	UG of *DA101 soil group*	0.58 ± 0.22	1.3 ± 0.3	1.8 ± 0.6
Rhizobium	1.9 ± 0.2	1.2 ± 0.6	UG of *Planctomycetaceae*	0.43 ± 0.13	0.72 ± 0.08	1.0 ± 0.2 *
Holophaga	0.54 ± 0.11	1.1 ± 0.5	*Collimonas*	0.41 ± 0.13	1.8 ± 0.5	1.3 ± 0.5
Rhizomicrobium	0.40 ± 0.04	1.1 ± 0.5	Other	12.4 ± 1.3	17.6 ± 1.3	23.8 ± 2.2
Acidovorax	4.4 ± 0.9 *	0.53 ± 0.47	**Bacterial genus_Year 1**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
Massilia	12.2 ± 2.5 *	0.45 ± 0.23	*Streptacidiphilus*	19.2 ± 9.5	14.7 ± 2.3	18.7 ± 5.5
Streptomyces	1.1 ± 0.3 *	0.21 ± 0.12	Unidentified	15.4 ± 4.2	13.6 ± 1.4	11.0 ± 1.6
UG of *Enterobacteriaceae*	3.4 ± 3.3	0.08 ± 0.06	*Burkholderia*	7.4 ± 1.3	14.3 ± 2.7	16.1 ± 4.9
Chryseobacterium	2.9 ± 2.8	0.05 ± 0.05	*Mycobacterium*	5.4 ± 2.9	1.6 ± 0.4	3.2 ± 0.5
Other	22.8 ± 1.8	23.1 ± 4.3	*Streptomyces*	4.4 ± 1.2	2.7 ± 1.6	2.0 ± 0.9
Bacterial genus_Year 1	**WT_E_T1**	**MiSSP7_E_T1**	*Actinospica*	3.7 ± 2.0	2.3 ± 0.8	1.0 ± 0.2
Unidentified	16.0 ± 3.1	10.6 ± 1.9	*Nocardia*	3.5 ± 2.3	0.68 ± 0.27	1.2 ± 0.5
Burkholderia	14.2 ± 5.1	12.5 ± 3.4	UG of *Streptomycetaceae*	3.1 ± 1.9	1.5 ± 0.8	2.4 ± 1.2
Streptacidiphilus	7.3 ± 2.4	8.2 ± 1.8	*Catenulispora*	2.9 ± 2.3	1.0 ± 0.3	1.6 ± 0.8
Collimonas	6.7 ± 4.9	4.7 ± 1.7	*Rhizobium*	2.4 ± 1.4	1.8 ± 0.6	0.69 ± 0.41
Mycobacterium	4.0 ± 0.4	2.1 ± 0.9	UG of *Chitinophagaceae*	1.5 ± 1.1	1.6 ± 0.5	2.1 ± 1.9
UG of Planctomycetaceae	3.7 ± 0.5	2.4 ± 0.6	UG of *Comamonadaceae*	1.2 ± 0.9	0.60 ± 0.29	0.31 ± 0.25
UG of DA101 soil group	2.9 ± 0.7	2.1 ± 0.6	*Mucilaginibacter*	1.2 ± 0.8	1.9 ± 0.6	0.59 ± 0.28
Bacillus	2.7 ± 1.1	1.4 ± 0.6	*Bradyrhizobium*	1.2 ± 0.3	2.3 ± 0.4	1.1 ± 0.3
Bradyrhizobium	2.2 ± 0.3	1.1 ± 0.1	*Sphingobium*	0.96 ± 0.92	0.32 ± 0.28	1.3 ± 1.3
Isosphaera	1.9 ± 0.6	1.3 ± 0.3	UG of *Planctomycetaceae*	0.86 ± 0.26	1.2 ± 0.1	1.2 ± 0.2
Actinospica	1.8 ± 1.1	0.85 ± 0.26	*Dyella*	0.78 ± 0.41	0.82 ± 0.20	4.8 ± 3.7
Azohydromonas	1.4 ± 1.2	0.0 ± 0.0	*Aquincola*	0.76 ± 0.52	2.6 ± 0.4	2.3 ± 0.6
Paenibacillus	1.2 ± 0.6	0.56 ± 0.18	*Bacillus*	0.69 ± 0.28	0.26 ± 0.06	1.1 ± 0.3
Aquincola	1.1 ± 0.3	4.2 ± 1.0 *	UG of *DA101 soil group*	0.67 ± 0.27	1.4 ± 0.3	0.80 ± 0.10
UG of DA111	1.0 ± 0.1 *	0.49 ± 0.13	*Chitinophaga*	0.66 ± 0.64	2.1 ± 1.9	0.02 ± 0.01
Halomonas	0.91 ± 0.04	1.5 ± 0.7	*Asticcacaulis*	0.49 ± 0.21	2.2 ± 0.6	0.64 ± 0.25
Rhizobium	0.86 ± 0.28	1.1 ± 0.2	*Collimonas*	0.43 ± 0.16	1.3 ± 0.4	1.7 ± 0.8
Pseudomonas	0.56 ± 0.27	2.5 ± 1.4	*Acidibacter*	0.31 ± 0.15	0.94 ± 0.11	1.8 ± 1.5
UG of Acidobacteriaceae (Subgroup 1)	0.55 ± 0.12	1.3 ± 0.5	UG of *Oxalobacteraceae*	0.21 ± 0.05	1.0 ± 0.48	0.62 ± 0.23
Massilia	0.46 ± 0.04	9.3 ± 2.6	*Phenylobacterium*	0.14 ± 0.08	1.2 ± 0.3	0.20 ± 0.07
UG of Oxalobacteraceae	0.44 ± 0.14	2.6 ± 1.3	*Rudaea*	0.06 ± 0.04	1.1 ± 0.8	0.05 ± 0.03
Mucilaginibacter	0.29 ± 0.06	2.0 ± 0.6 *	Other	19.5 ± 4.1	22.0 ± 2.2	20.5 ± 5.7
Streptomyces	0.27 ± 0.07	1.4 ± 0.7				
Rhizomicrobium	0.34 ± 0.09	0.34 ± 0.09				
Nocardia	0.32 ± 0.05	0.36 ± 0.27				
UG of Comamonadaceae	0.29 ± 0.05	0.34 ± 0.09				
Acidovorax	0.08 ± 0.00	1.4 ± 0.3 *				
Other	26.0 ± 3.6	23.3 ± 1.8				

Table S4 - Relative abundance of the fungal guilds detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 1. The asterisks denote fungal guild significantly different in relative abundance between WT and MiSSP7_High exp related samples. The crosses denote fungal guild significantly different in relative abundance between WT and MiSSP7 _Low exp related samples.

Fungal guild	**WT_BS_T1**	**MiSSP7_BS_T1**	**Fungal guild**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
AM fungi	0.55 ± 0.02 *	0.35 ± 0.04	AM fungi	0.24 ± 0.06	0.65 ± 0.31	0.23 ± 0.04
EcM fungi	25.8 ± 3.5	19.9 ± 1.3	EcM fungi	20.6 ± 9.4	28.3 ± 10.6	24.4 ± 8.0
Fungal endophyte	19.4 ± 1.5	17.0 ± 0.9	Fungal endophyte	21.7 ± 2.5	18.6 ± 2.2	15.7 ± 0.7
Other	0.64 ± 0.12	0.71 ± 0.08	Other	0.93 ± 0.23	0.66 ± 0.11	0.78 ± 0.26
Plant pathogen	3.3 ± 1.5	3.5 ± 0.8	Plant pathogen	3.0 ± 0.1	3.0 ± 0.5	3.0 ± 1.0
Unclassified	25.3 ± 1.1	28.7 ± 1.4	Unclassified	18.7 ± 1.8	15.6 ± 2.8	21.1 ± 4.9
Undefined Saprotroph	10.4 ± 1.6	13.8 ± 1.6	Undefined Saprotroph	15.2 ± 2.9	13.5 ± 3.5	15.1 ± 5.4
Unidentified	14.4 ± 2.0	15.8 ± 0.9	Unidentified	19.4 ± 3.6	19.4 ± 3.6	19.5 ± 4.3
Fungal guild	**WT_AS_T1**	**MiSSP7_AS_T1**	**Fungal guild**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
AM fungi	0.39 ± 0.11	0.37 ± 0.03	AM fungi	0.15 ± 0.04	0.21 ± 0.02	0.09 ± 0.01
EcM fungi	18.2 ± 2.4	18.3 ± 1.2	EcM fungi	17.2 ± 5.4	19.0 ± 6.4	20.3 ± 1.4
Fungal endophyte	21.2 ± 0.6 *	18.4 ± 0.6	Fungal endophyte	26.6 ± 1.9	22.8 ± 2.8	23.5 ± 2.6
Other	0.85 ± 0.18	0.64 ± 0.07	Other	0.98 ± 0.14	0.74 ± 0.12	0.67 ± 0.09
Plant pathogen	3.0 ± 0.5	3.4 ± 0.4	Plant pathogen	2.5 ± 0.6	1.9 ± 0.4	1.2 ± 0.1
Unclassified	23.5 ± 1.9	25.6 ± 0.9	Unclassified	22.5 ± 2.1	22.1 ± 1.9	16.9 ± 2.8
Undefined Saprotroph	16.3 ± 2.0	17.6 ± 1.6	Undefined Saprotroph	13.6 ± 1.6	20.2 ± 5.0	22.8 ± 3.1
Unidentified	16.3 ± 1.3	15.4 ± 0.4	Unidentified	16.3 ± 2.7	12.9 ± 1.2	14.2 ± 2.3
Fungal guild	**WT_Rh_T1**	**MiSSP7_Rh_T1**	**Fungal guild**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
AM fungi	0.22 ± 0.08	0.61 ± 0.14	AM fungi	0.06 ± 0.03	0.31 ± 0.25	0.04 ± 0.01
EcM fungi	19.8 ± 4.4	17.4 ± 2.8	EcM fungi	12.8 ± 5.9	29.8 ± 7.9	42.6 ± 17.1 *
Fungal endophyte	23.7 ± 2.0	25.1 ± 2.6	Fungal endophyte	39.3 ± 4.3 *x	23.8 ± 5.8	13.6 ± 4.7
Other	0.99 ± 0.37	2.23 ± 0.6	Other	5.0 ± 2.0	1.1 ± 0.4	2.5 ± 1.4
Plant pathogen	11.4 ± 4.6 *	3.9 ± 1.1	Plant pathogen	1.8 ± 0.8	0.52 ± 0.13	0.49 ± 0.35
Unclassified	23.6 ± 3.7	16.8 ± 1.7	Unclassified	19.5 ± 1.8	20.6 ± 2.7	18.1 ± 6.8
Undefined Saprotroph	8.9 ± 1.6	18.3 ± 4.3	Undefined Saprotroph	5.9 ± 0.7	13.5 ± 9.8	13.8 ± 11.1
Unidentified	11.0 ± 1.2	15.3 ± 1.9	Unidentified	15.5 ± 1.5	10.0 ± 2.6	8.6 ± 3.1
Fungal guild	**WT_E_T1**	**MiSSP7_E_T1**	**Fungal guild**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
AM fungi	0.11 ± 0.05	0.19 ± 0.07	AM fungi	0.06 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
EcM fungi	14.9 ± 1.6	38.8 ± 7.8	EcM fungi	13.5 ± 8.2	31.0 ± 9.7	38.0 ± 9.6
Fungal endophyte	15.5 ± 4.9	13.1 ± 2.1	Fungal endophyte	22.4 ± 6.2	16.2 ± 3.0	10.2 ± 1.6
Other	0.98 ± 0.27	0.62 ± 0.23	Other	1.1 ± 0.3	0.50 ± 0.20	2.6 ± 1.8
Plant pathogen	4.1 ± 0.8	2.2 ± 0.7	Plant pathogen	1.2 ± 0.4	0.62 ± 0.23	0.72 ± 0.22
Unclassified	18.1 ± 1.6	13.1 ± 1.5	Unclassified	35.4 ± 10.9	28.6 ± 8.7	25.6 ± 5.0
Undefined Saprotroph	14.2 ± 6.2	19.1 ± 7.9	Undefined Saprotroph	13.0 ± 3.7	11.2 ± 8.6	10.6 ± 5.0
Unidentified	31.8 ± 10.9	12.6 ± 2.1	Unidentified	12.9 ± 2.1	11.5 ± 2.4	12.0 ± 1.5

Table S5 - List of metabolites detected in roots of *Populus tremula x alba* expressing MiSSP7 line harvested after 10 days (T1) and 6.5 weeks (T2) of growth in the two years of experiment. The asterisks denote a significant difference of metabolite concentration which has been measured between Year 1 and Year 2 (* = P<0.05, one way ANOVA). Values indicate fold changes between Year 1 and Year 2. Metabolites highlighted in yellow and green are involved in primary, secondary metabolisms, respectively. The last columns indicate the relative abundance of each metabolite in the total root metabolome of *Populus tremula x alba* expressing MiSSP7 cuttings collected in Year 1 and Year 2 and of *Populus tremula x alba* WT cuttings collected in Year 1 and Year 2.

Metabolite (RT-m/z)	**Plant metabolite**	**Bacterial or fungal metabolite**	***Populus* roots collected in Year 1 vs. Year 2**		**% of all root metabolites of *P. tremula x alba* expressing *MiSSP7 line***	**% of all root metabolites of *P. tremula x alba WT* (Mangeot-Peter et al., 2020)**
			10 days of growth (T1)	**6.5 weeks of growth (T2)**		
sucrose	X		0.02 *	1.15	**22.38**	**30.72**
a-salicyloylsalicin	X		0.01 *	0.96	**12.14**	**14.15**
palmitic acid	X	X	0.18	0.83	**8.97**	**3.31**
malic acid	X	X	1.56	2.1	**7.57**	**9.74**
tremulacin	X		0.01 *	0.01 *	**6.81**	0.12
salicin	X		0.62 *	0.97	**6.67**	**6.67**
glucose	X	X	2.42	5.83 *	**6.37**	**8.11**
tremuloidin	X		0.09 *	1.55	**4.34**	**3.91**
B-sitosterol	X		0.8	2.30 *	**2.56**	**2.09**
fructose	X	X	1.16	3.63 *	**2.53**	**3.72**
7.69 169 101 75 68	?		ND	6.07 *	**1.81**	**1.65**
succinic acid	X	X	26.49	1.77	**1.50**	0.2
catechin	X		1.89	2.06	**1.44**	**1.68**
phosphate	X	X	2.64	2.19	**1.42**	**1.31**
galactose	X	X	0.99	2.43 *	**1.07**	**1.26**

Table S6 (1/3) - Relative abundance of the most abundant fungal phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 2. The asterisks denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_High exp exp related samples. The cross denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_Low exp exp related samples.

Fungal phylum_Year2	**WT_BS_T1**	**MiSSP7_Low exp_BS_T1**	**MiSSP7_High exp_BS_T1**	**Fungal phylum_Year2**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
Ascomycota	75.4 ± 1.5	81.7 ± 2.8	70.0 ± 8.8	Ascomycota	74.3 ± 3.2	75.3 ± 3.0 x	61.4 ± 2.9 *
Basidiomycota	18.0 ± 1.8	12.1 ± 1.7	22.9 ± 9.5	Basidiomycota	18.3 ± 1.9	18.1 ± 2.2	29.8 ± 2.5 *
Rozellomycota	2.7 ± 0.1	2.1 ± 0.3	3.2 ± 0.5	unidentified	3.1 ± 0.5	2.2 ± 0.2	3.8 ± 0.4
Zygomycota	0.12 ± 0.03	0.16 ± 0.06	0.08 ± 0.04	Zygomycota	0.41 ± 0.11	0.40 ± 0.12	0.64 ± 0.28
Chytridiomycota	0.02 ± 0.0	0.08 ± 0.03	0.09 ± 0.05	Glomeromycota	0.09 ± 0.04	0.14 ± 0.06	0.06 ± 0.01
Glomeromycota	0.18 ± 0.09	0.1 ± 0.04	0.25 ± 0.11	Chytridiomycota	0.0 ± 0.0	0.03 ± 0.01	0.01 ± 0.0
Unidentified	3.4 ± 0.8	3.6 ± 0.6	3.3 ± 0.7	Rozellomycota	3.6 ± 0.6	3.7 ± 0.7	4.2 ± 0.7
Fungal phylum_Year2	**WT_AS_T1**	**MiSSP7_Low exp_AS_T1**	**MiSSP7_High exp_AS_T1**	**Fungal phylum_Year2**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
Ascomycota	69.0 ± 1.2	67.4 ± 0.8	67.8 ± 4.5	Ascomycota	76.7 ± 2.7	77.5 ± 3.2	75.7 ± 2.1
Basidiomycota	21.2 ± 0.8	24.2 ± 0.8	22.2 ± 4.7	Basidiomycota	19.6 ± 2.4	16.6 ± 2.4	19.1 ± 1.9
Rozellomycota	2.9 ± 0.2	2.2 ± 0.2	3.4 ± 1.1	unidentified	2.2 ± 0.2	3.9 ± 0.8	3.8 ± 0.3
Zygomycota	0.3 ± 0.09	0.28 ± 0.12	0.13 ± 0.02	Zygomycota	0.14 ± 0.04	0.17 ± 0.07	0.21 ± 0.04
Chytridiomycota	0.08 ± 0.04	0.12 ± 0.05	0.06 ± 0.03	Glomeromycota	0.08 ± 0.03	0.07 ± 0.03	0.09 ± 0.02
Glomeromycota	0.28 ± 0.05	0.21 ± 0.05	0.06 ± 0.03	Chytridiomycota	0.0 ± 0.0	0.05 ± 0.01 x	0.0 ± 0.0
Unidentified	6.0 ± 1.0		6.2 ± 1.3	Rozellomycota	1.2 ± 0.1	1.6 ± 0.4	1.0 ± 0.1
Fungal phylum_Year2	**WT_Rh_T1**	**MiSSP7_Low exp_Rh_T1**	**MiSSP7_High exp_Rh_T1**	**Fungal phylum_Year2**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
Ascomycota	91.7 ± 2.1	89.9 ± 7.1	95.7 ± 1.26	Ascomycota	97.9 ± 0.6	97.3 ± 1.1	95.0 ± 1.3
Basidiomycota	1.2 ± 0.4	0.93 ± 0.51	0.37 ± 0.15	Basidiomycota	0.32 ± 0.13	0.19 ± 0.16	0.60 ± 0.37
Rozellomycota	0.13 ± 0.04	0.06 ± 0.02	0.09 ± 0.02	unidentified	1.6 ± 0.6	2.4 ± 0.9	3.5 ± 1.4
Zygomycota	0.08 ± 0.07	0.05 ± 0.01	0.06 ± 0.04	Zygomycota	0.08 ± 0.03	0.01 ± 0.01	0.76 ± 0.49
Chytridiomycota	0.0 ± 0.0	0.13 ± 0.11	0.0 ± 0.0	Glomeromycota	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Glomeromycota	0.05 ± 0.03	0.0 ± 0.0	0.04 ± 0.02	Chytridiomycota	0.0 ± 0.0	0.01 ± 0.01	0.0 ± 0.0
Unidentified	6.8 ± 1.2	8.9 ± 1.0	3.7 ± 0.3	Rozellomycota	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.1
Fungal phylum_Year2	**WT_E_T1**	**MiSSP7_Low exp_E_T1**	**MiSSP7_High exp_E_T1**	**Fungal phylum_Year2**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
Ascomycota	54.0 ± 10.4	63.0 ± 13.2	61.1 ± 11.4	Ascomycota	34.4 ± 12.3	33.4 ± 14.1	54.5 ± 10.3
Basidiomycota	44.6 ± 11.0	35.4 ± 12.6	38.1 ± 12.0	Basidiomycota	36.0 ± 13.1	65.4 ± 13.9	40.2 ± 11.4
Rozellomycota	0.0 ± 0.0	0.24 ± 0.24	0.0 ± 0.0	unidentified	29.1 ± 16.3	0.69 ± 0.55	4.8 ± 2.0
Zygomycota	0.19 ± 0.11	0.02 ± 0.02	0.02 ± 0.01	Zygomycota	0.16 ± 0.11	0.42 ± 0.16	0.10 ± 0.04
Chytridiomycota	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Glomeromycota	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Glomeromycota	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Chytridiomycota	0.16 ± 0.16	0.0 ± 0.0	0.0 ± 0.0
Unidentified	1.1 ± 0.2	1.2 ± 0.3	0.70 ± 0.10	Rozellomycota	0.0 ± 0.0	0.0 ± 0.0	0.16 ± 0.01

Table S6 (2/3) - Relative abundance of the most abundant fungal phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 2. The asterisks denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_High exp exp related samples. The cross denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_Low exp exp related samples.

Fungal genus_Year2	**WT_BS_T1**	**MiSSP7_Low exp_BS_T1**	**MiSSP7_High exp_BS_T1**	**Fungal genus_Year2**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
Unidentified	36.1 ± 2.0	39.8 ± 4.9	34.7 ± 3.9	Unidentified	38.6 ± 3.6	33.3 ± 3.5	30.8 ± 1.5
Cryptococcus	12.6 ± 1.0 x	9.0 ± 0.7	10.5 ± 1.3	*Cryptococcus*	14.6 ± 1.8	16.9 ± 1.8	27.4 ± 2.3 *
Laccaria	2.8 ± 1.6	1.4 ± 1.2	9.8 ± 9.2	*Cadophora*	6.3 ± 1.2	8.1 ± 3.2	4.8 ± 0.9
Cadophora	8.4 ± 1.3	8.1 ± 1.7	9.0 ± 1.8	*Oidiodendron*	2.5 ± 0.5	1.9 ± 0.2	2.7 ± 0.2
Fusicladium	3.5 ± 1.0	1.8 ± 0.4	3.4 ± 1.6	*Trichoderma*	3.1 ± 1.1	2.4 ± 0.3	2.4 ± 0.3
UG of *Venturiaceae*	1.9 ± 0.4	1.4 ± 0.5	2.5 ± 1.0	*Penicillium*	2.3 ± 0.4	2.2 ± 0.4	2.3 ± 0.2
Tuber	1.4 ± 0.5	3.2 ± 1.4	2.3 ± 0.9	*Fusicladium*	2.0 ± 0.7	2.0 ± 0.4	2.3 ± 0.5
Trichoderma	1.3 ± 0.3	0.98 ± 0.21	1.8 ± 0.6	UG of *Venturiaceae*	1.7 ± 0.6	2.0 ± 0.5	2.2 ± 0.7
Meliniomyces	0.72 ± 0.10	0.85 ± 0.28	1.6 ± 0.2	*Conlarium*	4.0 ± 2.5	7.1 ± 4.6	1.7 ± 0.7
Penicillium	1.8 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	*Chlorencoelia*	1.3 ± 0.2	1.2 ± 0.2	1.6 ± 0.3
Oidiodendron	2.4 ± 0.4	1.7 ± 0.4	1.5 ± 0.1	*Humicola*	1.2 ± 0.1	0.75 ± 0.22	1.4 ± 0.2
Exophiala	1.1 ± 0.1	0.72 ± 0.18	1.2 ± 0.3	*Hymenoscyphus*	2.8 ± 2.0	0.28 ± 0.13	1.2 ± 0.9
UG of *Pezizaceae*	0.84 ± 0.35	0.66 ± 0.32	1.1 ± 0.6	*Exophiala*	1.3 ± 0.2	1.1 ± 0.1	1.1 ± 0.2
UG of *Inocybaceae*	0.59 ± 0.18	0.39 ± 0.09	1.1 ± 0.6	*Wilcoxina*	1.1 ± 0.3	1.3 ± 0.2	1.0 ± 0.1
Chlorencoelia	1.3 ± 0.1 x	0.67 ± 0.18	1.0 ± 0.1	*Neobulgaria*	0.28 ± 0.09	3.8 ± 3.3	1.0 ± 0.4
Hymenoscyphus	4.1 ± 2.4	0.35 ± 0.1	1.0 ± 0.6	*Leptodontidium*	1.4 ± 0.5	1.2 ± 0.2	1.0 ± 0.1
Cladophialophora	2.0 ± 0.9	1.1 ± 0.3	0.99 ± 0.24	*Clathrosphaerina*	0.46 ± 0.16	1.3 ± 0.5	0.66 ± 0.15
Mycosphaerella	1.5 ± 0.3	3.9 ± 3.2	0.78 ± 0.13	*Cladophialophora*	1.2 ± 0.4	1.2 ± 0.4	0.32 ± 0.09
Conlarium	1.2 ± 0.2	0.92 ± 0.40	0.74 ± 0.09	Other	13.8 ± 1.9	12.0 ± 1.9	14.1 ± 3.0
Other	14.3 ± 2.2	21.5 ± 0.7	13.5 ± 1.9	**Fungal genus_Year2**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
Fungal genus_Year2	**WT_AS_T1**	**MiSSP7_Low exp_AS_T1**	**MiSSP7_High exp_AS_T1**	Unidentified	19.3 ± 1.7	20.2 ± 3.1	22.5 ± 1.2
Unidentified	31.2 ± 1.5	32.7 ± 0.6	36.2 ± 2.8	*Cadophora*	22.6 ± 3.4	35.5 ± 9.6	17.7 ± 5.0
Cryptococcus	18.1 ± 0.6	21.5 ± 0.7	19.9 ± 4.7	*Cryptococcus*	18.1 ± 2.4	13.3 ± 2.6	17.0 ± 1.7
Cadophora	3.6 ± 1.1	4.4 ± 0.4	3.9 ± 1.0	*Trichoderma*	13.0 ± 6.0	2.5 ± 1.0	9.5 ± 2.9
Humicola	1.1 ± 0.1	1.7 ± 0.5	2.8 ± 1.7	*Hymenoscyphus*	0.20 ± 0.05	0.12 ± 0.06	3.0 ± 2.5
Oidiodendron	2.8 ± 0.2	2.9 ± 0.3	2.5 ± 0.2	*Penicillium*	3.0 ± 0.55	1.8 ± 0.3	2.4 ± 0.1
Penicillium	3.8 ± 0.5	3.2 ± 0.1	2.3 ± 0.3	*Humicola*	0.71 ± 0.17	0.79 ± 0.26	2.3 ± 0.8
Chlorencoelia	2.5 ± 0.2	2.4 ± 0.3	2.1 ± 0.8	*Oidiodendron*	2.4 ± 0.4	1.9 ± 0.3	2.0 ± 0.2
Trichoderma	2.7 ± 0.3	2.3 ± 0.1	1.9 ± 0.1	*Chlorencoelia*	1.7 ± 0.3	0.98 ± 0.38	1.4 ± 0.2
Conlarium	0.7 ± 0.1	1.3 ± 0.2	1.8 ± 0.4	*Mycosphaerella*	0.91 ± 0.28	0.93 ± 0.47	1.2 ± 0.9
Mycosphaerella	6.2 ± 2.2	2.1 ± 0.3	1.3 ± 0.2	*Wilcoxina*	0.86 ± 0.13	0.95 ± 0.33	1.2 ± 0.2
UG of *Chaetomiaceae*	0.76 ± 0.46	0.67 ± 0.29	1.3 ± 0.8	*Leptodontidium*	0.50 ± 0.06	0.94 ± 0.11	1.1 ± 0.5
Exophiala	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	*Conlarium*	0.89 ± 0.17	1.3 ± 0.3	1.1 ± 0.2
Wilcoxina	1.1 ± 0.3	1.3 ± 0.3	1.1 ± 0.4	UG of *Chaetomiaceae*	0.24 ± 0.05	0.24 ± 0.09	1.1 ± 0.3
Clathrosphaerina	0.46 ± 0.13	1.2 ± 0.4	1.1 ± 0.4	UG of *Helotiaceae*	0.44 ± 0.13	0.22 ± 0.07	1.0 ± 0.9
Tuber	2.0 ± 1.6	0.97 ± 0.38	0.94 ± 0.15	*Exophiala*	1.2 ± 0.3	0.63 ± 0.10	0.98 ± 0.09
Fusicladium	0.83 ± 0.11	1.0 ± 0.3	0.90 ± 0.36	UG of *Venturiaceae*	0.39 ± 0.08	1.8 ± 1.0	0.91 ± 0.36
UG of *Trichocomaceae*	1.1 ± 0.2	1.2 ± 0.2	0.87 ± 0.09	*Meliniomyces*	1.2 ± 0.1	1.7 ± 0.6	0.83 ± 0.25
UG of *Inocybaceae*	1.1 ± 0.1	0.75 ± 0.2	0.70 ± 0.15	*Fusicladium*	0.51 ± 0.13	1.6 ± 0.7	0.79 ± 0.23
Hymenoscyphus	0.70 ± 0.40	1.5 ± 0.6	0.28 ± 0.1	*Peziza*	0.52 ± 0.32	1.2 ± 1.1	0.16 ± 0.16
Passalora	1.5 ± 0.5	0.35 ± 0.05	0.25 ± 0.08	Other	11.5 ± 3.1	11.4 ± 3.6	11.8 ± 1.9
Aureobasidium	1.1 ± 0.5	0.25 ± 0.06	0.12 ± 0.02	**Fungal genus_Year2**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
Other	15.3 ± 1.8	14.9 ± 1.8	16.3 ± 1.9	*Cadophora*	76.1 ± 2.6	70.9 ± 5.8	65.3 ± 5.2

Table S6 (3/3) - Relative abundance of the most abundant fungal phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 2. The asterisks denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_High exp exp related samples. The cross denote fungal phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_Low exp exp related samples.

Fungal genus_Year2	**WT_Rh_T1**	**MiSSP7_Low exp_Rh_T1**	**MiSSP7_High exp_Rh_T1**	Unidentified	8.0 ± 0.5	9.9 ± 1.8	12.0 ± 1.88
Cadophora	44.5 ± 2.9	38.5 ± 7.2	55.9 ± 4.8	*Meliniomyces*	5.5 ± 0.8	4.3 ± 0.9	5.3 ± 0.9
Unidentified	20.7 ± 2.8	21.0 ± 5.8	12.3 ± 2.0	*Peziza*	0.04 ± 0.04	2.7 ± 2.6	0.11 ± 0.10
Tuber	3.8 ± 1.2	7.3 ± 4.6	7.5 ± 3.4	*Leptodontidium*	4.2 ± 0.6	2.6 ± 1.1	5.2 ± 1.8
Wilcoxina	3.7 ± 1.3	0.44 ± 0.25	6.9 ± 6.7	*Trichoderma*	0.10 ± 0.05	0.43 ± 0.36	1.7 ± 1.1
UG of *Pezizaceae*	0.04 ± 0.01	6.5 ± 6.1	5.3 ± 4.9	*Wilcoxina*	0.43 ± 0.17	0.40 ± 0.27	2.3 ± 1.9
Meliniomyces	4.1 ± 0.6	4.4 ± 1.4	3.7 ± 1.3	UG of *Pezizaceae*	2.1 ± 2.1	0.05 ± 0.04	0.0 ± 0.0
Leptodontidium	7.2 ± 0.9 *	3.8 ± 1.0	2.9 ± 1.1	Other	3.6 ± 1.4	8.7 ± 2.0	8.2 ± 1.9
Gyoerffyella	3.3 ± 0.9	3.7 ± 2.8	1.2 ± 0.4	**Fungal genus_Year2**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
Conlarium	1.2 ± 0.4	0.64 ± 0.17	0.24 ± 0.02	Unidentified	44.7 ± 28.5	20.4 ± 18.3	29.1 ± 23.2
UG of *Leptosphaeriaceae*	0.12 ± 0.05	1.5 ± 1.5	0.05 ± 0.05	UG of *Ceratobasidiaceae*	21.0 ± 13.4	38.7 ± 13.7	17.5 ± 7.8
UG of *Ophiocordycipitaceae*	0.12 ± 0.06	3.4 ± 3.3	0.02 ± 0.02	*Conlarium*	3.9 ± 1.2	0.73 ± 0.31	9.8 ± 4.3
Colletotrichum	1.7 ± 1.6	0.11 ± 0.04	0.0 ± 0.0	*Colletotrichum*	0.26 ± 0.20	4.4 ± 4.3	9.3 ± 5.8
Other	9.5 ± 3.2	8.6 ± 1.2	3.8 ± 0.8	*Luellia*	0.35 ± 0.20	5.1 ± 5.0	9.2 ± 8.3
Fungal genus_Year2	**WT_E_T1**	**MiSSP7_Low exp_E_T1**	**MiSSP7_High exp_E_T1**	UG of *Thelephoraceae*	8.5 ± 6.1	14.6 ± 13.5	5.3 ± 2.9
UG of *Ophiocordycipitaceae*	0.25 ± 0.25	1.3 ± 1.3	26.9 ± 25.3	*Fusicladium*	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 2.2
UG of *Ceratobasidiaceae*	4.1 ± 3.9	16.4 ± 15.1	12.9 ± 5.1	*Pezicula*	7.3 ± 3.7	6.9 ± 3.0	3.3 ± 1.2
Mycosphaerella	13.0 ± 6.3	27.5 ± 8.7	12.6 ± 5.4	*Leptodontidium*	4.9 ± 2.4	5.2 ± 3.8	2.4 ± 1.7
Unidentified	17.4 ± 2.9	20.0 ± 5.0	10.7 ± 4.2	*Mycosphaerella*	0.95 ± 0.63	0.19 ± 0.07	1.8 ± 0.9
UG of *Thelephoraceae*	14.8 ± 11.8	0.60 ± 0.40	7.8 ± 5.5	*Alternaria*	0.0 ± 0.0	0.03 ± 0.03	1.5 ± 1.4
Chalastospora	5.1 ± 4.4	6.8 ± 5.2	4.5 ± 2.1	*Cadophora*	1.2 ± 0.5	0.46 ± 0.23	0.59 ± 0.22
Aspergillus	0.0 ± 0.0	0.01 ± 0.01	3.9 ± 3.6	Other	7.1 ± 3.8	3.3 ± 1.0	6.9 ± 2.3
Conlarium	3.8 ± 3.3	0.41 ± 0.41	3.0 ± 2.4				
UG of *Sebacinales_Group_B*	3.3 ± 3.2	0.0 ± 0.0	2.0 ± 1.4				
Pisolithus	0.0 ± 0.0	0.0 ± 0.1	1.9 ± 1.9				
Amanita	0.01 ± 0.01	0.0 ± 0.2	1.8 ± 1.8				
Phlebia	0.04 ± 0.04	0.0 ± 0.3	1.3 ± 1.3				
Tuber	0.26 ± 0.22	0.22 ± 0.22	1.2 ± 1.2				
Schizopora	0.01 ± 0.01	0.0 ± 0.0	1.1 ± 1.1				
Peniophora	2.1 ± 2.1	0.28 ± 0.28	0.91 ± 0.91				
Colletotrichum	12.3 ± 12.3	0.08 ± 0.08	0.78 ± 0.46				
Leptodontidium	1.2 ± 0.8	0.70 ± 0.54	0.71 ± 0.71				
Saccharomyces	2.9 ± 2.7	1.8 ± 1.7	0.20 ± 0.11				
Annulohypoxylon	0.08 ± 0.06	1.0 ± 1.0	0.18 ± 0.18				
Laetiporus	0.0 ± 0.0	4.4 ± 4.4	0.0 ± 0.0				
Pezicula	0.12 ± 0.12	3.1 ± 3.0	0.0 ± 0.1				
Diplodia	0.0 ± 0.0	2.3 ± 2.3	0.0 ± 0.2				
Mycoacia	0.03 ± 0.03	1.1 ± 1.1	0.0 ± 0.3				
Sporobolomyces	1.4 ± 1.4	0.23 ± 0.23	0.0 ± 0.4				
UG of *Corticiaceae*	1.0 ± 0.9	0.21 ± 0.21	0.0 ± 0.5				
Sistotrema	2.2 ± 2.2	0.14 ± 0.14	0.0 ± 0.6				
Terana	1.3 ± 1.3	0.0 ± 0.0	0.0 ± 0.7				
Ceriporia	1.3 ± 1.3	0.0 ± 0.1	0.0 ± 0.8				
Hydnotrya	1.1 ± 1.0	0.0 ± 0.2	0.0 ± 0.9				
Other	11.0 ± 1.2	11.3 ± 1.8	5.7 ± 1.0				

Table S7 (1/2) - Relative abundance of the most abundant bacterial phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 2. The asterisks denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_High exp exp related samples. The cross denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_Low exp exp related samples.

Bacterial phylum_Year2	**WT_BS_T1**	**MiSSP7_Low exp_BS_T1**	**MiSSP7_High exp_BS_T1**	**Bacterial phylum_Year2**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
WPS-2	16.6 ± 0.5	17.1 ± 0.8	17.0 ± 0.5	WPS-2	15.1 ± 0.4	15.7 ± 0.9	12.6 ± 0.2
Verrucomicrobia	49.9 ± 0.3	49.1 ± 1.2	49.8 ± 0.3	Verrucomicrobia	50.2 ± 0.8	51.1 ± 0.3	48.5 ± 0.4
Proteobacteria_*Alphaproteobacteria*	8.9 ± 0.2	8.7 ± 0.1	8.3 ± 0.1	Proteobacteria_*Alphaproteobacteria*	9.4 ± 0.4	9.2 ± 0.2	11.5 ± 0.7
Unidentified	6.1 ± 0.4	6.0 ± 0.4	6.0 ± 0.3	Unidentified	6.6 ± 0.2	5.9 ± 0.3	6.1 ± 0.6
Proteobacteria_*Gammaproteobacteria*	7.3 ± 0.3	7.5 ± 0.3	8.0 ± 0.4	Proteobacteria_*Gammaproteobacteria*	7.9 ± 0.4	7.1 ± 0.3	9.1 ± 0.4
Proteobacteria_*Deltaproteobacteria*	3.5 ± 0.2	3.8 ± 0.3	3.4 ± 0.2	Proteobacteria_*Deltaproteobacteria*	3.6 ± 0.1	3.5 ± 0.2	3.8 ± 0.3
Planctomycetes	1.6 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	Planctomycetes	1.6 ± 0.2	1.8 ± 0.2	2.0 ± 0.5
Acidobacteria	1.1 ± 0.1	1.0 ± 0.1	1.3 ± 0.2	Acidobacteria	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
Other	4.8 ± 1.0	4.8 ± 1.1	4.4 ± 0.5	Other	4.3 ± 0.6	4.5 ± 1.0	5.2 ± 1.2
Bacterial phylum_Year2	**WT_AS_T1**	**MiSSP7_Low exp_AS_T1**	**MiSSP7_High exp_AS_T1**	**Bacterial phylum_Year2**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
WPS-2	19.6 ± 1.0	19.4 ± 0.7	18.5 ± 1.6	WPS-2	21.3 ± 1.2	23.3 ± 1.1	19.2 ± 2.2
Verrucomicrobia	48.9 ± 0.6	48.6 ± 0.6	49.1 ± 1.0	Verrucomicrobia	47.8 ± 0.9	46.4 ± 0.4	47.5 ± 0.3
Proteobacteria_*Alphaproteobacteria*	8.8 ± 0.5	9.2 ± 0.6	8.8 ± 0.1	Proteobacteria_*Alphaproteobacteria*	8.9 ± 0.2	8.6 ± 0.5	9.1 ± 0.7
Unidentified	5.6 ± 0.3	5.4 ± 0.4	5.5 ± 0.2	Unidentified	5.5 ± 0.1	5.2 ± 0.2	5.8 ± 0.5
Proteobacteria_*Gammaproteobacteria*	7.1 ± 0.4	6.6 ± 0.2	6.8 ± 0.1	Proteobacteria_*Gammaproteobacteria*	7.0 ± 0.3	7.0 ± 0.1	7.6 ± 0.6
Proteobacteria_*Deltaproteobacteria*	3.3 ± 0.2	3.7 ± 0.3	3.6 ± 0.5	Proteobacteria_*Deltaproteobacteria*	2.9 ± 0.2	3.2 ± 0.3	3.4 ± 0.2
Planctomycetes	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	Planctomycetes	1.5 ± 0.2	1.4 ± 0.2	1.6 ± 0.3
Acidobacteria	1.0 ± 0.1	1.1 ± 0.2	1.2 ± 0.3	Acidobacteria	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
Other	4.2 ± 0.7	4.6 ± 0.9	5.0 ± 1.0	Other	4.0 ± 0.8	3.7 ± 1.0	4.3 ± 0.7
Bacterial phylum_Year2	**WT_Rh_T1**	**MiSSP7_Low exp_Rh_T1**	**MiSSP7_High exp_Rh_T1**	**Bacterial phylum_Year2**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
WPS-2	54.9 ± 2.3	53.6 ± 4.6	63.1 ± 2.2	WPS-2	55.8 ± 1.9	57.0 ± 3.2	53.3 ± 3.5
Verrucomicrobia	32.0 ± 2.3	31.9 ± 4.0	25.5 ± 1.6	Verrucomicrobia	30.8 ± 1.4	30.7 ± 2.5	31.9 ± 2.6
Proteobacteria_*Alphaproteobacteria*	4.2 ± 0.3	5.1 ± 0.7	3.6 ± 0.1	Proteobacteria_*Alphaproteobacteria*	3.9 ± 0.4	3.8 ± 0.2	4.3 ± 0.6
Unidentified	2.7 ± 0.3	2.5 ± 0.5	2.4 ± 0.1	Unidentified	3.5 ± 0.2	3 .1± 0.2	3.1 ± 0.4
Proteobacteria_*Gammaproteobacteria*	2.2 ± 0.2	2.7 ± 0.6	2.1 ± 0.4	Proteobacteria_*Gammaproteobacteria*	2.5 ± 0.1	2.1 ± 0.2	3.3 ± 0.3
Proteobacteria_*Deltaproteobacteria*	1.4 ± 0.2	1.3 ± 0.1	1.1 ± 0.2	Proteobacteria_*Deltaproteobacteria*	0.99 ± 0.07	0.97 ± 0.23	1.3 ± 0.2
Planctomycetes	0.7 ± 0.1	0.69 ± 0.12	0.4 ± 0.1	Planctomycetes	0.60 ± 0.06	0.50 ± 0.11	0.63 ± 0.07
Acidobacteria	0.37 ± 0.05	0.37 ± 0.06	0.5 ± 0.1	Acidobacteria	0.45 ± 0.10	0.41 ± 0.07	0.37 ± 0.05
Other	1.7 ± 0.2	1.8 ± 0.1	1.3 ± 0.2	Other	1.4 ± 0.3	1.4 ± 0.2	1.5 ± 0.5
Bacterial phylum_Year2	**WT_E_T1**	**MiSSP7_Low exp_E_T1**	**MiSSP7_High exp_E_T1**	**Bacterial phylum_Year2**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
WPS-2	51.3 ± 7.7	45.3 ± 8.3	77.7 ± 2.6	WPS-2	53.9 ± 6.0	65.2 ± 6.2	49.0 ± 6.9
Verrucomicrobia	35.7 ± 8.9	35.0 ± 5.19	12.5 ± 1.5	Verrucomicrobia	33.2 ± 5.2	22.6 ± 3.6	29.3 ± 4.3
Proteobacteria_*Alphaproteobacteria*	4.4 ± 0.8	5.6 ± 0.9	3.0 ±1.8	Proteobacteria_*Alphaproteobacteria*	3.7 ± 0.8	3.5 ± 0.6	8.0 ± 1.2 *
Unidentified	0.87 ± 0.30	2.4 ± 0.8	0.94 ± 0.84	Unidentified	3.5 ± 0.9	2.4 ± 0.1	2.8 ± 0.6
Proteobacteria_*Gammaproteobacteria*	2.1 ± 0.4	4.6 ± 1.8	2.9 ± 1.3	Proteobacteria_*Gammaproteobacteria*	1.5 ± 0.3	3.0 ± 1.6	3.3 ± 1.4
Proteobacteria_*Deltaproteobacteria*	2.7 ± 0.6	3.1 ± 0.9	0.72 ± 0.44	Proteobacteria_*Deltaproteobacteria*	1.6 ± 0.7	0.79 ± 0.22	2.4 ± 0.5
Planctomycetes	0.69 ± 0.21	1.0 ± 0.5	0.61 ± 0.18	Planctomycetes	0.40 ± 0.12	0.37 ± 0.16	1.4 ± 0.5
Acidobacteria	0.45 ± 0.09	0.4 ± 0.2	0.17 ± 0.09	Acidobacteria	0.42 ± 0.07	0.39 ± 0.01	0.82 ± 0.17
Other	1.8 ± 0.7	2.6 ± 1.1	4.4 ± 0.5	Other	1.6 ± 0.3	1.6 ± 0.2	2.6 ± 1.9

Table S7 (2/2) - Relative abundance of the most abundant bacterial phyla and genera (>1 % relative abundance in at least one of the two or three compared samples) detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 2. The asterisks denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_High exp exp related samples. The cross denote bacterial phyla and genera significantly different in relative abundance between WT and MiSSP7_MiSSP7_Low exp exp related samples.

Bacterial genus_Year2	**WT_BS_T1**	**MiSSP7_Low exp_BS_T1**	**MiSSP7_High exp_BS_T1**	**Bacterial genus_Year2**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
Unidentified	37.9 ± 0.8	38.4 ± 0.4	38.8 ± 1.0	Unidentified	37.0 ± 0.5	36.8 ± 0.7	35.5 ± 0.9
UG of *Pedosphaeraceae*	26.2 ± 0.5	26.7 ± 0.4	25.2 ± 0.2	UG of *Pedosphaeraceae*	25.0 ± 0.7	26.0 ± 0.7	23.5 ± 0.7
UG of *Verrucomicrobiaceae*	4.8 ± 0.2	4.8 ± 0.2	4.9 ± 0.5	UG of *Verrucomicrobiaceae*	4.8 ± 0.2	5.1 ± 0.2	4.3 ± 0.2
ADurb.Bin063-1	1.4 ± 0.1	1.4 ± 0.2	1.6 ± 0.1	*ADurb.Bin063-1*	1.5 ± 0.1	1.6 ± 0.1	1.8 ± 0.2
Chthoniobacter	1.9 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	*Chthoniobacter*	2.1 ± 0.3	1.9 ± 0.1	3.0 ± 0.3
UG of *Opitutaceae*	2.1 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	UG of *Opitutaceae*	2.3 ± 0.2	2.3 ± 0.2	1.9 ± 0.2
UG of *Xanthobacteraceae*	1.5 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	UG of *Xanthobacteraceae*	1.9 ± 0.1	2.0 ± 0.2	2.5 ± 0.3
UG of *Burkholderiaceae*	1.3 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	UG of *Burkholderiaceae*	1.2 ± 0.1 x	0.88 ± 0.12	1.6 ± 0.2 *
Pedosphaera	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.2	*Pedosphaera*	1.1 ± 0.1	1.1 ± 0.1	0.87 ± 0.08
Candidatus Udaeobacter	1.6 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	*Candidatus Udaeobacter*	1.9 ± 0.1	1.6 ± 0.2	1.5 ± 0.2
Lacunisphaera	1.1 ± 0.1	1.0 ± 0.1	0.97 ± 0.15	*Opitutus*	1.7 ± 0.2	1.9 ± 0.1	1.9 ± 0.2
Opitutus	1.3 ± 0.1	1.5 ± 0.3	1.5 ± 0.2	UG of *Methylacidiphilaceae*	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Other	17.7 ± 3.8	17.2 ± 1.9	17.8 ± 1.8	*LD29*	0.3 ± 0.1	0.33 ± 0.09	1.0 ± 0.3
Bacterial genus_Year2	**WT_AS_T1**	**MiSSP7_Low exp_AS_T1**	**MiSSP7_High exp_AS_T1**	Other	17.8 ± 1.9	17.2 ± 2.0	19.2 ± 3.0
Unidentified	40.2 ± 0.7	39.7 ± 1.1	39.9 ± 0.9	**Bacterial genus_Year2**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
UG of *Pedosphaeraceae*	25.8 ± 0.6	26.0 ± 0.4	25.4 ± 0.6	Unidentified	41.7 ± 1.1	43.4 ± 0.8	40.5 ± 2.0
UG of *Verrucomicrobiaceae*	4.6 ± 0.3	4.5 ± 0.5	4.6 ± 0.6	UG of *Pedosphaeraceae*	24.1 ± 0.7	23.1 ± 0.1	22.7 ± 0.6
ADurb.Bin063-1	1.4 ± 0.1	1.6 ± 0.2	1.5 ± 0.3	UG of *Verrucomicrobiaceae*	3.7 ± 0.2	3.7 ± 0.2	3.4 ± 0.1
Chthoniobacter	1.8 ± 0.1	2.2 ± 0.1	1.8 ± 0.1	*ADurb.Bin063-1*	1.9 ± 0.2	1.6 ± 0.1	1.9 ± 0.2
UG of *Opitutaceae*	2.6 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	*Chthoniobacter*	2.2 ± 0.2	1.7 ± 0.2	1.9 ± 0.1
UG of *Xanthobacteraceae*	1.6 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	UG of *Opitutaceae*	2.4 ± 0.2	2.4 ± 0.3	2.6 ± 0.2
UG of *Burkholderiaceae*	1.2 ± 0.2	1.0 ± 0.1	1.2 ± 0.2	UG of *Xanthobacteraceae*	1.4 ± 0.1	1.6 ± 0.2	1.7 ± 0.1
Pedosphaera	1.2 ± 0.1	0.89 ± 0.12	1.1 ± 0.1	UG of *Burkholderiaceae*	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
Candidatus Udaeobacter	1.2 ± 0.1	1.7 ± 0.2	1.5 ± 0.1	*Candidatus Udaeobacter*	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
Opitutus	1.1 ± 0.1	1.1 ± 0.3	1.3 ± 0.1	*Opitutus*	1.4 ± 0.2	1.6 ± 0.1	2.2 ± 0.5
Other	17.2 ± 3.2	17.3 ± 2.9	17.9 ± 2.8	Other	18.1 ± 1.8	17.8 ± 6.0	19.9 ± 6.7
Bacterial genus_Year2	**WT_Rh_T1**	**MiSSP7_Low exp_Rh_T1**	**MiSSP7_High exp_Rh_T1**	**Bacterial genus_Year2**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
Unidentified	66.1 ± 2.3	65.4 ± 2.8	72.6 ± 1.6	Unidentified	67.1 ± 1.1	69.2 ± 2.1	66.1 ± 1.8
UG of *Pedosphaeraceae*	16.4 ± 0.9	17.7 ± 2.2	13.5 ± 0.7	UG of *Pedosphaeraceae*	16.0 ± 0.6	16.3 ± 1.2	16.3 ± 1.1
UG of *Verrucomicrobiaceae*	5.2 ± 2.4	3.6 ± 0.7	2.5 ± 0.3	UG of *Verrucomicrobiaceae*	3.6 ± 0.8	2.2 ± 0.3	2.7 ± 0.3
Other	12.1 ± 7.1	13.1 ± 3.8	11.2 ± 1.3	*ADurb.Bin063-1*	1.0 ± 0.1	0.84 ± 0.14	1.1 ± 0.1
Bacterial genus_Year2	**WT_E_T1**	**MiSSP7_Low exp_E_T1**	**MiSSP7_High exp_E_T1**	*Chthoniobacter*	0.89 ± 0.10	1.0 ± 0.1	0.86 ± 0.11
Unidentified	61.9 ± 4.5	80.9 ± 2.3	56.7 ± 6.0	UG of *Opitutaceae*	0.83 ± 0.09	0.60 ± 0.11	1.0 ± 0.2
UG of *Pedosphaeraceae*	16.5 ± 7.2	5.1 ± 2.8	12.6 ± 3.8	*Lacunisphaera*	0.56 ± 0.13	0.77 ± 0.23	1.2 ± 0.2
UG of *Verrucomicrobiaceae*	0.36 ± 0.19	0.91 ± 0.91	2.5 ± 1.0	Other	9.9 ± 0.9	8.9 ± 1.8	10.5 ± 2.9
Chthoniobacter	1.7 ± 0.8	0.56 ± 0.31	2.0 ± 0.4	**Bacterial genus_Year2**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
UG of *Opitutaceae*	1.1 ± 0.4	0.18 ± 0.18	4.7 ± 3.1	Unidentified	67.3 ± 3.5	62.1 ± 6.3	75.4 ± 4.8
UG of *Xanthobacteraceae*	0.72 ± 0.31	2.1 ± 2.0	1.6 ± 0.4	UG of *Pedosphaeraceae*	19.4 ± 2.7	17.3 ± 3.9	11.0 ± 1.4
UG of *Burkholderiaceae*	0.85 ± 0.30	0.20 ± 0.15	1.7 ± 1.0	*ADurb.Bin063-1*	1.2 ± 0.6	0.36 ± 0.16	0.31 ± 0.13
Candidatus Udaeobacter	2.5 ± 0.7	4.9 ± 3.4	2.0 ± 0.9	*Chthoniobacter*	0.42 ± 0.22	3.3 ± 1.3 *	0.62 ± 0.23
Candidatus Xiphinematobacter	2.3 ± 0.5	0.15 ± 0.13	1.8 ± 1.0	UG of *Xanthobacteraceae*	0.72 ± 0.21	2.2 ± 0.8	0.84 ± 0.61
Opitutus	1.4 ± 0.7	0.03 ± 0.01	2.6 ± 0.7	*Candidatus Udaeobacter*	1.2 ± 0.5	0.69 ± 0.37	0.36 ± 0.28
UG of *Chthoniobacteraceae*	2.4 ± 1.1	0.56 ± 0.23	1.0 ± 0.8	*Candidatus Xiphinematobacter*	1.5 ± 0.2	1.7 ± 0.4	1.7 ± 0.6
Other	8.1 ± 1.0	4.3 ± 0.3	9.8 ± 0.3	*FukuN18 freshwater group*	0.0 ± 0.0	1.1 ± 0.8	2.2 ± 1.9
				Other	8.2 ± 1.8	11.1 ± 8.4	7.4 ± 1.9

Table S8 - Relative abundance of the fungal guilds detected in the bulk soil (BS), the rhizosphere (AS), the rhizoplan (Rh) and the endosphere (E.) of the *Populus* WT and *Populus* expressing MiSSP7 cuttings collected in Year 2. The asterisks denote fungal guild significantly different in relative abundance between WT and MiSSP7_MiSSP7_High exp exp related samples. The cross denote fungal guild significantly different in relative abundance between WT and MiSSP7_MiSSP7_Low exp exp related samples.

Guild_Year2	**WT_BS_T1**	**MiSSP7_Low exp_BS_T1**	**MiSSP7_High exp_BS_T1**	**Guild_Year2**	**WT_BS_T2**	**MiSSP7_Low exp_BS_T2**	**MiSSP7_High exp_BS_T2**
EcM	5.3 ± 1.5	5.4 ± 2.5	14.1 ± 7.7	EcM	2.6 ± 0.4	2.1 ± 0.2	2.4 ± 0.5
Endophyte	1.6 ± 0.2	1.1 ± 0.3	1.2 ± 0.2	Endophyte	3.2 ± 1.3	1.6 ± 0.3	1.5 ± 0.3
Other	2.5 ± 0.3	1.9 ± 0.4	1.8 ± 0.2	Other	2.2 ± 0.3	2.0 ± 0.3	2.7 ± 0.3
Plant pathogen	5.8 ± 1.2	6.2 ± 3.2	4.7 ± 1.5	Plant pathogen	2.9 ± 0.8	2.4 ± 0.5	2.9 ± 0.5
Saprotroph	8.4 ± 0.4	17.2 ± 8.1	8.4 ± 0.9	Saprotroph	9.6 ± 1.1	15.8 ± 4.4	10.1 ± 0.8
Unclassified	76.4 ± 1.9	68.2 ± 6.5	69.7 ± 6.1	Unclassified	79.5 ± 1.5	75.9 ± 3.7	80.2 ± 1.0
Guild_Year2	**WT_AS_T1**	**MiSSP7_Low exp_AS_T1**	**MiSSP7_High exp_AS_T1**	**Guild_Year2**	**WT_AS_T2**	**MiSSP7_Low exp_AS_T2**	**MiSSP7_High exp_AS_T2**
EcM	4.8 ± 1.5	3.1 ± 0.7	2.9 ± 0.7	EcM	1.8 ± 0.4	2.1 ± 0.4	1.9 ± 0.4
Endophyte	1.1 ± 0.1	1.1 ± 0.2	1.3 ± 0.1	Endophyte	11.6 ± 6.0	2.4 ± 0.9	6.7 ± 3.3
Other	3.1 ± 0.3	3.1 ± 0.3	2.6 ± 0.4	Other	2.1 ± 0.3	2.2 ± 0.3	2.2 ± 0.2
Plant pathogen	7.8 ± 2.2	3.8 ± 0.4	3.0 ± 0.5	Plant pathogen	2.0 ± 0.2	3.2 ± 1.2	2.5 ± 1.0
Saprotroph	12.2 ± 0.8	12.4 ± 0.5	11.6 ± 0.4	Saprotroph	9.5 ± 1.2	8.7 ± 2.2	10.2 ± 2.7
Unclassified	70.9 ± 1.1	76.4 ± 0.9	78.5 ± 0.5	Unclassified	73 ± 5.1	81.5 ± 3.7	76.5 ± 3.5
Guild_Year2	**WT_Rh_T1**	**MiSSP7_Low exp_Rh_T1**	**MiSSP7_High exp_Rh_T1**	**Guild_Year2**	**WT_Rh_T2**	**MiSSP7_Low exp_Rh_T2**	**MiSSP7_High exp_Rh_T2**
EcM	8.1 ± 1.7	8.4 ± 4.8	14.7 ± 5.5	EcM	0.53 ± 0.18	0.94 ± 0.32	2.9 ± 1.7
Endophyte	8.0 ± 0.9 *	4.6 ± 1.0	3.6 ± 0.8	Endophyte	5.3 ± 0.6	3.9 ± 1.4	6.7 ± 2.5
Other	0.32 ± 0.24	0.11 ± 0.05	0.02 ± 0.02	Other	0.22 ± 0.07	0.04 ± 0.03	0.80 ± 0.69
Plant pathogen	1.8 ± 0.5	1.8 ± 0.7	0.61 ± 0.14	Plant pathogen	0.20 ± 0.09	0.18 ± 0.09	0.36 ± 0.16
Saprotroph	2.9 ± 0.5	6.2 ± 3.2	1.2 ± 0.4	Saprotroph	0.47 ± 0.08	0.37 ± 0.07	1.5 ± 0.5
Unclassified	78.8 ± 1.4	78.9 ± 4.1	79.8 ± 6.3	Unclassified	93.3 ± 0.6	94.4 ± 1.5	87.6 ± 3.4
Guild_Year2	**WT_E_T1**	**MiSSP7_Low exp_E_T1**	**MiSSP7_High exp_E_T1**	**Guild_Year2**	**WT_E_T2**	**MiSSP7_Low exp_E_T2**	**MiSSP7_High exp_E_T2**
EcM	16.2 ± 12.8	0.82 ± 0.61	12.6 ± 6.7	EcM	9.7 ± 6.2	14.7 ± 13.6	5.4 ± 2.9
Endophyte	1.4 ± 1.0	0.70 ± 0.54	0.71 ± 0.71	Endophyte	5.1 ± 2.4	5.4 ± 3.8	2.4 ± 1.7
Other	0.0 ± 0.0	0.49 ± 0.43	0.40 ± 0.20	Other	0.0 ± 0.0	0.02 ± 0.02	0.0 ± 0.0
Plant pathogen	19.7 ± 6.6	36.2 ± 9.4	17.6 ± 7.8	Plant pathogen	1.6 ± 0.7	0.47 ± 0.2	7.2 ± 2.4
Saprotroph	12.2 ± 4.8	13.1 ± 2.2	37.8 ± 21.1	Saprotroph	4.7 ± 1.4	6.6 ± 4.7	20.0 ± 8.2
Unclassified	50.3 ± 8.6	48.6 ± 10.3	30.6 ± 6.7	Unclassified	78.9 ± 8.8	72.7 ± 11.3	64.9 ± 7.5

Table S9 (1/2) - List of metabolites detected in roots of *Populus tremula x alba* WT and *Populus tremula x alba* expressing MiSSP7 roots harvested in Year 2 after 10 days (T1) and 6.5 weeks (T2). Values indicate fold changes between WT and MiSSP7 line at each sampling time. The asterisks denote significant difference of concentration which has been measured between WT and MiSSP_High and between WT and MiSSP7_Low (* = P<0.05, one way ANOVA). Metabolites highlighted in yellow and green are involved in primary, secondary metabolisms, respectively.

Metabolite (RT-m/z)	Plant metabolite	Bacterial or fungal metabolite	WT/MiSSP7_ Low (T1)	WT/MiSSP_ High (T1)	WT/MiSSP7_ Low (T2)	WT/MiSSP_ High (T2)
1,2,3-benzenetriol	X	X	ND	ND	1,45	2,05
1,2,4-benzenetriol	X	X	ND	ND	1,48	1,63
10.68 217 391 411	?		0,65	0,58	1,33	1,44
10.90 450 dehydro sugar	?		ND	ND	1,78	1,80
11.22 450 dehydro sugar	?		1,43	2,16	1,40	1,54
11.29 393 303 257	?		ND	ND	2,71	2,08
13.84 183 256 167	?		0,70	0,09	ND	ND
14.09 375 292 217	?		1,21	1,38	ND	ND
14.25 331 263 233 258 M+ glycoside	?		0,35 *	0,20	1,25	2,02
14.38 254 inositol conj	?		0,54	0,34	ND	ND
15.18 284 glycoside	?		1,18	0,38	1,66	2,33 *
15.24 284 glycoside	?		1,30	0,41	1,44	2,00 *
16.04 guaiacyl lignan	X		0,69	1,17	1,53	1,87
16.11 guaiacyl lignan	X		0,83	3,10	1,24	1,67 *
16.37 guaiacyl lignan	X		ND	ND	1,31	1,81
17.65 418 179 193 91 glycoside	?		0,69	2,77	1,69	2,28
19.00 219 171 331	?		0,19	1,81	1,84	2,16
19.18 171 coumaroyl glycoside	?		0,26	0,32	2,02	2,71
19.69 171 caffeoyl glycoside	?		0,33	2,02	1,14	2,09
2-hydroxypentanedioic acid	X	X	1,13	0,53	1,04	1,11
2,5-dihydroxybenzoic acid-2-O-glucoside	X	X	ND	ND	3,75	1,85
2,5-dihydroxybenzoic acid-5-O-glucoside	X		ND	ND	2,41	1,45
4-hydroxybenzoic acid	X	X	1,16	0,18	0,75	1,05
5-oxo-proline	X	X	1,57	0,34	1,70	1,82
6-hydroxy-2-cyclohexenone alcohol	X		0,66	0,56	1,42	1,24
6-hydroxy-2-cyclohexenone-1-carboxylic acid	X	X	0,77	0,32	2,14 *	2,40 *
6.94 225 240 332 278	?		0,70	0,04	1,45	1,14
7.69 169 101 75 68	?		ND	ND	1,43	2,67
8.34 256 167	?		ND	ND	1,66	2,09 *
9.98 98 288 390	?		ND	ND	0,89	0,54
a-linolenic acid	X	X	2,09	3,22	0,64	1,19
a-salicyloylsalicin	X		0,46	2,05	1,44	2,29
a-tocopherol	X	X	0,70	1,81	0,87	2,59
alanine	X	X	1,06	0,04	1,15	1,25
arabinose	X	X	0,99	0,19	ND	ND
arabitol	X	X	1,25	0,10	1,67	1,46
arbutin	X		0,86	0,18	2,24	2,74
B-sitosterol	X		1,98	0,37	1,28	1,59
caffeic acid	X		0,78	0,16	1,61	1,43
catechin	X		1,65	1,40	1,63 *	1,78 *
catechol	X		0,38	0,97	1,49	2,37 *
cis-aconitic acid	X	X	1,39	6,52	1,59	2,11 *
citric acid	X	X	1,33	0,84	2,03	2,02
digalactosylglycerol	X	X	0,54	0,81	2,12	2,20
erythronic acid	X		1,06	0,34	1,10	0,98
ethyl-phosphate	?		2,11	0,70	0,86	1,24

Table S9 (2/2) - List of metabolites detected in roots of *Populus tremula x alba* WT and *Populus tremula x alba* expressing MiSSP7 roots harvested in Year 2 after 10 days (T1) and 6.5 weeks (T2). Values indicate fold changes between WT and MiSSP7 line at each sampling time. The asterisks denote significant difference of concentration which has been measured between WT and MiSSP_High and between WT and MiSSP7_Low (* = P<0.05, one way ANOVA). Metabolites highlighted in yellow and green are involved in primary, secondary metabolisms, respectively.

fructose	X	X	0,74	0,95	1,99	1,07
fumaric acid	X	X	1,48	0,99	0,81	1,06
GABA	X	X	1,65	0,44	1,06	1,97
galactose	X	X	0,78	0,72	1,15	0,99
gallocatechin	X		0,93	2,59	1,44	1,68
glucose	X	X	0,71	1,17	1,47	1,63
glutamic acid	X	X	ND	ND	2,22	1,72
glyceric acid	X	X	1,59	0,42	0,93	0,92
glycerol	X	X	1,15	0,16	1,88	1,46
glycerol-1/3-P	X	X	2,02	0,60	1,91	2,22
hydroquinone	X		0,60	0,05	2,26	2,39
lactic acid	X	X	0,85	0,07	1,08	1,37
linoleic acid	X	X	1,67	1,63	0,74	1,58
maleic acid	X	X	1,38	0,63	1,10	0,82
malic acid	X	X	1,33	1,57	1,02	1,03
mannitol		X	0,88	0,30	1,50	1,82 *
monogalactosylglycerol	X	X	0,73	0,84	1,17	1,98
myo-inositol	X		1,24	0,95	4,38	2,23
nonanoic acid	X		0,90	0,05	1,97	1,22
oxalomalic acid	X	X	0,80	2,39	3,05 *	3,72 *
palmitic acid	X	X	1,01	0,06	1,28	1,19
phluoroglucinol	X	X	0,21	0,01	1,02	1,02
phosphate	X	X	1,74	0,37	1,25	1,56
quinic acid	X	X	1,29	1,17	2,27	1,69
ribitol	X	X	ND	ND	1,03	1,48
salicin	X		0,41 *	0,98	1,25	2,01 *
salicortin	X		1,45	0,73	7,09	5,18
salicyl alcohol	X		0,48	0,23	1,10	1,70
salicyl-salicylic acid-2-O-glucoside	X	X	1,15	1,80	11,64	4,82
salicylic acid	X	X	1,00	0,25	1,80	2,12
salicyltremuloidin	X		0,38	1,42	1,72	2,98
salireposide	X		0,53	1,01	1,02	1,37
shikimic acid	X	X	1,49	0,69	1,13	1,30
succinic acid	X	X	1,19	0,19	0,90	1,31
sucrose	X		1,12	1,30	1,23	2,12 *
threonic acid	X		2,27	1,00	1,33	1,52
threono-1,4-lactone	X	X	1,24	0,03	2,14 *	1,78
trehalose		X	1,45	0,04	0,66	1,70
tremulacin	X		0,00 *	0,01	0,02	0,02 *
tremuloidin	X		0,51	3,23	0,98	1,92
xylitol	X	X	1,08	0,30	3,19	4,23 *
xylono-1,4-lactone	X		1,43	0,79	1,88 *	2,20 *

Table S10 - Relative distribution of the most active fungal genera detected in the roots of *Populus*-expressing MiSSP7 and *Populus* WT cuttings collected at T2 in Year 2 (average of 5 or 9 replicates ± SE).

Active fungal genera	MiSSP7_High exp	MiSSP7_Low exp	WT
Thelephora	0.503 ± 0.101	0.537 ± 0.130	0.310 ± 0.112
Serendipita	0.173 ± 0.091	0.071 ± 0.025	0.128 ± 0.055
Others	0.108 ± 0.004	0.110 ± 0.005	0.121 ± 0.016
Tulasnella	0.091 ± 0.053	0.055 ± 0.055	0.203 ± 0.082
Meliniomyces	0.060 ± 0.009	0.094 ± 0.032	0.085 ± 0.013
Ceratobasidium	0.043 ± 0.025	0.074 ± 0.033	0.103 ± 0.066
Laccaria	0.014 ± 0.007	0.043 ± 0.035	0.040 ± 0.033
Tuber	0.010 ± 0.004	0.014 ± 0.012	0.008 ± 0.004
Terfezia	<0.010	0.002 ± 0.002	0.001 ± 0.001

Résumé

Les micro-organismes jouent un rôle essentiel dans le fonctionnement des écosystèmes forestiers. L'ensemble de ces micro-organismes associés aux racines est appelé « microbiote racinaire » et est connu pour promouvoir la croissance de l'arbre, améliorer sa résistance face aux stress biotiques et abiotiques et participer au cycle des nutriments. Etudier les facteurs de structuration et de régulation du microbiote racinaire est essentiel pour mieux comprendre les mécanismes impliqués dans les interactions arbres/micro-organismes et le rôle du microbiote racinaire de l'arbre en réaction aux contraintes environnementales actuelles et futures. Au cours de ma thèse, j'ai étudié la dynamique de colonisation des racines du peuplier gris par les communautés bactériennes et fongiques du sol en combinant approches métagénomique et microscopique. Parallèlement, une étude réalisée en mésocosme a été menée afin de connaître l'impact du génotype de l'hôte et des facteurs environnementaux tels que le climat et le type de sol sur le microbiote racinaire du peuplier noir, espèce colonisant les écosystèmes ripisylves et particulièrement touchée par le changement climatique. Enfin, par des approches de métagénomique et de métabolomique, j'ai étudié, d'une part, l'impact des variations du microbiote du sol et, d'autre part, celui de la voie de signalisation de l'acide jasmonique, sur le métabolome et les communautés du microbiote racinaire du peuplier gris. L'ensemble de mes résultats met en lumière l'impact significatif de l'arbre et des facteurs environnementaux sur la composition et la structure taxonomique et fonctionnelle du microbiote racinaire ainsi que la nécessité de considérer l'arbre et son microbiote comme un « méta-organisme » à part entière.

Mots clés: microbiote, communautés microbiennes, champignons, bactéries, sol, climat, phytohormone, peuplier.

Abstract

Microorganisms play an essential role in the functioning of forest ecosystems. The pool of the root-associated microorganisms is called "root microbiome" and is known to promote tree growth, improve tree resistance to biotic and abiotic stresses and participate in nutrient cycling. Studying the factors that structure and regulate the root microbiome is essential to better understand the mechanisms involved in tree-microorganism interactions and the role of the tree root microbiome in response to current and future environmental constraints. During my thesis, I studied the colonization dynamic of grey poplar roots by bacterial and fungal communities in the soil by combining metagenomic and microscopic approaches. In parallel, a mesocosm study was carried out to determine the impact of the host genotype and environmental factors such as climate and soil type on the root microbiome of black poplar, a species that colonizes riparian ecosystems and is particularly affected by climate change. Finally, through metagenomic and metabolomic approaches, I studied, on the one hand, the impact of soil microbiome variations and, on the other hand, the signalling pathway of jasmonic acid, a phytohormone involved in defence, on the metabolome and communities of the root microbiome of grey poplar. The results of my thesis highlight the significant impact of the tree and environmental factors on the composition and taxonomic and functional structure of the root microbiome as well as the need to consider the tree and its microbiota as a "meta-organism" in its own right.

Key words: microbiome, microbial communities, fungi, bacteria, soil, climate, phytohormone, poplar.