Thesis

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by **Charlotte Grossiord**

Impact of tree species diversity on water and carbon relations in European forests

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Impact of tree species diversity on carbon and water relations in European forests

To manage a system effectively, you might focus on the ***interactions*** *of the parts rather than their behavior taken separately*

Russell Ackoff, *On purposeful systems*, 1972

Acknowledgments

I would like to start by thanking the two major contributors to this work, my two supervisors, Dr Damien Bonal and Dr Arthur Gessler. I am extremely grateful for the great opportunity they gave me by selecting me for this PhD and thereby allowing me to discover the fascinating and often challenging field of ecophysiological science. I consider them both to be my mentors as I enjoyed and learnt a great deal from the time that we have worked together. I would specifically like to thank Damien with whom I interacted the most during these three years. His implication, perspicacity, great ideas, constructive criticism and council make him one of the major actors in the success of this work and I am extremely grateful.

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Baeten L., K. Verheyen, C. Wirth, H. Bruelheide, F. Bussotti, L. Finér, B. Jaroszewicz, F. Selvi, F. Valladares, E. Allan, E. Ampoorter, H. Auge, D. Avăcăriei, L. Barbaro, I. Bărnoaiea, C.C. Bastias, J. Bauhus, C. Beinhoff, R. Benavides, A. Benneter, S. Berger, F. Berthold, J. Boberg, D. Bonal, W. Brüggemann, M. Carnol, B. Castagneyrol, Y. Charbonnier, E. Chećko, D. Coomes, A. Coppi, E. Dalmaris, G. Dănilă, S.M. Dawud, de Vries W., De Wandeler H., Deconchat M., Domisch T., Duduman G., Fischer M., Fotelli M., Gessler A., Gimeno T.E., Granier A., **Grossiord C.**, Guyot V., Hantsch L., Hättenschwiler S., Hector A., Hermy M., Holland V., Jactel H., Joly F.-X., Jucker T., Kolb S., Koricheva J., Lexer M.J., Liebergesell M., Milligan H., Müller S., Muys B., Nguyen D., Nichiforel L., Pollastrini M., Proulx R., Rabasa S., Radoglou K., Ratcliffe S., Raulund-Rasmussen K., Seiferling I., Stenlid J., Vesterdal L., von Wilpert K., Zavala M.A., Zielinski D. & Scherer-Lorenzen M. 2013. A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. *Perspectives in Plant Ecology, Evolution and Systematics* **15**, 281-291.

II. **Grossiord, C.**, A. Granier, A. Gessler, M. Scherer-Lorenzen, M. Pollastrini, & D. Bonal. 2013. Application of Loreau & Hector's (2001) partitioning method to complex functional traits. *Methods in Ecology and Evolution* **4**, 954–960

III. **Grossiord, C.**, A. Granier, A. Gessler, M. Pollastrini, & D. Bonal. 2013. The influence of tree species mixture on ecosystem-level carbon accumulation and water use in a mixed boreal plantation. *Forest Ecology and Management* **298**, 82-92

IV. **Grossiord, C.**, A. Granier, A. Gessler, F. Bussotti, M. Pollastrini, & D. Bonal. 2014 - Interspecific competition influences the response of oak transpiration to increasing drought stress in a mixed Mediterranean forest. *Forest Ecology and Management* **318**, 54-61.

V. **Grossiord, C.**, A. Forner, A. Gessler, A. Granier, M. Pollastrini, F. Valladares, D. Bonal. 2014. Influence of species interactions on transpiration of Mediterranean tree species during a summer drought. *European Journal of Forest Research*, DOI: 10.1007/s10342-014-0857-8.

VI. **Grossiord, C.**, A. Granier, A. Gessler, T. Jucker & D. Bonal. 2014. Does Drought Influence the Relationship between Biodiversity and Ecosystem Functioning in Boreal Forests? *Ecosystems* **17**, 394-404.

VII. **Grossiord, C.**, A. Granier, S. Ratcliffe, O. Bouriaud, H. Bruelheide, E. Chećko, L. Finér, M. Pollastrini, M. Scherer-Lorenzen, F. Valladares, D. Bonal, A. Gessler. 2014. Higher tree diversity reduces the effect of drought in European forest ecosystems. *PNAS*, 111, 14812–14815.

VIII. **Grossiord, C.**, S. Berger, C. Bréchet, A. Gessler, A. Granier, R. Henschel, R. Hommel, M. Scherer-Lorenzen, D. Bonal. 2014. Impact of interspecific competition on the depth of soil water extraction in a young temperate mixed plantation. *Journal of Hydrology*, in press.

1. General Introduction

Forests cover 31% of the world's land area and 40 % of Europe's and include a great diversity of biotic and abiotic conditions across regions (FAO, 2010; EC, 2013). These ecosystems are considered multifunctional because they host habitats for animals and plants, they play a major role in mitigating climate change, and they offer us many benefits such as clean freshwater, improved health, and recreation and touristic opportunities. However, in order for forests to deliver these functions and services in a balanced way, it is essential to ensure a sustainable management of these ecosystems. During the last decades, forests policies in Europe have largely evolved in this direction and management objectives no longer favor wood production only, be it oriented toward quantity or quality (EC, 2013). To protect forests from the significant effects of storms, fires, scarce water resources and pests, forest management strategies also aim to maintain natural forest dynamics and to favor the conservation of species diversity. A conversion from pure- to mixed-species stands has already been put into practice by forest administrations and has led to an overall increase in the proportion of mixed species forests in Europe (Knoke et al. 2008).

The general interest in the relationship between species diversity and ecosystem functions and services originally rose from the important loss of biodiversity occurring in most natural ecosystems world-wide (MEA 2005). More than 30 years of ecological studies have led to a consensus that ecosystem performance is highly dependent on species richness and/or functional diversity (Loreau et al. 2001; Hooper et al. 2005). Until recently, most of the research carried out on this subject concerned simple communities like grasslands (e.g. Tilman & Downing 1994; Hector et al. 2010). The ability to experimentally manipulate species in these ecosystems and the reasonable timeframe in which results are available explain ecological researchers' enthusiasm for studies in these ecosystems (Scherer-Lorenzen et al 2007; Hector et al. 2010). Woody species, however, were largely excluded from these early experiments, despite the ecological and socioeconomic importance of forest ecosystems. Nevertheless, over the last 15 years, a body of evidence demonstrating the benefits provided by higher tree species diversity in forest ecosystems has started to accumulate as well. Greater tree species diversity has been found to be related to improvement in some vital forest ecosystem functions such as wood production (e.g. Zhang et al. 2012), efficiency of nutrient use (e.g. Menalled et al. 1998) and resistance to insect pests or diseases (e.g. Jactel & Brockerhoff 2007).

Several mechanisms have been put forward in the literature to explain how species composition and functional diversity can influence ecosystem functions and services. For example, higher species diversity can have a negative influence resulting in lower ecosystem performance driven by direct competition for the available resources among co-existing species. These “negative” interactions commonly arise when ecological niches overlap and neighboring species with the same functional characteristics are functionally redundant in the ecosystem (Vila & Sardans 1999). In contrast, higher species diversity can also result in improved ecosystem performance; here, two distinct mechanisms are commonly involved: complementarity and selection. Firstly, the complementary acquisition and use of resources among species includes both ecological niche partitioning, where species coexist without major interspecific competition for resources, and facilitation processes whereby the presence of a given species will be beneficial for the functioning of a neighboring species (Loreau & Hector 2001). Secondly, the selection effect states that a highly-performing species, i.e. one leading to improved ecosystem functioning, is more likely to occur in ecosystems with high species diversity (Loreau & Hector 2001). Complementarity and selection mechanisms are not mutually exclusive; both can simultaneously affect ecosystem functions. Therefore, separately quantifying complementarity and selection effects is important to understand what underlying mechanisms are driving tree species diversity effects. In 2001, Loreau & Hector proposed a convenient method to partition the net biodiversity effect observed on ecosystem-level productivity into complementarity and selection effects. However, the application of this method has so far been limited to the study of only a few ecosystem functions because many ecological properties such as respiration, photosynthesis or the efficiency to acquire and use resources, are not additive as is productivity, and therefore cannot be calculated at the ecosystem level in the same way.

In addition to competition and the complementarity and selection processes, local environmental conditions are also known to play a role in species diversity effects on ecosystem functions and services (Hooper & Dukes 2004; Belote et al. 2011). Indeed, the interactions between any given pair of species are dynamic, changing as resource availability or climatic conditions change. It is not unusual for “positive” interactions that enhance ecosystem functioning to turn into “negative” interactions under new conditions, thereby reducing ecosystem performance. The reverse is also true. Under the assumptions of the ‘‘stress gradient’’ hypothesis, which predicts that the net outcome of biotic interactions shifts from negative to positive along gradients of limiting abiotic conditions (Bertness & Callaway

1994), beneficial species diversity effects are expected to be more common under severely resource-limited conditions. This conceptual framework is of great interest because it suggests that the relationships between species diversity and ecosystem functioning that exist today are likely to change in the future with the more intense and severe drought episodes forecasted by climate models (**Figure 1**). A large body of evidence supporting such a shift in the outcome of species interactions under more stressful environmental conditions has already been accumulated for marine, freshwater and terrestrial habitats (He et al. 2013; Herbert et al. 2004; Jucker & Coomes 2012; Steudel et al. 2012; Wang et al. 2013). However, observations which do not support the predictions of the "stress-gradient" hypothesis have also been found (e.g. Maestre & Cortina 2004; Maestre et al. 2005; Tielbörger & Kadmon 2000). This indicates that the exact role of environmental conditions in the relationship between species interactions and ecosystem functioning remains unclear.

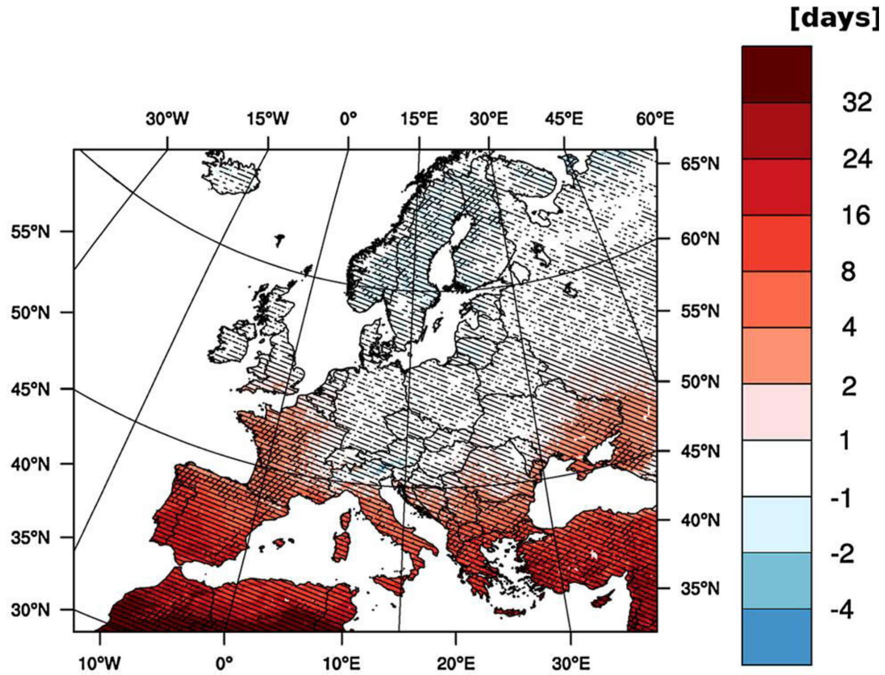


Figure 1: Increased frequency of drought by the end of the century in Europe. Projected changes in the 95th percentile of the length of dry spells (days) for 2071–2100 compared to 1971–2000. Mean of 20 regional EUROCODEX climate models (extracted from Jacob et al. 2014).

The expected increase in the frequency and severity of drought episodes (IPCC 2013, **Figure 1**) is one of the main changes in climatic conditions which could severely affect species interactions and consequently, ecosystem functioning in mixed habitats. The combined effects of changing temperatures and precipitation patterns will modify evaporative demand, gas exchange, carbon allocation, nutrient mineralization, and the growth and survival of plants in terrestrial ecosystems, among them forests (Saxe et al. 2000; Bréda et al. 2006; Rennenberg et al. 2006; McDowell et al. 2008). Tree species have a wide range of water use strategies in order to cope with very dry environmental conditions involving leaf- or plant-level structural and/or physiological adaptations (e.g. Kramer 1983). These different strategies have been widely studied, but much less is known about how the interactions among species with similar (or contrasting) water-use strategies during drought stress will affect their physiological responses to water depletion in forest ecosystems. In recent years, some evidence of the influence of tree species composition and richness on functions related to the water and carbon cycle has been found. For example, in dry-climate mixed forests, combinations of species with different functional traits and/or higher tree species richness have been shown to improve water use and influence water use efficiency (Forrester et al. 2010; Kunert et al. 2012). In a temperate wet-climate forests on the other hand, tree species richness itself was shown to have no direct impact on water use rates (Gebauer et al. 2012; Moore et al. 2011). These few case studies do not allow us to generalize these effects, but at least they have demonstrated the important influence of local climatic conditions on how tree species diversity affects the water and carbon cycle. Complementary studies are necessary to allow us to predict how tree species diversity will influence the response of forest ecosystems to drier climatic conditions and to prepare climate-smart management practices for the future.

The overall aim of this thesis was to analyze the role of tree species diversity on forest ecosystem functions related to the water and carbon cycle at both tree and stand-level under contrasted environmental conditions. The study area was located along a North-South gradient in Europe and includes Europe's main forest types and climatic conditions. Field measurements of tree transpiration, estimation of the depth of water uptake by trees and measurements of carbon isotope composition (a proxy for carbon and water trade-off) in foliar and woody material were conducted in forested sites including both young tree plantations and adult forest stands. The work was done within the framework of the European project "FunDivEUROPE" (FP7/2007-2013).

This thesis presents three years of work through a global overview and eight original papers (published or submitted). The different parts of this document complement each other although certain repetitions could not be avoided. A brief description of the material and methods is given before the main discussion. More detailed information on the experimental designs can be found in each related paper.

The specific aims of the different papers were to:

1. Present the design and implementation of the FunDivEUROPE exploratory platform consisting of forest stands along tree species diversity gradients in six major European forest types (**Paper I**). Note here that my personal contribution to this paper was only minor. However, the paper is referred to in this overview since it explains the experimental approach and design used for this work.
2. Adapt the original method proposed by Loreau & Hector (2001) in order to apply it to complex functional traits like stand-level water use efficiency (**Paper II**).
3. Determine the impact of tree species diversity on tree- and stand-level transpiration, water use efficiency and carbon accumulation under favorable soil water conditions (**Paper III**).
4. Determine the impact of tree species diversity on tree-level transpiration under dry soil conditions (**Papers IV & V**).
5. Compare the impact of tree species diversity on stand-level drought exposure across the main European forest types (**Papers VI & VII**).
6. Determine whether below-ground stratification in the depth of soil water uptake and a potential resulting complementarity for water use occurs in temperate mixed-species forests (**Paper VIII**).

2. Overview of the Material & Methods

2.1 Study sites

One major aim of the “FunDivEUROPE” project was to show the need for and subsequently implement a new generation of research that brings functional biodiversity research into the complex realm of the forest and examines ecosystem processes that provide important goods and services to humanity. More specifically, the scientists working within the framework of the “FunDivEUROPE” project are trying to quantify the effects of forest tree species diversity on ecosystem functions and services in major European forest types (www.fundiveurope.eu). To do so, two research platforms were established in order to include both experimental and observational approaches. This thesis includes studies conducted in both platforms (**Figure 2**).

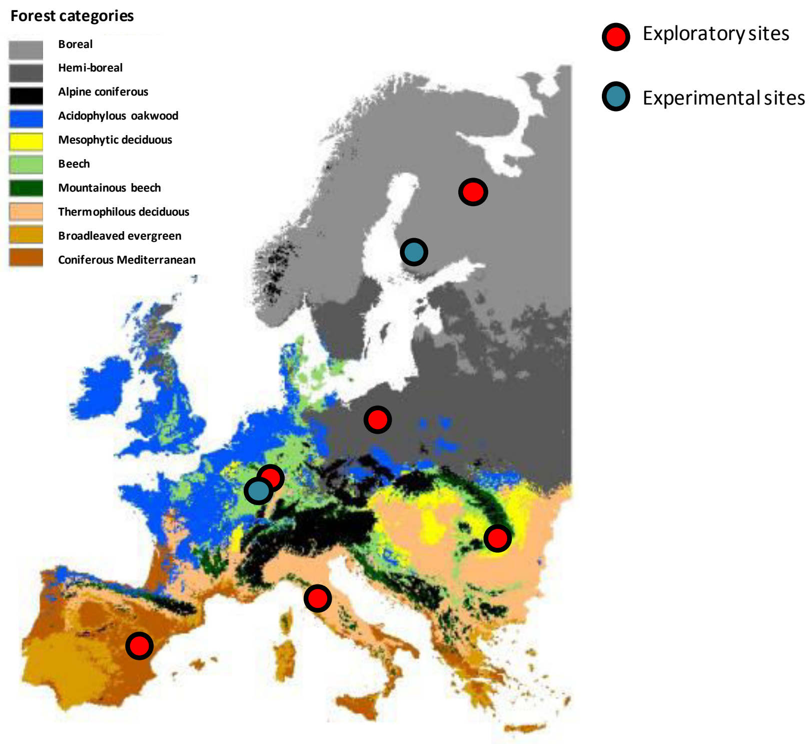


Figure 2: Geographical distribution of the study sites included either in the **experimental platform** (plantations) or in the **exploratory platform** (adult forests) of the FunDivEUROPE project. The experimental sites are the boreal plantation in Satakunta (Finland) and the BIOTREE temperate plantation (Germany). Going from North to South, the exploratory sites are a boreal forest (Finland), a hemi-boreal forest (Poland), a temperate beech forest (Germany), a mountainous temperate beech forest (Romania), a thermophilous deciduous forest (Italy) and a Mediterranean broadleaved-coniferous forest (Spain). The colored background in the figure shows the distribution of the European forest categories (extracted from Casalegno et al. 2011).

The **experimental platform** relies on tree diversity experiments conducted within the TreeDiv_Net network, where tree plantations differing in tree species diversity were established since 1999. The present work was conducted at only two experimental sites in the experimental platform: a mixed boreal plantation in Satakunta (Finland) and the BIOTREE and BIOTREE-simplex mixed temperate plantations in Kaltenborn (Germany) (**Figures 2 & 3**). More information on these sites can be found in **Papers II**, **III & VIII**.

Figure 3: Pictures of the experimental sites: (a) the mixed boreal plantation in Satakunta (Finland), (b) the BIOTREE-simplex temperate mixed plantation and (c) the BIOTREE temperate mixed plantation, both in Kaltenborn (Germany).

The **exploratory platform** consists of a specifically designed network of approximately 300 stands in mature forests in six different focal regions in Europe, replicated across wide gradients of tree species diversity. The six focal regions represent important European forest types along the gradient from boreal forests to Mediterranean forests (**Figures 2 & 4**). This approach maximizes representativeness at the continental scale. Within each focal region, the forest stands primarily differ in tree species richness (natural or management driven). Variations in other factors such as soil type, topography, land use history and spatial clustering, which may profoundly influence functions and services, were kept at a minimum. Some variability in these factors could not be avoided however, and they were therefore included in the statistical analyses as confounding factors. A detailed description of the exploratory platform can be found in **Paper I** while less detailed descriptions are presented in **Papers IV**, **V**, **VI**, **& VII**.

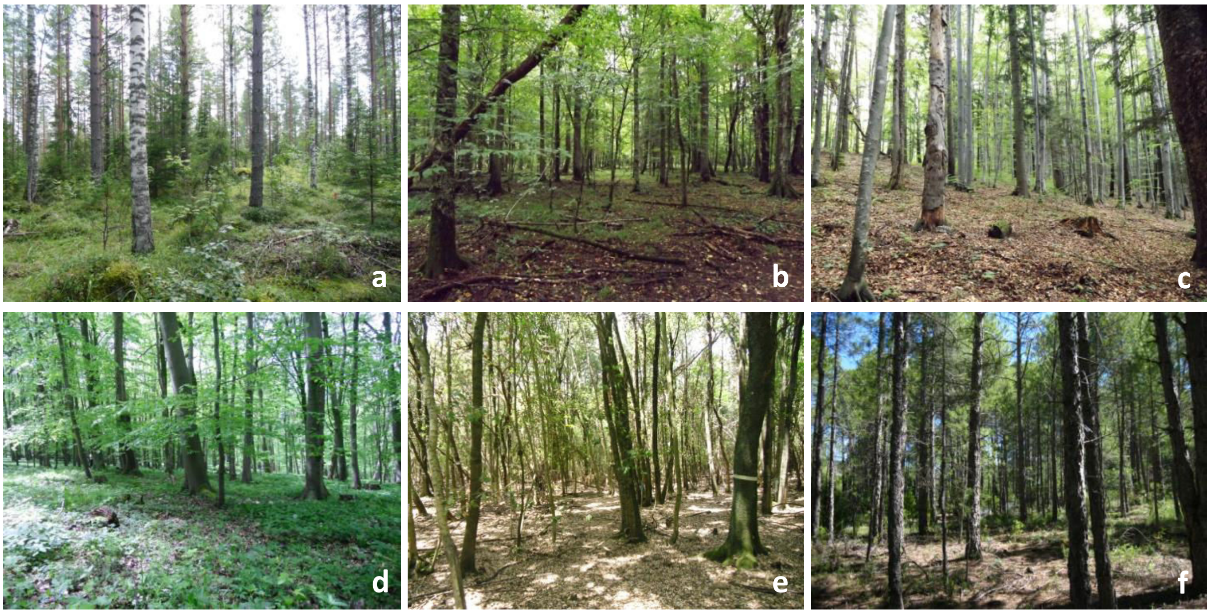


Figure 4: Pictures of the exploratory sites: (a) boreal forest in Finland, (b) hemi-boreal forest in Poland, (c) mountainous beech forest in Romania, (d) temperate beech forest in Germany, (e) thermophilous deciduous forest in Italy and (f) Mediterranean broadleaved-coniferous forest in Spain.

2.2 Sap flux measurements

At all sites included in the exploratory platform as well as in the experimental "Satakunta" and BIOTREE sites, we used the thermal dissipation method to measure sap flux density. In the Satakunta plantation, this work was conducted by A. Granier, D. Bonal and L. Bes De Berc during the summer of 2011 and data were made available for my PhD. At the other sites, the work was conducted by myself and A. Granier, with the help of D. Bonal at some sites, and the occasional help of students, local technicians, or other scientists. Depending on the study site, a subsample of dominant and/or co-dominant trees, for a given species and in a given number of forest stands was equipped with sap flux sensors. For each selected tree, the two 20-mm-long probes of the thermal dissipation sap flux sensors (UP-Gmbh, Cottbus, GE) (Granier et al. 1987) were installed under the bark and the cambium at around 1.3 m above the ground with a 10-cm vertical spacing between the probes (**Figure 5**).

Figure 5: (a) Sap flux sensor insertion into the trunk of a *Quercus cerris* tree in the thermophilous deciduous forest in Italy (June 2012) and (b) into a *Pinus sylvestris* tree in the Mediterranean broadleaved-coniferous forest in Spain (May 2013). (c) Two *Quercus cerris* trees in the thermophilous deciduous forest in Italy (June 2012) and (d) two *Pinus nigra* trees in the Mediterranean broadleaved-coniferous forest in Spain (May 2013) equipped with sap flux sensors after the covers had been put in place.

Each sensor was supplied with a power of 0.2 W. Data were collected every five or ten seconds on CR800 and/or CR1000 data loggers (Campbell Scientific Inc, Loughborough, UK) for periods ranging from two to five days. These measurement periods were repeated over several months at some sites, when technical issues (power supply, for instance) could be easily solved. Volumetric sap flux density (F_D, L dm^{-2} h^{-1}) was calculated following Granier (1987). Daily tree-level transpiration values based on F_D where converted into stand-level transpiration rates; however, because of the limited number of stands studied, no statistical analyses could be conducted on stand-level data. More information on the experimental design for the "Satakunta" site (Finland), the thermophilous deciduous (Italy) and Mediterranean broadleaved-coniferous forest sites (hereafter called Mediterranean, Spain) can be found in **Papers III**, **IV & V**, respectively. At the other study sites, a protocol similar to the one at the Italian site was applied (**Paper IV**). However, as the data obtained at these other sites were not sufficiently reliable, these results are not presented in this overview and nor are they referred to in any paper.

2.3 Foliar analyses

At all exploratory sites and in the experimental "Satakunta" and BIOTREE-simplex sites, the FundivEUROPE "Leaf Team" and I sampled ten to twenty fully expanded sunlit leaves for broadleaved species, or three to five 30-to-40-cm-long branches supporting fully mature needles for conifers, during the 2012 or 2013 summers on a subsample of dominant and/or co-dominant trees in each selected stand. All samples were collected during summers when no water stress occurred or before water stress had started (i.e. thermophilous deciduous and Mediterranean forests). Leaf samples were collected with tree clippers connected to extension loppers, by shooting down branches (Romania only), or by professional tree climbers who were hired to work on the project (**Figure 6**).

We used isotopic ratio mass spectrometers (IRMS) to measure the carbon isotope composition (δ^{13}C, ‰) and nitrogen contents (*N%, %*) on these foliar samples, and in addition, at the Satakunta site in Finland, the oxygen isotope composition (δ^{18}O, ‰). Sample preparation (grinding, micro-weighing) was conducted by myself with the occasional help of local technicians at INRA Nancy. Isotopic analyses were done either at the PTEF Isotope Facility of INRA Nancy or at the Isotope Facility of UC Davis (CA, USA).

δ^{13}C is positively related to leaf intrinsic water use efficiency (Farquhar et al. 1982), which is defined as the ratio of net CO_2 assimilation over stomatal conductance to water vapour. Therefore δ^{13}C provides information on intrinsic water use efficiency (WUE_{int}) integrated over various time periods depending on the turnover time of the organic matter pools assessed (Fotelli et al. 2003). However, the δ^{13}C of organic matter is influenced by a large range of environmental factors including light intensity, atmospheric CO_2 concentrations or water availability (reviewed in Ferrio et al. 2003b). To avoid effects related to gradients of light and CO_2 concentrations within forest canopies, we only sampled sunlit leaves or needles at the canopy top.

Figure 6: (a) Sampling of canopy leaves with tree clippers connected to extension loppers in the Mediterranean forest in Spain (June 2013) and (b) sampling of canopy leaves by a tree climber in the thermophilous deciduous forest in Italy (June 2012).

The combined measurement of δ^{18}O and δ^{13}C provides further insight into the changes in leaf δ^{13}C (Farquhar et al. 1998; Saurer et al. 1997). Indeed, variations in δ^{18}O are mainly driven by source water and changes in stomatal conductance (Farquhar et al. 1998). Measuring both δ^{18}O and δ^{13}C allowed us to distinguish between the stomatal and the photosynthetic origin of the changes in δ^{13}C (**Paper III**).

At the BIOTREE-simplex site, we used two Scholander-type pressure chambers (PMS Instruments, Corvallis, USA and UP Analytics, Cottbus, Germany) to measure predawn leaf water potential on the sampled leaves or twigs of the studied species. More information on specific measurements conducted for each site on the leaf samples can be found in **Papers II**, **III**, **IV**, **V & VIII**.

2.4 Wood analyses

At all the exploratory sites and with the help of the FunDivEUROPE "Core Team", I extracted one 5-mm-diameter wood core at breast height (1.3 m) on a subsample of dominant and/or co-dominant trees for each species and in each forest stand (**Figure 7**).

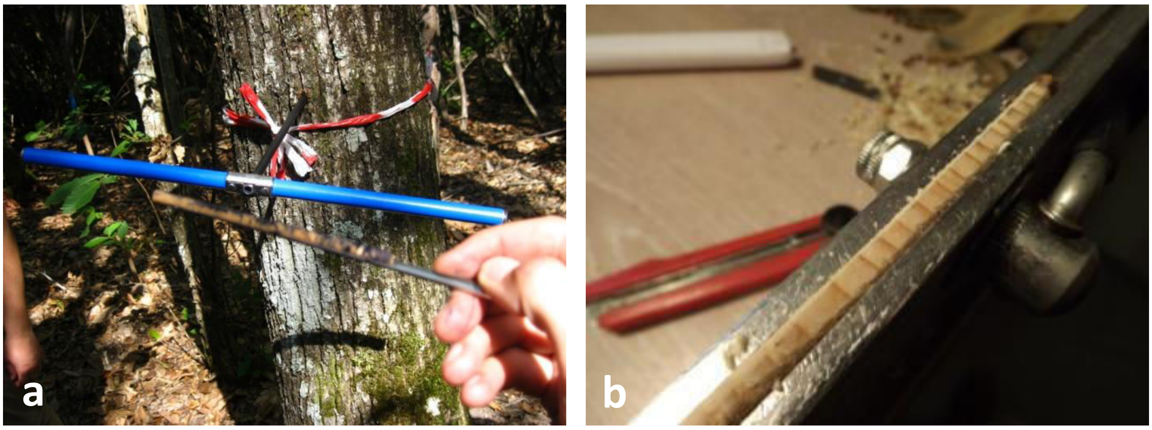


Figure 7: (a) Extraction of a wood core from a *Castanea sativa* tree in the thermophilous deciduous forest in Italy and (b) the wood core from a *Pinus sylvestris* tree from the boreal forest in Finland after being filed with a scalpel for tree-ring dating.

For each site, A. Granier and I selected two years with contrasting soil water limitations during the summer (dry *vs.* wet year) based on local climatic date and a water balance modeling approach (BILJOU model, Granier et al. 1999). Daily meteorological data for each forest study site were obtained from the CGMS database of interpolated meteorological data (AGRI4CAST, http://mars.jrc.ec.europa.eu/mars). Then, from each wood core, I extracted the late-wood corresponding to the two selected years for each site and prepared the samples for isotope analysis. The samples from a given species within a given stand were pooled together before isotope analysis. Samples from *Quercus ilex* in the Mediterranean forest were not analyzed for isotope composition as there was insufficient material in the late wood for the isotope analyses, particularly for the dry year. Furthermore, tree-ring detection was very difficult for this species. δ^{13}C values were measured at the Isotope Facility of PTEF (INRA Nancy) or UC Davis (CA, USA). More information on sample preparations and year selection for each site can be found in **Papers VI & VII**.

Plants typically react to a decrease in water availability by closing their stomata. Although carboxylation rates may also decline under water shortage, leaf conductance is usually affected to a larger extent, and this leads to a reduction in intercellular CO_2 concentrations and a concomitant increase in δ^{13}C (Farquhar et al. 1982). Therefore, the functional response of plants (at least in terms of CO_2 and H_2O exchange) to soil water deficit can easily be tracked by means of δ^{13}C analyses during dry conditions. This strong increase in δ^{13}C under dry soil conditions has been observed in many studies conducted either in growth-chambers or in the field (Ehleringer & Cooper 1988; Dupouey et al. 1993; Araus et al. 1997; Warren et al. 2001; Ferrio et al; 2003a). We therefore used the increase in δ^{13}C from wet to dry conditions ($\Delta\delta^{13}$C) as a proxy for the level of soil drought exposure undergone by each tree species and forest stand (**Papers VI & VII**).

2.5 Deuterium labelling

At the BIOTREE-simplex site, we conducted a labelling experiment in order to compare soil water extraction depth among species under different diversity levels (**Paper VIII**). This experiment consisted of spraying highly enriched deuterium water to the superficial soil layers in order to create an artificial vertical profile of soil water deuterium isotope composition (δ^2H, ‰). One day prior and three days after spraying the highly enriched solution, we extracted soil cores up to 150-m-depth and sampled twigs from a subsample of selected trees in the plantation. Water was extracted from the twig and soil samples with a cryogenic vacuum distillation system and the deuterium isotopic composition was measured on these water samples at the Isotope Facility of PTEF (INRA, Nancy). We then used a simple modelling approach to estimate the mean depth of soil water uptake for a given tree. More information on the experimental design and the overall labelling experiment can be found in **Paper VIII**.

Specific measurements that were conducted for each site are presented in **Table 1**.

Table 1: List of measurements conducted for each site: maximum number of interacting species (NB_{max}), sap flux density (F_D), foliar or wood carbon and oxygen isotope composition ($\delta^{13}C$ and $\delta^{18}O$, respectively), foliar nitrogen content (N%), predawn leaf water potential (ψ_{pd}), depth of water uptake (D_W), total number of sampled trees (NB_{tree}) and paper(s) corresponding to each study site.

Forest type	**Experimental**			**Exploratory**					
	Boreal	Temperate BIOTREE Simplex	Temperate BIOTREE	Boreal	Hemi-boreal	Mountainous beech	Temperate beech	Thermophilous deciduous	Mediterranean
NB_{max}	5	4	4	3	5	4	5	5	3
F_D	×		×	×	×	×	×	×	×
Foliar $\delta^{13}C$	×	×	×	×	×	×	×	×	×
Foliar $\delta^{18}O$	×								
Wood $\delta^{13}C$				×	×	×	×	×	×
Foliar N%	×	×	×	×	×	×	×	×	×
ψ_{pd}		×							
D_W		×							
NB_{tree}	255	122	100	180	372	206	275	259	252
Paper(s)	**III**	**VIII**	**II**	**I & VI**	**I & VII**	**I & VII**	**I & VII**	**I, IV & VII**	**I, V & VII**

3. Discussion

3.1 Functional diversity in water and carbon relations in European forests

Forests host tree species that are known to exhibit a wide range of functional characteristics and features which dictate their patterns of growth and survival. These species-level differences in ecophysiological strategy are deemed to play a crucial role in the coexistence of tree species and in the overall community structure of forest ecosystems (Kraft et al. 2008). Beyond species richness per se, there is growing consensus that the effects of diversity on ecosystem functions and services can be largely attributed to the functional diversity of co-existing species in diverse ecosystems (Grime 1997). Therefore, before discussing tree species diversity effects on carbon and water relations in European forest ecosystems, I will first describe the functional diversity in water and carbon relations which exist in these forests. To do so, I will use direct measurements as well as information on functional traits extracted from the literature. As this thesis mainly focuses on species diversity effects under conditions of water stress, the functional traits discussed here are mostly those known to influence the response of tree species to drought.

Tree species differ in growth rate, root architecture, gas exchange regulation, vulnerability to cavitation or seed dispersal, thus allowing their classification into different plant functional types. There is no universal functional type classification. Rather, the classification depends on the aim of the study, its scale (from local to global) and the ecosystem processes considered (Gitav & Noble 1997; Lavorel et al. 1997). For experimental studies and modeled predictions of community responses to a drier environment, certain tree species features such as life span or seed dispersal seem unrelated; instead, information on stomatal conductance, photosynthetic rates and carbon allocation patterns seems more suitable.

A convenient way to characterize the functional diversity of tree species in relation to their water and carbon fluxes in mixed forest ecosystems is to use the carbon isotope composition (δ^{13}C) of photosynthetically assimilated organic material. Indeed, δ^{13}C integrates information about how a plant regulates carbon acquisition and water fluxes since it relates to the ratio of net CO_2 assimilation during photosynthesis and to stomatal conductance for water vapour (i.e. WUE_{int}, Farquhar et al. 1982). The mean values obtained for the foliar δ^{13}C of the studied species in the pure stands of the exploratory platform are presented in **Figure 8**. These values show a large variability in foliar δ^{13}C (between – 24.9 ‰ for *Quercus faginea* and – 29.1 ‰ for *Betula pendula*) and thus in the compromise between carbon acquisition and stomatal regulation among European tree species. This large variability is, of course, partly explained by differences in environmental conditions among sites. Nevertheless, for a given forest type, the maximum differences in foliar δ^{13}C between species range from 0.5 ‰ in the boreal forest to 2.0 ‰ in the thermophilous deciduous forests (**Figure 8**). Furthermore, we observed that tree species with different phenology (conifers *vs.* broadleaved) exhibited important differences in δ^{13}C, although this observation does not hold true for all forest types and clear variations can also be found within a given species and/or phenological group (**Figure 8**).

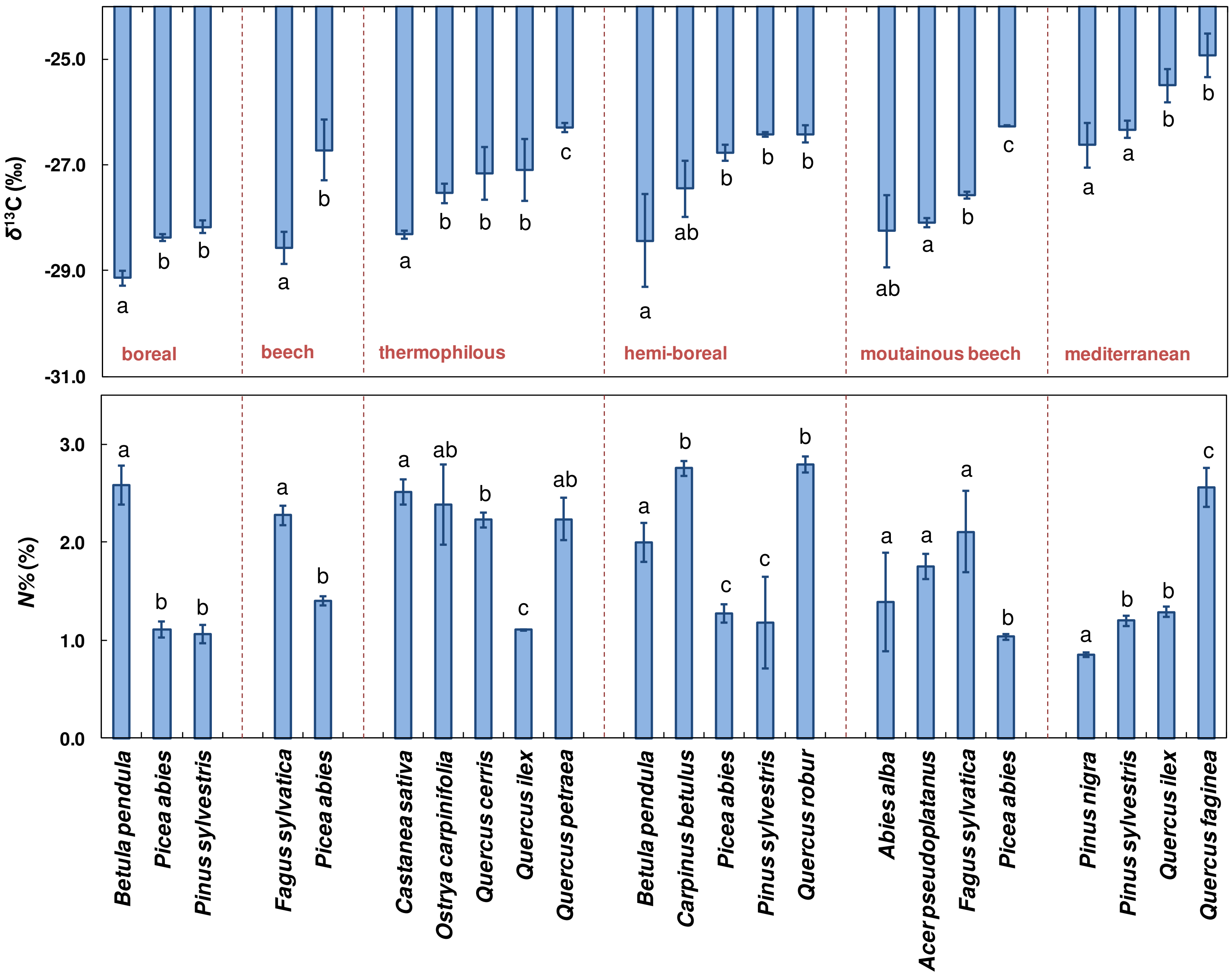


Figure 8: Mean foliar carbon isotope composition ($\delta^{13}C$, ‰) and nitrogen content (*N%*, %) for each sampled species in the pure forest stands in the boreal, temperate beech (beech), thermophilous deciduous (thermophilous), hemi-boreal, mountainous beech and Mediterranean broadleaved-coniferous (Mediterranean) exploratory platform forest sites. Note that three more species are present in the temperate beech forest type but no pure stands for these species exist within the region. Letters denote differences among species for a given forest type. Vertical bars are standard errors of the mean.

A wide interspecific variability was also found for foliar *N%* content (between 0.8 % for *Pinus nigra* and 2.8 % for *Quercus robur*) (**Figure 8**). The variability in foliar *N%* within a given forest type ranged from 0.88 % in the temperate beech forest to 1.71 % in the Mediterranean forest (**Figure 8**). The most important differences within a given forest type seem to be between coniferous and broadleaved tree species. Additionally, we identified strong differences between broadleaved-deciduous and broadleaved evergreen species (i.e. *Quercus ilex* in the thermophilous deciduous and Mediterranean forests, **Figure 8**). *N%* content is generally known to affect the photosynthetic performance of plants across a wide range of life-forms (Reich et al. 1992); these results are consistent with the wide variability in growth performance we observed among these tree species (FundivEurope data, not shown here).

I also researched the literature for functional traits related to water and carbon relations and to the response of our studied tree species to drought (**Table 2**). Some of these traits are either quantitative and corrected for variability (i.e. ψ_{50}), or simple observations (i.e. phenology). However, it should be noted that some traits could be influenced by both biotic and abiotic conditions (e.g. rooting depth) and therefore might not always represent the potential values encountered in our experimental forest stands.

Table 2: Species functional characteristics in the exploratory platform. Forest types in which the tree species occur are presented in Section 1 of the Supplementary Information (SI). Description of light preference is given in Section 2 of SI. ψ_{50} represents the water potential level causing a 50% loss of conductivity in stems and reflects a measure of vulnerability to xylem embolism (Choat et al. 2012). Corresponding references for each trait are given in Section 3 of the SI. Blanks represent traits for which information could not be found (e.g. ψ_{50} of *C. sativa*).

Species	Light preference	Initial growth rate	Phenology*	Rooting depth	ψ_{50} (MPa)
A. alba	3	Slow	C	Deep	-3.6
A. pseudoplatanus	4	Fast	DB	Medium	-2.2
B. pendula	7	Fast	DB	Medium to deep	-2.4
C. betulus	4	Fast	DB	Medium	-3.8
C. sativa	5	Fast	DB	Deep	
F. sylvatica	3	Slow	DB	Medium	-3.2
F. excelsior	4	Fast	DB	Deep	-2.8
O. carpinifolia	4	Fast	DB	Shallow	
P. abies	5	Slow	C	shallow	-3.9
P. nigra	7	Fast	C	Medium to deep	-2.8
P. sylvestris	7	Slow	C	Shallow to medium	-3.6
Q. cerris	6	Fast	DB	Deep	
Q. faginea		Slow	DB	Deep	
Q. ilex	4	Slow	DE	Deep	-2.2
Q. petraea	6	Slow	DB	Deep	-3.5
Q. robur	7	Slow	DB	Deep	-2.8

The information shown below clearly points out potential contrasted functional responses to drought in the studied species within a given forest type (Table 2). In the **boreal**, **hemi-boreal, mountainous beech, temperate beech** and **Mediterranean forests**, drought intolerant conifers (*Picea abies* and *Pinus sylvestris*) are interacting in mixed stands with drought tolerant conifers (*Abies alba* and *Pinus nigra*) and drought tolerant broadleaved species (*Acer pseudoplatanus*, *Betula pendula*, *Carpinus betulus*, *Fraxinus excelsior*, *Fagus* *sylvatica*, *Quercus faginea*, *Quercus petraea* and *Quercus robur*). However, even the broadleaved species have well known differences in ecophysiological strategies. We can hypothesize that the deepest-rooted species such as *Fraxinus excelsior*, *Quercus faginea*, *Quercus petraea* and *Quercus robur* will probably have a different response to drought stress than the other more shallow-rooted broadleaved species. The **thermophilous deciduous forest** has the particularity of hosting only broadleaved tree species. However, even these species seem to present considerable variability in their ecophysiological strategies. Here, drought tolerant broadleaved deciduous species (*Castanea sativa*, *Quercus cerris* and *Quercus petraea*) are interacting with a drought tolerant evergreen species (*Quercus ilex*) and a rather drought intolerant broadleaved deciduous species (*Ostrya carpinifolia*).

In brief

Within a given forest type, the tree species studied here present important functional dissimilarities and overall have different responses to fluctuating environmental conditions. One can therefore expect these species to show some degree of complementarity for resource acquisition and use. For example, we may expect shallow-rooted conifers to occupy different above- and below-ground ecological niches than do deep-rooted broadleaved species (e.g. *Pinus sylvestris* and *Quercus faginea* in the Mediterranean forest type). If each species occupies a different ecological niche in a given forest stand, the rate of ecosystem processes measured should depend linearly on species richness and evenness (i.e. the Shannon diversity index). Inversely, if most or even all species occupy the same ecological niche, the rate of ecosystem processes measured should not be dependent on the Shannon diversity index.

3.2 Effects of tree species diversity on water and carbon relations under non-limiting soil water conditions

The important dissimilarities in functional characteristics observed among co-existing tree species in European forests presupposes an important effect of species diversity on ecosystem functioning. Under non-limiting soil water conditions, previous studies have successfully demonstrated that higher tree species diversity improves ecosystem productivity (e.g. Morin et al. 2011; Paquette & Messier 2011; Vilà et al. 2013). These studies did, however, raise the question of whether higher productivity in more diverse forests could partially be due to higher/better water use in these ecosystems. Such a response could be expected in species diverse forests since enhanced productivity is often associated with increased transpiration rates (Law et al. 2002). Simultaneously, we could hypothesize that changes in productivity and transpiration rates could themselves impact the water use efficiency of tree species. In this chapter, I will discuss how transpiration, productivity and water use efficiency are influenced by tree species diversity under non-limiting soil water conditions based on my own results and on previous observations from the literature.

Two recent studies have shown that higher tree species diversity (Kunert et al. 2012) or the interaction of two species with highly contrasting functional traits (Forrester et al. 2010) improves stand productivity. These studies expand on previous ones by demonstrating that this enhanced above-ground wood production was mainly a consequence of improved transpiration rates. The authors mainly attributed changes in ecosystem functioning to both above- and below-ground niche stratification in mixed stands (i.e. complementarity processes). However, during periods when water resources are sufficiently available throughout the soil profile, complementarity in water acquisition and use through below-ground processes does not seem to be the most probable cause for such improved transpiration rates. Instead, above-ground canopy stratification may explain these results.

3.2.1 Effects at the tree-level

Above-ground stratification in mixed-species forests takes place because tree species can have different phenology and/or canopy structures (Kelty 2006). As a consequence, different light gradients occur both vertically and horizontally in mixed *vs.* pure forest stands. These light gradients lead to different levels of light interception for trees in pure and mixed-species stands (Kelty 2006). Increasing light interception promotes plant photosynthesis and water use and is therefore often considered to be one of the positive mechanisms underlying higher productivity and transpiration in mixed-species forest ecosystems (e.g. Forrester et al. 2010; Morin et al. 2011; Kunert et al. 2012; Zhang et al. 2012).

The results from the study we conducted on the sap flux density of three tree species in a mixed boreal plantation (**Paper III**) suggest that even if above-ground stratification of the canopy occurs in mixed stands, it does not always lead to improved functioning for all the interacting species. Above-ground stratification clearly occurred in the young plantation at Satakunta, with *Betula pendula* trees occupying the highest layers of the canopy and all the other species, particularly *Picea abies,* occupying a dominated and slightly shaded position (**Figure 9**).

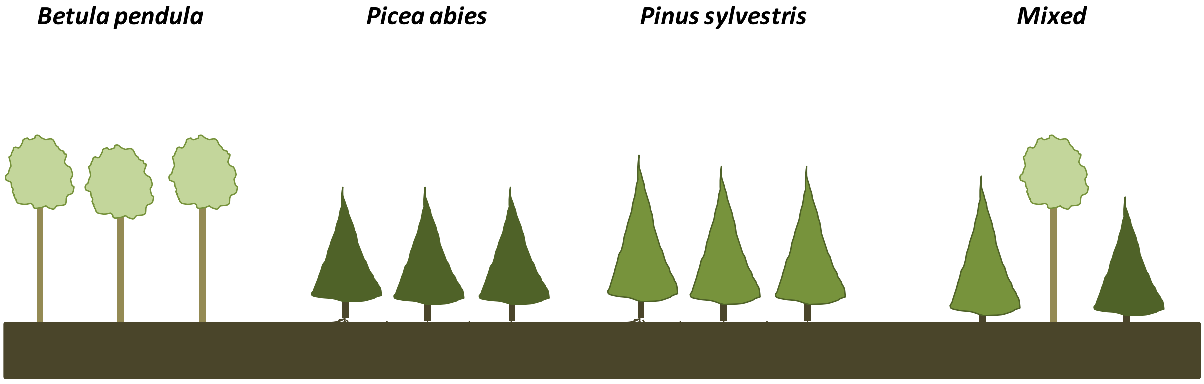


Figure 9: Schematic representation of the pure and the 3-species mixture studied for sap flux density in the mixed boreal plantation at Satakunta (Finland) (**Paper III**). *Betula pendula* trees occupied the top layers of the canopy while the two conifers occupied a dominated and slightly shaded position in the mixed stands.

We found that transpiration for *Picea abies* was lower in the mixed than in the pure stand, and a similar, although not significant, pattern was observed for *Pinus sylvestris* trees (Fig. 4 in **Paper III**). On the contrary, *Betula pendula* trees did not exhibit a change in sap flux density between the mixed and pure stands. As water availability was not limiting for tree transpiration during these measurements, we attributed the differing levels of sap flux density to changes in light regimes between mixed and pure stands. This hypothesis was confirmed by patterns in foliar $\delta^{13}C$ (Fig. 6 in **Paper III**). Higher radiation is known to increase $\delta^{13}C$ values, mainly through its direct effect on photosynthetic activity (Farquhar et al. 1982). The conifers exhibited lower $\delta^{13}C$ (a proxy for WUE_{int}) in the mixtures, which we attributed to impaired photosynthetic activity. In contrast, the reverse pattern was found for *Betula pendula*: *Betula pendula* trees encountered less competition for light in mixed stands since the interacting trees of the companion species were shorter (**Figure 9**), and *Betula pendula* WUE_{int} was higher in mixed stands than in pure ones. It should be noted, however, that the differences in WUE_{int} and transpiration among stands were rather small and did not lead to significant differences among stands in above-ground biomass production (Fig. 2 in **Paper III**). Similar contrasted responses among species to diversity were also found by Forrester et al. (2010) in Australia. These authors found that mixing light-demanding Eucalyptus trees with shade-tolerant Acacia trees improved the transpiration of Eucalyptus but seemed to decrease that of Acacia. Furthermore, similar to our observations in the boreal plantation, differences among species in their response to diversity were also reflected in water use efficiency levels: the water use efficiency of Eucalyptus improved in mixed stands whereas that of Acacia decreased slightly.

In the mature forests of the exploratory platform, we measured both foliar $\delta^{13}C$ under non-limiting soil water conditions and $\delta^{13}C$ of late wood samples during a year when no water stress occurred. For both measurements, we found no evidence of an effect of tree species diversity on $\delta^{13}C$ (i.e. WUE_{int}) for any species except one (*Castanea sativa* in the thermophilous deciduous forest). Rather than species diversity, we found that other environmental factors that could not be controlled for when selecting the stands (leaf area index, competition intensity, altitude, soil C/N ratio) explained part of the variability in $\delta^{13}C$ among forest stands (Section 4 in SI). In any case, our results suggest that no clear above-ground stratification occurred in these mature forests in comparison to the young boreal plantation. Even if above-ground stratification had occurred in these stands, the mechanism would have been of such low intensity that it would not have affected the trees' water and carbon acquisition and use. The only species that showed any significant effect of species diversity on $\delta^{13}C$ (with a negative relationship) was *Castanea sativa* in the thermophilous deciduous forest. It is quite difficult to interpret this result since *Castanea sativa* is suffering from large die-off events in this region caused by *Phytophthora*, also called "ink disease" (H. Jactel and V. Guyot, perso. comm., **Figure 10**). Although we only sampled trees that looked healthy, we cannot exclude that some of them might have been affected by the disease; indeed, infected trees usually suffer from extensive necrosis of the root system before any apparent above-ground symptoms occur (Vannini & Vettraino 2001).

Figure 10: (a) Branch sample of a *Castanea sativa* tree affected with ink disease, (b) extraction of a wood core from a *Castanea sativa* tree affected with ink disease, (c) dead *Castanea sativa* trees killed by ink disease near the exploratory platform in the thermophilous deciduous forest (Italy).

3.2.2 Effects at the stand-level

At the stand-level, neither of the studied traits (productivity and δ^{13}C) were influenced by species diversity in any of our experiments (Fig. 3 & 5 in **Paper III**).

The lack of any effect on stand δ^{13}C in the young boreal plantation (Fig. 3 in **Paper III**) suggests that individual species responses to species diversity compensated for each other at the stand-level. Interestingly, this was consistent with the lack of effect on productivity which was less surprising since no significant effect of diversity on productivity had been detected at the tree-level. Statistical analyses could not be performed on stand-level transpiration because of the limited number of stands where transpiration measurements were taken. It seems, however, that the more diverse stands did not exhibit higher transpiration rates than what would have been expected based on pure stands (Fig. 5 in **Paper III**).

In the adult forest stands of the exploratory platform, variations in foliar and wood δ^{13}C among stands were not explained by species diversity levels under non-limiting water conditions (**Table 3**). However, they were explained to some extent by differences in nutrient availability (i.e. C/N ratio in the boreal and Mediterranean forests) or light regime (i.e. leaf area index in the hemi-boreal, temperate beech and Mediterranean forest). Our results in mature forests for these six European regions do not, therefore, confirm the findings of Forrester et al. (2010) for stand-level water use efficiency. It should be noted, however, that in Forrester et al.'s study (2010), an important part of the improved water use efficiency was explained by the presence of a nitrogen-fixing species in the mixture (i.e. Acacia). Furthermore, Kunert et al. (2012), who also observed higher transpiration and productivity in mixed species stands, could not identify a clear pattern of response for stand-level water use efficiency. Whether species diversity deeply affects ecosystem functions under non-limiting environmental conditions remains an open question. FunDivEUROPE colleagues (i.e. T. Jucker, D. Coomes and O. Bourriaud) are currently analysing these effects on productivity.

In brief

These observations suggest that under favourable soil water conditions, species diversity has no clear direct effect on species- or stand-level δ^{13}C - and consequently, no clear effect on water-use efficiency - in mature forests across Europe. Nevertheless, the data from the boreal plantation indicates that some effects on δ^{13}C and transpiration might appear at a young developmental stage and at the tree-level only. This single case does not allow me to draw any conclusions as to whether the occurrence of species effects under non limiting soil water conditions could be dependent on ontogeny; conducting further studies on a chronosequence could help fill in this gap.

In the boreal plantation, diversity effects seem to be driven by above-ground complementarity processes that affect light acquisition and use by the co-existing tree species. In a recent study, del Rio et al. (2014) suggested that under high soil water conditions, species diversity effects are mostly explained by differences in light supply. However, these differences can only occur if the neighbouring species have very different canopy structures which result in important above-ground space partitioning.

Table 3: Results from the statistical tests on stand-level foliar and wood carbon isotope composition for each forest type under non-limiting soil water conditions. Mixed linear models were used to determine the effects of the Shannon diversity index, altitude, leaf area index (LAI), competition intensity (i.e. stand basal area) and soil C/N ratio on stand-level foliar carbon isotope composition ($\delta^{13}C_{SF}$) and on stand-level wood carbon isotope composition ($\delta^{13}C_{SW}$). Stand was used as a random factor. Slopes of the fitted relationships between $\delta^{13}C_{SF}$ or $\delta^{13}C_{SW}$ and the factors are given when the effect is significant ($P < 0.05$). Significant *P*-values for each factor included in the model are presented in bold characters.

Factors	**Boreal**			**Hemi-boreal**			**Mountainous beech**			**Temperate beech**			**Thermophilous deciduous**			**Mediterranean**		
$\delta^{13}C_{SF}$	*F-value*	*P-value*	Slope	*F-value*	*P-value*	Slope	*F-value*	*P-value*	Slope	*F-value*	*P-value*	Slope	*F-value*	*P-value*	Slope	*F-value*	*P-value*	Slope
Shannon	2.52	0.127		0.33	0.564		4.10	0.069		0.01	0.975		4.93	0.061		0.03	0.850	
Altitude	1.80	0.193		0.51	0.476		0.23	0.632		0.03	0.862		4.02	0.068		3.72	0.067	
LAI	3.12	0.092		2.04	0.161		0.02	0.872		0.99	0.262		0.06	0.801		5.25	**0.029**	**-0.28**
Basal area	4.77	0.073		1.05	0.310		0.01	0.909		2.04	0.165		1.50	0.230		3.71	0.069	
C/N	20.27	**< 0.001**	**0.09**	1.99	0.167		0.43	0.518		0.11	0.740		0.01	0.955		4.92	**0.034**	**0.05**
$\delta^{13}C_{SW}$																		
Shannon	0.48	0.498		2.43	0.127		4.04	0.059		0.62	0.437		0.03	0.873		4.52	0.056	
Altitude	1.04	0.318		1.28	0.266		0.08	0.776		0.10	0.750		2.57	0.120		0.01	0.936	
LAI	0.34	0.568		14.26	**< 0.001**	**-0.28**	2.42	0.135		7.06	**0.012**	**-0.31**	1.70	0.203		6.46	**0.022**	**-0.16**
Basal area	0.55	0.467		0.35	0.560		0.43	0.520		0.01	0.909		3.09	0.089		0.53	0.476	
C/N	0.39	0.541		0.97	0.331		0.43	0.520		0.01	0.993		6.90	0.013	0.06	1.41	0.253	

3.3 Effects of tree species diversity on water and carbon relations under limiting soil water conditions

The influence of climatic conditions on species diversity effects has received growing interest by ecologists in the last decade. It is now well agreed that climatic conditions are directly influencing the outcome of species interactions, and therefore the relationship between diversity and ecosystem functioning. Considering the forecasted drier and warmer climatic conditions that will occur in the next decades worldwide (IPCC, 2013), we may expect to observe a change in species diversity effects on ecosystem functioning in the future. Whether interactions among species will translate into beneficial or detrimental effects on tree and ecosystem functioning remains unclear. In this chapter, based on observations acquired during my thesis and on results from the literature, I discuss whether tree species diversity influences tree-level transpiration and tree- and stand-level drought exposure in forest ecosystems under limiting soil water conditions.

3.3.1 Effects at tree-level

3.3.1.1 Transpiration

I conducted two experiments to test the impact of species interactions on the decrease in transpiration during a summer drought (**Papers IV & V**). These experiments were carried out in the two southernmost sites of the exploratory platform (Spain & Italy) where severe soil drought occurs frequently during the summer months (Fig. S1 & Table S4 in Supplementary Information of **Paper VII**).

In the thermophilous deciduous forest (Italy) during a dry summer, we followed the seasonal variations in sap flux density of two co-existing drought-tolerant oak species (*Quercus cerris* & *Quercus petraea*) in pure and mixed stands. *Quercus cerris* suffered from higher drought exposure (i.e. a greater decrease in sap flux density) when interacting with *Quercus petraea* than when growing in monospecific conditions (Fig. 3 in **Paper IV**), whereas *Quercus petraea* was unaffected by inter-specific interactions (Fig. 3 in **Paper IV**). We suspect that the negative effect of the mixture on *Quercus cerris* transpiration is in part explained by the fact that the roots of *Quercus petraea* might out-compete those of *Quercus cerris* in the deep soil layers when the two species are interacting. Differences in the lateral spread of the roots in the two oak species could also have played a role in the observed responses. We were not able to directly study this effect during our research, but *Quercus petraea* is known to have a rooting system that explores both shallow and deep soil layers, and to extend its shallowest roots long distances from its trunk (Bréda et al.1995). Such a mechanism can allow this species to better acquire soil resources and can be detrimental to co-existing species that do not spread their root systems so widely. However, very little information on *Quercus cerris* can be found in the literature and even less concerning its rooting capacity and structure. Although any of the processes cited above could have partially explained the response we found for the two oak species, no clear conclusion can be drawn here on these mechanisms.

In the study that we conducted in the Mediterranean forest (Spain), we followed the variations in sap flux density of one drought-tolerant broadleaved species (*Quercus faginea*), one drought-tolerant conifer (*Pinus nigra*) and one less drought-tolerant conifer species (*Pinus sylvestris*) during the 2013 summer drought. We found that *Quercus faginea* clearly benefited from the interactions with the two other species (i.e. less marked decrease in sap flux density) while *Pinus sylvestris* was more exposed to drought stress when interacting with non-conspecific neighbors (i.e. a greater decrease in sap flux density) (Fig. 4 in **Paper V**). *Pinus nigra* showed only a benefit from the interaction with a shallower tree species such as *Pinus sylvestris* (Fig. 4 in **Paper V**). The important differences among these three species in water requirements and rooting strategy could explain part of their different responses to species interactions. Shallow-rooted species such as *Pinus sylvestris* seem to be negatively impacted by the interactions with deeper-rooting and more water-consuming species such as *Quercus faginea* and *Pinus nigra*. Inversely, more drought-tolerant species (i.e. *Quercus faginea* & *Pinus nigra*) should experience less competition for water in the deep soil layers when they are mixed with shallower rooting species that take up less soil water.

Taken together, these two studies demonstrate that the effect of species interactions in response to water deficit can be beneficial, detrimental or non-existent. Our results suggest that species interaction effects at the tree-level during drought periods can vary widely depending on local site characteristics and the species considered.

3.3.1.2 Drought exposure levels

The very few studies interested so far in the relation between tree species diversity and forest ecosystem response to drought have focused on productivity. Among them, Lebourgeois et al.

(2013) observed beneficial species mixture effects for *Abies alba* in the Vosges mountains where interactions with *Fagus sylvatica* slightly reduced the decrease in growth of *Abies alba* under dry conditions. The authors attributed this effect to a below-ground root stratification and a lower canopy rainfall interception in the mixed stands. Similarly, in southern Germany, Pretzsch et al. (2013) found that mixing *Fagus sylvatica* with *Quercus petraea* improved the former species' resistance and resilience to drought as compared to pure situations. During the severe drought in 1976, *Fagus sylvatica* showed a 72% reduction in growth in pure stands compared to a reference period, but only a 63% reduction in the mixtures. The authors attributed this effect mainly to hydraulic lift performed by *Quercus petraea* which benefited the *Fagus sylvatica* trees. When studying the same species combination, Zapater et al. (2011) also found that *Quercus petraea* performed hydraulic lift; however, they were unable to detect any direct benefit to *Fagus sylvatica* during a summer drought.

My PhD research objective was to test whether tree species in more diverse stands are less subjected to water depletion during intense droughts than when they occur in less diverse stands, applied to as broad a range of tree species and diversity levels as possible. The exploratory platform of the FunDivEUROPE project was a great opportunity. As a proxy for drought exposure level for all species and all stands, we used the increase in δ^{13}C in tree-rings between wet and dry years ($\Delta\delta^{13}$C) (see **Papers VI & VII** for results at stand-level). Under the assumption that complementarity for water use takes place, one should observe a smaller $\Delta\delta^{13}$C with increasing tree diversity (i.e. a negative relationship between $\Delta\delta^{13}$C and diversity). Inversely, if tree species occupy redundant ecological niches and highly compete for water resources, one would expect to observe a similar or even greater $\Delta\delta^{13}$C with increasing diversity (i.e. a null or positive relationship between $\Delta\delta^{13}$C and diversity).

When considering all six focal regions across Europe, we found a large variability in the response of species $\Delta\delta^{13}$C to tree species diversity under drought conditions (Section 5 in SI). Some species exhibited a clearly detrimental effect of diversity (i.e. a higher increase in δ^{13}C in the more diverse stands) while others exhibited a beneficial effect (i.e. a lower increase in δ^{13}C in the more diverse stands) or no effect at all (**Table 4**; Section 5 in SI). Furthermore, an important variability in the intensity of this effect was found among species (slopes between the Shannon index and $\Delta\delta^{13}$C varying from – 0.78 ‰ for *Fagus sylvatica* in the temperate beech forest to 1.10 ‰ for *Picea abies* in the mountainous beech forest, **Table 4**).

Table 4: Results from the statistical tests on species-level increase in carbon isotope composition between wet and dry conditions. Mixed linear models were used to determine for each species within each forest type the effects of the Shannon diversity index (calculated with the basal area of each species) on species-level increase in carbon isotope composition from a wet to a dry year ($\Delta\delta^{13}C$). The global model also included the effects of altitude, leaf area index, competition intensity and soil C/N ratio (Section 5 in SI). Significant *P*-values are shown in bold.

Forest type	Species	***F*- values for the Shannon index**	***P*- values for the Shannon index**	Slope
Boreal	*B. pendula*	0.05	0.813	
	P. abies	5.94	**0.034**	**0.88**
	P. sylvestris	4.17	**0.040**	**0.72**
Hemi-boreal	*B. pendula*	1.48	0.241	
	C. betulus	0.03	0.859	
	P. abies	1.27	0.275	
	P. sylvestris	2.03	0.173	
	Q. robur	0.32	0.578	
Mountainous beech	*A. alba*	0.01	0.962	
	A. pseudoplatanus	0.01	0.907	
	F. sylvatica	3.81	0.076	
	P. abies	1.46	0.271	
Temperate beech	*A. pseudoplatanus*	0.24	0.633	
	F. sylvatica	10.14	**0.007**	**- 0.78**
	F. excelsior	7.74	**0.016**	**- 0.18**
	P. abies	0.09	0.776	
	Q. petraea	1.35	0.274	
Thermophilous deciduous	*C. sativa*	5.55	**0.041**	**- 0.66**
	O. carpinifolia	0.80	0.393	
	Q. cerris	4.06	0.061	
	Q. ilex	10.11	**0.006**	**- 0.14**
	Q. petraea	12.59	**0.004**	**- 0.38**
Mediterranean	*P. nigra*	0.04	0.833	
	P. sylvestris	0.29	0.607	
	Q. faginea	5.21	**0.045**	**- 0.05**

Within a given site, a large range of responses was observed among species, pointing to an important variability in the responses of co-existing tree species to species interactions during drought stress. We also observed that a given tree species can exhibit contrasting responses to species interactions in terms of drought exposure in different forest types. For example, *Pinus sylvestris* showed higher drought exposure with increasing tree diversity in the boreal forest (where it is interacting with *Betula pendula* and *Picea abies*), but was unaffected by species interactions in the Mediterranean and hemi-boreal forests (interaction with *Pinus nigra* and *Quercus faginea* or with *Abies alba*, *Fagus sylvatica* and *Acer pseudopltanus*, respectively). This result highlights the importance of interacting species identity and local environmental conditions in species diversity effects.

Our results confirmed that under limiting soil water conditions, some species clearly benefit from the presence of other species in the stands and are subsequently less affected by drought conditions (lesser increase in $\Delta\delta^{13}$C). This pattern is consistent with previous findings (Lebourgeois et al. 2013; Pretzsch et al. 2013). In contrast, we also found that some species clearly do not benefit from these interactions, or even underwent more severe drought impact in species diverse stands (*Picea abies* & *Pinus sylvestris* in the boreal forest). Therefore, one cannot conclude that there is a general pattern for beneficial species diversity effects. The response of a given tree species seems to be highly context-dependent and to vary with local climatic, and undoubtedly edaphic conditions and with the identity of the other co-existing species. How these different responses are reflected at the stand-level when the individual species responses are combined and what underlying mechanisms potentially drive these responses are discussed in the following chapter.

3.3.2 Effects at stand-level

We found considerable variability among forest types in the effects of drought on the relationship between species diversity and forest drought exposure levels (**Papers VI & VII**). For some forest types (i.e. temperate beech and thermophilous deciduous forests), more diverse stands are less subjected to intense droughts (Fig. 1 in **Paper VII**; **Figure 11**). This result implies that at stand level, water availability was higher in diverse stands than in pure ones under drought conditions in these regions. In contrast, other sites showed no effect (i.e. hemi-boreal, mountainous beech and Mediterranean forests) or even showed a higher drought exposure with increasing diversity (i.e. boreal forest) (Fig. 1 in **Paper VII**; **Figure 11**). As for the species level, no general pattern in the effect of tree species diversity on drought exposure at stand-level was detected.

In the forest types that showed no response to diversity levels, we hypothesize that most, if not all the species, are functionally redundant for water acquisition and use. Indeed, it seems that interactions among different species in these forests have the same effect on drought exposure levels than interactions among conspecific neighbours. However, for both the hemi-boreal and mountainous beech forests, the variability among stands in $\Delta\delta^{13}C_S$ was partly explained by competition intensity, which reflects a higher competition for water (i.e. basal area, Fig. S3 in **Paper VII**). Stands with higher basal area showed the greatest increase in $\Delta\delta^{13}C_S$, and consequently, the most severe soil water limitation.

Beneficial or detrimental effects of species diversity on drought exposure levels are driven by various interaction mechanisms among species. As I did not directly measure these underlying processes, the explanatory patterns I present hereafter are purely hypothetical.

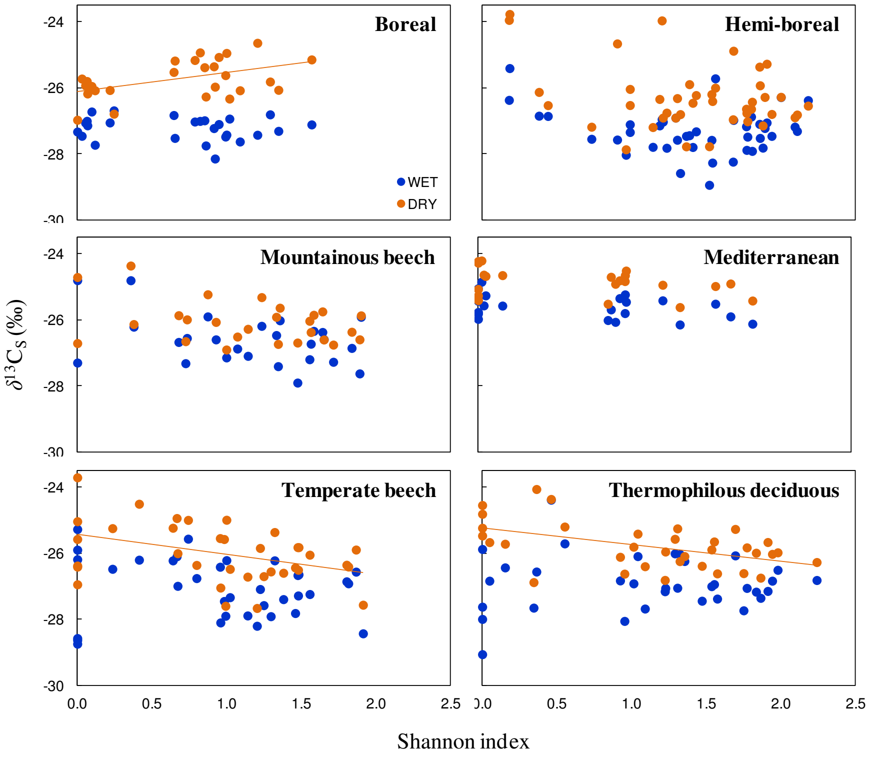


Figure 11: Stand-level carbon isotope composition under dry and wet conditions in relation to tree species diversity for each forest type. Relationships between stand-level carbon isotope composition ($\delta^{13}C_S$, ‰) for wet (blue) and dry (red) years and the Shannon diversity index for the boreal, hemi-boreal, mountainous beech, Mediterranean, temperate beech and thermophilous deciduous forest types. Solid lines show the statistically significant relationships ($P < 0.05$).

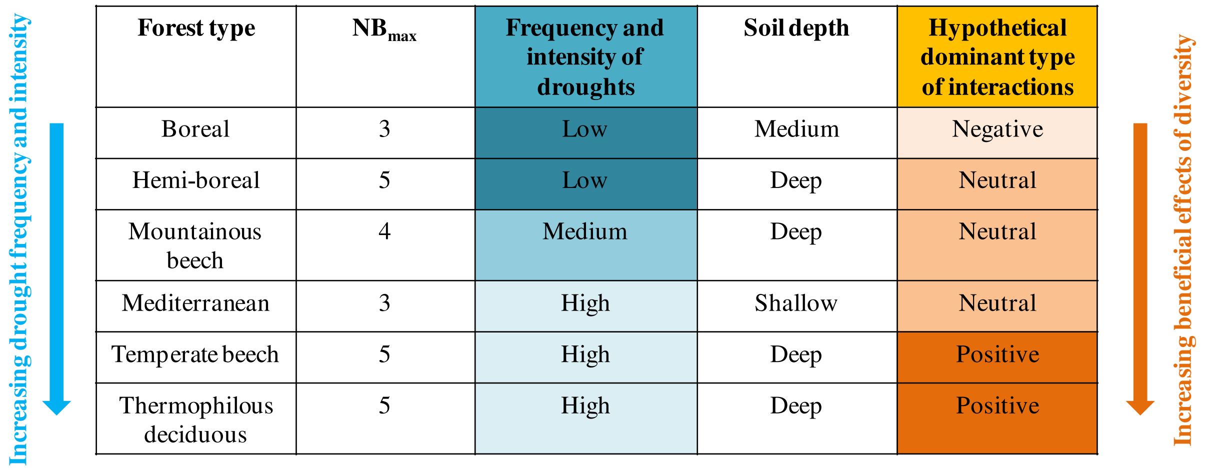
3.3.2.1. Potential underlying mechanisms

Among potential explanatory mechanisms, one can suppose that the lower drought exposure in more diverse stands for the temperate beech and thermophilous deciduous forests might be partially explained by below-ground complementarity processes. Indeed, evidence of below-ground stratification among tree species has already been found in temperate European forests (e.g. Bolte & Villanuena, 2006; Hendricks & Bianchi, 1995; Reyer et al., 2010; Schmid & Kazda, 2002). In **Paper VIII,** we studied the impact of species interactions on water extraction depth in a young temperate plantation. Though the results from this experiment must be taken with caution since they were obtained in a young plantation, we showed that a vertical stratification due to rooting habits among broadleaved and coniferous species takes place and leads to different water extraction depths among different plant functional types (Fig. 4b & 5 in **Paper VIII**). From these observations, it seems that below-ground complementarity for water uptake could occur when species with important functional differences co-exist. Furthermore, in the same study we showed a difference in the water uptake depth for *Fagus sylvatica* when competing with a varying percentage of conifers (Fig. 5 in **Paper VIII**): the soil water extraction depth for *Fagus sylvatica* was deeper when surrounded with a large proportion of conifers than when it was mainly surrounded by broadleaf trees. This result points to the existence of plasticity-mediated responses to inter-specific interactions.

Apart from stratification in below-ground space occupancy, other structural and functional differences among stands could explain both the positive and negative responses to species diversity. I briefly recall them here: i) Differences in species composition between mixtures and monocultures could lead to dissimilar rain water interception levels. ii) Soil water storage could be modified if mixed stands develop a thicker or thinner organic horizon. iii) Hydraulic lift could take place in mixed stands if a deep rooting species takes up water and redistributes it to drier superficial soil layers. iv) As tree water uptake is partially dependent on mycorrhizal symbiosis (Lehto & Zwiazek 2011) and species diversity can positively influence the fungal community (Buée et al. 2011), we could also envisage that a change in mycorrhizal composition in mixtures *vs.* monocultures could influence tree species water availability as well.

3.3.2.2. Variability of diversity effects across Europe

Environmental conditions are highly variable along the North-South gradient considered in this study. Here I address whether differences in climatic and environmental conditions among study sites could account for the inconsistency of response among the different forest types. Negative relationships (i.e. beneficial effects of diversity on drought exposure) occurred in the temperate beech and thermophilous deciduous forests, the two sites that showed the highest mean drought stress intensity and highest frequency of drought stress over the past 15 years (Table S4 in **Paper VII**). In contrast, drought events in the boreal and hemi-boreal forests are moderate and rather rare (Table S4 in **Paper VII**), and these are the sites where we observed either a positive relationship (i.e. detrimental diversity effects on drought exposition) or no effect of species interaction. Although this study was not designed to test the "stress-gradient hypothesis" directly (He & Bertness 2014), this global pattern seems consistent with the hypothesis: potential complementarity and facilitation processes should prevail under stressful conditions while competition should be more important in favorable conditions. However, the Mediterranean forest site, which is also characterized by a high drought stress intensity and frequency (Table S4 in **Paper VII**), showed no effect of species interaction. It therefore seems that the local climatic conditions at each site do not solely explain the variability of responses to diversity across Europe. The Mediterranean site is situated on very shallow soils which could prevent the establishment of below-ground complementarity and facilitation processes such as root stratification or hydraulic lift. Finally, local tree species associations probably interact with local climatic and edaphic conditions to explain the complexity of the relationship between biodiversity and forest adaptation to drought stress across Europe (**Figure 12**).



Forest type	NB_{max}	Frequency and intensity of droughts	Soil depth	Hypothetical dominant type of interactions
Boreal	3	Low	Medium	Negative
Hemi-boreal	5	Low	Deep	Neutral
Mountainous beech	4	Medium	Deep	Neutral
Mediterranean	3	High	Shallow	Neutral
Temperate beech	5	High	Deep	Positive
Thermophilous deciduous	5	High	Deep	Positive

Figure 12: Maximum number of interacting species in mixed stands (NBmax), frequency and intensity of drought episodes over the last 15 years, approximate soil depth and hypothetical dominant type of inter-specific interactions under conditions of water stress (**Paper VII**)

In brief

The important finding here is that higher diversity offers benefits during extreme drought events for some European tree species and/or forest types but no general pattern for the impact of species diversity on tree-level transpiration and tree- and stand-level drought exposure can be derived for forest ecosystems. Depending on the identity of the interacting species, environmental site characteristics (soil type, texture, porosity…), climatic conditions (dry regions from the Mediterranean basin *vs.* wet regions in north and central Europe) or forest management practices (which influence canopy openings, stand basal area, tree density), beneficial, detrimental or no effects of species interactions on tree water acquisition and use during drought stress can be expected. Local site characteristics, climatic conditions and tree species composition are obviously interacting, thus adding to the complexity of the "diversity signal" in forest ecosystems.

4. Conclusions

Several important findings can be drawn from this work that contribute to improving our knowledge of the impact of tree species diversity on forest ecosystem water and carbon relations under close to optimum or stressful soil water conditions.

First, our results highlight that diversity can have an important influence on the functioning of tree species in young forest ecosystems in terms of water and carbon acquisition and use under non-stressful conditions. Nonetheless, this effect is characterized by considerable variability in the responses of co-existing tree species. In mature forest ecosystems, in contrast, no species diversity effect on water and carbon relations were found under these same conditions. Even though one should be cautious when generalizing, taken together, these results suggest that ontogenic stage may affect the interactions among species in forest ecosystems. Positive or negative interactions may occur during the establishment phase in plantations or naturally regenerating stands, then these mechanisms may evolve, change, or disappear as the ecosystems mature.

My second important finding is that water availability plays an important role in shaping tree species response to diversity. Drawing hypotheses based on observations made under non-stressful conditions can be misleading. This is not surprising if one considers the concept of the stress-gradient hypothesis developed in the 90’s (Bertness & Callaway 1994). This concept has been widely discussed since then, and our work and the experimental design we used across Europe was not designed to test it. Nevertheless, I believe that it is not coincidental that we detected beneficial interactions in the very sites in Europe that have undergone the most frequent and severe droughts over the past 15 years, while detrimental interactions occurred at a northern site with very rare or weak soil drought conditions. My work suggests that forest management practices that favour mixed forest ecosystems should be preferred in the context of climatic changes and the expected increase in the intensity and frequency of drought events in some regions in Europe and North America, not only to preserve biodiversity but also to reduce the risk or delay the negative effect of drought stress in dry ecosystems.

For further research, I would suggest the following areas of investigation:

First, in order to improve our knowledge on species diversity effects, future experiments will have to focus specifically on the processes that are at work in mixed-species forests rather than simply measuring ecosystem functions. For example, certain below-ground mechanisms like root stratification and hydraulic lift can easily be measured in forests with simple labeling approaches which do not necessitate work-intensive excavation. The information obtained will undoubtedly enlighten our understanding of why certain species or forest types show a strong beneficial response to species diversity while others exhibit the opposite trend in terms of water and carbon acquisition and use.

Second, future research should also explore the consequences of the significant effects found in the present work in relation to ecosystem functions such as productivity which are important in forest management. In my opinion, responses should not only be looked at during extreme climatic events themselves, but should also be investigated over time after important drought stresses to determine long-term ecosystem resilience.

Third, the ecological and ecophysiological research focusing on drought stress and overall climate change has begun to look into tree mortality and considerable effort is currently being made to understand the underlying mechanisms driving mortality events. Whether widely observed tree mortality events differ between monocultures and mixtures also remains to be studied.

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6. Supplementary Information

6. Supplementary Information

Section 1: Species information

Latin name	**Common name**	**Forest type**
Abies alba Mill.	Silver fir	Hemi-boreal
Acer pseudoplatanus L.	Sycamore maple	Temperate beech, mountainous beech
Betula pendula Roth.	Silver birch	Boreal, hemi-boreal
Carpinus betulus L.	Common hornbeam	Hemi-boreal
Castanea sativa Mill.	Sweet chestnut	Thermophilous deciduous
Fagus sylvatica L.	European beech	Temperate beech, mountainous beech
Fraxinus excelsior L.	European ash	Temperate beech
Ostrya carpinifolia Scop.	Hop hornbeam	Thermophilous deciduous
Picea abies (L.) H.Karst.	Norway spruce	Boreal, hemi-boreal, temperate beech
Pinus nigra J.F.Arnold	Black pine	Mediterranean broadleaved-coniferous
Pinus sylvestris L.	Scots pine	Boreal, hemi-boreal, Mediterranean broadleaved-coniferous
Quercus cerris L.	Turkey oak	Thermophilous deciduous
Quercus faginea Lam.	Portuguese Oak	Mediterranean broadleaved-coniferous
Quercus ilex L.	Holm oak	Thermophilous deciduous
Quercus petraea (Matt.) Liebl.	Sessile oak	Temperate beech, thermophilous deciduous
Quercus robur L.	Pedunculate oak	Hemi-boreal

Section 2: Light preferences.

Presence of a plant in relation to the relative light intensity (translated from Ellenberg & Leuschner, 2001).

1	**Deep shade plants.** Less than 1% of light interception and rarely more than 30%.
2	**Between 1 and 3**
3	**Shade plants.** Mostly with less than 5% of light interception.
4	**Between 3 and 5**
5	**Medium-shade plants.** Exceptionally in direct light and mostly with more than 10% of light interception.
6	**Between 5 and 7.** Rarely with less than 20% of light interception.
7	**Medium-light plants.** Mostly in direct light but exceptionally in the shade with 30% of light interception.
8	**Light plants.** Only exceptionally with less than 40% of light interception
9	**Full-light plant.** Only in direct light, never less than 50% of light interception

Section 3: References for each functional trait and each species in Table 2.

Species Abbr.	**Initial growth rate**	**Rooting depth**	**ψ_{50} (MPa)**
A. alba	Escher et al. 2004	Elling et al. 2009	Cochard 1992
A. pseudoplatanus	Hein et al. 2009	Čermák & Fér 2007	Lens et al. 2011
B. pendula	Hester et al. 2004	Kalliokoski et al. 2008	Herbette & Cochard 2010
C. betulus	Rameau et al. 2008	Čermák & Fér 2007	Herbette & Cochard 2010
C. sativa	Mousseau et al. 1996	Martins et al. 2010	
F. sylvatica	Mousseau et al. 1996	Čermák & Fér 2007; Rust & Savill 2000	Cochard et al. 1999
F. excelsior	Dobrowolska et al. 2011	Bréda et al. 2002; Rust & Savill 2000	Cochard et al. 1997
O. carpinifolia	Bernetti 1995	Bernetti 1995	
P. abies	Li et al. 2003	Kalliokoski et al. 2008	Mayr et al. 2006
P. nigra	Vallet et al. 2009	Peñuelas & Filella 2003	Cochard 2006
P. sylvestris	Hester et al. 2004	Kalliokoski et al. 2008	Martínez-Vilalta et al. 2009
Q. cerris	Rameau et al. 2008	Epron & Dreyer 1993	
Q. faginea	Rameau et al. 2008	Rameau et al. 2008	
Q. ilex	Faria et al. 1998	Pemán et al. 2006	Martínez-Vilalta et al. 2002
Q. petraea	Vallet et al. 2009	Bréda et al. 1993	Cochard et al. 1992
Q. robur	Pons & Westbeek 2004	Čermák & Fér 2007	Cochard & Tyree 1990

Section 4: Results from the statistical tests (*P*-values) on foliar and wood tree-level carbon isotope composition for each species in each forest type under non-limiting soil water conditions.

For each species in each forest type, mixed linear models were used to determine the effects of the Shannon diversity index calculated with the basal area of each species, altitude, leaf area index (LAI), competition intensity (i.e. stand basal area) and soil C/N ratio on tree-level foliar carbon isotope composition ($\delta^{13}C_F$) and wood carbon isotope composition ($\delta^{13}C_W$). Stand was used as a random factor. *P*-values are given for each factor. The slopes of the fitted relationships between $\delta^{13}C_F$ or $\delta^{13}C_W$ and the factors are given when the effect is significant ($P < 0.05$; bold values).

Site	**Species**	**Shannon index**		**Altitude**		**LAI**			**Basal area**			**C/N**		
		$\delta^{13}C_F$	$\delta^{13}C_W$	$\delta^{13}C_F$	$\delta^{13}C_W$	$\delta^{13}C_F$	$\delta^{13}C_W$	Slope	$\delta^{13}C_F$	$\delta^{13}C_W$	Slope	$\delta^{13}C_F$	$\delta^{13}C_W$	Slope
Boreal	*B. pendula*	0.94	0.39	0.27	0.11	0.49	**0.02**	**- 0.37**	0.82	0.07		0.74	0.11	
	P. abies	0.16	0.43	0.46	0.48	0.82	0.07		0.24	0.38		0.58	0.89	
	P. sylvestris	0.06	0.31	0.31	0.37	0.15	0.79		0.86	0.82		0.12	0.53	
Hemi-boreal	*B. pendula*	0.54	0.51	0.75	0.11	0.28	0.71		0.17	0.33		0.14	**<0.001**	**-0.11**
	C. betulus	0.18	0.26	0.99	0.38	0.42	0.07		0.28	0.53		0.36	0.63	
	P. abies	0.19	0.31	0.51	0.86	0.40	0.87		0.34	**0.03**	**-0.89**	0.86	0.29	
	P. sylvestris	0.69	0.44	0.57	0.61	0.43	0.49		0.90	0.18		0.65	0.72	
	Q. robur	0.63	0.15	0.96	0.63	0.29	0.63		0.44	0.98		0.82	0.69	
Mountainous beech	*A. alba*	0.81	0.35	0.94	0.56	0.30	0.80		0.11	0.11		0.18	0.87	
	A. pseudoplatanus	0.41	0.45	0.69	0.48	0.45	0.24		0.79	0.45		0.46	0.40	
	F. sylvatica	0.51	0.21	0.24	0.86	0.53	0.44		0.12	0.78		0.51	0.61	
	P. abies	0.09	0.06	0.80	0.98	0.59	0.29		0.53	0.24		0.16	0.24	

Site	**Species**	**Shannon index**			**Altitude**		**LAI**			**Basal area**			**C/N**		
		$\delta^{13}C_F$	$\delta^{13}C_W$	Slope	$\delta^{13}C_F$	$\delta^{13}C_W$	$\delta^{13}C_F$	$\delta^{13}C_W$	Slope	$\delta^{13}C_F$	$\delta^{13}C_W$	Slope	$\delta^{13}C_F$	$\delta^{13}C_W$	Slope
Temperate beech	*A. pseudoplatanus*	0.72	0.90		0.34	0.34	0.76	0.89		0.87	0.94		0.62	0.67	
	F. sylvatica	0.79	0.87		0.55	0.70	0.44	0.88		0.05	**0.03**	**0.25**	**0.04**	0.12	**0.72**
	F. excelsior	0.25	0.88		0.59	0.64	0.12	**0.01**	**-0.69**	0.05	**0.02**	**-0.46**	0.53	0.78	
	P. abies	0.29	0.94		0.99	0.81	0.86	0.97		0.71	0.35		0.89	0.84	
	Q. petraea	0.35	0.28		0.68	0.44	0.98	0.73		0.34	0.84		0.45	0.95	
Thermophilous deciduous	*C. sativa*	0.05	**0.04**	**-1.27**	0.56	0.36	0.29	0.34		0.52	0.24		0.57	0.45	
	O. carpinifolia	0.79	0.41		0.21	0.08	0.41	0.06		0.23	0.09		0.58	0.87	
	Q. cerris	0.06	0.47		0.43	0.53	0.13	0.71		0.33	**0.01**	**-0.35**	0.12	0.26	
	Q. ilex	0.54	0.06		0.80	0.56	0.55	**0.04**	**-0.31**	0.11	0.54		0.53	0.08	
	Q. petraea	0.47	0.76		0.42	0.17	0.51	0.15		0.86	0.15		0.14	0.22	
Mediterranean forest	*P. nigra*	0.22	0.17		0.89	0.52	0.30	0.71		0.67	0.26		0.05	0.86	
	P. sylvestris	0.07	0.14		0.43	0.17	0.60	0.16		0.84	0.51		0.42	0.10	
	Q. faginea	0.94	0.10		0.32	0.37	0.79	**0.03**	**-0.04**	0.70	0.34		0.38	0.31	
	Q. ilex	0.48			0.08		0.95			0.52			0.56		

Section 5: Results from the statistical tests on species-level increase in carbon isotope composition between wet and dry conditions.

For each species within each forest type, mixed linear models were used to determine the effects of the Shannon diversity index calculated with the basal area of each species, altitude, leaf area index (LAI), competition intensity (i.e. stand basal area) and soil C/N ratio on the species-level increase in carbon isotope composition from a wet to a dry year ($\Delta\delta^{13}C$) (**Papers VI & VII**). Stand was used as a random factor. *P*-values are given for each factor. The slopes of the fitted relationships between $\Delta\delta^{13}C$ and the factors are given when the effect is significant ($P < 0.05$; bold values).

Site	**Species**	**Shannon index**		**Altitude**		**LAI**		**Basal area**		**C/N**	
		P-value	Slope	*P*-value	Slope	*P*-value	Slope	*P*-value	Slope	*P*-value	Slope
Boreal	*B. pendula*	0.81		0.86		0.13		0.43		0.06	
	P. abies	**0.03**	**0.88**	0.43		0.26		0.25		0.40	
	P. sylvestris	**0.04**	**0.72**	0.94		0.91		0.66		0.83	
Hemi-boreal	*B. pendula*	0.24		0.74		0.93		0.09		0.49	
	C. betulus	0.85		0.79		0.98		0.05		0.51	
	P. abies	0.27		0.60		0.74		**0.03**	**0.10**	0.19	
	P. sylvestris	0.17		0.39		0.49		**0.03**	**0.68**	0.14	
	Q. robur	0.57		0.67		**0.005**	**-0.57**	0.07		0.87	
Mountainous beech	*A. alba*	0.96		0.09		0.35		0.78		0.12	
	A. pseudoplatanus	0.90		0.85		0.56		**0.005**	**0.12**	0.14	
	F. sylvatica	0.07		0.42		0.83		0.20		0.12	
	P. abies	0.27		0.43		0.11		**0.007**	**1.10**	0.15	

Site	**Species**	**Shannon index**		**Altitude**		**LAI**		**Basal area**		**C/N**	
		P-value	Slope	*P*-value	Slope	*P*-value	Slope	*P*-value	Slope	*P*-value	Slope
Temperate beech	*A. pseudoplatanus*	0.63		0.30		0.39		**0.04**	**0.12**	0.45	
	F. sylvatica	**0.007**	**-0.78**	0.06		0.47		0.21		0.61	
	F. excelsior	**0.01**	**-0.18**	0.36		0.42		0.10		0.06	
	P. abies	0.77		0.72		0.69		0.81		0.82	
	Q. petraea	0.27		0.33		0.72		0.41		0.75	
Thermophilous deciduous	*C. sativa*	**0.04**	**-0.66**	0.47		0.22		0.93		0.59	
	O. carpinifolia	0.39		0.28		0.76		0.76		0.91	
	Q. cerris	0.06		0.13		0.27		0.33		0.38	
	Q. ilex	**0.006**	**-0.14**	0.79		0.82		0.39		0.98	
	Q. petraea	**0.004**	**-0.38**	0.73		0.19		0.74		0.98	
Mediterranean	*P. nigra*	0.83		0.82		0.50		0.54		0.62	
	P. sylvestris	0.60		0.36		0.83		**0.009**	**0.05**	0.97	
	Q. faginea	**0.04**	**-0.05**	0.57		0.96		0.65		0.91	

7. Publications

Paper I

Perspectives in Plant Ecology, Evolution and Systematics 15 (2013) 281–291

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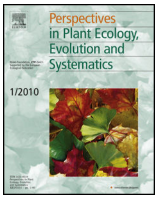

Forum

A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests

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ABSTRACT

One of the current advances in functional biodiversity research is the move away from short-lived test systems towards the exploration of diversity-ecosystem functioning relationships in structurally more complex ecosystems. In forests, assumptions about the functional significance of tree species diversity have only recently produced a new generation of research on ecosystem processes and services. Novel experimental designs have now replaced traditional forestry trials, but these comparatively young experimental plots suffer from specific difficulties that are mainly related to the tree size and longevity. Tree species diversity experiments therefore need to be complemented with comparative observational studies in existing forests. Here we present the design and implementation of a new network of forest plots along tree species diversity gradients in six major European forest types: the FunDivEUROPE Exploratory Platform. Based on a review of the deficiencies of existing observational approaches and of unresolved research questions and hypotheses, we discuss the fundamental criteria that shaped the design of our platform. Key features include the extent of the species diversity gradient with mixtures up to five species, strict avoidance of a dilution gradient, special attention to community evenness and minimal covariation with other environmental factors. The new European research platform permits the most comprehensive assessment of tree species diversity effects on forest ecosystem functioning to date since it offers a common set of research plots to groups of researchers from very different disciplines and uses the same methodological approach in contrasting forest types along an extensive environmental gradient.

Introduction

The past two decades of research on biodiversity and ecosystem functioning (BDEF) have shown that biodiversity is not only a passive consequence of the environmental conditions and interactions between species (Naeem, 2002; Loreau, 2010), but that differences in biodiversity can largely affect ecosystem functioning. Several hundreds of published effects have provided ample evidence that plant biodiversity can influence key ecosystem processes such as biomass production, nutrient cycling and pest regulation (Cardinale et al., 2012). While it is now generally accepted that plant diversity can affect function, the importance of BDEF relationships in complex natural ecosystems and their relevance to ecosystem management and conservation have been debated (Srivastava and Vellend, 2005; Duffy, 2009; Hillebrand and Matthiessen, 2009). Many of these concerns have arisen because early experiments and models on functional biodiversity lacked environmental and biological complexity and did not consider ecosystem multifunctionality, i.e., the maintenance of multiple functions (Hillebrand and Matthiessen, 2009). A new generation of studies therefore attempts to investigate BDEF relationships in real-world settings (Solan et al., 2009). Short-lived test systems such as microcosms, mesocosms and grasslands have dominated previous research (Balvanera et al., 2006), but much greater emphasis is now being placed on structurally more complex systems containing long-lived plants, including forests (Scherer-Lorenzen et al., 2005a).

Trees are text book examples of ecosystem engineers capable of modifying aspects of their environment, yet surprisingly little is known about the functional significance of tree species diversity in forests (Nadrowski et al., 2010). Experimental forestry trials comparing monocultures with two-species mixtures have existed for many decades (Pretzsch, 2005), but large-scale experiments with more species richness levels were only installed during the past decade (e.g., Scherer-Lorenzen et al., 2005b; www.treedivnet.ugent.be), meaning that these systems are still far from maturity. While important diversity-functioning patterns have already been reported from these experiments (Vehviläinen and Koricheva, 2006; Potvin et al., 2011; Lei et al., 2012), the results might differ considerably from those found in mature forests, when trees are older and the species diversity affected the environment for several decades. Furthermore, experimental tree communities face several specific limitations including small plot size, simplified age distributions and simplified stand structure compared with mature forest (reviews: Scherer-Lorenzen et al., 2005b; Leuschner et al., 2009). Studying existing, mature forests in real landscapes would therefore complement the results from these experiments. To this end, two approaches seem to be promising. First, regional or national forest inventory databases are ready-to-use and have now also been explored to examine BDEF relationships in mature forest (Caspersen and Pacala, 2001; Vilà et al., 2005; Paquette and Messier, 2011; Gamfeldt et al., 2013). However, they suffer from the disadvantages that tree diversity is generally confounded with many environmental variables and that usually only a few functions are measured. The second approach, comparative observational plots set up along tree diversity gradients in mature forests while maximally controlling other environmental factors, would allow to better isolate potential diversity effects from confounding environmental conditions. This approach would complement the existing networks of experimental research sites and inventories.

A first case study in a central European beech forest compared forest patches on similar soils, in which past ownership and forest use had created a small-scale mosaic of tree species diversity (Leuschner et al., 2009). However, this study used a complete dilution design (Nadrowski et al., 2010; see Glossary) where the only monocultures were the ones of *Fagus sylvatica* and this tree species was present in all mixtures. This does not allow for separating effects of increasing species diversity from the confounding effects of decreasing dominance of *F. sylvatica*. At present few other local-scale comparative research platforms exist and most do not allow for disentangling the diversity signal from confounding factors such as environmental gradients and species identity (see review by Nadrowski et al., 2010). A more extensive comparative approach, which goes considerably beyond typical observational studies, was recently adopted in the German Biodiversity Exploratories, which focus on the effects of land-use change on biodiversity and ecosystem processes in forest and grassland (Fischer et al., 2010). A similar comparative approach could be used to allow for comparisons of stands of different tree species diversity in mature forest, in analogy to the land-use comparisons in the German Biodiversity Exploratories.

A comparative approach with study sites varying in tree species diversity and replicated at the continental scale would address many of the deficiencies in forest functional biodiversity research outlined above. Here we present the design and implementation of the FunDivEUROPE Exploratory Platform, a network of forest plots along tree species diversity gradients in six major European forest types (FunDivEUROPE: Functional significance of forest biodiversity; www.fundiveurope.eu). With the additional asset of studying forest types of very different European regions, the platform will contribute to answering some of the most important unsolved questions in BDEF research: does tree species diversity affect ecosystem functioning and the provisioning of ecosystem services? Do the effects of differences in resource use and facilitation among species (i.e., complementarity effects) vary along broad environmental (climatic, soil) gradients? Using forest inventory data bases, Paquette and Messier (2011) showed that complementarity may be more important for tree productivity in boreal compared with temperate forests, while Zhang et al. (2012) found similar complementarity effects across biomes. Furthermore, there are virtually no data available on ecosystem processes other than productivity. In the FunDivEUROPE project we will therefore measure a large and comprehensive spectrum of different functions and related services in each plot of our platform. The Exploratory Platform provides further added value because it is combined with two complementary platforms that are studied in parallel (Fig. 1): (i) a network of European tree species diversity experiments (Experimental Platform) and (ii) a compilation of national and regional forest inventories (Inventory Platform). As the Exploratory Platform was set up as a hypothesis-driven network of plots, we begin this contribution with an overview of the specific research questions and hypotheses we are aiming to test. The rationale of the platform and the design are subsequently discussed.

Guiding research questions and hypotheses

The research platform was designed to test five general hypotheses that have been highlighted as unresolved in recent review and opinion papers (e.g., Hillebrand and Matthiessen, 2009; Nadrowski et al., 2010; Cardinale et al., 2012). The first four hypotheses have been explored extensively in other systems but need further attention in forest ecosystems, while the fifth is especially relevant to trees and forests:

(1) Tree species mixtures outperform ecosystem functioning of monocultures, including the stocks and fluxes of energy and materials and their stability over time (Pacala and Kinzig, 2002). Mixtures have a higher probability of containing key species with a large impact on ecosystem functioning (i.e., identity and sampling/selection effect) and resource partitioning and positive interactions among heterospecific neighbouring trees further influence function (i.e., complementarity effect).

(2) The relative importance of species identity effects and complementarity effects is not consistent for forests at different positions along extensive environmental gradients. For instance, the strength of BDEF relationships is expected to increase with environmental stress (Paquette and Messier, 2011; Jucker and Coomes, 2012).

(3) Mixtures of tree species enhance associational resistance to pests and diseases via reduced host colonization and increased top down regulation by natural enemies. Likewise, their resistance and resilience towards abiotic environmental stressors and disturbances such as drought are increased compared with monocultures (the relative importance of the potential mechanisms are debated; Griffin et al., 2009).

(4) The number of species needed to support a single ecosystem function is lower than the number of species needed to support multiple functions simultaneously. The significance of forest tree species diversity for ecosystem functioning increases when multiple functions are considered.

(5) Mixtures create larger environmental heterogeneity at the scale of several individual trees compared with monospecific stands because trees are autogenic ecosystem engineers and individuals may modify their immediate environment (e.g., via root processes, litter input, light penetration and rain interception). Diversity effects are therefore not only expressed in terms of mean values, but also in terms of variability within forest stands.

A test of this set of hypotheses will provide the most comprehensive assessment of BDEF relationships in forests to date. Furthermore, interactions among coexisting species are at the heart of any diversity effect on ecosystem functioning. Testing the importance of species interactions therefore requires recognizing individuals, not only species, as a relevant level of observation. The size of trees makes them preferred study objects to explicitly explore the interactions between individuals. We are thus also interested in how tree performance and a tree's impact on its immediate environment are influenced by the diversity and structure of its neighbourhood.

Rationale: maximizing three fundamental design criteria

A research platform designed to answer the questions above should comprise a systematic network of research plots in existing forests that maximizes three fundamental criteria: comprehensiveness, representativeness and orthogonality (Nadrowski et al., 2010; see Glossary). First, comprehensiveness refers to the spectrum of ecosystem functions and services that can be quantified. Ecosystems inherently exhibit a multitude of functions, so increasing the relevance of BDEF studies requires a multifunctional perspective (Gamfeldt et al., 2008; Hillebrand and Matthiessen, 2009). Since functions are expressed at different spatial scales, the challenge is to establish plots with a layout matching the particular measurement requirements of a diverse set of functions, some of which need a certain number of trees while others need particular plot sizes. Low comprehensiveness is generally a limitation of existing observational (inventory) networks, which focus on a limited number of ecosystem processes such as productivity. The second criterion, representativeness, is achieved for plots established in characteristic forest types as we find them in landscapes, allowing a credible translation of results to the real world. Representativeness thus relates to the relevance of the results for managers and policy makers. This is an important design element and is the fundamental reason for complementing planned experiments with comparative studies in existing forest. Representativeness in our platform is maximized in three directions: (i) the forests are at least historically managed for forestry and are currently in the

Fig. 1. Locations of the study sites that form the different research platforms in the FunDivEUROPE project: the Exploratory Platform (filled circles), the Experimental Platform (stars) and Inventory Platform (dark grey countries). The six exploratory regions were selected to represent major European forest types (see Table 1) and are described in detail in this paper. Some of the experiments were in fact established in several localities within a region (FORBIO, BIOTREE) and the figure indicates their approximate location.

mid to late stem exclusion, understory reinitiation or old-growth development stage (i.e., excluding very young stands; Oliver and Larson, 1996), (ii) six major European forest types (EEA, 2007) are studied and (iii) focus is on sets of target tree species that are regionally common and/or important from a silvicultural point of view. The third criterion, an orthogonal design, permits separating diversity effects from other variables that influence ecosystem functioning. The general idea is to include plots that primarily differ in (stochastic or management driven) tree species diversity while keeping the variation in confounding factors (topography, soil, disturbances) at a minimum. For example, a design where the more diverse species mixtures are, by chance, situated on the most productive soils would be problematic (Vilà et al., 2005). Along this species diversity gradient, the compositional variation among plots should include true species turnover, not species dilution. These three fundamental criteria were fully integrated in the design and implementation of the platform and will emerge in the subsequent sections. This rationale is also reflected in the term “exploratory” (coined for the German Biodiversity Exploratories; Fischer et al., 2010), which was explicitly chosen to illustrate the hybrid nature of the platform, combining strengths of true observatories (representativeness) and experiments (comprehensiveness and orthogonality).

Design and implementation of the diversity oriented Exploratory Platform

Six European forest types

The Exploratory Platform covers the major forest regions of Europe that extend from southern Mediterranean Europe (‘Alto Tajo’ in Spain and ‘Colline Metallifere’ in Italy) to the northern Boreal (subarctic) region ‘North Karelia’ in Finland (Fig. 1). The European Environment Agency classification, which is based on the distribution and classification of the natural vegetation and ecological site conditions, was used to select the different types (EEA, 2007; Table 1). The selected focal regions cover large soil and climatic gradients as they occur across Europe. The mean annual temperatures range from ca. 2 °C in North Karelia (Finland) at 62.6°N, to ca. 13 °C in Colline Metallifere (Italy) at 43.2°N (Table 1). Together, the regions host many important European broadleaved (e.g., *F. sylvatica*, *Quercus petraea/robur*, *Quercus ilex*, *Betula pendula/pubescens*) and coniferous tree species (e.g., *Pinus sylvestris*, *Picea abies*), with most tree species occurring in several exploratory regions. Certain less representative forest types such as mire, swamp and floodplain forests and alpine coniferous forests were excluded, but they may be included later on.

Table 1
Description of the six exploratory regions and region-specific design of the new research platform, including the pool of species used to create a gradient in species diversity.

	North Karelia	Białowieża	Hainich	Râşca	Colline Metallifere	Alto Tajo
(a) Site description						
Country	Finland	Poland	Germany	Romania	Italy	Spain
Latitude/longitude (°)	62.6, 29.9	52.7, 23.9	51.1, 10.5	47.3, 26.0	43.2, 11.2	40.7, −1.9
Forest type[a]	Boreal	Hemiboreal, nemoral coniferous, mixed broadleaved-coniferous	Beech	Mountainous beech	Thermophilous deciduous	Mediterranean mixed
Ownership	State, large private forest companies	State	State	State	State	State
MAT, MAP[b]	2.1 °C, 700 mm	6.9 °C, 627 mm	6.8 °C, 775 mm	6.8 °C, 800 mm	13 °C, 850 mm	10.2 °C, 499 mm
Topography, altitude[c]	Flat, 80–200 m	Flat, 135–185 m	Mainly flat, 500–600 m	Medium-steep slopes, 600–1000 m	Medium-steep slopes, 260–525 m	Flat-medium slopes, 960–1400 m
Study area size (km × km)	150 × 150	30 × 40	15 × 10	5 × 5	50 × 50	50 × 50
Responsible site manager	L. Finér	B. Jaroszewicz	H. Bruelheide	O. Bouriaud	F. Bussotti	F. Valladares
Stand developmental stage[d]						
(1) Mid/late stem exclusion	x	x		x	x	x
(2) Understory reinitiation	x	x	x	x		x
(3) Old growth			x			
Tree cohorts	Single	Multiple	Multiple	Single	Multiple	Multiple
(b) Platform design						
Species richness levels	3	5	4	4	5	4
Number of plots	28	43	38	28	36	36
Plots per richness level	11/14/3	6/11/13/11/2	6/14/14/4	8/10/8/2	9/10/9/7/1	11/18/4/3
Species pool						
(1) Coniferous						
Abies alba				×		
Picea abies	×	×	×	×		
Pinus nigra						×
Pinus sylvestris	×	×				×
(2) Broadleaved						
Acer pseudoplatanus			×	×		
Betula pendula/pubescens	×	×				
Carpinus betulus		×				
Castanea sativa					×	
Fagus sylvatica			×	×		
Fraxinus excelsior			×			
Ostrya carpinifolia					×	
Quercus robur/petraea		×	×		×	
Quercus cerris					×	
Quercus faginea						×
Quercus ilex (evergreen)					×	×

[a] Categories of the European Environment Agency (EEA, 2007).
[b] MAT: mean annual temperature, MAP: mean annual precipitation.
[c] Altitude in metres above sea level.
[d] Stand developmental stages according to Oliver and Larson (1996).

A nested platform design

An appropriate design for the Exploratory Platform that allows determining how species identity and species interactions influence ecosystem functioning should control for the variation in species composition along the tree species diversity gradient. If every species in the pool is not present in about the same number of plots at every species richness level – with a complete dilution design being the most extreme example – there is the risk that the effect of the presence of a particular species cannot be separated from the effect of species diversity. Similarly, testing for tree species diversity effects has to be done against different community compositions, i.e., including different species combinations at every species richness level, to allow distinguishing the effects of particular species combinations from species diversity (Schmid et al., 2002). To create a sound design, the basic setup of experiments in terms of the compositional variation between the plots was mimicked in the Exploratory Platform. This means we aimed to include the monocultures of all the tree species, replicate each species richness level with different mixtures and have every species represented at each richness level, preferably in similar frequencies (ORPHEE experiment: Castagneyrol et al., 2013; FORBIO experiment: Verheyen et al., 2013; BIOTREE experiment: Scherer-Lorenzen et al., 2007). Such a design avoids complete dilution, but comprises a series of dilution gradients that can be used, among others, to look at pathogen or herbivore effects on focal species. For the majority of species combinations we included two or more “realizations” (not strict replicates, because species abundances may differ), which will allow comparing the importance of species diversity with that of species composition for this subset of plots (e.g., model by Hector et al., 2011). The strength of the interactions among particular species can be quantified for the full design, i.e., also including the species combinations with only one realization (e.g., model by Kirwan et al., 2009).

A fixed pool of target species needed to be selected for each region to establish the species diversity gradient. With the design that was put forward, the maximum size of this species pool was constrained by the total sample size. Biological and logistic realities impose a natural limit to the number of plots that can be measured within each of the six regions and desired time window (e.g., phenological period versus the 3200 km travelling distance between the Finnish and Spanish region). We proposed ca. 40 plots per region as a feasible sample size. Preliminary power analyses using simulated data (Gelman and Hill, 2007) were performed to determine the maximum number of species richness levels that still allows for the detection of a diversity effect with about 40 plots, i.e., a longer gradient would have less realizations at each richness level, reducing the statistical power. For instance, using the 'species identity model' from Kirwan et al. (2009) and realistic productivity differences of ±20% between five species (coefficient of variation of 15%), the probability to detect the species identity effects was >95%. Designs with larger species pools would require even stronger species effects and not all of the other ecosystem properties and processes we will measure are expected to vary that much between species. Furthermore, similar analyses using 'species interactions models' showed that the diversity effects become small and indiscernible at very unequal relative abundances of the species in the community, i.e., at low evenness (Kirwan et al., 2007, 2009; see below).

A pool of five regionally important species co-occurring in similar forest habitats were selected in three regions (Białowieża, Hainich and Colline Metallifere; Table 1). The naturally restricted species pool in North Karelia limited the pool to three species, while in Alto Tajo and Râşca four species were included as we would have needed to move to quite different environmental conditions if a fifth species was to be included. Some species are represented in several regions (e.g., 3 × *P. sylvestris*, 3 × *Q. robur/petraea*, 4 × *P. abies*), so their identity and interaction effects can be compared between bioclimatic regions. Furthermore, each region except Colline Metallifere combines one or two evergreen coniferous species with broadleaved species. To allow for direct comparisons between the six regions differing in the size of their species pool, we opted for a nested platform design. The rationale is that the design of a region with a smaller species pool is a hierarchically nested subset of the more species rich regions, i.e., the design (not the species combinations) of the regions with large species pools can always be reduced to the low-species regions simply by leaving out certain plots. This was put into practice by searching for each possible species combination at each species richness level. For North Karelia this means for instance that the three monocultures, the three different two-species combinations and the full three-species mixture were included. The optimal number of realizations per combination was adjusted to have adequate sample sizes in the more species poor regions (e.g., four in North Karelia, two or three in the other regions) and balance sample sizes between species richness levels (Table 1; Appendix 1). While for experiments it is initially no problem to establish and maintain all possible mixtures (Scherer-Lorenzen et al., 2007), the challenge of working in existing forests is to find rare combinations (e.g., *Castanea sativa/Ostrya carpinifolia* mixtures in the Italian study area) and, at the same time, to meet all other selection criteria. Ultimately, we selected 209 plots across Europe.

Selection criteria

Focus of this research platform is on forest stands that were at least historically managed for forestry and are currently either managed by low frequency thinning or minimal intervention. They are at least in the late to mid stem exclusion stage, the understory reinitiation or old-growth stage (Oliver and Larson, 1996). All sites are considered ancient forest, i.e., they have been continuously forested at least since the oldest available land-use maps. The implementation of the platform further required a rigorous set of criteria to evaluate the suitability of forest stands in the field. The central principle for selection was to have silvicultural management and/or pure random species assembly to be the key drivers of the diversity gradient. Covariation between an environmental factor (e.g., soil variation, topography) and species richness levels was strictly avoided, whereas covariation with species composition was avoided as much as possible. Because the spatial clustering of particular species richness levels and species combinations would increase the probability of covariation, no clustering was allowed.

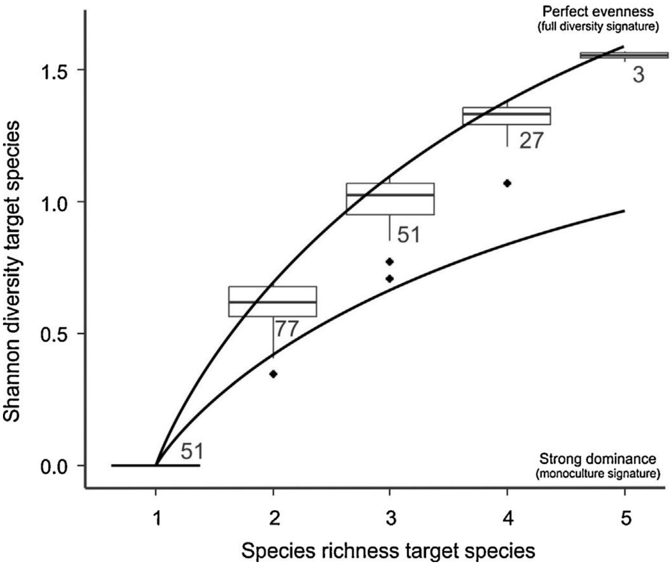

Fig. 2. Boxplots of the Shannon index of diversity for each of five species richness levels in the 209 selected plots. The number of plots in each species richness level is shown for each box. Here we used the total basal area of each species as a measure of abundance and omitted (low abundance) admixed species. The upper line shows the maximum theoretical Shannon diversity for each richness level attained at perfect evenness (i.e., basal areas of the species are equal); the lower line delineates 60% of this maximum. At low Shannon values one of the species dominates and the mixture rather has a monoculture signature. Evenness was a central selection criterion during plot selection.

There were three important criteria. First, we have put much emphasis on the evenness of the community composition, which expresses the distribution of the relative abundances of the tree species in a mixed stand. Few experiments have investigated the importance of evenness for ecosystem processes directly and the outcomes are still inconclusive (Mulder et al., 2004; Kirwan et al., 2007; Hillebrand et al., 2008). Zhang et al. (2012) showed in a meta-analysis that forests productivity increases with evenness, but the specific mechanisms still need to be tested. Evenness is considered an important design issue here because tree species that are present in low numbers or as small individuals probably do not influence the ecosystem processes much either directly or via interspecific interactions (Mulder et al., 2004). Mixtures strongly dominated by a single species rather exhibit a monoculture signature, which would be especially problematic if a species with high monoculture performance also tends to be the dominant in most mixtures (i.e., a high selection effect). Whereas experiments can manipulate relative species abundances quite easily, one or few tree species usually dominate forest stands. This is why we did not apply random or grid-based plot selection, but particularly looked for sites where the target species had similar abundances, setting a lower limit of 60% of maximum evenness based on basal area (Fig. 2). The species also needed to be represented by at least two trees in the mixtures allowing measurements that use individuals as the level of observation to have replicates of 'species identity'.

The second criterion related to the presence of non-target species. Admixture of such species was unavoidable, but we aimed to keep the summed basal area of the admixed species below 5% of the total basal area (with a maximum of ca. 10%). Across the regions, only 18 out of the 209 plots slightly exceeded the 10% admixture criterion and 43 exceeded the 5% criterion.

The third criterion was to ensure that the effect of tree species diversity was not confounded with site-, soil- and stand-related factors. These factors were operationalized in a list of basic descriptors, which was used as a checklist during field exploration (Appendix 2). Regarding the stand-related criteria, shrubs lower than 1.3 m were considered to be response variables, i.e., not contributing to the desired mixtures, whereas larger individuals followed the same rules of admixed species outlined above. The age distribution and forest structure were allowed to covary to some degree with the species diversity gradient, e.g., more uneven-aged and multi-layered sites at the higher species richness levels. We consider this covariation as an integral part of the diversity effect. Yet, along the diversity gradient, the tree populations had to be in the same developmental stage.

Stepwise selection and establishment of research plots

An important issue in the implementation of a research platform is plot size. Relatively small plots (e.g., <2000 m^2) are criticized for containing few trees of each species at high levels of diversity, may represent atypical habitat islands in the larger forest matrix creating edge effects and may poorly represent particular larger-scale ecosystem properties such as resilience after disturbance (Leuschner et al., 2009). Large plots are on the other hand more likely to be environmentally heterogeneous and to contain the dominant species in every plot. We performed a preliminary test with a circular plot shifted at 10 m intervals over the tree maps of two full forest inventories (Hainich National Park, Germany, 28 ha; Liedekerke, Belgium, 9 ha). The tested plot sizes were 500 m^2, 1000 m^2 and 2000 m^2, corresponding to plot radii of 12.6 m, 17.8 m and 25.2 m. At a plot size of 2000 m^2, it was impossible to avoid a complete dilution design, with the dominant species present in every potential site (Hainich: *F. sylvatica*; Liedekerke: *B. pendula*). We decided to use a 900 m^2 core plot size (30 m × 30 m) for the Exploratory Platform, i.e., small enough to avoid a complete dilution design and at least the minimum size for several measurements to be ecologically meaningful (e.g., herbivory, litter input, water quality). To account for potential edge effects, a 10 m wide buffer zone around each plot was requested during plot selection (selected zone 50 × 50 m). This buffer zone had to be similar to the core plot in terms of the forest type, structure and composition, but the evenness and admixture criteria were less strict.

The selection of plots basically occurred in two steps, each of which also included part of the plot characterization. Once the design and selection criteria were fixed, the species pool was selected for each exploratory region and the focal regions were screened to delineate subareas with a good probability to find those species in all the desired combinations (Fig. 3). This screening was done using regionally available data such as forest management plans and soil maps, complemented with exploratory field visits. Then, local teams searched several potentially suitable sites within these subareas for each of the desired species combinations during intensive field campaigns. The idea was to initially have more sites than actually needed in the final design (“oversample”) and select the final set of sites randomly from this larger pool. This approach introduced an important randomization step into the design. In some cases (e.g., rare species combinations) only one site was ultimately available for selection, of course. The suitability of each site was checked in the field using a standardized list of quick descriptors of site conditions, soil properties and forest stand structure that could be estimated without establishing plots (Appendix 2). Actual dendrometric measurements were performed where needed to verify the suitability of the mixture in terms of the evenness or the degree of admixture of non-target species.

After this first selection phase, we analyzed the variation among the selected sites based on the three groups of descriptors (site, soil, stand) to identify undesired covariation with species richness levels and to check for clearly deviating sites (e.g., one plot on calcareous bedrock when all others were on sandstone) (Fig. 4). The final set of sites was selected after removing potentially problematic ones. In a second field campaign, 900 m^2 plots (30 m × 30 m), subdivided into nine 100 m^2 subplots, were established in the selected sites using wooden poles (Fig. 5a). The position (±0.5 m) and diameter at breast height (dbh, ±0.5 cm) of each tree >7.5 cm dbh was measured in these plots. Based on these tree position data, plot-specific plans were drawn allocating each measurement to particular locations within the plot, including strict no-go areas and preferred walking tracks (Fig. 5b).

Outlook for scientists and managers

With this Exploratory Platform we move away from earlier research on the effects of environmental factors on forest biodiversity to a new research paradigm focussing on the effects of biodiversity on ecosystem functioning. Together with the experiments and inventory data, the Exploratory Platform provides an important new European research infrastructure for long-term monitoring of the effects of tree species diversity on forest ecosystem functions and the ecosystem services provided by forests. With its diversity-oriented design, the Exploratory Platform definitely complements and provides added value to existing networks that were based on a systematic sampling grid such as national and regional forest inventories or the pan-European network of forest monitoring plots (ICP Forests Level I and II; www.icp-forests.org), originally designed to monitor air pollution effects on forest vitality. We focussed particularly on a design that allows separating tree species identity from complementarity effects, improving on more common dilution schemes. With mixtures up to five species, the species diversity gradient is longer than common two-species mixture trials, and the special attention to community evenness ensures that no single species dominates the mixtures.

The platform uses the same methodological approach in contrasting forest types along an extensive environmental gradient and offers a common set of suitable research plots to groups of researchers from very different disciplines (cf. Fischer et al., 2010). Various measurements characterizing a wide array of ecosystem properties, processes and functions will be performed and, in turn, related to the provisioning of ecosystem services. Measurements related to supporting and provisioning services include the quantification of nutrient stocks and cycling, net above and belowground productivity, photosynthesis parameters and the water balance. Measurements related to regulating services include the quantification of the load of insect pests, mammal herbivory, fungal pathogens and invasive plants. Comprehensive syntheses across many response variables and modelling efforts to forecast BDEF relationships under changing environmental conditions such as climate change heavily rely on such data sets collected on common plots. It is therefore the platform’s philosophy to make sure that all measurements are performed on all plots. This philosophy calls for indicators and proxies that can be measured quickly and at low cost, instead of relying on the monitoring of only few processes in great detail, which is the core activity of other networks (e.g., CarboEUROPE, ICP Forests Level II). Only few labour intensive and

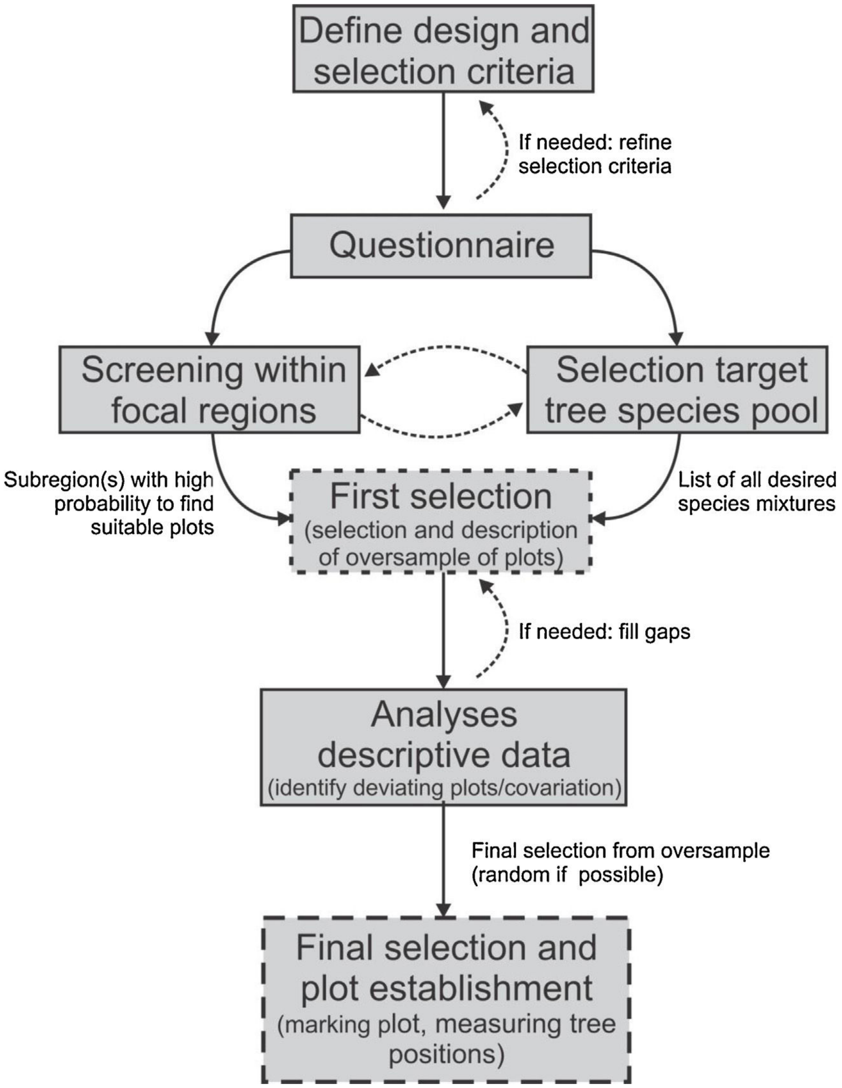

Fig. 3. Schematic overview of the plot selection procedure. Dashed lines show iterative steps and the two dashed boxes indicate plot characterization phases. After designing the research platform and defining the plot selection criteria, a questionnaire was sent to every local manager to verify potential regional incompatibilities and make an inventory of available data for plot selection. The target tree species pool to create a diversity gradient was defined and the six focal regions were explored to find potentially suitable sites (e.g., based on land-use maps, forest management plans, field visits). In the first selection phase, we searched for many more sites in the field than were actually needed and characterized those sites with quick descriptors (e.g., stand structural features, soil properties; Appendix 2). After the analyses of the site descriptors and filtering out the deviating sites, we performed a final randomized subsetting from the larger pool of sites. Plots were further characterized during plot establishment.

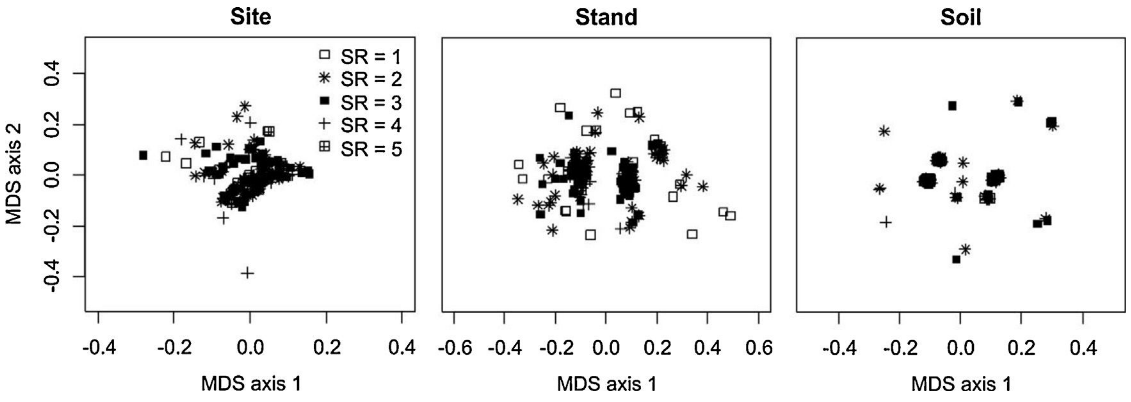

Fig. 4. Result of a non-metric multidimensional scaling ordination on soil, stand and site descriptors of 164 preselected sites in Białowieża (Poland) (descriptors in Appendix 2). We used Gower dissimilarities with the Podani (1999) extension to ordinal variables. Permutational analysis of variance (PERMANOVA; Anderson, 2001) showed that soil and site conditions did not significantly differ between the species richness levels ($F = 0.36$, $P = 0.91$ and $F = 1.53$, $P = 0.20$). Stand descriptors differed between species richness levels ($F = 2.85$, $P = 0.003$), mainly due to differences in forest structure and current management between monocultures and two species mixtures on the one hand (generally single/double layered canopies, no management) and the higher diversity levels on the other hand (generally multi layered, low frequency thinning).

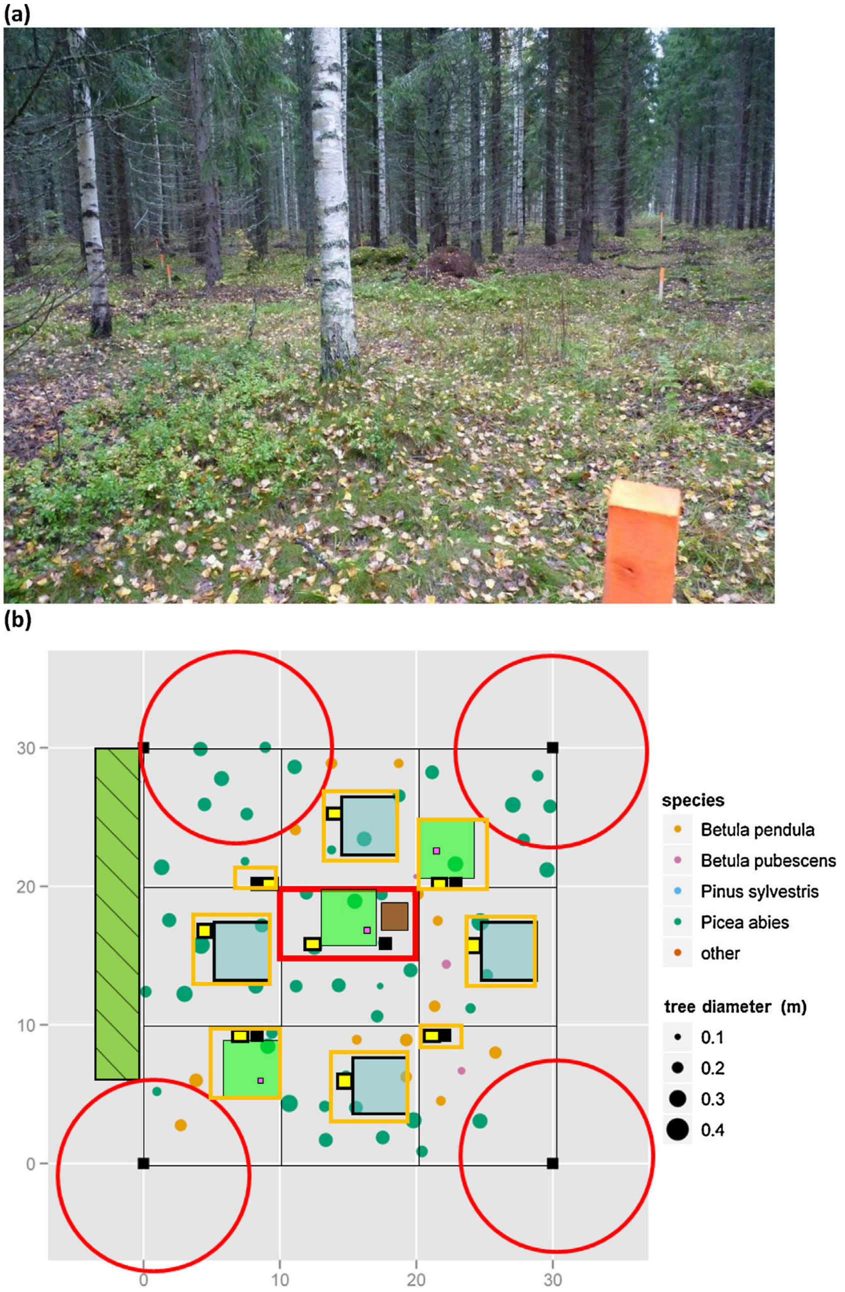

Fig. 5. (a) Photograph of an established plot in North Karelia, Finland (two-species mixture with *P. abies* and *B. pendula/pubescens*). The photo was taken from a corner post and shows a plot border and a plot diagonal; the wooden poles are at every 10 m (Photograph by Timo Domisch). (b) Example of a detailed plot scheme for this 30 m × 30 m plot. Dots represent trees, with dot sizes proportional to the tree diameter at breast height. Shapes with different colours indicate the various locations for sampling and setting up experiments. The large red circles are for instance the areas for dead wood sampling and the small yellow squares indicate the sites for root biomass and soil sampling. The green shaded area left of the plot is used to plant small trees with different provenances.

expensive measurements (e.g., water quality, sapflow) are carried out on a subset of Highly Instrumented Plots (HIPs). The lifetime of the platform is intended to be stretched far beyond the duration of the project funding (2014), e.g., by continuing and improving the research in close collaboration with the further development of the LTER Europe network (www.lter-europe.net). In this way, it can be continued as a long-term network open to other scientists ready to work within the same philosophy.

The knowledge generated within the Exploratory Platform will contribute to new views on sustainable forest management and nature conservation. This input is coming at a critical time, when strategies to adapt European forests to global changes, such as climate change (Kolström et al., 2011) and the transition towards more biobased economies (Schulze et al., 2012), are required. It is key that these strategies are based on an in-depth understanding of the relationships between forest structure, composition and function. Since most of the economically important European timber tree species are represented in the Exploratory Platform, it has great potential for translating the forest diversity-functioning relationships into guidelines that are of real interest for forest management. These guidelines will, among others, list which forest types, tree species or tree species combinations will lead to the optimization of multiple ecosystem services or create important trade-offs between services (Gamfeldt et al., 2013). Finally, knowledge on the resistance of different forest types to perturbations as well as their adaptive capacity will be generated, which is crucial in an era of global change.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ppees.2013.07.002.

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Glossary

Complementarity effect: The complementarity effect quantifies the combined effects of species interactions on mixture performance after accounting for changes in the relative abundance of species (selection effect – see below). Positive values result when mixtures do better than expected based on the relative abundance of species and their monoculture performances and are consistent with 'niche differentiation' in terms of resource partitioning, facilitation or diversity-dependent effects of natural enemies (although other explanations are possible – see Turnbull et al., 2013). Negative values are thought to equate to interference competition.

Comprehensiveness: The spectrum of ecosystem functions and services quantified in a study (Nadrowski et al., 2010).

Dilution gradient: A design with monoculture stands of only one species, combined with a species diversity gradient including this monoculture species in all mixtures (Nadrowski et al., 2010). Increasing species diversity is confounded with decreasing dominance of this species.

Species identity effect: The expected effect of a component species on mixture performance as derived from its monoculture performance, i.e., purely additive effects as opposed to complementarity effects (Kirwan et al., 2009). A strong identity effect may cause the sampling/selection effect if this species becomes dominant at the expense of others, although particular species may be associated with positive complementarity too. Identity effects are due to the (binary) presence/absence of particular species across mixtures and are distinct from species composition effects which distinguish the effects between different species combinations (Hector et al., 2011).

Orthogonality of species diversity: Orthogonality means the statistical independence of species diversity from other factors. Only orthogonal designs allow the effect of species diversity to be completely uncorrelated with that of other variables and covariates (e.g., species identity, environment, management) (Nadrowski et al., 2010). Unfortunately, it is often not possible to design studies where diversity is completely orthogonal from other variables due to unavoidable biological correlations, which can be seen as either 'hidden treatments' (Huston, 1997), or the mechanisms by which diversity has its effects.

Representativeness: The degree to which the units of a study represent the larger population, such as randomly drawn forest stands. High representativeness allows for generalization of results and increases the relevance of the results for the study system as we find it in the real-world landscapes (Nadrowski et al., 2010).

Sampling/selection effect: The sampling effect recognizes that more diverse communities are more likely to contain high-performing species, which can generate a positive BDEF relationship if they then dominate the community (Huston, 1997; Tilman et al., 1997). The selection effect is similar, but allows for negative as well as positive effects. Selection occurs when changes in the relative abundances of species covary with their monoculture performances (Loreau and Hector, 2001). Dominance of species with high (or low) monoculture performance generate positive (or negative) BDEF relationships.

Paper II

Methods in Ecology and Evolution

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Application of Loreau & Hector's (2001) partitioning method to complex functional traits

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Summary

1. In 2001, Loreau and Hector proposed a method to calculate the effect of biodiversity on ecosystem-level properties that distinguished selection effects (SE) from complementarity effects (CE). The approach was designed and has been widely used for the study of yield in mixed-species situations taking into account the relative abundance of each species in ecosystem-level yield. However, complex functional traits commonly used to integrate ecosystem-level properties that cannot be analysed like yield data because the weighted contribution of each species is not determined by its relative abundance.

2. We adapted the original method by clearly identifying ecologically meaningful weighting coefficients to represent species-specific contributions to ecosystem function.

3. We applied the adapted method of analysis to tree foliar carbon isotope composition in an experimental plantation in order to test the influence of species richness on plot water use efficiency (WUE_{plot}). The appropriate weights for the WUE_{plot} of each species are leaf CO_2 assimilation rate.

4. We observed a large range of WUE_{plot} and biodiversity effects among plots. The absence of a significant SE on WUE_{plot} indicated that the overall net biodiversity effect was primarily driven by a CE. The net biodiversity effect and CE were mostly negative, suggesting that interspecific interactions resulted in a decrease in the ratio between carbon acquisition and transpiration at the ecosystem level.

5. The application of the method to complex components of ecosystem functioning provides important new insights into the practical and conceptual aspects of functional biodiversity research.

Key-words: biodiversity effect, BIOTREE, carbon isotope composition, complementarity effect, FunDivEUROPE, method, selection effect, water use efficiency

Introduction

The loss of biodiversity occurring in most natural environments world-wide has sparked an interest among the scientific community in the relationship between biodiversity and ecosystem functioning (Hooper *et al.* 2012). More than 20 years of ecological studies have led to a consensus that ecosystem performance is highly dependent on species richness and on species functional characteristics (Loreau *et al.* 2001; Hooper *et al.* 2005; Zhang, Chen & Reich 2012). However, the mechanisms underlying biodiversity–function relationships have been hotly debated. Two major groups of mechanisms were initially proposed to explain positive effects of biodiversity: (i) a sampling or selection effect (SE) which arises, as species richness increases, from the increasingly probable occurrence of one or several species that strongly contribute to the ecosystem function observed (Aarssen 1997; Huston 1997; Tilman 1997), and (ii) a complementarity effect (CE) driven either by niche differentiation among species, which tends to increase the efficiency with which coexisting species use the available resources or to facilitation or other mutualistic interactions among species (Tilman *et al.* 1997; Loreau 1998). Recently, the combination of evenness, richness and life-history variations was also successfully linked to the mechanisms producing positive biodiversity effects (Zhang, Chen & Reich 2012).

To quantitatively evaluate this biodiversity – ecosystem functioning relationship and partition the underlying mechanisms, Loreau & Hector (2001) proposed a convenient method to calculate the influence of species mixture on ecosystem productivity: the net biodiversity effect on the yield (ΔY) of a given mixture can be calculated as the difference between the observed total yield in the mixture (Y_O) and the expected total yield in the mixture (Y_E) under the null hypothesis that intraspecific and interspecific interactions are identical. The original method was extended by Fox (2005) to include trait-dependent

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*Correspondence author. E-mail: bonal@nancy.inra.fr and trait-independent CE in addition to the dominance effect (tripartite partitioning).

Loreau and Hector's method has been widely used and so far more than 100 peer-reviewed papers analysing the relationships between species richness and ecosystem functioning in highly diverse biomes have been published. Even though the method has proven to be very popular, existing studies have focused on a limited number of ecosystem functions, mostly on standing biomass.

One explanation for this limited application could stem from the fact that some ecological functions in mixed species stands cannot be treated at the ecosystem level in the same way as observed yield. For complex functional properties, ecosystem-level values correspond to the mean value of the species present in the community weighted by the contribution of each species to the given function; this weighted contribution can be totally different from the relative abundance of these species in terms of frequency or biomass, which the original method does not imply. These complex functions include, among others, any measurement related to the efficiency of individuals to acquire and use resources (e.g. water use efficiency, photosynthesis efficiency, nutrient use efficiency), whatever the ecosystem (plant or aquatic ecosystems, bacterial communities…). The isotope composition of organic or mineral elements in biological material or the density of any gas flux (e.g. sap flow density, density of CO_2 respiration) is also an example of such complex functional traits.

In this paper, we extend Loreau & Hector's (2001) method to complex functional traits by clearly identifying ecologically meaningful weighting coefficients, which represent species-specific contributions to ecosystem functioning. We illustrate the usefulness of the adapted equations for leaf carbon isotope composition. More precisely, we analyse the effect of species richness on foliar carbon isotope composition ($\delta^{13}C$) at the ecosystem level in a temperate mixed-species tree plantation. Foliar $\delta^{13}C$ is a convenient proxy for time-integrated intrinsic water use efficiency ($\mathrm{WUE}_{\mathrm{int}}$; Farquhar, O'Leary & Berry 1982) and reflects the trade-off between CO_2 acquisition and stomatal regulation of transpiration at the leaf level. Foliar $\delta^{13}C$ and $\mathrm{WUE}_{\mathrm{int}}$ can only be obtained at the individual level, and ecosystem-level $\delta^{13}C$ cannot be calculated by simply taking into account the summed contribution of the individuals in terms of biomass or frequency. Instead, the proportional amount of CO_2 assimilated by each species in the plot needs to be considered to correctly weight each species' contribution to ecosystem-level functioning.

Application of Loreau & Hector's (2001) method to complex functional traits

According to Loreau & Hector (2001), the net biodiversity effect on the yield (ΔY) of a given mixture of species is the difference between the observed total yield in the mixture (Y_O) and the expected total yield in the mixture (Y_E) calculated as the sum of the products between the yield of the different species in their corresponding monocultures and the proportion of the species in the mixture (defined in terms of individual frequency or biomass):

$$\begin{aligned}\Delta Y &= Y_O - Y_E\\ &= \sum\nolimits_{i=1}^{N} Y_{Oi} - \sum\nolimits_{i=1}^{N} Y_{Ei} \qquad \text{eqn 1}\\ &= \sum\nolimits_{i=1}^{N} (\mathrm{RY}_{Oi} \times M_i) - \sum\nolimits_{i=1}^{N} (\mathrm{RY}_{Ei} \times M_i),\end{aligned}$$

where N is the number of species in the mixture, Y_{Oi} and Y_{Ei} denote the observed and expected yield of species i in the mixture, M_i is yield of species i in the monoculture, RY_{Oi} is the observed relative yield of species i in the mixture and RY_{Ei} is the expected relative yield of species i in the mixture. RY_{Oi} is calculated as the ratio of the observed yield of species i in the mixture and the yield of species i in the monoculture, whereas RY_{Ei} is simply the proportion of species i seeded or planted in the mixture.

For complex functional properties where the contribution of each species to a given ecosystem-level function is not simply proportionate to the frequency of these species or their proportion in biomass, we introduce a weighting coefficient (W_{Oi}) to calculate the contribution of species i to the complex function (F) in the mixed plots. W_{Oi} is normalised to one and therefore represents a proportional contribution. Thus, the net biodiversity effect on a complex function (ΔF) is written as:

$$\Delta F = F_O - F_E = \sum\nolimits_{i=1}^{N} (F_{Oi} \times W_{Oi}) - \sum\nolimits_{i=1}^{N} (F_{Ei} \times W_{Oi}), \qquad \text{eqn 2}$$

where F_{Oi} and F_{Ei} denote the observed and expected value of the function of species i in the mixture. This equation is a generalisation of the equation proposed by Loreau & Hector (2001). If one considers that F_{Oi} is the observed biomass of species i in the mixed plot and W_{Oi} is the proportion of species i in the mixed plot in terms of the number of seeded individuals or in terms of biomass, then $F_{Oi} \times W_{Oi}$ equals Y_{Oi}. Similarly, if one considers that F_{Ei} is the observed biomass of species i in the monoculture and W_{Oi} is the proportion of species i in the mixed plot in terms of the number of seeded individuals or in terms of biomass, then $F_{Ei} \times W_{Oi}$ equals Y_{Ei}.

The weighting coefficients are specific to each studied complex property and must take into account the underlying biological and ecological mechanisms to correctly estimate the contribution of each species to ecosystem-level functioning. In Table 1, we have listed some weighting factors that could be used in plant ecological studies. Let us illustrate this point with sap flow density. Sap flow density ($\mathrm{L_{sap}}\ \mathrm{dm}^{-2}_{\mathrm{sapwood}}$ per h) represents the density of the flow of raw sap circulating in the

Table 1. Examples of complex functional properties used in plant ecological studies, with units and suitable corresponding weighting coefficients

Properties	Units	Weighting coefficient
Sap flow density	L dm^{-2} per h	Sapwood area
Bark CO_2 efflux	μmol m^{-2} per s	Trunk surface
Photosynthesis	μmol m^{-2} per s	Leaf area
Leaf water use efficiency	μmol mol^{-1}	Leaf CO_2 exchange
Carbon isotope composition	‰	Leaf CO_2 exchange
Plant water use efficiency	kg L^{-1}	Leaf area
Nutrient uptake rates	μmol g^{-1} per h	Root surface area

xylem vessels of trees and can be directly measured with sap flow sensors at the single tree level. However, sap flow density cannot simply be added among the trees to calculate total plot sap flow density and to estimate the influence of biodiversity on this ecosystem-level property, because the proportion in biomass or tree frequency among species in the mixture does not give the proportional contribution of each species to total plot sap flow density. Rather, sapwood area (the cross-sectional, water conducting area in the trunk) of each tree is the correct weighting coefficient and should be used as the quantity W_{Oi} in eqn 2.

The goal of Loreau & Hector's (2001) method is to partition ΔY into two effects generated by species interactions in mixtures: SE and CE. SE arises from interspecific competition leading to the dominance of a given species with particular functional traits. CE reflects the degree to which niche differences and facilitation outweigh interference competition and other negative species interactions (Loreau *et al.* 2012). As when calculating ΔY, partitioning the effects of complex functions into *SE* and *CE* must also take W_{Oi} into account. The equation to calculate CE can be rewritten as:

$$\mathrm{CE} = N \times \overline{\frac{(F_{Oi} \times W_{Oi}}{F_{Ei}} - W_{Oi})} \times (\overline{F_{El}}) \qquad \text{eqn 3}$$

and the equation for SE is:

$$\mathrm{SE} = \sum\nolimits_{i=1}^{N} \left[\left(\frac{F_{Oi} \times W_{Oi}}{F_{Ei}} - W_{Oi} \right) - \overline{\left(\frac{F_{Ol} \times W_{Ol}}{F_{El}} - W_{Ol} \right)} \right] \times \left[F_{Ei} - \overline{F_{El}} \right]. \qquad \text{eqn 4}$$

As our approach is intended to be a generalisation of the original method and can be applied to complex functions, which are not directly related to yield, changes in species contribution with time are not taken into account in eqn 4. The SE thus here stresses the dominance of a one or more species for the considered complex ecosystem function at a given time.

Application of the modified equations to ecosystem-level carbon isotope composition

A positive effect of species mixture on forest ecosystem productivity (Paquette & Messier 2011; Zhang, Chen & Reich 2012) and transpiration (Forrester *et al.* 2010; Kunert *et al.* 2012) has previously been reported. Complementarity effects and SE for these ecosystem-level parameters were highlighted with Loreau & Hector's (2001) method, thus making it possible to explain differences in ecosystem functioning among species richness levels. Promoting a mixture of tree species to enhance the ratio of ecosystem-level productivity to transpiration (i.e. high water use efficiency) has been advocated for sustainable forest management (McCarthy *et al.* 2011), of particular importance in a context of climate change. To further investigate this relationship, we applied the widely used carbon isotope approach (Farquhar, O'Leary & Berry 1982) to study the impact of tree species mixtures on ecosystem-level water use efficiency ($\mathrm{WUE_{plot}}$) in a temperate mixed plantation.

At leaf level, intrinsic water use efficiency ($\mathrm{WUE_{int}}$) represents the ratio between photosynthetic assimilation of CO_2 by the leaf (A) and stomatal conductance for water vapour (g_s) and depends on the molar fraction of CO_2 in the air (C_a) and in the leaf intercellular spaces (C_i) following this equation:

$$\mathrm{WUE_{int}} = \frac{A}{g_s} = \frac{C_a - c_i}{1 \cdot 6}. \qquad \text{eqn 5}$$

During photosynthetic assimilation of CO_2, plants discriminate against molecules of CO_2 containing ^{13}C because $^{13}CO_2$ diffuses more slowly from the atmosphere to the site of carboxylation (stomatal diffusion) than does $^{12}CO_2$. $^{13}CO_2$ also reacts less with the primary carboxylating enzyme (Rubisco; Fig. 1). Farquhar, O'Leary & Berry (1982) showed that foliar carbon isotope composition ($\delta^{13}C$, ‰) is strongly negatively correlated with WUE_{int} following this simplified equation:

$$\delta^{13}C = \delta^{13}C_{\mathrm{air}} - b + (b - a) \times \frac{1 \cdot 6 \times \mathrm{WUE_{int}}}{C_a}, \qquad \text{eqn 6}$$

where $\delta^{13}C_{\mathrm{air}}$ is the carbon isotope composition of the air, and a and b are factors characterising the discrimination against

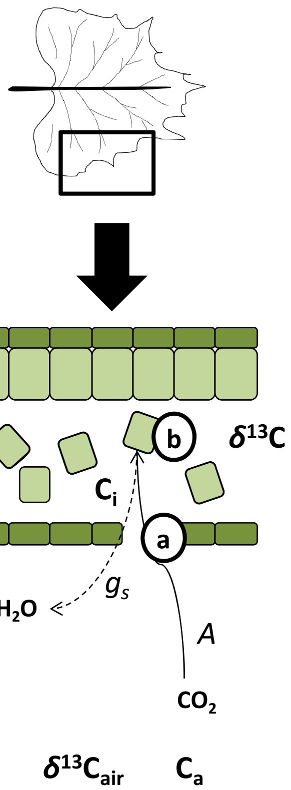

Fig. 1. Schematic representation of the processes involved in leaf carbon isotope discrimination during photosynthesis. The carbon isotope composition ($\delta^{13}C$) of total leaf organic matter is determined by the carbon isotope composition of CO_2 in the air ($\delta^{13}C_{\mathrm{air}}$) and the CO_2 concentration in the air (C_a) and in the leaf intercellular spaces (C_i). Discrimination processes against $^{13}CO_2$ occur during photosynthetic CO_2 assimilation (A) when CO_2 passes through the stomata (g_s) from the outside air (fractionation factor a) and during the carboxylation process by the Rubisco enzyme inside the chloroplasts (fractionation factor b). The plain arrow represents A and the dotted arrow represents the transpiration flux.

$^{13}CO_2$ during stomatal diffusion and carboxylation, respectively. Therefore, $\delta^{13}C$ provides a convenient time-integrated estimate of WUE_{int}. Values for $\delta^{13}C$ are obtained at the individual tree level by sampling representative subsets of leaves or needles, but individual values cannot simply be added to represent ecosystem-level carbon isotope composition ($\delta^{13}C_{plot}$). Instead, the contribution of a single tree to the carbon isotope composition of the whole population depends on tree-specific CO_2 assimilation rates that control carbon isotope fractionation during photosynthesis (Lloyd & Farquhar 1994). Consequently, when $\delta^{13}C$ values are scaled up from tree or species level to ecosystem level, tree or species $\delta^{13}C$ values should be weighted by these assimilation rates (Fig. 2). Since direct measurements of CO_2 assimilation rates cannot easily be made for each tree in the field, we used a convenient proxy for canopy-level, species CO_2 assimilation. In a given environment, the quantum yield for reduction in end electron acceptors at the PSI acceptor side is strongly positively correlated with time-integrated leaf CO_2 exchanges (Genty, Briantais & Baker 1989). Thus, the proportions of the measured quantum yield of species i can therefore be used as the weighting coefficient (W_{Oi}) for $\delta^{13}C$ values.

Materials

We conducted our study at the BIOTREE tree biodiversity experimental site in Germany (Kaltenborn site, Scherer-Lorenzen *et al.* 2007), which was planted in winter 2003–2004. This plantation is located on acidic sandy soils and includes four species: *Fagus sylvatica* (L.), *Quercus petraea* (Matt.), *Picea abies* (L.) Karst. and *Pseudotsuga menziesii* (Mirb.) Franco. The plantation was designed to assure maximum above- and below-ground interactions among species at the adult stage. Therefore, in the mixed plots of 120 × 48 m, each species was planted in monospecific rectangular patches of 8 × 8 m, arranged in a regular pattern in order to reduce out-competition of slow-growing species at an early stage and to maximise interspecific interactions along borders and corners (Scherer-Lorenzen *et al.* 2007). In summer 2011, we sampled leaves and needles from four trees per species and per plot in monocultures (n = four plots), two-species mixtures (n = 6), three-species mixtures (n = 4) and four-species mixtures (n = 1). In each plot, we only took samples from trees at the corners of the patches. The samples were oven-dried at 60°C for 48 h, then finely ground. $\delta^{13}C$ analysis was carried out at the Stable Isotope Facility of UC Davis, USA. The $\delta^{13}C$ (‰) values are expressed relative to the Vienna Pee Dee Belemnite standard. The quantum yield for reduction in end electron acceptors at the PSI acceptor side was measured with a HandyPea fluorimeter (Hansatech Instruments, Pentney-Norfolk, UK) on leaves or needles in close vicinity to the ones harvested for elemental and isotope analyses following the procedure described by Strasser *et al.* (2010). This value was then used as a weighting coefficient (W_{Oi}) when calculating biodiversity effects on $\delta^{13}C_{plot}$. We used the nonparametric Wilcoxon test to check for complementarity, selection and net effects among mixture levels and t-tests to evaluate whether all the indices differed significantly from zero (SAS 9.3; SAS Institute, Cary, NC, USA).

Results and discussion

We found large differences in ΔF, CE and SE among plots, with either positive or negative values (Fig. 3, Table 2). Positive or negative values confirmed that in this plantation,

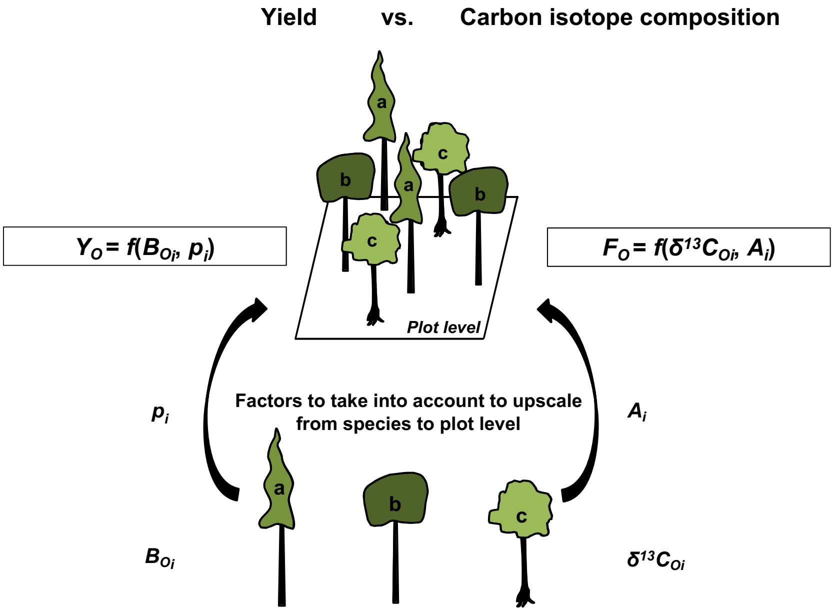

Fig. 2. Comparison of the factors taken into account to calculate the observed trait at plot level for yield (Y_O) and carbon isotope composition (F_O). For yield, the observed biomass of each species i in the mixture (B_{Oi}) and the proportion of each species in the mixture (p_i) are taken into account to calculate Y_O. For carbon isotope composition, F_O is dependent on the observed carbon isotope composition of species i in the mixture ($\delta^{13}C_{Oi}$, i.e. F_{Oi}) and the corresponding CO_2 assimilation rate during photosynthesis (A_i) representing the species-specific contribution to plot level carbon isotope composition. Letters on the trees denote different species.

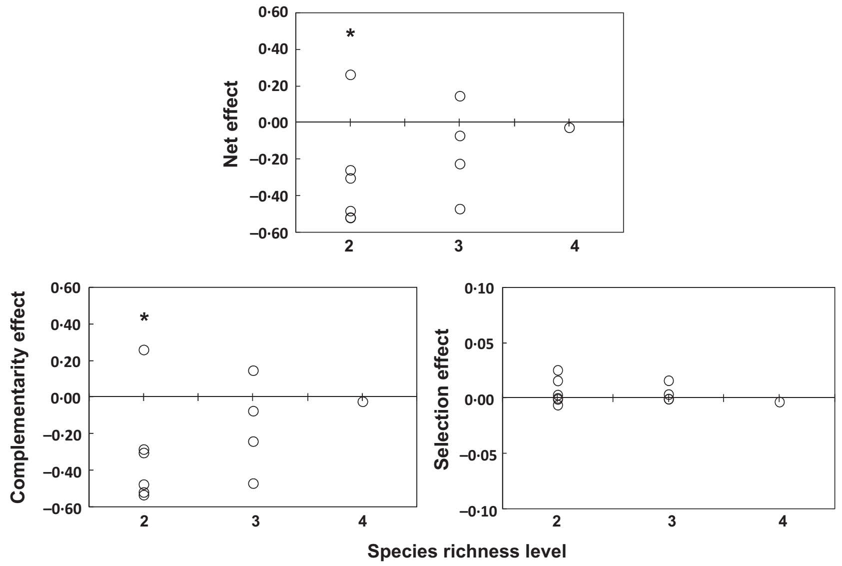

Fig. 3. Application of the adapted method to plot carbon isotope composition ($\delta^{13}C_{plot}$). Net, complementarity and selection effects calculated for $\delta^{13}C_{plot}$ for the different richness levels. Asterisks denote significant differences from zero for each effect (*t*-test, $^{*}P < 0{\cdot}05$).

Table 2. Mean species foliar carbon isotope composition measured in the mixed plots (F_{Oi}, ‰), expected mean species foliar carbon isotope composition measured in the monoculture plots (F_{Ei}, ‰) and proportional weighting coefficient (W_{Oi}) of species *i*, in each studied plot for the four studied species: *Fagus sylvatica* (Fs), *Pseudotsuga menziesii* (Pm), *Quercus petraea* (Qp) and *Picea abies* (Pa). Species richness, net biodiversity effect (ΔF), complementarity effect (CE), selection effect (SE) and calculated plot carbon isotope composition (F_O) are shown for each plot

Plot	Richness level	Species	F_{Oi}	F_{Ei}	W_{Oi}	ΔF	CE	SE	F_O
3	2	Fs	−28·46	−28·82	0·46	0·26	0·26	$3{\cdot}11 \times 10^{-3}$	−26·99
		Pm	−25·74	−25·91	0·54				
6	2	Qp	−27·43	−26·60	0·50	−0·48	−0·47	$-5{\cdot}91 \times 10^{-3}$	−26·64
		Pa	−25·84	−25·72	0·50				
7	2	Pm	−26·36	−25·91	0·51	−0·52	−0·52	$-7{\cdot}38 \times 10^{-4}$	−26·77
		Qp	−27·19	−26·60	0·49				
10	2	Pm	−26·13	−25·91	0·58	−0·30	−0·30	$2{\cdot}14 \times 10^{-4}$	−26·13
		Pa	−26·15	−25·72	0·42				
12	2	Fs	−28·99	−28·82	0·41	−0·52	−0·53	$1{\cdot}59 \times 10^{-2}$	−28·02
		Qp	−27·36	−26·60	0·59				
16	2	Fs	−28·58	−28·82	0·36	−0·26	−0·28	$2{\cdot}54 \times 10^{-2}$	−27·11
		Pa	−26·26	−25·72	0·64				
4	3	Fs	−28·60	−28·82	0·25	−0·22	−0·24	$1{\cdot}61 \times 10^{-2}$	−27·06
		Qp	−26·73	−26·60	0·39				
		Pa	−26·35	−25·72	0·36				
5	3	Fs	−28·57	−28·82	0·29	0·15	0·15	$-5{\cdot}72 \times 10^{-4}$	−26·84
		Pm	−25·57	−25·91	0·36				
		Qp	−26·75	−26·60	0·35				
9	3	Fs	−28·82	−28·82	0·27	−0·07	−0·07	$3{\cdot}58 \times 10^{-3}$	−26·70
		Pm	−25·70	−25·91	0·34				
		Pa	−26·09	−25·72	0·39				
15	3	Pm	−26·25	−25·91	0·38	−0·47	−0·47	$2{\cdot}54 \times 10^{-2}$	−27·11
		Qp	−27·20	−26·60	0·30				
		Pa	−26·23	−25·72	0·31				
8	4	Fs	−28·92	−28·82	0·18	−0·03	−0·02	$-3{\cdot}15 \times 10^{-3}$	−26·61
		Pm	−25·24	−25·91	0·25				
		Qp	−26·95	−26·60	0·30				
		Pa	−26·00	−25·72	0·27				

interactions among species drive $\delta^{13}C_{plot}$, and thus WUE_{plot}. When considering individual species richness levels, we found that both the net effect and CE were significantly different from zero ($P < 0{\cdot}05$) for the two-species mixtures; however, we found no SE ($P = 0{\cdot}27$). Furthermore, the net effect and CE were mostly negative, suggesting lower observed $\delta^{13}C_{plot}$ than what would have been expected based on the monoculture values. Since $\delta^{13}C$ and water use efficiency are positively related (Farquhar, O'Leary & Berry 1982), our findings point towards lower $\mathrm{WUE}_{\mathrm{plot}}$ when several different species coexist. This result contrasts with previous patterns of enhanced water use efficiency found in species mixtures (Forrester *et al.* 2010; Kunert *et al.* 2012).

The absence of a significant SE on $\mathrm{WUE}_{\mathrm{plot}}$ indicates that the overall negative net biodiversity effect observed in the two-species mixtures was primarily driven by a CE. Our interpretation is that the species coexisting in the mixed plots are in direct competition for the same resources because they still share the same ecological niche at the early establishment stage (7 years after planting, at the time of our measurements). This competition most likely caused a decrease in the ratio between carbon acquisition and transpiration at the ecosystem level in the two-species mixtures. As no overshading was observed, the competition among species is presumably occurring below-ground. This assumption is consistent with the strong competition among fine roots observed by Lei, Scherer-Lorenzen & Bauhus (2012) in this plantation.

Furthermore, we did not observe any significant effect of richness level for any of the biodiversity effects ($P > 0{\cdot}05$). This indicates that the number of species competing for resources does not significantly affect the difference in $\delta^{13}C_{\mathrm{plot}}$ between observed and expected values. The $\delta^{13}C$ value for a given species in the two-, three- and four-species mixtures did not greatly change.

Conclusion

Applying the version of Loreau & Hector's (2001) method to complex components of ecosystem functioning will provide important new practical applications as well as conceptual insights into functional biodiversity research. We have shown here that, with the appropriate weighting factors for specific, complex functional properties, the method can be applied to a broad range of functional properties, rather than to yield alone. In our case, we used the quantum yield for reduction in end electron acceptors at the PSI acceptor side as the weighting factor for ecosystem-level carbon isotope composition. This combination of weights and functions provides an estimate of intrinsic water use efficiency in mixed species plots. For other complex traits, the selection of the most pertinent weighting factor should make it possible to determine the contribution of each species to the studied ecosystem property. Some of the weighting factors may be difficult to measure precisely with currently available equipment, as in the case of CO_2 assimilation rates in our study. Nevertheless, if appropriate measurements are not easily obtainable, proxies could be found that provide the same proportional contributions; modelling approaches may be of help in this case. In the young mixed temperate plantation in our study, complementarity rather than SE were the substantial drivers of plot water use efficiency. As the plantation ages and taller trees with broader root systems begin to compete for light and soil resources, CE might increase and SE might arise due to more frequent interspecific interactions. It will thus be interesting to follow the changes in the relative importance of these two components of net biodiversity effects for a multitude of ecological processes and functions.

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Author contributions

CG and DB elaborated the adaptation of the method to complex functional traits. DB, AG^1, AG^2 and MSL designed the experimental study. DB, MP and AG^1 performed the experimental work. CG, DB, AG^1 and AG^2 analysed the results. CG and DB wrote the first draft of this manuscript and all authors substantially contributed to revisions.

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Paper III

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The influence of tree species mixture on ecosystem-level carbon accumulation and water use in a mixed boreal plantation

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Water use efficiency

ABSTRACT

Throughout the world, huge areas have been occupied by monospecific tree plantations in order to fulfil the increasing demand for industrial wood products. The use of mixed-species plantations has recently been advocated as a potential compromise between maintaining high-volume wood production and conserving other ecosystem services. Yet little is known about the impact of tree species mixture on species- and ecosystem-level carbon accumulation and water use in mixed plantations. We combined data on above-ground biomass, xylem sap flux density, and the carbon and oxygen isotope composition of leaves and needles in a boreal plantation to test whether different levels of species mixture would impact tree- and plot-level biomass production, transpiration and water use efficiency. Data were recorded in 22 20-x-20-m plots (7 for transpiration) randomly allocated to either monocultures or to two-, three-, and five-species mixtures of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), Silver birch (*Betula pendula*), Common alder (*Alnus glutinosa*) and Siberian larch (*Larix sibirica*). At tree level, for a given species, we found significant differences among mixtures in transpiration and water use efficiency, though species mixture had no impact on above-ground biomass. At plot level, a large variability in functioning among mixtures was observed, but increasing the number of species in the mixture did not enhance productivity, transpiration or water use efficiency. The presence in mixtures of a high performing species like birch brought about changes in the canopy structure which in turn may have led to changes in micro-environmental conditions. Such changes could have contributed to explain differences in transpiration and water use efficiency among mixtures. We concluded that mixing locally-adapted species under non-limiting soil water conditions did not provide any benefit for ecosystem-level carbon accumulation and water use at this young ontogenic stage, but our study does not preclude that some benefits might arise at older stages.

1. Introduction

While forest plantations only cover 5% of forested lands at a global scale, they represent 15% of the world's wood production (Carnus et al., 2006). Depending on management practices, site fertility, or species, tree plantations can produce up to 40 m^3 ha^{-1} y^{-1} of usable wood products (Paquette and Messier, 2010), much more than natural forests. Therefore, to fulfil the increasing demand for industrial wood products over the last decades, industrial tree plantations have replaced large areas originally occupied by natural forests throughout the world. In Europe, the second largest area in the world where forest plantations exist, about a quarter of the forested land is managed in the form of plantations, with *Pinus sylvestris*, *Picea abies*, and *Larix decidua* as the most common species (Del Lungo et al., 2006).

However, such forest plantations have been highly criticized (Paquette and Messier, 2010) because they are usually associated with reduced species diversity (Erskine et al., 2006), decreased soil fertility (Merino et al., 2005), or an increase in water consumption (Lane et al., 2004). To overcome these drawbacks and to adapt plantation practices to changing environmental conditions, new management strategies have been proposed. Mixed-species plantations have been advocated as a potential compromise between high-yield wood production and biodiversity conservation (Kelty, 2006). Unfortunately, mixed-species plantations represent less than 0.1% of the industrial plantations around the world to date (Nichols et al., 2006).

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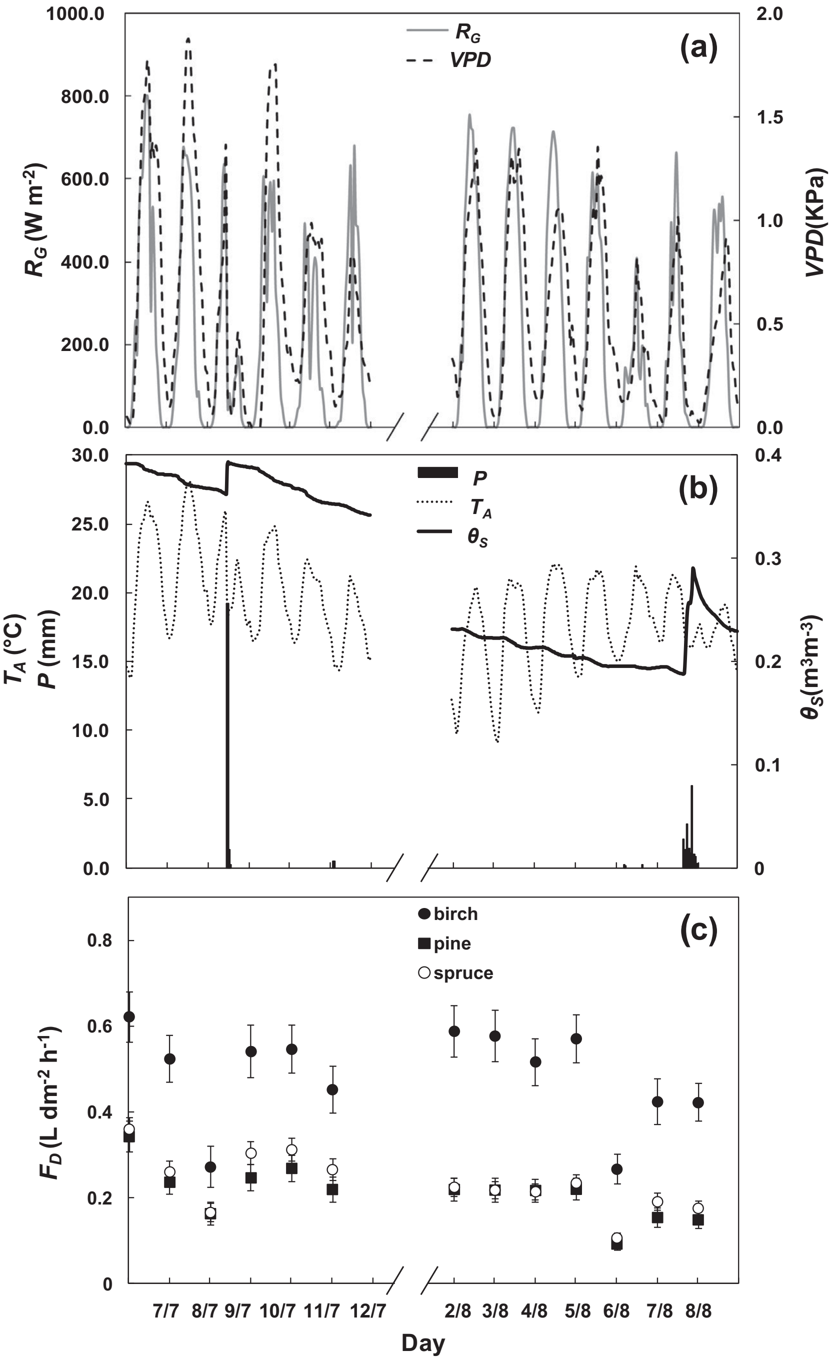

Fig. 1. Daily patterns of (a) global radiation (R_G, W m^{-2}), vapour pressure deficit (VPD, kPa), (b) atmospheric temperature (T_A, °C), precipitation (P, mm), soil water content (θ_S, m^3 m^{-3}), and (c) daily mean sap flux density of birch, pine and spruce in monocultures during the two experimental periods (7–12 July, 2011 and 2–8 August, 2011). Bars are standard errors of the mean for the 3–4 sampled trees per species.

More diverse tree communities have been shown to have a positive impact on a wide range of ecosystem functions and services. Greater species diversity in forest ecosystems has been related to increased productivity (Zhang et al., 2012), more efficient nutrient use (Menalled et al., 1998), better resistance to insects or diseases (Jactel and Brockerhoff, 2007), and improved biodiversity conservation (Paquette and Messier, 2011), though some studies have also found no significant direct effect of species richness on ecosystem functioning (Nguyen et al., 2012). Little is known about the impact of species mixture on carbon accumulation and water use in forest ecosystems. Two studies conducted in tree plantations in the tropics concluded that transpiration and water use efficiency (the ratio of carbon acquisition over transpiration) increased with species richness (Forrester et al., 2010; Kunert et al., 2012). In contrast, a study conducted in an old-growth, species-rich temperate forest showed that species-specific traits rather than species richness mainly explained the variations in plot-level transpiration (Gebauer et al., 2012).

Higher performance in species-rich stands as compared to monocultures indicates that interspecific interactions due to differences in ecological and functional characteristics among species can be less detrimental than intraspecific interactions. Several mechanisms have been described to explain such positive interactions among species (e.g. Loreau and Hector, 2001). Complemen-

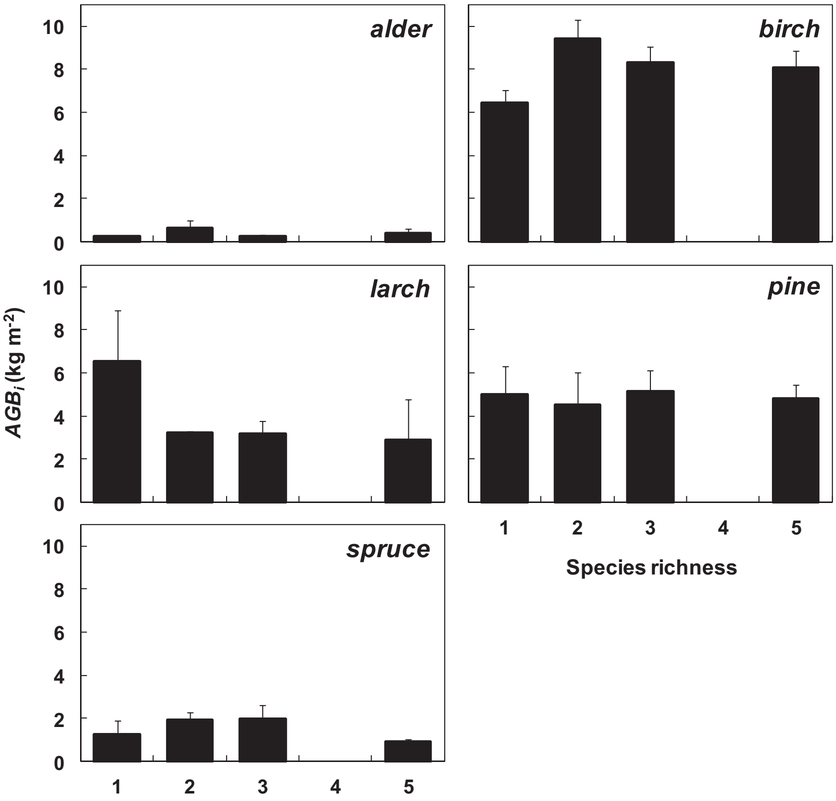

Fig. 2. Mean above ground biomass per unit of ground surface area (AGB_i, kg m^{-2}) for each species richness level for alder, birch, larch, pine and spruce. Vertical bars are standard errors of the mean for 2–4 plots per richness level.

tarity, for example, can be explained by niche differentiation among species. Due to interspecific differences in resource use, ecosystem-level use of the available resources becomes more efficient. For example, species may use water from different soil layers because of different root stratification (Forrester et al., 2010; Kunert et al., 2012), which results in higher ecosystem-level productivity. Complementarity also includes facilitation and other mutualistic interactions among species (Tilman et al., 1997). For example, species-rich stands have higher resistance to wind disturbance because of a more complex canopy structure (Papaik and Canham, 2006). Another important performance-enhancing mechanism in species-rich stands is called "the selection effect" (Loreau and Hector, 2001). This effect occurs when a high-performing dominant species in a mixed stand is clearly driving the performance of the stand. For example, Gebauer et al. (2012) showed that plot-level transpiration in a mixed temperate forest was strongly related to the abundance of a given species (European beech).

Even though differences in species ecological characteristics can lead to these mechanisms, these effects may not always be sufficient to significantly influence ecosystem functioning or performance (Nguyen et al., 2012). Particularly in the context of young forest plantations, the relationship between biodiversity and ecosystem functioning can be altered, directly or indirectly, by other factors such as abiotic-stress, successional stage, or management practices that concomitantly influence tree size and vegetation structure (Hooper and Dukes, 2004). Such confounding factors could positively or negatively influence species interactions and thus affect the overall performance of the ecosystem. We could therefore expect that in young plantations, increasing the number of species in mixture would lead to enhanced tree- and ecosystem-level productivity, transpiration and water use efficiency, but would as well increase the occurrence of a selection effect and of changes in micro-environmental conditions that would interact with the biodiversity effects.

In order to test this hypothesis, we conducted an experiment in a 12-year-old boreal plantation in Finland implemented with species that differ greatly in growth and functional characteristics. This plantation includes five tree species (Scots pine (*P. sylvestris*), Norway spruce (*P. abies*), Silver birch (*Betula pendula*), Common alder (*Alnus glutinosa*) and Siberian larch (*Larix sibirica*)) planted at different species richness levels. We focused our work on the influence of tree species richness level on tree- and ecosystem-level carbon and water acquisition and regulation by (i) comparing total aboveground biomass per unit of ground surface among species and plots, (ii) estimating water use efficiency at the tree and plot level based on the carbon isotope composition (δ^{13}C) in leaves or needles, (iii) using the oxygen isotope composition (δ^{18}O) in leaves and needles to distinguish between the effects of photosynthesis and/or stomatal regulation processes on the variations in δ^{13}C, (iv) measuring sap flux density in a subsample of individual trees, (v) and calculating plot-level canopy transpiration.

2. Materials and methods

2.1. Site description

The study was conducted in one of the three areas of the Satakunta forest diversity experiment established in 1999 in south-western Finland (Scherer-Lorenzen et al., 2005). The site is a 1.5-ha plantation on a clear-cut area near Pomarkku (61.42°N, 21.58°E, 35-m elevation) which contains 38 plots (20 × 20 m) randomly allocated to monocultures and to two-, three- and five-species mixtures of Scots pine (*P. sylvestris*), Norway spruce (*P. abies*), Silver birch (*B. pendula*), Common alder (*A. glutinosa*) and Siberian

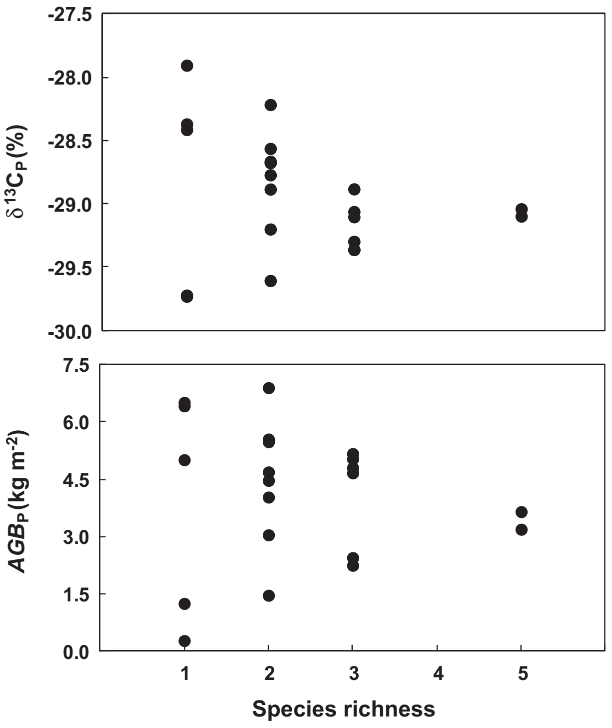

Fig. 3. Plot carbon isotope composition ($\delta^{13}C_P$, ‰) and total plot above-ground biomass per unit of ground surface area (AGB_P, kg m^{-2}) for each species richness level.

larch (*L. sibirica*). The trees are spaced 1.5 m apart both between and within rows with 169 trees per plot. Mixed plots were planted with an equal number of seedlings per species but mortality, though low, changed the original proportion slightly. In the mixed plots, the position of the different tree species was randomized within the plots.

In 2011, the mean annual precipitation at the nearby meteorological station in Pori (20 km from the site, 13-m elevation) was around 700 mm; mean air temperature was 6.4 °C with high seasonal variability. The soils are shallow Podzols (Food and Agriculture Organization of the United Nations classification) on granodiorite bedrock.

2.2. Biometric measurements

Diameter at breast height of a subsample of 10 randomly chosen trees per species and per plot was measured with a calliper in summer 2011. For the plots equipped with sap flow sensors, all the trees in the plot were measured for diameter at breast height. Basal area of each species in each plot was calculated based on the diameter at breast height measured on the subsample of trees and by applying the mean diameter value to all trees belonging to the same species present in the given plot. In order to express basal area as a function of surface occupied (m^2 ha^{-1}), we considered that all species present in a mixture plot occupied an equal area in the plot as the plantation was designed with full evenness.

Mean dry-weight above-ground biomass over a ground surface area unit per species (AGB_i, kg m^{-2}) and per plot (AGB_P, kg m^{-2}) was calculated thanks to allometric relationships for each species extracted from the literature (Jenkins et al., 2004) by applying the mean diameter at breast height of the subsample of measured trees to all trees.

2.3. Sap flux measurements and stand transpiration

Sap flux was measured following the thermal dissipation method (Granier 1987) in July and August 2011 in seven plots that included Silver birch, Scots pine and Norway spruce as monocultures and in two- and three-species mixtures. These three species were selected because they represent the most widely-planted commercial species in Finland. In each plot, three or four trees per species were equipped with sap flow sensors for a total of 34 studied trees. The trees were randomly selected among the dominant ones for each species. Due to technical constraints, the sap flux measurements were restricted to two periods (July 7–12, 2011 and August 2–8, 2011) (Fig. 1).

The two 20-mm-long probes on the sensors (UP-Gmbh, Cottbus, Germany) were installed under the bark and the cambium at around 1.3 m above soil level with a 10-cm vertical spacing between the probes. Data were collected every five seconds with a CR1000 data logger (Campbell Scientific Inc, Loughborough, Leicestershire, UK) and 30-min averages were recorded. The volumetric sap flux density (F_D, L dm^{-2} h^{-1}) was calculated as (Granier, 1987):

$$F_D = 4.28\left[\frac{\Delta T(0)}{\Delta T(u)} - 1\right]^{1.23}, \tag{1}$$

where $\Delta T(0)$ is the maximum temperature difference between the two temperature probes during the night and $\Delta T(u)$ is the current temperature difference. F_D was converted into transpiration (L day^{-1}) by multiplying the values obtained by sapwood area. Sapwood area was estimated through allometric linear relationships for each species taken from the literature (Pokorny, 2000; Poyatos et al., 2005; Vincke et al., 2005). As Granier-type probes only measure sap flux density radially along a 20-mm cylinder, a proportion of total tree sapflow is not measured when sapwood width is wider than the sensor length. To extrapolate this measurement to the whole cross-sectional area of the sapwood, the radial profile of sap flux density has to be estimated. For pine, the corrections were based on a modal distribution (Nadezhdina et al., 2002). For birch, as it is a diffuse-porous species like beech, the corrections were made with the assumption of a linear decrease from the bark to the trunk centre as observed for beech by Gessler et al. (2005). For spruce, sapwood width was either equal or smaller than 20 mm. When it was smaller, we applied the correction proposed by Clearwater et al. (1999). Whole plot transpiration was then calculated by summing all tree transpiration values in each plot and was expressed as a function of plot surface area (T_P, mm day^{-1}).

2.4. Carbon and oxygen isotope composition of leaves and needles

Ten to twenty fully expanded sunlit leaves for broadleaf species or 3–5 30-to-40-cm-long branches supporting fully mature, sunlit needles for conifers were sampled with 18-m-long pruning shears at the beginning of July 2011 in 22 plots covering all mixture combinations (monocultures, two-, three- and five-species mixtures) (Appendix A). For the conifers, both current and previous year needles were sampled and bulked. The samples were oven dried at 40 °C for 48 h at the Pomarkku laboratory. They were then shipped to INRA Nancy, France, where they were further dried in an oven for 72 h at 60 °C and then finely ground. About 1.8 mg of the powdered material of each sample was placed into tin capsules for analysis of carbon isotope composition ($\delta^{13}C$, ‰), total nitrogen content (N%, %) and total carbon content (C%, %). Furthermore, two replicates of about 1.3 mg of powdered material of each sample were placed into silver capsules for analysis of oxygen isotope composition ($\delta^{18}O$, ‰). All analyses were conducted at the Stable Isotope Facility of UC Davis, California, USA. $\delta^{13}C$ values were obtained with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK), while δ^{18}O values were obtained with an PyroCube (Elementar Analysen Systeme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). All isotopic measurements are reported in the standard delta notation (δ, ‰):

$$\delta‰ = \frac{R_{sample}}{R_{standard}} - 1, \quad (2)$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratios of the samples and the Vienna Pee Dee Belemnite (VPDB) and Vienna Mean Ocean Water (V-SMOW) standards for carbon and oxygen, respectively.

δ^{13}C is positively related to leaf intrinsic water use efficiency (WUE) (Farquhar et al., 1982), which is defined as the ratio of net photosynthesis over stomatal conductance to water vapour. Therefore, δ^{13}C provides information on WUE integrated over various time periods depending on the turnover time of the organic matter pools assessed (Fotelli et al., 2003). The combined measurement of δ^{18}O and δ^{13}C provides further insight into the changes in leaf δ^{13}C (Farquhar et al., 1998; Saurer et al., 1997). Indeed, variations in δ^{13}C are explained by a trade-off between stomatal conductance, which represents the supply of CO_2, and photosynthetic activity, which defines the demand for CO_2. On the other hand, variations in δ^{18}O are mainly driven by δ^{18}O of source water and changes in stomatal conductance (Farquhar et al., 1998). Measuring both δ^{18}O and δ^{13}C allowed us to distinguish between the stomatal and the photosynthetic origin of the changes in δ^{13}C.

Based on species individual δ^{13}C values in each plot ($\delta^{13}C_i$), we calculated the carbon isotope composition at plot level ($\delta^{13}C_P$). In

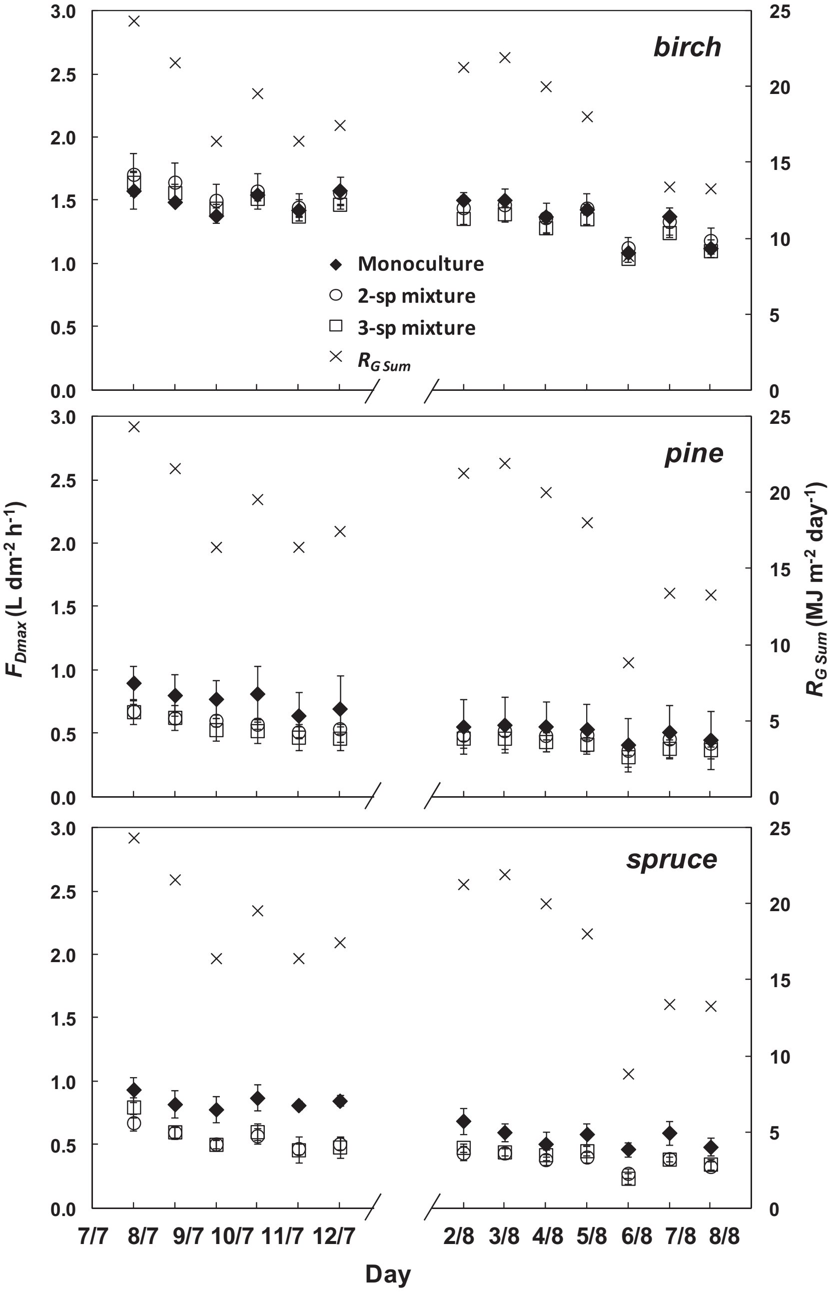

Fig. 4. Mean of daily maximum sap flux density (F_{Dmax}, L dm^{-2} h^{-1}) of the measured trees by species (birch, pine and spruce) and daily sum of global radiation (R_{GSum}, MJ m^{-2} day^{-1}) during the two measurement periods (7–12 July, 2011 and 2–8 August, 2011). Vertical bars are standard errors of the mean for $n = 3–4$ trees.

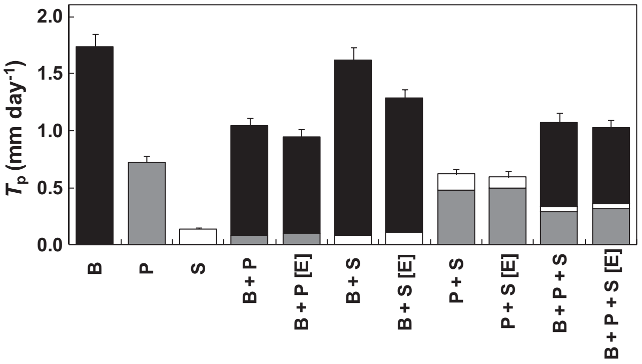

Fig. 5. Mean observed and expected ([E]) plot transpiration (T_P; mm day^{-1}) in monocultures and mixtures of birch (B), pine (P) and spruce (S) during the two measurement periods (7–12 July, 2011 and 2–8 August, 2011). Vertical bars are standard errors of the mean for the 13 days of sap flux density measurements.

order to take into account the contribution of each species to $\delta^{13}C_P$, we used a proxy for the time-integrated CO_2 assimilation of each species in order to weight species average leaf $\delta^{13}C_i$ in the canopy (Lloyd and Farquhar, 1994). As stated by Bonal et al. (2000), it can be assumed that differences among species in canopy-level, time-integrated, species CO_2 assimilation are proportional to differences among species in basal area. Therefore, we calculated $\delta^{13}C_P$ as:

$$\delta^{13}C_P = \frac{\sum_{i=1}^{N}(\delta^{13}C_i \times BA_i)}{\sum_{i=1}^{N} BA_i} \qquad (3)$$

where BA_i is the basal area of species i in the mixture and N the number of species in a given plot. We also compared $\delta^{13}C_P$ values calculated according to Eq. (3) with values calculated from the maximum quantum yield of electrons reaching the acceptor side, another convenient proxy of canopy-level, time-integrated, species CO_2 assimilation. Indeed, in a given environment, the latter parameter is positively correlated with time-integrated leaf CO_2 exchanges (Cascio et al., 2010). This parameter was measured with a HandyPea fluorimeter (Hansatech Instruments, Pentney-Norfolk, UK) following the procedure described by Cascio et al. (2010). Fluorimeter measurements were performed on leaves or needles in close vicinity to the ones harvested for elemental and isotope analyses. As no significant differences were observed between the two different methods of calculation for $\delta^{13}C_P$ (data not shown), only $\delta^{13}C_P$ values from basal area were considered in the data analyses presented hereafter.

2.5. Meteorological measurements

Soil volumetric water content in the 0–25 cm soil layer (θ_S, m^3 m^{-3}) was determined with TDR Probes (CS616, Campbell Scientific Inc.) inserted to a depth of 10 cm at three locations in the plantation (i.e. three plots measured for sap flux) and recorded with the data loggers used for the sap flow sensors. Thirty-minute means of global radiation (R_G, W m^{-2}), air temperature (T_A, °C), air humidity (H, %) and cumulative precipitation (P, mm) were obtained from a meteorological station located in Pori, 20 km from the site. T_A and H were then used to calculate air vapour pressure deficit (VPD, kPa).

2.6. Data analysis

The analyses were performed with the statistical software R 2.14.1 (R Development Core Team, 2011). Mixed-effect models were used to estimate the fixed effects of richness level and species on maximum sap flux density (F_{Dmax}), with "time" as a repeated measure and 'tree' as a random effect. Mixed-effect models were also used to estimate the fixed effects of richness level and species on $\delta^{13}C$, $\delta^{18}O$, C(%) and N(%), with 'tree' as a random effect. Non

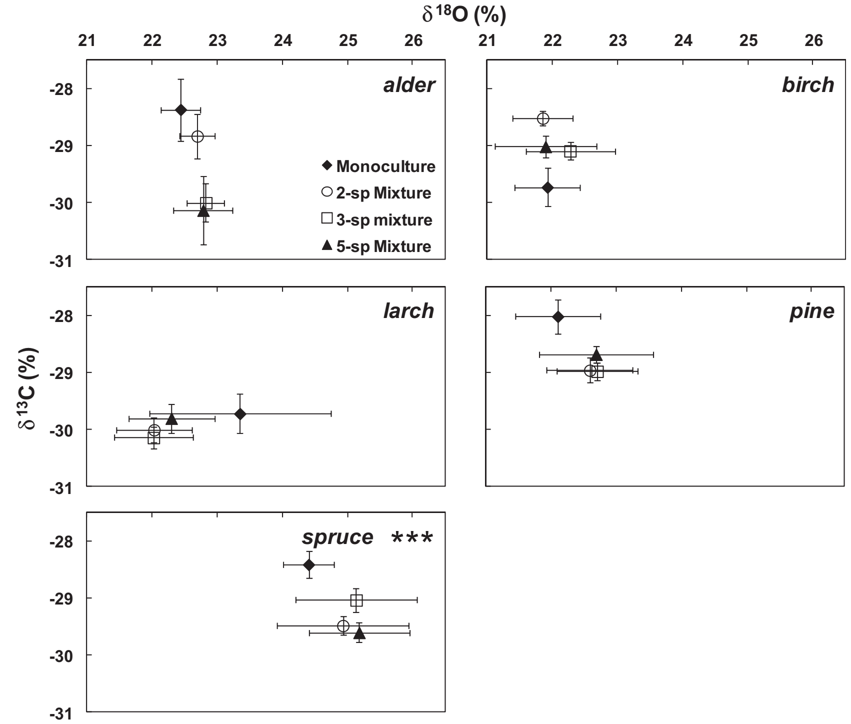

Fig. 6. Relationship between mean leaf or needle carbon and oxygen isotope composition ($\delta^{13}C$ and $\delta^{18}O$, ‰) for alder, birch, larch, pine and spruce in monocultures, two-species mixtures, three-species mixtures and five-species mixtures. Bars are standard errors of the mean for 10 sampled trees. Asterisks denote significant linear relationships between $\delta^{13}C$ and $\delta^{18}O$ (type II regression; $^{***}P < 0.001$).

parametric tests (Wilcoxon) were used to assess the effect of richness level on ABG_i for each species. Contrasts were used to test relevant differences among the different richness levels for a given species or among the different species for a given richness level. Orthogonal regressions were used to test the relationships between individual tree values of δ^{13}C and δ^{18}O.

We followed the approach proposed by Loreau and Hector (2001) to estimate the expected plot transpiration (T_{EP}) in all mixture treatments:

$$T_{EP} = \sum_{i=1}^{N} (\overline{F_{Dim}} \times SA_i) \tag{4}$$

where N is the number of species in the plot, $\overline{F_{Dim}}$ is the mean sap flux density of species i in a monoculture and SA_i is the sapwood area of species i in a mixture.

General linear models were used to test the effect of measurement period on F_{Dmax}, daily mean θ_S and daily sum of R_G, or to test the impact of the daily sum of R_G and the daily mean of VPD on F_{Dmax} or T_P, then to test the effect of daily mean θ_S on the residues from these previous tests.

In order to calculate the net biodiversity effect (NE), the complementarity effect (CE), and the selection effect (SE) (*sensu* Loreau and Hector, 2001) of $\delta^{13}C_P$ and AGB_P, we used the additive partition equation proposed by Loreau and Hector (2001). Following this method, NE is calculated as:

$$NE = CE + SE = N \times \overline{\Delta RY} \times \overline{Y_M} + N \times cov(\Delta RY, Y_M), \tag{5}$$

where N is the number of species in mixture, ΔRY is the deviation from the expected relative value of $\delta^{13}C_P$ or AGB_P, Y_M is the value of $\delta^{13}C_P$ or AGB_P in the monoculture, the horizontal bars above a given parameter denote the mean of this parameter, and 'cov' corresponds to the covariance of the two variables in the parentheses. ΔRY represents the difference between the observed relative value of a given parameter in the mixture and the expected relative value of the parameter based on species monoculture values. We used one-way ANOVA and post hoc Tukey tests to check for differences in $\delta^{13}C_P$ and AGB_P and in NE, CE and SE among richness levels. T-tests were used for all three biodiversity effects to check whether these parameters differ from 0 for a given richness level.

3. Results

3.1. Above-ground biomass at species and plot level

Mean above-ground biomass per unit of ground surface area (AGB_i) ranged from 0.1 kg m^{-2} for alder in the mixture with birch and pine to 10.9 kg m^{-2} for birch in the mixture with alder (Fig. 2). For a given species, no significant impact of species richness on AGB_i was found ($P > 0.050$).

Mean plot above-ground biomass per unit of ground surface area (AGB_P) varied from 0.3 kg m^{-2} in the alder monoculture to 6.9 kg m^{-2} in the pine/birch mixture (Fig. 3). There were no significant differences in AGB_P among richness levels ($P = 0.253$) (Fig. 3).

3.2. Sap flux density and plot transpiration

Sap flux density measurements were conducted both during very clear, sunny days and during rainy days (Fig. 1a and b). The daily mean θ_S remained high during both measurement periods; it was, however, generally lower during the second period ($P < 0.001$). The daily sum of R_G did not change significantly between the first period and the second ($P = 0.900$), even though we observed a seasonal decrease in day length (19 h and 17 h on average for periods 1 and 2, respectively).

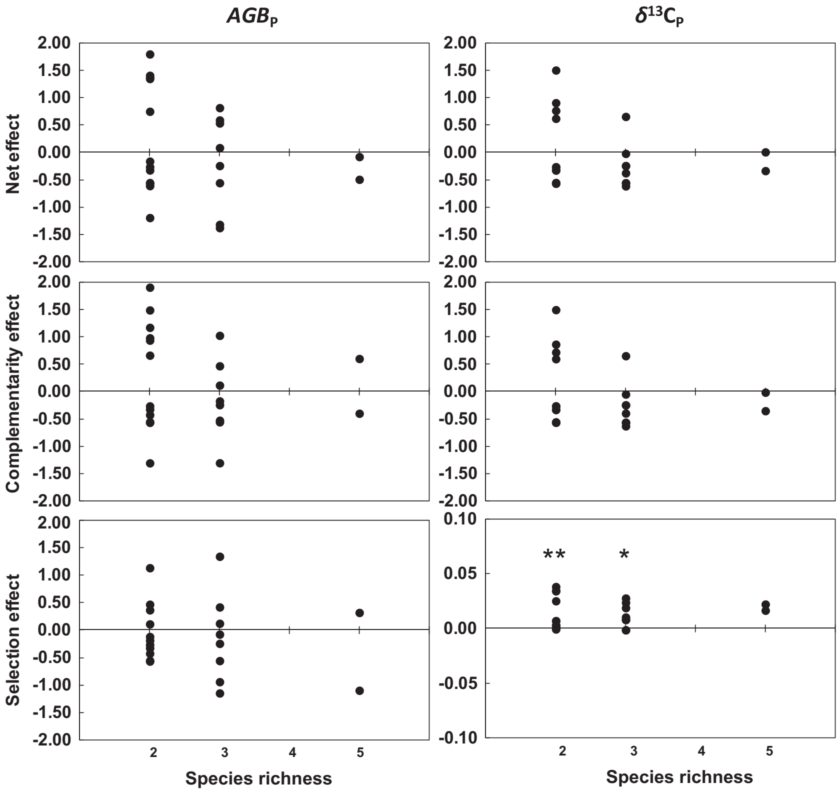

Fig. 7. Net, complementarity and selection effect on plot total above-ground biomass per unit of ground surface area (AGB_P, kg m^{-2}) and plot carbon isotope composition ($\delta^{13}C_P$, ‰) for each species richness level. Asterisks denote significant differences from zero for a given richness level (t-test, $^{*}P < 0.05$; $^{**}P < 0.01$).

Daily variations in F_D followed the daily time course of R_G and *VPD* (Fig. 1). In the monocultures, daily maximum sap flux density (F_{Dmax}) was significantly higher for birch than for spruce and pine ($P < 0.001$), with no significant differences between the two coniferous species (Fig. 4). No richness level effect was found for birch or pine ($P > 0.050$), while spruce showed significantly higher values in the monoculture than in the species-rich plots ($P < 0.001$), with no significant difference between two- or three-species mixtures ($P > 0.050$) (Fig. 4). Significantly lower F_{Dmax} values were observed for all species in the second measurement period compared to the first period, independently of the mixture ($P < 0.001$) (Fig. 4). This change can partly be explained by variations in the daily cumulative R_G and in the daily mean *VPD* ($P < 0.001$). Furthermore, the residues from these relationships were not significantly affected by changes in mean daily θ_S ($P = 0.787$).

Plot transpiration (T_P) differed among plots and was highly dependent on the species present (Fig. 5). T_P varied from 0.21 mm day^{-1} in the spruce monoculture to 2.22 mm day^{-1} in the birch monoculture during the day with the highest sum of R_G (July 7, 2011). T_P was always highest in the mixtures with birch. Expected plot transpiration (T_{EP}) was lower than the observed transpiration in all mixtures except for the pine/spruce mixture for which expected and observed transpiration levels were similar (Fig. 5). Plots containing birch always had the greatest differences between observed and expected values.

While a significant effect of the daily cumulative R_G on T_P was found for all plots ($P < 0.001$), no effect of daily mean *VPD* or θ_S was found on T_P.

3.3. Carbon and oxygen isotope composition at species and plot level

Mixed models revealed significant differences in δ^{13}C among species ($P < 0.001$) and richness levels ($P = 0.003$). In the monocultures, δ^{13}C was significantly different among species ($P = 0.004$), ranging from −29.7‰ for birch and larch to −28.0‰ for pine (Fig. 6, Appendix). Birch had significantly higher δ^{13}C in the mixed plots than in the monoculture ($P = 0.002$). In contrast, pine and spruce had significantly higher δ^{13}C in the monocultures than in any mixed plots ($P = 0.040$ and $P < 0.001$, respectively). Alder showed significantly higher δ^{13}C values in the monoculture than in the three- and five-species mixtures ($P = 0.040$). Finally, we did not observe any mixture effect on δ^{13}C for larch.

Mixed models revealed significant differences in δ^{18}O among species ($P < 0.001$) but no differences among richness levels ($P = 0.56$). In the monocultures, δ^{18}O was significantly different among species ($P < 0.001$), ranging from 22.0‰ for birch to 24.4‰ for spruce (Fig. 6, Appendix). Spruce had significantly higher values than all the other species ($P < 0.001$); alder, birch and pine had significantly lower values than larch ($P < 0.001$). There was no significant effect of richness level on δ^{18}O except for larch for which the values were higher in the monoculture than in the mixtures ($P < 0.001$) (Fig. 6).

The relationship between δ^{13}C and δ^{18}O was significantly negative for spruce ($P < 0.001$) but not significant for the four other species ($P > 0.050$) (Fig. 6). Based on the outputs of the Scheidegger model (2000), the decrease in δ^{13}C with increasing species mixture for alder, pine and spruce corresponded to a decrease in photosynthetic assimilation. For birch, the outputs from this model indicated that the increase in δ^{13}C corresponded to an increase in photosynthetic assimilation with increasing species mixture. Finally for larch, the outputs from this model indicated both a change in photosynthetic assimilation and stomatal conductance.

Plot-level carbon isotope composition ($\delta^{13}C_P$) varied from −28.2‰ to −29.6‰ (Fig. 3) and no significant differences among richness levels were found ($P = 0.550$).

3.4. Carbon and nitrogen content

In the monocultures, C% and N% content was significantly different among species ($P = 0.007$ and $P < 0.001$, respectively) (Appendix). Larch displayed significantly lower C% than the four other species, which were not significantly different among each other. Birch and alder had significantly higher N% values than the other species; pine and spruce had significantly lower N% values than larch ($P < 0.001$).

No impact of richness level was found for C%, except for spruce which displayed significantly higher values in the monoculture than in the mixed plots ($P < 0.001$). The impact of species richness level on N% was significant for larch ($P = 0.008$) and spruce ($P = 0.004$), but not significant for the other species ($P > 0.050$). While N% was significantly lower in the monoculture than in the mixed plots for larch, the reverse was observed for spruce (Appendix B).

3.5. Biodiversity effects

We found a large variability among plots in *NE*, *CE* and *SE* for AGB_P and $\delta^{13}C_P$ (Fig. 7). For AGB_P, *NE* ranged from −1.4 kg m^{-2} for the birch/larch/pine mixture to +1.8 kg m^{-2} for the alder/birch mixture (Fig. 7). For $\delta^{13}C_P$, *NE* ranged from −0.6‰ for the birch/spruce/pine mixture to +1.5‰ for the alder/birch mixture (Fig. 7). *CE* values of AGB_P and $\delta^{13}C_P$ were in the same range as those for *NE*. *SE* values were in the same range as those for *NE* for AGB_P, whereas those for $\delta^{13}C_P$ were much lower than the ones for *NE* or *CE* (Fig. 7).

Generally speaking, the three biodiversity effects decreased with increasing species richness levels for $\delta^{13}C_P$ and AGB_P, even though we did not observe any significant effect of richness level for the three parameters ($P > 0.050$). When testing whether each effect for each species richness level differed from zero, we found no statistical difference for AGB_P for any richness level ($P > 0.050$). For $\delta^{13}C_P$, a significant positive selection effect for the two- ($P = 0.010$) and three-species richness levels was found ($P = 0.020$), whereas there was no statistical difference from zero for *NE* and *CE* for any richness level ($P > 0.050$) (Fig. 7).

4. Discussion

4.1. Species mixture effect at the tree level

An unexpected finding was the absence of a strong effect of richness level on species-specific AGB_i for all species and on F_D for birch and pine. This suggests that intra- and inter-specific interactions in these plots did not strongly differ (Figs. 2 and 4). Actually, only F_D of spruce was lower in the mixtures than in the monoculture, pointing to positive inter-specific interactions for transpiration for this species. However, leaf-level physiological adjustments clearly occurred in the different richness levels for most species: for three species (alder, pine and spruce), a general tendency toward decreasing leaf δ^{13}C (and thus WUE) with increasing levels of species richness appeared, while the reverse pattern was observed for birch and there was no significant change for larch (Fig. 6). The influence of richness level on species WUE has already been observed. As an example, in a mixed plantation, Forrester et al. (2010) found higher WUE for eucalypt trees whereas it was lower for acacia trees. These differences clearly point to distinct responses among species in the trade-off between CO_2 assimilation and transpiration at leaf level in different mixture conditions.

Differences in foliar δ^{18}O are mainly driven by differences in stomatal conductance and δ^{18}O of source water (Farquhar et al., 1998). We considered that δ^{18}O of source water was equal for all species in the studied plots as (i) the water table remained near the soil surface throughout the year (Jussila, pers. comm.), (ii) measured soil water content was high in all plots (Fig. 1), and (iii) water from different soil horizons was well mixed with rain water. Thus, differences in foliar δ^{18}O among richness levels were mainly driven by stomatal conductance in our study. We applied the dual δ^{13}C and δ^{18}O approach (Scheidegger et al., 2000) to understand whether contrasted leaf gas exchange patterns could explain differences in δ^{13}C and WUE among richness levels. Although care must be taken when interpreting the relationships of this dual isotope approach (Roden and Farquhar, 2012), we concluded that changes in δ^{13}C were mainly due to a decrease in photosynthetic activity for alder, pine and spruce and an increase in photosynthetic activity for birch. Furthermore, N% is positively correlated with photosynthetic capacity (Evans, 1989) and while there was no significant difference in N% for alder, birch and pine among richness levels, the decrease in N% for spruce was consistent with the interpretation of the dual isotope approach. Our results thus point to strongly differing leaf physiological responses among species to richness levels that reflect the response of these species to below- and/or above-ground interspecific interactions.

4.2. Species mixture effect and biodiversity effects at the plot level

Ecosystem functional parameters (AGB_P, T_P, $\delta^{13}C_P$) and biodiversity effects (*NE*, *CE*, *SE*) displayed a large variability within and among richness levels (Figs. 3, 5 and 7), showing that interactions among species clearly affected plot-level carbon accumulation and water use. Nevertheless, we did not detect any clear statistical trends in the relationship between these functions and richness levels (Figs. 3 and 7). The lack of a clear trend for AGB_P was consistent with the absence of an effect of richness levels on species AGB_i; nevertheless, this result contrasts with most studies published to date which show a positive response of AGB_P to richness levels (e.g. Morin et al., 2011; Nguyen et al., 2012; Zhang et al., 2012). The main reason for this discrepancy could be the young age of the studied plantation and consequently the potential weak below-ground interactions among trees at this ontogenic stage. However, we evaluated the effect of species mixture on above-ground biomass but did not consider its effect on the below-ground components. A study conducted in a young mixed temperate plantation showed that fine root production increased with tree species diversity (Lei et al., 2012) and those effects might have also occurred in the plantation studied here. For $\delta^{13}C_P$, the lack of a general trend was explained by the opposite response patterns of species δ^{13}C to richness levels, as previously discussed (Fig. 6).

Disentangling the net biodiversity effect for AGB_P and $\delta^{13}C_P$ into their additive components did not reveal any significant complementarity effect (Fig. 7). This indicated no facilitation or niche differentiation mechanisms for wood production, water acquisition and water use efficiency among species. This pattern contrasts with the two studies published to date for mixed plantations on the impact of tree species mixture on plot level water use efficiency and productivity (Forrester et al., 2010; Kunert et al., 2012). In these studies, a complementarity effect explained the observed increase in plot level water use efficiency and woody biomass production with species richness. The discrepancy between our observations and the two previous studies could be explained not only by plantation age, but also by differences in environmental conditions. Indeed, soil water depletion or nutrient stress was the main argument given by Forrester et al. (2010) and Kunert et al. (2012) to explain the observed complementarity effects, while no such limiting conditions occurred at our site. The absence of a positive complementarity effect in our study would thus be consistent with the conceptual framework of the "stress-gradient hypothesis" (Malkinson and Tielbörger, 2010) which states that the more stressful the environmental conditions, the higher the chance that facilitative mechanisms among species may occur and drive ecosystem functioning.

A positive selection effect over $\delta^{13}C_P$ was found (Fig. 7) which indicates the dominance of one species characterized by higher WUE in mixed plots. In the study plantation, we suggest that the selection effect was driven by birch trees: indeed, the basal area of this species was always among the highest in the mixed plots and an increase in δ^{13}C from the monoculture to the mixed plots was only observed for birch trees (Fig. 6). However, it is worth mentioning that the selection effect related to birch was not strong enough to induce a clear positive relationship between $\delta^{13}C_P$ and richness level (Fig. 3).

Even though the small number of plot repetitions did not allow us to make statistical tests, observed plot transpirations were similar to or slightly higher than expected values. The lack of a significant mixture effect on AGB_i, and thus on sapwood area, suggested that differences in sap flux density were driving the differences between expected and observed T_P values. However, not all species showed an increase in F_D with mixture levels (Fig. 5). We therefore suggest that the strong contribution of birch to basal area in mixed plots and the small differences in F_D for birch (though non-significant) are the main origins of these differences. This again suggests that birch was the keystone species in this mixed plantation.

4.3. Origin of the differences in tree- or plot-level functioning

Differing physiological response patterns among species to richness levels could be explained by distinct local micro-environmental conditions in the mixtures. As the studied species are characterized by different growth rates, dynamics and development characteristics, the canopy structure of this young plantation may vary according to species composition and interactions, and this in turn could directly affect micro-environmental conditions in mixed plots (Kelty, 2006). Varying environmental conditions in this boreal plantation could include lateral shading by fast growing species onto slower growing ones, vertical or horizontal gradients in air temperature, air humidity, CO_2 concentrations, or δ^{13}C of ambient CO_2, as well as differences in soil water and nutrient content. We were not able to precisely measure those micro-environmental conditions in the studied plots, but nevertheless discuss below their potential influence on tree- or plot-level functioning.

As water content remained high during the measurement period (Fig. 1), observed lower F_D values for spruce in mixtures as compared to monocultures – true also to a lesser extent for pine – could not be explained by soil water limiting conditions. We thus suspect that the lower F_D might be due to a large proportion of their crown being subjected to lower radiation, lower temperatures and vapour pressure deficit, induced by competing species that had modified the micro-environmental conditions (Bréda et al., 2006). Indeed, birch is a fast-growing species with high primary growth rates at early stages and in our plantation this species clearly had the highest AGB_i values and was taller than the other species in the mixed plots, thus inducing lateral shading for the competing species.

With regards to the differences in δ^{13}C among richness levels, both CO_2 assimilation and stomatal conductance are dependent on micro-environmental conditions (e.g. Dawson et al., 2002). To avoid any effect of CO_2 concentrations and δ^{13}C in the ambient CO_2 within the canopy, we sampled all leaves and needles from the top of the crown of each tree; we therefore consider these two confounding factors to be minor (Buchmann et al., 2002). Lower radiation, lower temperatures and vapour pressure deficit resulting from lateral shading due to the dominance of one species in the canopy (here birch) could, however, reduce photosynthesis and result in more negative foliar δ^{13}C values for the other species in the mixture. This was exactly the response we observed for spruce, pine and alder (Fig. 6) and this confirms the key role birch plays in this plantation on species interaction and plot functioning.

In conclusion, this study reveals that in a young boreal plantation with highly favourable soil water conditions throughout the growing season, plot level above-ground biomass, transpiration and water use efficiency were affected by species mixture and associated above-ground interactions. However, we demonstrated that species response to these interactions was too variable to deduce a general relationship between richness level and plot-level carbon accumulation and water use. This means that mixing locally-adapted species in boreal plantations provides no clear benefit in terms of wood production and water use at such a young ontogenic stage. No positive net biodiversity effect for these ecosystem-level functions is likely for such young plantations. Our results suggest that when a highly performing species at the young development stage such as birch is planted with other species that grow slower, the resulting changes in canopy structure may induce changes in species- and plot-level functioning. These changes involve gradients in light or in air temperature and humidity. Our study, however, does not preclude the usefulness of young mixed-species plantations in terms of provisioning (genetic resources), regulating (resistance to pathogens and herbivory) or supporting (nutrient cycle) services. Furthermore, this work was conducted in a 12-year-old plantation where interactions between species and canopy structure will continue to change considerably over time. The selection effect we observed caused by one dominating species might be dampened in the future when competition for soil resources becomes greater; furthermore, complementarity effects for carbon and water acquisition and use may very well arise at different ontogenic stages.

Table A1
Plot basal area ($m^2\ ha^{-1}$) and individual species contribution to plot basal area (%). Lines written in bold characters correspond to the plots studied for sap flux measurements.

		Basal area ($m^2\ ha^{-1}$)	Species contribution to basal area (%)				
Richness	Plot	Total	Alder	Birch	Larch	Pine	Spruce
1	4	1.7	100				
1	17	24.2				100	
1	**20**	**16.1**				**100**	
1	**25**	**15.1**		**100**			
1	30	23.6			100		
1	**34**	**3.1**					**100**
2	1	18.0			34	66	
2	8	19.8		65		35	
2	23	6.2	42				58
2	29	11.9			56		44
2	**31**	**15.4**				**83**	**17**
2	**32**	**13.6**		**80**			**20**
2	36	14.0	5	95			
2	**37**	**14**		**76**		**24**	
3	6	10.5			35	38	27
3	15	14.4	1	53		46	
3	21	17.7		34	22	44	
3	**27**	**11.3**		**43**		**50**	**7**
3	33	13.7	5	60	35		
3	35	8.6	6		53		41
5	18	11.3	7	36	12	37	8
5	22	12.2	2	36	27	29	6

Table A2
Mean leaf or needle values of carbon isotope composition (δ^{13}C, ‰), oxygen isotope composition (δ^{18}O, ‰), carbon content (C%, %), nitrogen content (N%, %) and mean above-ground biomass per unit of ground surface area (AGB_i, $kg\ m^{-2}$), with standard errors of the mean, for alder, birch, larch, pine and spruce and for each mixture.

Species	Mixture	δ^{13}C (‰)	δ^{18}O (‰)	C% (%)	N% (%)	AGB_i ($kg\ m^{-2}$)
Alder	Monoculture	−28.4 ± 0.5	22.4 ± 0.3	48.6 ± 0.5	2.5 ± 0.2	0.3 ± 0.0
	2-Species	−28.8 ± 0.4	22.7 ± 0.3	48.4 ± 0.2	3.2 ± 0.2	0.6 ± 0.3
	3-Species	−30.0 ± 0.3fcopy	22.9 ± 0.3	47.9 ± 0.3	3.1 ± 0.2	0.2 ± 0.1
	5-Species	−30.1 ± 0.6	22.8 ± 0.5	47.6 ± 0.1	3.3 ± 0.2	0.4 ± 0.2
Birch	Monoculture	−29.7 ± 0.3	22.0 ± 0.2	49.4 ± 0.1	2.7 ± 0.1	6.4 ± 0.6
	2-Species	−28.5 ± 0.1	21.9 ± 0.1	49.6 ± 0.1	2.6 ± 0.1	9.4 ± 0.9
	3-Species	−29.1 ± 0.2	22.2 ± 0.1	49.3 ± 0.1	2.4 ± 0.1	8.3 ± 0.7
	5-Species	−29.0 ± 0.2	21.9 ± 0.2	49.0 ± 0.2	2.3 ± 0.1	8.1 ± 0.8
Larch	Monoculture	−29.7 ± 0.3	23.3 ± 0.6	47.8 ± 0.4	2.0 ± 0.1	6.5 ± 0.3
	2-Species	−30.0 ± 0.2	22.0 ± 0.2	48.0 ± 0.2	1.9 ± 0.1	3.5 ± 1.5
	3-Species	−30.1 ± 0.2	22.0 ± 0.1	48.3 ± 0.2	2.1 ± 0.1	3.4 ± 0.9
	5-Species	−29.8 ± 0.2	22.2 ± 0.2	48.3 ± 0.2	2.4 ± 0.1	2.9 ± 1.6
Pine	Monoculture	−28.0 ± 0.3	22.0 ± 0.2	48.7 ± 0.2	1.8 ± 0.1	5.1 ± 1.3
	2-Species	−28.9 ± 0.2	22.6 ± 0.1	48.5 ± 0.2	1.8 ± 0.1	4.5 ± 1.5
	3-Species	−29.0 ± 0.1	22.7 ± 0.1	48.6 ± 0.1	1.9 ± 0.1	5.1 ± 0.9
	5-Species	−28.7 ± 0.1	22.7 ± 0.3	48.6 ± 0.3	2.0 ± 0.1	4.9 ± 0.6
Spruce	Monoculture	−28.4 ± 0.2	24.4 ± 0.1	49.3 ± 0.1	1.5 ± 0.1	1.2 ± 0.6
	2-Species	−29.4 ± 0.2	24.9 ± 0.2	47.9 ± 0.1	1.7 ± 0.1	1.9 ± 0.3
	3-Species	−29.0 ± 0.2	25.1 ± 0.3	48.2 ± 0.1	1.7 ± 0.1	1.9 ± 0.6
	5-Species	−29.6 ± 0.1	25.1 ± 0.2	47.7 ± 0.2	1.8 ± 0.1	1.3 ± 0.1

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Appendix A.

Table A1.

Appendix B.

Table A2.

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Paper IV

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Interspecific competition influences the response of oak transpiration to increasing drought stress in a mixed Mediterranean forest

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ABSTRACT

Increasingly severe droughts are expected to negatively impact forest functioning in the future, especially in the Mediterranean region. Favoring mixed species stands has been advocated as a compromise between wood production and biodiversity conservation, but whether such management practices would allow forest ecosystems to acclimate to future climate conditions remains to be addressed. We tested whether the transpiration of *Quercus cerris* (Qc) and *Quercus petraea* (Qp) during droughts differ when they grow in pure or mixed forests. We measured sap flux density (F_D) and leaf carbon isotope composition (δ^{13}C), as a proxy for intrinsic water use efficiency (WUE_{int}), in pure and mixed Qc and/or Qp forest plots in Italy during the summer 2012. Qc and Qp showed a drop in F_D of 41% and 52% respectively during the summer drought independently of the mixtures, but with different seasonal trends. Qp transpiration response to soil drought did not differ between pure and mixed plots. In contrast, Qc transpiration was reduced by 56% in mixed plots at the maximum of the drought and only by 31% in the pure plot. Furthermore, we observed higher WUE_{int} for Qc in the mixed plot and no change for Qp, supporting the higher water stress intensity in the mixed plot for Qc. Our study illustrated the negative impact of Qp on Qc when these species compete for water resources. We suggest that managing for mixed stands in the Mediterranean region might, in some cases, increase the detrimental effect of drought on species functioning.

1. Introduction

Both fundamental and applied questions are emerging about potential changes in the functioning of European forest ecosystems in regions subjected to severe climatic changes. Scenarios predict a global increase in the frequency and intensity of atmospheric and soil drought during the growing season throughout Europe (IPCC, 2007) and whether today's forest ecosystems will acclimate and survive in the upcoming decades remains to be seen. The Mediterranean zone will be one of the regions most affected during the next decades, with a considerable reduction in rainfall during the growing season accompanied by more frequent and more intense heat-waves (Giorgi, 2006; Somot et al., 2008). Ecological stability and productivity of Mediterranean forests is particularly sensitive to climatic conditions (Scarascia-Mugnozza et al., 2000). In this context, forest management practices adapted to future climatic change must be implemented (Fabbio et al., 2003). Specifically, these practices should be able to counteract the likely decrease in productivity and increase of catastrophic events such as fires and pests outbreaks so that acceptable levels of goods and services can continue to be provided.

Mixed forests have been advocated as a potential compromise between high wood production and biodiversity conservation (Kelty, 2006) and seem to be an interesting management option to help forest ecosystems acclimate to future climatic conditions. The two mechanisms underlying the positive effects of biodiversity on ecosystem services in mixed forests are: complementarity of resource use arising from facilitation and/or niche stratification

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Abbreviations: F_D, sap flux density (L dm^{-2} h^{-1}); $F_{D\ Mean}$, mean daily sap flux density (L dm^{-2} h^{-1}); $F_{D\ Mean\text{-}S}$, mean daily sap flux density standardized by potential evapotranspiration (L dm^{-2} h^{-1} mm^{-1}); *PET*, daily potential evapotranspiration (mm); Qc, *Quercus cerris* L.; Qp, *Quercus petraea* (Matt) Liebl; *REW*, relative extractable water (unitless); *VPD*, vapor pressure deficit (kPa); WUE_{int}, intrinsic water use efficiency (μmol^{-1} mol^{-1}); δ^{13}C, leaf carbon isotope composition (‰).

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between species with different functional traits, and selection effects explained by a higher probability of including one or several highly productive species in more diverse stands (Loreau and Hector, 2001). Positive effects of biodiversity on productivity in mixed Mediterranean forests have already been demonstrated (i.e. Vilà et al., 2007); however, species interactions in mixed forests may also strongly influence other ecosystem functions, particularly related to water use under soil water deficit conditions, though studies to date are lacking for these ecosystems.

The influence of tree species diversity on ecosystem transpiration has already been observed (Forrester et al., 2010; Gebauer et al., 2012; Grossiord et al., 2013; Kunert et al., 2012). In tropical and temperate tree plantations, higher water use seems to be triggered by a complementary behavior between the interacting species through differential canopy and/or root stratification in mixed and pure stands (Forrester et al., 2010; Kunert et al., 2012). Conversely, in a mature temperate forest and a boreal tree plantation, higher transpiration of mixed-species stands was due to the presence of a species with particularly high water use per canopy projection area (Gebauer et al., 2012; Grossiord et al., 2013). Including species with high transpiration rates in mixed species stands subjected to periods of reduced precipitation may therefore exhaust soil water reserves earlier during drought as compared to pure stands. In addition, soil water limiting conditions may have negative consequences on species survival, ecosystem productivity and ecosystem resistance to soil drought.

This paper presents a field study on the impact of soil water deficit on the tree-level transpiration of two oak species (*Quercus cerris* L., turkey oak, Qc, and *Quercus petraea* (Matt) Liebl., sessile oak, Qp) growing in pure or mixed conditions in Tuscany in central Italy. We tested whether the transpiration response of these species to severe soil water depletion would differ in pure and mixed plots. We also analyzed whether these differences were related to differing intrinsic water use efficiencies (WUE_{int}). We hypothesized that mixing the two oak species would lead to a better utilization of available resources during a summer drought and that, consequently, transpiration rates would decrease less than in the pure plots. Indeed, these two oak species are known to have different water use strategies (Tognetti et al.,, 1996) and to differ in their rooting systems (Nardini and Tyree, 1999). Turkey oak is considered to be highly opportunistic for water uptake and keeps stomata open during summer drought periods, whereas sessile oak has a more conservative strategy of controlling transpiration earlier during drought.

2. Materials and methods

2.1. Site description

The study was conducted in a thermophilous deciduous forest situated in south-central Tuscany in the natural forest of Alto Merse, Italy (43.08°N, 11.19°E). One pure plot of turkey oak (*Q. cerris* L., Qc), one of sessile oak (*Q. petraea* (Matt) Liebl., Qp) and one mixed plot with both oaks (Qc/Qp) were selected. The 30 m × 30 m plots were no more than 2 km from one another and are included in the exploratory platform of the FP7- FunDivEUROPE project ("Functional Significance of Forest Biodiversity in Europe") (Baeten et al.,, in press). They are situated on Cambisol soils (World Reference Base for Soil Resources, FAO classification) above quartzite and anagenite bedrock and were selected to maximize soil type homogeneity and reduce as much as possible differences in diameter distribution, basal area, tree height, tree density, or canopy structure. The plots were composed of 50- to 60-year-old trees at altitudes ranging from 250 to 450 m a.s.l. The past management of all plots is coppice with standards and the last cut was about 20 years ago. Mean annual rainfall in the region is 950 mm, occurring mainly during the winter, and mean annual temperature is around 13.5 °C. Detailed plot characteristics are presented in Table 1.

2.2. Drought intensity

In order to characterize the drought intensity experienced by the study trees, we estimated the daily relative extractable water in the soil over the whole root zone (*REW*, unitless) in each plot over 2012. *REW* is defined as the ratio between available soil water and maximum extractable water. It varies between 1.0 (field capacity) and 0.0 (permanent wilting point). When *REW* is higher than 0.4, soil water conditions are non-limiting for transpiration of a large range of tree species and stomatal conductance mainly depends on air vapor pressure deficit, irradiance and forest phenology (Granier et al., 1999). When *REW* is below 0.4, the resulting soil water shortage induces a drop in stomatal conductance and thus in leaf gas exchange, causing most tree species to cease radial growth (Granier et al., 1999). In order to simulate *REW* at a daily time-scale, we ran the BILJOU water balance model (Granier et al., 1999) with measurements of daily climate conditions (rainfall, global radiation, air temperature and humidity, wind speed) from a nearby meteorological station in Pentolina (43.12°N, 11.10°E, 414 m a.s.l.), 1 km from the study plots. This generic model calculates the different water fluxes at daily time-scale in the ecosystem: tree transpiration, understory evapotranspiration, rainfall interception and drainage. These fluxes are dependent on plant area index and evaporative demand, i.e. the potential evapotranspiration, calculated using the Penman equation (*PET*, mm). The difference between rainfall and evapotranspiration fluxes refills the soil reservoir, whereas excess of water is drained out. All the functions implemented in the model were calibrated using various field experiments in different forests, climate conditions, and soil types. In order to parameterize the model to our study site, the plant area index (*PAI*, $m^2\ m^{-2}$) of each selected plot was measured in September 2012.We used a LAI 2000 Plant Canopy Analyzer (PCA, LI-COR, Lincoln, NE, USA) to measure *PAI* at five locations in each plot and then calculated plot average (Table 1). Maximum extractable water (i.e. the water holding capacity) in this soil type was assumed to be 140 mm (Badeau V, pers. comm.). Sensitivity tests using lower (100 mm) and higher (160 mm) water holding capacities did not change the seasonal patterns of *REW* (data not shown).

2.3. Tree transpiration

We followed the thermal dissipation method described by Granier (1985) to measure volumetric sap flux density (F_D, $L\ dm^{-2}\ h^{-1}$) in the three selected plots at four periods in the 2012 growing season (P1 = June, P2 = July, P3 = August, and P4 = September). In the pure plots, five trees were equipped with sapflow sensors; in the mixed plot, five trees per species were equipped with sapflow sensors, for a total of 20 trees (Table 2). This number of replicates was considered representative of the response of single tree species to soil drought and it is currently used in the literature on tree-level transpiration research. All sapflow sensors were installed on the North faced side of trees in order reduce the effect of direct sunshine that can be responsible of thermal gradients that would alter measurements. Each sensor was supplied with a power of 0.2 W.

Studied trees were randomly selected among the dominant and co-dominant tree classes (canopy stage) for each species in each plot. These classes were defined according to the diameter of the trees in each plot and an index of light interception (Dawkins index) that was visually assigned for each tree. Together, the dominant and co-dominant classes represented 86%, 93% and 80% of the sapwood area in the Qc, Qp and Qc/Qp plots, respectively. Measurements were restricted to trees of the dominant and

Table 1
Elevation (m), tree density (ha^{-1}), mean tree height (m) and tree diameter at breast height (DBH, m) with standard errors, plant area index (*PAI*, $m^2\ m^{-2}$) with standard errors ($N = 5$), basal and sapwood area ($m^2\ ha^{-1}$), and species' contribution to basal area (%) for each study plot. Qc and Qp stand for turkey and sessile oak, respectively.

Plot	Species combination	Elevation (m)	Tree density (ha^{-1})	Mean tree height (m)	Mean DBH (m)	*PAI* ($m^2\ m^{-2}$)	Basal area ($m^2\ ha^{-1}$)	Sapwood area ($m^2\ ha^{-1}$)	Species contribution to basal area (%)	
									Qc	Qp
1	Qc	254	933	16.4 ± 0.7	0.22 ± 0.01	3.5 ± 0.1	28.0	19.9	100	
2	Qp	421	867	17.5 ± 0.5	0.20 ± 0.01	4.0 ± 0.1	27.7	12.3		100
3	Qc/Qp	436	689	15.1 ± 0.6	0.17 ± 0.01	2.9 ± 0.2	27.2	7.9	45	55

Table 2
Number of trees, mean tree height (m), mean tree diameter at breast height (DBH, m), and sapwood area (cm^2) for trees equipped with sapflow sensors.

Number of species in mixture	Species	*Number of trees*	Mean tree height (m)	Mean DBH (m)	Sapwood area (cm^2)
1	Qc	4	23.9 ± 1.6	0.40 ± 0.03	802 ± 109
1	Qp	5	22.5 ± 0.6	0.30 ± 0.02	210 ± 19
2	Qc	5	20.3 ± 0.2	0.25 ± 0.03	380 ± 66
2	Qp	5	20.2 ± 0.3	0.27 ± 0.02	171 ± 23

co-dominant classes in order to minimize other confounding factors on transpiration such as sun exposure. Because of sensor failure, one tree in the Qc pure plot had to be removed from the analyses (Table 2). Due to technical constraints (power supply system), the sap flux density measurements were restricted to 4 or 5 consecutive days in each period (Fig. 1).

The two 20-mm-long probes of the sapflow sensors (UP-Gmbh, Cottbus, Germany) were installed under the bark and the cambium at around 1.3 m above soil level with a 10-cm vertical spacing between the probes. Data were collected every ten seconds with a CR1000 or a CR800 data logger (Campbell Scientific Inc, Loughborough, Leicestershire, UK) and thirty-minute averages were recorded. F_D was calculated according to Granier (1985):

$$F_D = 4.28\left[\frac{\Delta T(0)}{\Delta T(u)} - 1\right]^{1.23}, \tag{1}$$

where $\Delta T(0)$ is the maximum temperature difference between the two temperature probes during the night and $\Delta T(u)$ is the current temperature difference. Although this equation was not originally validated for Qc and Qp directly, literature shows validation of this method for a large number of species with great physiological differences (e.g. Braun and Schmid, 1999; Mc Culloh et al., 2007; Liu et al., 2010).

Allometric equations to calculate tree sapwood area and thus sapwood thickness were established for both species by extracting wood cores from the North aspects of the stems on 21 and 48 Qc and Qp trees selected right outside the study plots with a Pressler increment borer (Suunto, Finland) in July 2012 (Table 3). Sapflow sensors integrate sap flux density over a 20-mm-long section of sapwood. As only a small proportion of sap flux density beyond 20 mm-depth has been observed in *Quercus* species (Cermak et al., 1992), we did not apply any radial distribution corrections

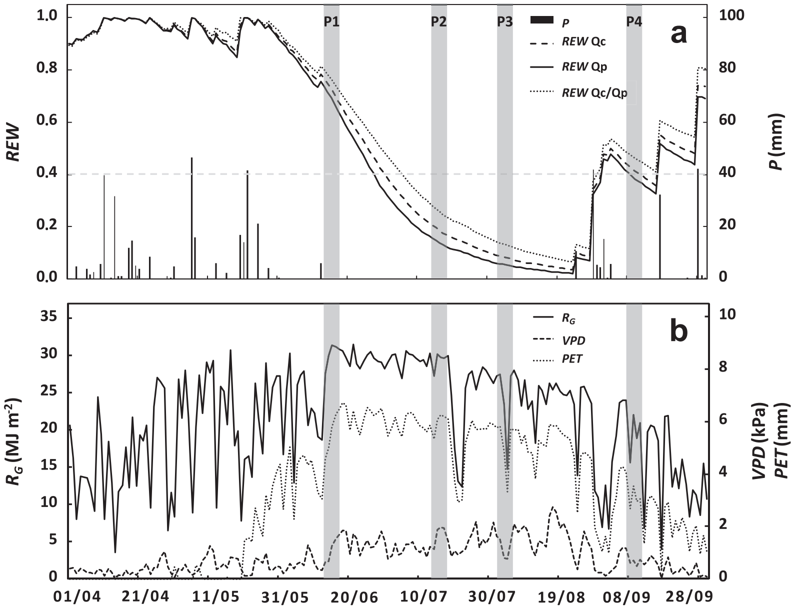

Fig. 1. Seasonal pattern of (a) modelled relative soil extractable water (*REW*) for each plot, daily sum of precipitation (*P*, mm), (b) daily sum of global radiation (R_G, MJ m^{-2}), daily evapotranspiration (*PET*, mm) and daily mean vapor pressure deficit (*VPD*, kPa) during the summer 2012 (01/04/2012 to 31/09/2012). Periods indicated in grey represent the periods of sap flux density measurements (P1, P2, P3 and P4). The dashed grey line stand for the threshold of 0.4 of *REW* under which the resulting soil water shortage induces a drop in stomatal conductance and thus in leaf gas exchange.

Table 3
Number of trees sampled, diameter range of the sampled trees at breast height (m), and allometric equations between sapwood area (SA, m^2) and tree diameter at breast height (DBH, m) with coefficient of determination (R^2) established for turkey and sessile oak (Qc and Qp respectively).

	Sampled trees	DBH range (m)	Equation	R^2
Qc	21	0.15–0.47	$SA = 1.843 \times (DBH^{1.6351})$	0.90
Qp	48	0.16–0.52	$SA = 0.3653 \times (DBH^{1.8483})$	0.94

for F_D. For two Qp trees, the sapwood length was smaller than 20 mm and we applied the correction proposed by Clearwater et al. (1999).

As sapflow sensors signals were stable during nights among the 4 investigated periods, we concluded that there was no substantial occurrence of night-time transpiration. We then calculated mean daily sap flux density (F_{DMean}, L dm^{-2} h^{-1}) and mean daily sap flux density standardized by *PET* ($F_{DMean\text{-}s}$, L dm^{-2} h^{-1} mm^{-1}) for each tree.

2.4. Foliar carbon isotope composition

To estimate leaf intrinsic water use efficiency (WUE_{int}), 20 fully expanded sunlit leaves were sampled once from each of the selected trees by tree climbers at the beginning of July 2012. The samples were oven dried at 60 °C for 48 h at INRA Nancy and then finely ground. About 1.0 mg of the powdered material from each sample was placed into tin capsules for analysis of leaf carbon isotope composition (δ^{13}C, ‰). Analyses were performed by the Technical Platform of Functional Ecology (OC 081) at the INRA Forest Ecology and Ecophysiology Unit with an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany; ±0.2‰). Isotopic measurements are reported in the delta notation (δ, ‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard. δ^{13}C is positively related to WUE_{int} (Farquhar et al., 1982) and provides information on WUE_{int} integrated over the leaf life-span.

2.5. Data analysis

The analyses were performed with the statistical software R 2.14.1 (R Development Core Team, 2011). For the pure plots, mixed linear models were used to determine the fixed effects of *REW*, *PET*, species, and their interactions on F_{DMean}. The day of measurement and tree identity were included in these tests as a repeated measure and a random effect, respectively. Tests were conducted separately for the first three measurement periods and for all four periods together in order to test the impact of the decreasing soil water content (first three periods) separately from the recovering period (fourth period). For all plots, mixed linear models were used (1) to determine the effect of mixture, measurement period, species, and their interactions on $F_{DMean\text{-}s}$, with “days” as a repeated measure and “tree” as a random effect, and (2) to analyze the effect of mixture and species on δ^{13}C values, with “tree” as random effect. Finally, contrasts (Tukey test) were used to test for relevant differences between fixed effects when the overall models were significant.

3. Results

3.1. Model simulation of drought intensity

On June 1st, i.e. just before the first measurement period (P1), soil water content was close to field capacity (Fig. 1). During P1, *REW* was well above 0.4 for all plots, suggesting that transpiration was not yet limited by soil water (Fig. 1). From P1 to P3, *REW* decreased progressively in all three plots (Fig. 1a). During P2, *REW* was already lower than 0.4 in all plots. P3 revealed even lower *REW* values in all three plots; furthermore, *REW* was still decreasing during this period. Over the whole study period, *REW* reached minimum value in all plots on August 23rd, 2012, i.e. between P3 and P4. *REW* was lower than 0.4 for a minimum period of 45 days in the mixed plot and a maximum period of 65 days in the Qc pure plot. Finally, rain events between August 23rd and September 9th 2012 resulted in a sharp increase in *REW*. During the last period (P4), *REW* was higher than 0.4 in all plots. Radiation, *VPD*, and *PET* were high during P1 and P2 and then decreased in P3 and P4 (Fig. 1b).

3.2. Tree transpiration

As the drought intensified during the summer months, F_{DMean} of both species and in all plots progressively decreased to a minimum during P3. Thereafter, F_{DMean} increased from P3 to P4 for both species and in all plots (Fig. 2).

In the pure plots, the mixed-effect models revealed significant species ($P = 0.001$), *PET* and *REW* effects ($P < 0.001$) on F_{DMean} when considering the four periods individually or combined. For all plots and all periods combined, we observed significant species ($P = 0.012$) and measurement period ($P < 0.001$) effects on $F_{DMean\text{-}s}$, but no effect of the mixture ($P = 0.081$). The interactions between mixture and measurement period or species and measurement period were significant ($P < 0.001$) (Fig. 3), whereas the interaction between mixture and species was not ($P = 0.068$). Qc always had significantly higher $F_{DMean\text{-}s}$ values than Qp in all plots.

The Tukey contrast tests revealed that $F_{DMean\text{-}s}$ values did not differ from P1 to P2 in the Qc pure plot (Fig. 3), whereas values in P3 were significantly lower (Fig. 3). Conversely, in the Qp pure plot, $F_{DMean\text{-}s}$ was already lower in P2 than in P1 (Fig. 3), and no further decrease in $F_{DMean\text{-}s}$ was observed between P2 and P3.

After the rain events at the end of the summer (Fig. 1), $F_{DMean\text{-}s}$ values strongly increased during P4 for both Qc and Qp in pure plots. The values reached higher levels than during P1 for Qc and similar levels for Qp (Fig. 3).

The Tukey contrast tests also revealed that the two oak species showed different trends in $F_{DMean\text{-}s}$ values between pure and mixed conditions. For any given period, Qp did not show any significant differences in $F_{DMean\text{-}s}$ values between pure and mixed plots. In contrast, for any given period, significant differences were observed for Qc (Fig. 3). Furthermore, for Qc, the difference in $F_{DMean\text{-}s}$ values between pure and mixed plots increased from P1 to P2, then remained similar during P2 and P3, resulting in very low values in $F_{DMean\text{-}s}$ in the mixed plot in P3 (Fig. 3).

3.3. Foliar carbon isotope composition

For Qc, mean tree leaf δ^{13}C was −26.1‰ (±0.2 ‰) in the mixed plot and −27.2‰ (±0.4‰) in the pure one. For Qp, mean tree leaf δ^{13}C was −27.5‰ (±0.4 ‰) in the mixed plot and −27.4‰ (±0.3‰) in the pure one. Overall, there was no species ($P = 0.063$) nor mixture ($P = 0.051$) effect and no interaction between species and mixture ($P = 0.203$) on δ^{13}C. However, when considering each species separately, a significantly higher δ^{13}C value was observed for Qc in the mixed plot compared to the pure one ($P = 0.038$) (Fig. 4). For Qp, no significant differences were found between plots ($P = 0.991$) (Fig. 4).

The absence of any difference in δ^{13}C between plots for Qp was consistent with the absence of any significant percentage change in $F_{DMean\text{-}s}$ values between the periods P1 and P3, whereas higher δ^{13}C values in the mixed plot for Qc were consistent with a clear reduction in $F_{DMean\text{-}s}$ values (Fig. 4).

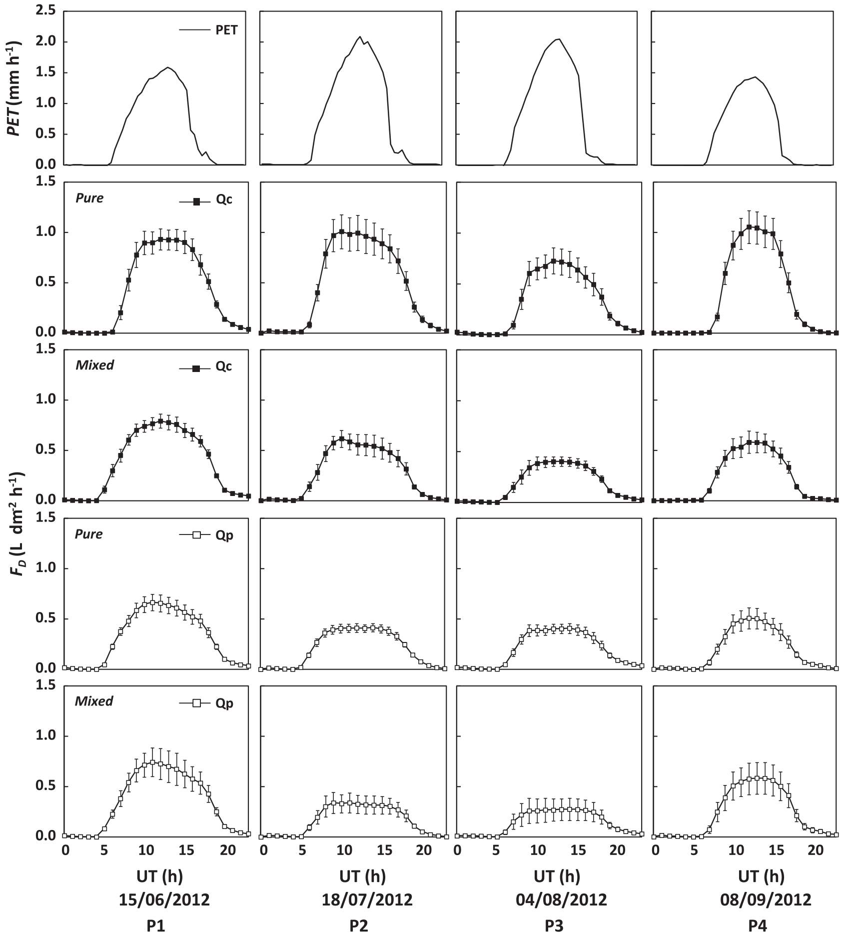

Fig. 2. Daily variations in potential evapotranspiration (*PET*, mm h^{-1}) and mean sap flux density (F_D, L dm^{-2} h^{-1}) of sampled turkey and sessile oak trees (Q$_C$ and Qp respectively) in pure and mixed plot with standard mean errors for four sunny days, during the four measurement periods.

4. Discussion

4.1. Response to drought of the two oak species in pure conditions

Before any soil water deficit occurred (P1), daily sap flux density of Qc was higher than that of Qp in the pure plots (Figs. 2 and 3); this is consistent with previous measurements conducted by Tognetti et al., (1996) on the same species in Italy.

Both oak species displayed a marked decrease in *PET* standardized sap flux density ($F_{D\text{Mean-s}}$) throughout the summer (from P1 to P3) in the pure plots (Figs. 2 and 3), but with different intensities and timing. The decrease in $F_{D\text{Mean-s}}$ for Qc occurred between P2 and P3 whereas it occurred between P1 and P2 for Qp (Figs. 2 and 3). The pattern observed for Qc, a "drought tolerant" species *sensu* Kramer (1983), was consistent with observations by Tognetti et al. (2007) who concluded that this species has a non-conservative water use strategy. Indeed, Qc transpiration regulation occurred late during the dry period. This strategy allows the tree to avoid limiting its carbon assimilation during medium soil water deficit periods, as has previously been observed for other *Quercus* species (Epron and Dreyer, 1993). Conversely, the pattern observed for Qp corresponded to a "water conservative" strategy, which is consistent with previous studies conducted on this species (Bréda et al., 1993). Physiological and morphological mechanisms potentially supporting this strategy include rapid stomatal regulation when soil water deficit increases and developing extensive lateral and deep rooting systems (Kramer, 1983). It is noteworthy that these different water use strategies actually led to a similar relative decrease in transpiration from P1 to P3 for these species in our study (Fig. 4). A differential influence of atmospheric conditions (radiation, *VPD*) on species transpiration is not a likely explanation for this pattern since we were able to show that these conditions impacted transpiration similarly for the two species; furthermore, we used *PET* standardized daily sap flux density means in our calculations. Our results indicate that under similar environmental conditions, contrasted water use strategies may not necessarily confer any clear advantage to a given species in terms of transpiration response to drought.

Nevertheless, the rainy episodes at the end of August (between P3 and P4) were clearly more beneficial in terms of transpiration for Qc than for Qp (Fig. 3); this can be explained by the shallower rooting system in Qc (Nardini and Tyree, 1999) than for Qp. As evaporative demand (*VPD* and *PET*) was very low during P4 in comparison to P1, the lower transpiration rates of Qc during P1could be explained by stricter stomatal control against atmospheric drought at the beginning of the summer than at the end of the drought.

4.2. Impact of species interactions on the response to drought

In this study, we revealed the differential impact of competition for water resources between the two species on their response to soil water deficit. Indeed, while tree transpiration rates for Qp did not differ between the mixed and the pure plot, Qc had much lower transpiration rates in the mixed plot (Fig. 3). Furthermore, Qc response to drought was timed differently between pure and mixed conditions (Fig. 3): $F_{D\text{Mean-s}}$ of Qc decreased much earlier

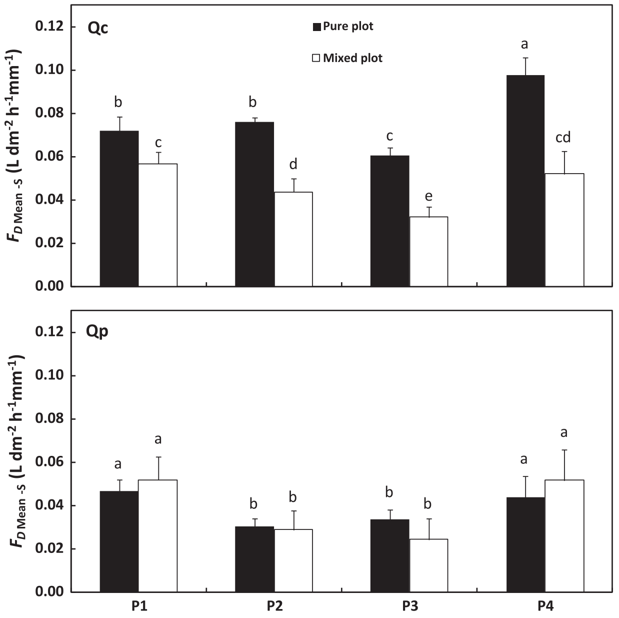

Fig. 3. Mean of the daily mean sap flux densities standardized for potential evapotranspiration ($F_{DMean\text{-}S}$, L dm^{-2} h^{-1} mm^{-1}) for each measurement period and for each study plot for turkey and sessile oak (Q_C and Qp respectively). Letters denote significant differences between plots and periods revealed by contrast tests for each species after mixed effect models had been applied.

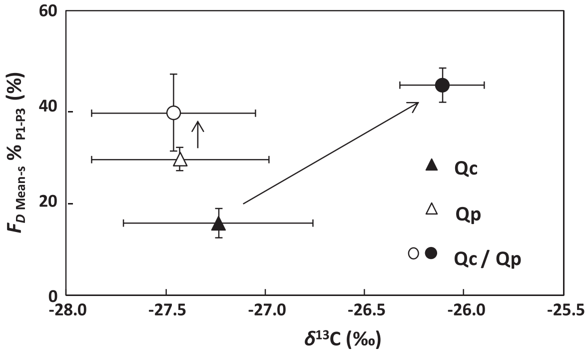

Fig. 4. Mean leaf carbon isotope composition (δ^{13}C, ‰) in July 2012 for turkey (black symbols, Qc) and sessile (white symbols, Qp) oak as a function of the percentage of decrease in mean daily sap flux density between P1 and P3 ($F_{DMean\text{-}s}$ % $_{P1\text{-}P3}$, %) for each plot and each species. Bars are standard errors of the mean. Arrows indicate the change in δ^{13}C and $F_{DMean\text{-}s}$ % $_{P1\text{-}P3}$ from pure to mixed plots for each species.

during the summer in the mixed plot than in the pure plot, even though the decrease in *REW* was slightly lower in the mixture (Fig. 1). We have thus demonstrated a clearly detrimental effect of interspecific competition for water resources on Qc transpiration under soil drought in a Mediterranean forest.

Several morphological and/or physiological adaptations may explain the observed patterns. Sap flow measurements were conducted on dominant and co-dominant trees in each plot so differences in crown exposure to atmospheric conditions can be excluded as a potential cause for the patterns we found for $F_{D\text{-}Mean\text{-}s}$. However, a different vertical distribution of the Qc root system in pure and mixed plots could lead to a different mean depth of water extraction, thus explaining the patterns observed for Qc. The response of stomatal conductance is assumed to be linked to the soil water deficit experienced by the majority of the roots, even if water is being extracted from deeper depths (Irvine et al., 1998). We can therefore assume that the Qc roots are mostly restricted to the upper soil horizons in the mixed plot. We were unable yet to directly evaluate the mean rooting depth of the sampled trees or to estimate it through indirect approaches such as soil water labelling experiments (e.g. Zapater et al., 2011). Contrary to Qc, Qp typically develops a deep root system when competing with other species, thus allowing the tree to maintain high levels of transpiration during soil water deficit periods (Bréda et al., 1993; Leuschner et al., 2001; Zapater et al., 2013). We therefore hypothesize that in mixed plots, where the limiting soil water conditions occur every year, Qp roots consistently outcompeted Qc roots in the deeper and wetter soil horizons, which explained the absence of a negative response in terms of transpiration of Qp although mixed with a higher water consumer such as Qc (Fig. 3). Interspecific competition thus resulted in a negative impact on Qc transpiration as compared to the pure plot situation. For Qp, on the other hand, usable water resources were neither reduced nor improved when the species was mixed with Qc. Our results thus show a negative interaction between the two oak species for soil water use in this Mediterranean ecosystem and, contrary to our initial hypothesis, do not support any complementarity effect in Qc/Qp mixture. This result contrasts with previous studies conducted on tropical or temperate forest ecosystems (Forrester et al., 2010; Kunert et al., 2012). It should be noted that, even though the plots were selected in order to minimize differences in soil characteristics, some minor differences in soil depth, texture and porosity might also partly explain the observed differences in transpiration between pure and mixed plots. Furthermore, differences in transpiration of Qc between the pure and mixed plots might be partly due to differences in tree density and diameter (Table 2). Some studies indeed reported a positive relationship between tree sap flux density and tree size (Mc Jannet et al., 2007; Jung et al., 2011) although some works also found negative relationships (Meinzer et al., 2001). However, those differences would contribute to explain the distinct seasonal patterns we observed for Qc in the pure vs. mixed plot only if they significantly affected their root depth. A clear relationship between tree size and root depth in mature Mediterranean forests has not yet been demonstrated.

4.3. Leaf physiological adjustments in pure vs. mixed conditions

Differences between Qc and Qp in the response of tree-level transpiration to mixture conditions were accompanied by different leaf-level physiological adjustments. Indeed, while no difference in δ^{13}C was found between pure and mixed trees for Qp, higher δ^{13}C values (and thus WUE_{int}) were found for Qc in the mixed plot (Fig. 4). Higher δ^{13}C values for Qc is consistent with the large body of literature showing higher δ^{13}C and WUE_{int} under higher drought conditions (e.g., Galle et al., 2010; Zhang and Marshall, 1994). Enhanced δ^{13}C and WUE_{int} for Qc in the mixed plot was consistent with reduced transpiration and supported our hypothesis of reduced water resource availability in the mixed plot for this species. It should be noted that our δ^{13}C values were obtained on leaves sampled in July 2012, i.e. before any intensive soil water deficit occurred (Fig. 1). As Qc is a deciduous species, these data therefore might reflect a strong, long-term physiological acclimation of Qc trees to the different soil water deficit conditions encountered every year in the different plots. For Qp, the similar δ^{13}C values between plots was also consistent with transpiration data and further indicated that this species neither benefited nor suffered from the interactions with Qc in terms of water use.

In conclusion, under severe soil water deficit conditions, we highlighted an interesting differential response pattern in terms of transpiration and water use efficiency between *Q. cerris* (Qc) and *Q. petraea* (Qp) when they are growing in pure plots or when they are competing for water resources in mixed species conditions. Qp sap flux density was neither negatively affected nor favored by species interactions as would have been expected had complementarity in water resource use occurred. On the contrary, Qc was negatively impacted in its water use when growing in mixed conditions with Qp, an oak species that is known to be able to extract water from deep soil layers. Whether carbon fluxes and productivity of Qc were also negatively influenced still remains to be evaluated. Our results suggest that management practices in the Mediterranean region that favor mixing of Qp and Qc could be detrimental for Qc in terms of water use and survival in the expected context of more frequent and more severe droughts. Nevertheless, whether interactions between these oak species and other broadleaf or coniferous species would be beneficial for them, or at least would have no negative effects, remains to be addressed.

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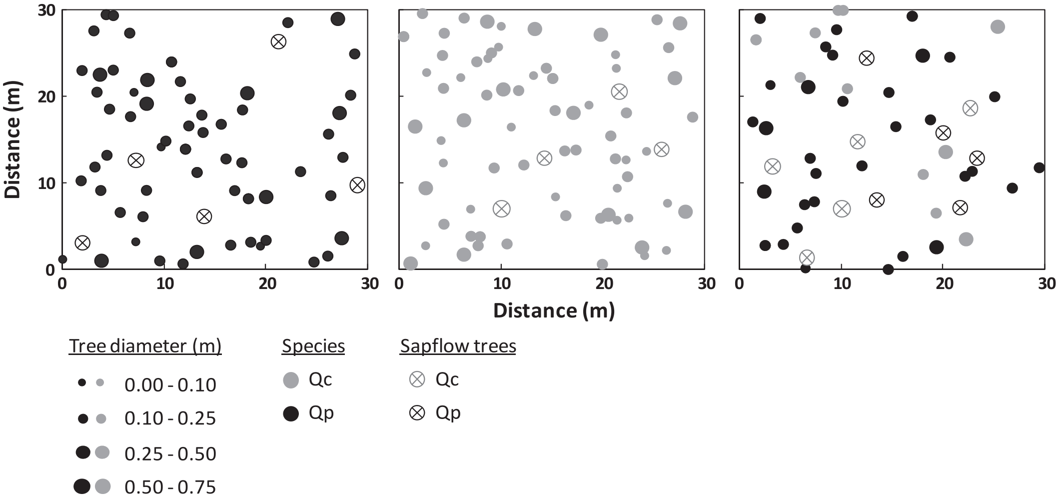
Appendix A

Maps of the three selected plots with tree diameter class distribution (m). Grey circles stand for turkey oak (Qc) and black circles stand for sessile oak (Qp). Circles that contain a cross are selected trees for sap flux density measurements.



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Paper V

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ORIGINAL PAPER

Influence of species interactions on transpiration of Mediterranean tree species during a summer drought

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Abstract Recent research has shown that interactions between species with different functional traits can promote forest ecosystem processes. In the context of climate change, understanding whether species interactions in mixed-species ecosystems can improve the adaptation of these ecosystems to extreme climatic events is crucial to developing new management strategies. In this study, we investigated the impact of species interactions on the sap flux density of three Mediterranean tree species (*Quercus faginea*, *Pinus nigra* and *Pinus sylvestris*) during a summer drought. Measurements of foliar carbon isotopic composition (δ^{13}C) were also conducted on the same trees. The decline in transpiration during drought was the greatest for *P. sylvestris* and the least pronounced for *Q. faginea*. For *P. nigra* and *Q. faginea,* the decrease in transpiration as the drought progressed was lower when these species where interacting with another tree species, particularly with *P. sylvestris*. In contrast, the decrease for *P. sylvestris* was higher when this species was interacting with another species. Differing drought effects were consistent with the δ^{13}C values. We showed that the identity of the species present in the direct neighbourhood of a given tree can differentially influence water availability and water-use of these three co-existing Mediterranean tree species during a summer drought. Our findings suggest that species interactions play an important role in modulating the response of tree species to drought. Favouring tree species diversity in this region does not seem to be systematically beneficial in terms of soil water availability and water-use for all the interacting species.

Keywords Complementarity · Competition · Facilitation · Species interaction · Drought · Transpiration · δ^{13}C

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Introduction

Climate models forecast a decrease in annual mean precipitation in large areas in the northern hemisphere in the future, accompanied by an increase in air temperatures (IPCC 2013). As a result, the evaporative demand over these regions will increase and drought episodes will not only become more frequent, but will also increase in severity and duration. Such expected conditions may lead to gradually increasing tree mortality rates and rapid forest die-off events during the next century (Allen et al. 2010).

Tree species in drought-prone forest ecosystems have a wide range of water-use strategies involving leaf- or plant-level structural and/or physiological adaptations (e.g. Kramer 1983). The major physiological adjustment trees undergo to preserve leaf water status, and thus leaf—and even whole tree—functionality during drought, is related to stomatal aperture. Leaf stomatal conductance is driven by atmospheric water pressure deficit and soil water supply. By closing their stomata during drought, trees reduce leaf transpiration and therefore water-use, but in the mean time, CO_2 uptake and assimilation rates are restricted.

Differences in structural characteristics among tree species contribute to the considerable variability in their responses to water depletion in drought-exposed forests. Among these features, the vertical development of rooting systems plays a large role in tree functional response to drought stress (Burgess 2006). Some species develop a deep-rooting system which allows them to maintain high rates of transpiration by using water from deep soil layers as superficial layers dry out (e.g. Zapater et al. 2013). Other species mainly rely on a superficial rooting system, and they suffer sooner from soil water exhaustion as drought conditions worsen. However, a shallow rooting pattern can also sometimes translate into a competitive advantage since the species can absorb water from summer rainy episodes more efficiently (Xu and Li 2006).

These different strategies have been widely studied, but less is known about how the interactions among species with contrasting water-use strategies during drought stress affect their physiological responses to water depletion in natural forest ecosystems. Since the early nineteenth century, ecologists have been trying to understand how ecosystem processes are influenced by community assembly and species interactions. In many cases, studies have shown that species interactions can be beneficial for ecosystem functions and services involving the forest carbon and water cycles (Pretzsch et al. 2013a, b; Zhang et al. 2012), even though a negative influence of species interactions can also be found (e.g. Grossiord et al. 2014a). Different mechanisms of species interactions have been suggested to explain these positive effects: Facilitation among species whereby one species will benefit from the presence of another species can take place in mixed-species ecosystems and have an overall positive effect on ecosystem functioning (Loreau et al. 2001). Furthermore, complementarity among co-occurring species can lead to a more effective use of available resources and may also be a driver of higher performance in mixed-species ecosystems (Loreau et al. 2001). This mechanism occurs when functional traits enable plant species in the community to exploit resources unavailable to others or to use the same resource at a different place or time. This resource partitioning in mixed-species communities can take place both aboveground and belowground. Indeed, species interactions optimize the aboveground stratification of the canopy and improve overall light interception (Kelty 2006; Pretzsch 2014), resulting in higher gross and net ecosystem carbon uptake rates (Forrester et al. 2010). Inversely, belowground complementarity can impact the accessibility of different water reservoirs and may impact plant and ecosystem water uptake (Schmid and Kazda 2001).

Among various biotic and abiotic factors, positive species interactions (i.e. facilitation and/or complementarity) have been suggested to contribute to the higher transpiration in mixed forests as compared to mono-specific ones (Forrester et al. 2010; Kunert et al. 2012). However, other studies also demonstrated no impact of species diversity on tree- or stand-level transpiration (Gebauer et al. 2012, 2013), or contrasting results according to species identity (Grossiord et al. 2014b). The discrepancy between these results can partially be explained by differences in climatic conditions during measurements periods: Studies were conducted under dry soil conditions tended to show higher transpiration in mixed forests in comparison to mono-specific ones (Forrester et al. 2010; Kunert et al. 2012), whereas the others that were conducted under wet soil conditions showed no effect of species diversity (Gebauer et al. 2012, 2013). Consequently, one could expect that in ecosystems frequently subjected to extreme soil water conditions (i.e. drought), like Mediterranean forests, tree species interactions, are likely to have a positive effect on water availability and/or accessibility. The underlying mechanisms that could drive such an effect could be both facilitation processes such as hydraulic lift (Zapater et al. 2011) and/or complementarity processes such as the differentiation in belowground rooting patterns among species (e.g. Schmid and Kazda 2001) that would lead to a better sharing of the available soil water among tree species.

Here, we assessed tree species identity in the local neighbourhood of three co-existing Mediterranean tree species known to present important differences in functional traits related to drought tolerance (rooting strategy, stomatal regulation under water shortage, phenology…): *Quercus faginea* a drought-adapted, broadleaved, deciduous/semi-evergreen, oak species with a deep-rooting system, *Pinus nigra* a drought-adapted, Mediterranean, evergreen, conifer species and *Pinus sylvestris* an evergreen conifer tree species with a shallow rooting system and a rather sensitive stomatal response to soil drought. We tested whether interactions among these species would influence their transpiration response to a summer drought. We also tested whether species interactions would influence the carbon isotope composition ($\delta^{13}C$) of leaf or needle samples. Indeed, differences in $\delta^{13}C$ of organic material are related to changes in forest soil moisture and/or air humidity (Saurer et al. 2004). We hypothesized that

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(1) community assembly of tree species with differing functional characteristics in this Mediterranean forest ecosystem would affect soil water availability and (2) processes of complementarity and/or facilitation would develop when species compete with non-conspecific neighbours. This would be reflected by a lesser decline in transpiration of target trees and by lower δ^{13}C values of target trees in interaction with non-conspecific neighbours as compared to target trees in interaction with conspecific ones.

Materials and methods

Study site

The study was conducted in a continental Mediterranean mixed forest situated in the Alto Tajo natural park (Guadalajara, Castilla La Mancha, 40.66°N, 02.27°W) in central Spain. A total of ten 30 m-by-30 m plots including monocultures and mixtures of Portuguese oak (*Q. faginea* Lam.), black pine (*Pinus nigra* J.F. Arnold) and Scots pine (*Pinus sylvestris* L.) were selected in the natural park. Three monocultures (one for each species) and seven two-species mixtures were included, with characteristics as described in Table 1. The selected stands are all within 20 km of each other and are included in the exploratory platform of the FP7-FunDiv EUROPE project (www.fundiveurope.eu). Plots were selected to reduce soil type heterogeneity and differences in diameter distribution, basal area, tree height, tree density and canopy structure (Table 1; for more information, see also Baeten et al. 2013). Understory vegetation in the selected plots is mainly composed of shrub species (*Arctostaphylos uva-ursi*, *Buxus sempervirens* and *Genista Scorpius*) and juveniles of the dominant tree species. The ten selected plots are situated on calcic cambisol soils (Food and Agriculture Organization of the United Nations classification) on a cracked limestone bedrock. Soil depth is very shallow in the selected plots and varied between 20 and 40 cm, but roots may extend down to several metres through the cracked bedrock (Peñuelas and Filella 2003). Mean annual rainfall in this region is 499 mm and mean annual temperature is 11 °C. Plot altitudes range from 980 to 1,300 m a.s.l.

Our objective was to characterize two types of species interactions: conspecific or non-conspecific ones. Selected trees of a given species should be in interaction with trees of one of the two other species or with conspecific neighbours (i.e. three neighbourhood levels). To select the trees, we retained in each plot only dominant or co-dominant trees (crown illumination index strictly higher than four, Clark and Clark 1992) with a diameter at breast height of more than 0.10 m that were located at least 5 m from the edge of the plots. We chose dominant or co-dominant trees to avoid any confounding effects caused by differing light acquisition levels. We then characterized species interactions within the immediate neighbourhood around each tree. The neighbourhood of a given tree was defined as all the other trees located within a 5 m radius of its trunk (Fig. 1). This radius was chosen because (1) the majority of the lateral roots of a given tree are supposed to be largely confined within its crown width projection (Schenk and Jackson 2002) and (2) the mean radius of the crown projection of the trees in the selected plots was 1.9 m. The root systems of trees distant by 3.8 m are thus supposed to be competing for resources. We arbitrarily extended this radius to 5 m to ensure other potential interactions.

The size of the interacting trees within an immediate neighbourhood and their distance to the target tree have a strong effect on the water availability of a given tree during a drought event. To take these effects into account, we calculated the distant-dependent Hegyi competition index (CI) (Hegyi 1974) within the 5 m radius of a tree. This

Table 1 Species combination, elevation (m), tree density (ha^{-1}), leaf area index (LAI, m^2 m^{-2}), mean tree diameter at breast height (DBH, cm), and mean tree height (m), with standard errors of the mean (SE), for the 10 plots selected in this study

Plot	Species combination	Elevation (m)	Tree density (ha^{-1})	LAI (m^2 m^{-2})		DBH (cm)		Tree height (m)	
				Mean	SE	Mean	SE	Mean	SE
1	QF	1,187	889	1.6	0.0	11.4	0.9	6.4	0.3
2	PN	980	1,244	1.9	0.3	17.1	1.1	11.2	0.3
3	PS	1,310	789	2.3	0.1	24.4	1.4	12.8	0.3
4	PS–QF	1,224	1,689	1.9	0.1	11.6	0.9	7.4	0.3
5	PS–QF	1,325	644	1.9	0.5	19.2	1.5	8.5	0.4
6	PN–QF	1,010	1,289	1.9	0.4	14.1	1.3	5.1	0.2
7	PN–QF	1,228	422	1.3	0.1	24.0	1.7	10.1	0.6
8	PN–QF	1,207	1,589	1.8	0.1	15.7	2.0	7.3	0.2
9	PN–PS	1,311	778	2.3	0.4	18.9	1.1	10.3	0.3
10	PN–PS	1,304	811	2.6	0.3	23.1	1.7	12.8	0.5

PN, *Pinus nigra*; PS, *Pinus sylvestris*; QF, *Quercus faginea*

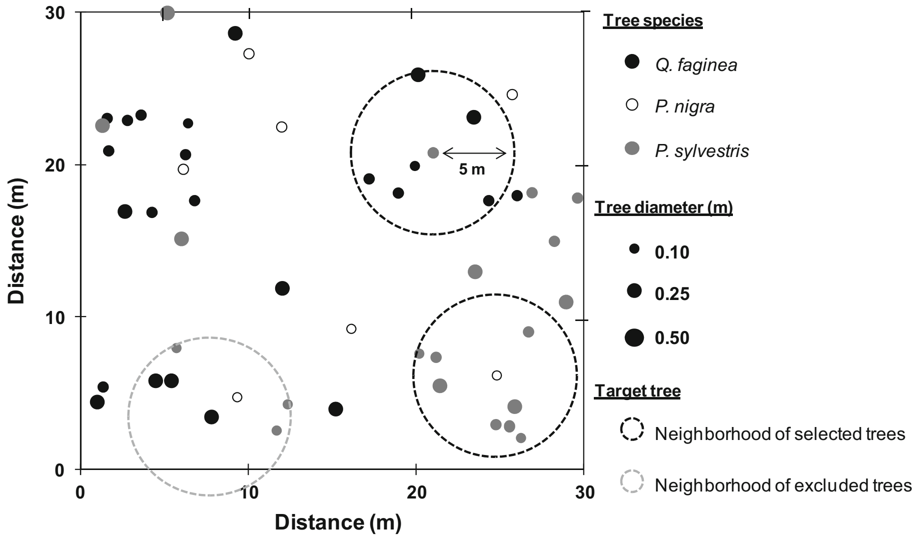

Fig. 1 Schematic representation characterizing the local neighbourhood for selected and excluded trees in a given stand. The local neighbourhood of a given selected tree was used to characterize the identity of the interacting species and to calculate the Hegyi competition index

index uses the ratio of the basal area of the competitor trees and the subject tree weighted by the distance between the subject tree and the competitors. We also characterized the species identity of the trees in the neighbourhood of each tree.

Finally, we randomly selected six trees per species for each of the three species interaction levels (i.e. interaction with conspecific neighbours and with each one of the two other tree species) that covered the full gradient of CI.

Soil water balance

In order to characterize the drought intensity potentially encountered by the selected trees, we calculated the relative extractable water (REW, unitless) in the soil of each plot daily from January 2013 to November 2013 (Fig. 2). REW varies between 1.0 (field capacity) and 0.0 (permanent wilting point). When REW remains between 1.0 and 0.4, tree transpiration depends only on air humidity, irradiance and leaf area index (LAI) (Granier et al. 1999). When REW falls below the threshold of 0.4, water shortage induces a drop in stomatal conductance and leaf gas exchange for most tree species (Granier et al. 1999).

In order to calculate REW, we used the generic model for forest water balance "BILJOU" (Granier et al. 1999). The model calculates the different water fluxes in the ecosystem at a daily time scale: tree transpiration, understory evapotranspiration, rainfall interception and drainage. These fluxes are mainly dependent on LAI and evaporative demand, i.e. potential evapotranspiration (PET). The difference between rainfall and evapotranspiration fluxes refills the soil reservoir, whereas the excess of water is drained out. All the functions in the "BILJOU" model were calibrated through various field experiments in different forests, climates and soil types (Granier et al. 1999). This model was applied by using above-canopy measurements of rainfall, global radiation, air temperature and humidity, and wind speed, which were recorded at a nearby meteorological station (40.66°N, 02.27°W, 1,000 m a.s.l.) no more than 15 km from any of the study plots. These data were also used to calculate daily PET following the Penman formula (Fig. 2). In order to parameterize the model, in July 2013, we used an LAI-2000 leaf area metre (LI-COR, Nebraska, USA) to measure the LAI at five locations within each plot and then averaged the five values. Mean plot LAI was $1.9\ m^2\ m^{-2} \pm 0.3$. Maximum extractable soil water (i.e. the soil water-holding capacity) of calcic cambisols is low so we set the reference value at 80 mm. However, to address uncertainties concerning the water-holding capacity in the different plots, two additional simulations per plot were performed with higher (100 mm) and lower (60 mm) water-holding capacities (REW_{max} and REW_{min}, respectively). REW_{mean} is the modelled value for 80 mm of extractable water (Fig. 2).

Sap flux density

We used the thermal dissipation method (Granier 1987) to measure sap flux density (F_D, $L\ dm^{-2}\ h^{-1}$) over the 2013 summer with five distinct collection periods (Fig. 2). The five periods were selected to characterize tree transpiration before drought and to assess the effect of decreasing REW on tree transpiration once drought conditions had appeared (Table 2). Due to technical constraints (power supply), sap flux measurements were restricted to three to five consecutive days in each measurement period (Fig. 2). Climate within each selected periods was rather homogenous, with warm and sunny conditions. The five periods were selected

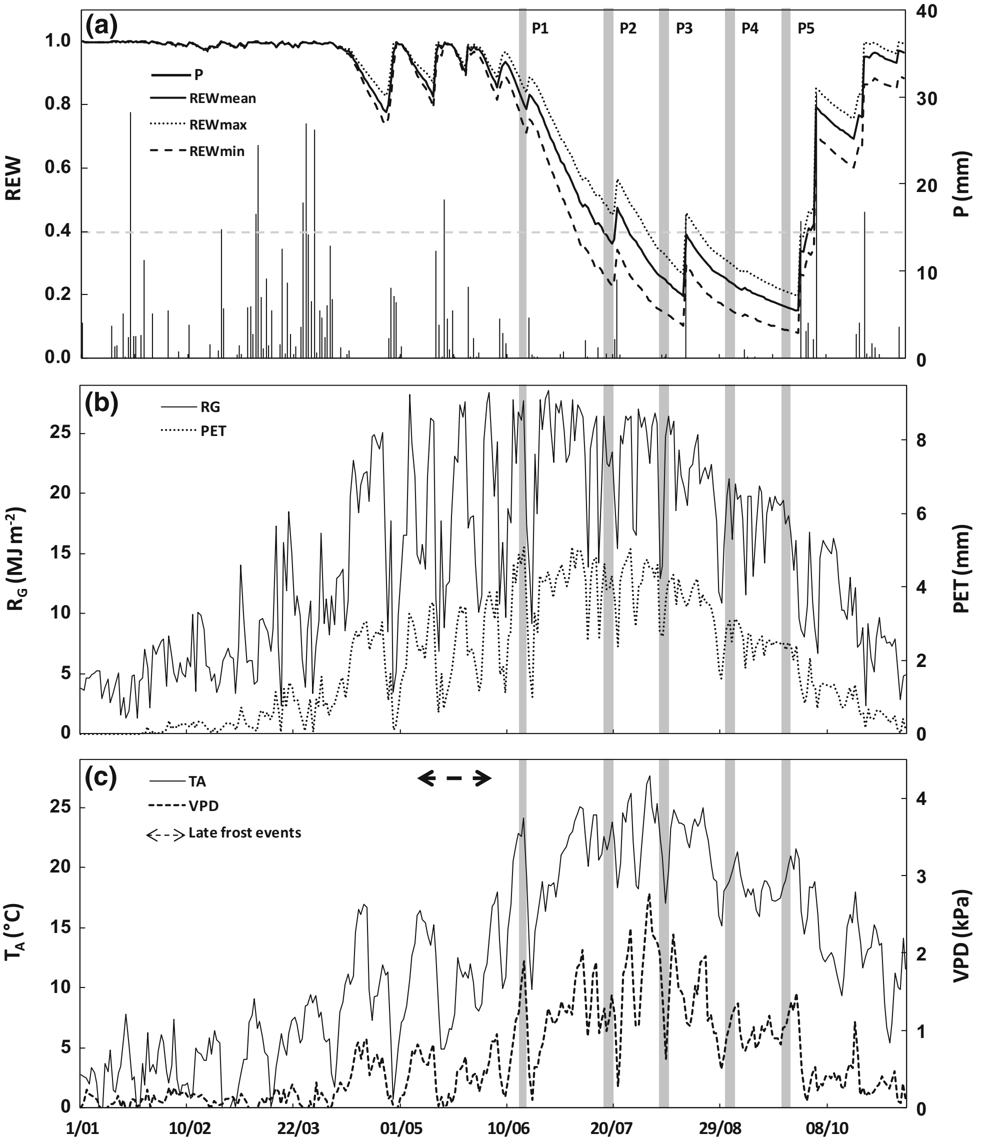

Fig. 2 Seasonal pattern of modelled relative extractable water and climatic conditions for the summer 2013. **a** maximum, minimum and mean modelled relative soil extractable water averaged for all plots (REW_{max}, REW_{min} and REW_{mean}, respectively) and daily cumulative precipitation (*P*, mm), **b** daily cumulative global radiation (R_G, MJ m^{-2}) and daily potential evapotranspiration (PET, mm), and **c** daily mean vapour pressure deficit (VPD, kPa) and daily mean atmospheric temperature (T_A, °C). Periods indicated in *grey* represent the five sap flux measurement periods (P1, P2, P3, P4 and P5 in chronological order). The *horizontal arrow* indicates the period when late frosts occurred in May 2013. The *dashed grey line* shows the REW threshold of 0.4 under, which the resulting soil water shortage induces a drop in stomatal conductance and thus in leaf gas exchange

Table 2 Mean daily relative extractable water modelled using a water balance model (Granier et al. 1999) during the five measurement periods (P1, P2, P3, P4 and P5) for a water-holding capacity of 60 mm (REW_{min}), 80 mm (REW_{mean}) and 100 mm (REW_{max}) with standard errors from the mean (SE)

Period	*N*	REW_{min}		REW_{mean}		REW_{max}	
		Mean	SE	Mean	SE	Mean	SE
P1	3	0.76^a	0.02	0.82^a	0.01	0.89^a	0.01
P2	4	0.26^b	0.01	0.39^b	0.01	0.49^b	0.01
P3	3	0.14^c	0.01	0.25^c	0.01	0.33^c	0.01
P4	5	0.16^c	0.01	0.24^c	0.01	0.34^c	0.01
P5	4	0.09^d	0.01	0.17^d	0.01	0.22^d	0.01

N are the total number of days for each period of measurement

Letters denote significant differences among periods for a given REW

according to weather forecast and spaced by 16 to a maximum of 31 days (P1, P2, P3, P4 and P5) (Fig. 2). Finally, because of sensor failures, data for a few trees had to be removed from the final analyses (Table 3).

The two 20-mm-long probes on the sap flux sensors (UP-GmbH, Cottbus, Germany) were installed under the bark and the cambium at ca. 1.3 m aboveground level with a 10-cm vertical spacing between the probes. All sap flux sensors were installed on the North facing side of the tree to reduce the risk of direct sunlight causing thermal gradients and distorting the measurements. Each sensor was supplied with 0.2 W of constant power. Data were collected every 10 s with a CR800 or CR1000 data logger (Campbell Scientific Inc, Leicestershire, UK), and 30 min averages were recorded. F_D was calculated following the equation proposed by Granier (1987).

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Table 3 Number of trees analysed for sapflow measurements (N), mean diameter at breast height (DBH, cm), mean tree height (m) and mean competition index (CI) for each species and each level of species interactions tested with standard errors from the mean (SE)

Type of interaction	*Pinus nigra*							*Pinus sylvestris*							*Quercus faginea*						
	N	DBH (cm)		Tree height (m)		CI		N	DBH (cm)		Tree height (m)		CI		N	DBH (cm)		Tree height (m)		CI	
		Mean	SE	Mean	SE	Mean	SE		Mean	SE	Mean	SE	Mean	SE		Mean	SE	Mean	SE	Mean	SE
PN–PN	5	26.9	3.3	14.3	0.8	2.5	1.0														
PN–PS	6	35.5	3.0	16.1	0.9	1.9	0.7	5	27.4	2.0	12.3	0.8	2.1	1.5							
PN–QF	5	32.4	3.9	12.6	0.3	1.4	0.3								6	20.8	3.4	9.9	1.1	2.5	1.4
PS–PS								4	32.2	4.7	15.6	1.3	2.4	1.0							
PS–QF								5	26.6	2.4	13.7	0.3	2.4	0.7	5	20.6	0.5	12.1	0.5	2.2	0.7
QF–QF															6	16.8	0.9	7.7	0.4	1.6	0.5

PN, *Pinus nigra*; PS, *Pinus sylvestris*; QF, *Quercus faginea*

As Granier-type probes can only measure F_D along a radial cylinder 20 mm in width, if the sapwood is thicker than the sensor length, part of the total tree sap flux is not measured. As we were unable to find any allometric equations between tree diameter and sapwood thickness for *Q. faginea* in the literature, we established an equation to calculate sapwood thickness (ST, mm) and sapwood area (SA, cm^2) for this species. To do so, we used a Pressler increment borer (Suunto, FI) to extract wood cores from the North side of the stems of 21 *Q. faginea* trees (diameter at breast height (DBH) between 10 and 36 cm) selected outside the study plots in July 2013. The sapwood–heartwood transition was determined with visual colouration differences (Rybnicek et al. 2006; Herrero de Aza et al. 2011). We then related SA to tree DBH through the following equation ($R^2 = 0.92$):

$$\mathrm{SA} = 0.64 \times DBH^{1.81} \quad (1)$$

For both pine species, we used equations from the literature to calculate SA of each tree (Poyatos et al. 2005). This assumption may lead to some slight overestimation of *SA*, but as our objective was to compare trees within a given tree species, these slight errors had no significant consequences on our results.

Based on SA, we calculated ST of all trees. For five of the *Q. faginea* trees, ST was <20 mm, so we applied the correction proposed by Clearwater et al. (1999). For the remaining *Q. faginea* trees, we did not apply corrections for changes in F_D radial patterns since it has been shown that F_D decreases to zero after the first 20 mm for *Quercus* species (Cermak et al. 1992). For the pines, ST was always above 20 mm, so we applied corrections for radial changes in F_D based on the modal distribution provided by Nadezhdina et al. (2002):

$$\text{Total } F_D = F_D\text{m}*\left[1+\frac{\frac{2}{3}*\pi*(\mathrm{ST}-20)^2}{\frac{2}{3}*\pi*ST^2}\right] \quad (2)$$

where Total F_D represents the sap flux density along the whole sapwood, F_Dm represents the sap flux density measured with the sensor, and ST represents the sapwood thickness of the tree.

During the five measurement periods, sap flux sensor signals were stable throughout the night and from night to night, showing that there was no occurrence of night-time transpiration. This was consistent with the fact that the vapour pressure deficit (VPD) at night during these periods was very low (between 0.0 and 0.1 kPa).

Based on F_D values, we calculated the mean daily sap flux density (F_{Dmean}) for each tree and each measurement day. We then defined the maximum value of mean daily sap flux density (F_{Dmax}) for each tree over the whole measurement campaign (i.e. all five periods) as the value of

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F_{Dmean} on the day with the highest amount of global radiation (i.e. DOY 165). We also calculated the daily relative mean sap flux density ($F_{\mathrm{D\%}}$) for each tree as the ratio of F_{Dmean} to F_{Dmax} in order to compare the decline in transpiration among trees along the drought period.

Foliar analyses

For each selected tree, we used 18-m-long pruning shears to sample 20–30 fully expanded sunlit leaves (*Q. faginea*) and three to five 30–40-cm-long branches supporting fully mature, sunlit needles in June 2013. For conifers, current- and previous-year needles were sampled and bulked. The samples were oven-dried at 65 °C for 48 h at INRA Nancy and finely ground (CB2200, Sodemi, St-Ouen l'Aumône, FR). About 1.0 mg of the powdered material from each tree was weighed out (MX5, Mettler Toledo, Viroflay, FR) and placed into tin capsules (Elemental Microanalysis Limited, Devon, UK) for carbon isotope composition (δ^{13}C, ‰) analysis at the Technical Platform of Functional Ecology (OC 081) at the INRA Forest Ecology and Ecophysiology Unit; an EA-GC/IRMS (Delta S, Finnigan MAT, Bremen, Germany; ±0.2 ‰) was used for the analyses. Isotopic measurements are reported in the delta notation (δ, ‰) according to the Vienna Pee Dee Belemnite (VPDB) standard.

Data analyses

To test the effect of the period of measurements (P1–P5) on $\mathrm{REW_{min}}$, $\mathrm{REW_{mean}}$ and $\mathrm{REW_{max}}$, one-way ANOVAs were used followed by Tukey-type post hoc tests to determine differences among periods. To determine differences among species in their response to drought, we used mixed linear models where the fixed effects of *species*, *period* and their interaction were tested on F_{Dmean} with "tree" as a random factor. Prior to these tests, we employed Bartlett's tests to confirm homogeneity of variance. To determine the influence of the identity of species in the local neighbourhood (*neighbourhood*), the competition intensity (CI), the size of the trees (DBH) and the drought (*period*) on the decrease in sap flux density for each species, we used mixed linear models where the fixed effects of *neighbourhood*, CI, DBH and period were tested on $F_{\mathrm{D\%}}$ with "tree" as a random factor. The interactions between *neighbourhood* and *period* and between CI and *period* were also included in the model to determine whether the effects of species interactions and competition intensity changed during the drought. Finally, we used mixed linear models to analyse the effect of *neighbourhood*, DBH and CI on $\delta^{13}C$ values of each species, with "tree" as a random factor. All analyses were performed with the statistical software R 2.14.1 (R Development Core Team 2011). For the mixed models, we used the package *lme4* (Bates et al. 2013). Tukey-type post hoc tests were used to reveal significant differences when a given factor was significant by using the package *multcomp* (Hothorn et al. 2008).

Results

Seasonal variations in climate and soil water

At the beginning of June, i.e. at the first measurement period (P1), REW was high in all the study plots, indicating that soil water was close to field capacity and thus not yet limiting transpiration. From P1 to P5, REW significantly decreased ($P < 0.001$) (Fig. 2, Table 2). At P2, REW was close to 0.4, possibly indicating a first effect of restricted soil water availability on tree transpiration (Granier et al. 1999). REW then continued to drop and remained below 0.4 during P3, P4 and P5. Between P2 and P3, and between P3 and P4, light rain events occurred that induced slight increases in REW (Fig. 2). Over the whole study period, REW reached its lowest value on September 26, 2013, i.e. just after the end of P5. REW was lower than 0.4 for a minimum of 78 days and a maximum of 87 days depending on the water-holding capacity. Finally, rain events at the end of September, 2013 resulted in a sharp increase in REW in all the plots (Fig. 2). Radiation, VPD and PET were high during P1 and P2 and then decreased steadily throughout the summer (Fig. 2). Late frosts were observed in May 2013 that induced late leaf establishments in several broadleaf species in the region, including *Q. faginea* (Valladares unpubl.).

Sap flux density

We observed no significant species effect on F_{Dmean} ($P = 0.202$) but a significant effect of the period of measurement ($P < 0.001$) and of the interaction between species and the period of measurement ($P < 0.001$), indicating that the three tree species differently responded to the drought (Fig. 3). Before the start of the drought (P1), *P. nigra* had the highest F_{Dmean} values and *Q. faginea* the lowest (Fig. 3). As the drought progressed, F_{Dmean} for the two conifers decreased steadily to the minimum values in September (P5), while *Q. faginea* values decreased more slowly (Fig. 3).

For *P. nigra*, we found a significant effect of the identity of species in the immediate neighbourhood and the period of measurements on $F_{\mathrm{D\%}}$, ($P < 0.001$), but no effect of CI and of DBH (Table 4). The mixed model also revealed significant interactions between the level of species interaction and the period ($P < 0.001$) as well as between CI and the period ($P = 0.001$) (Table 4). $F_{\mathrm{D\%}}$ of *P. nigra*

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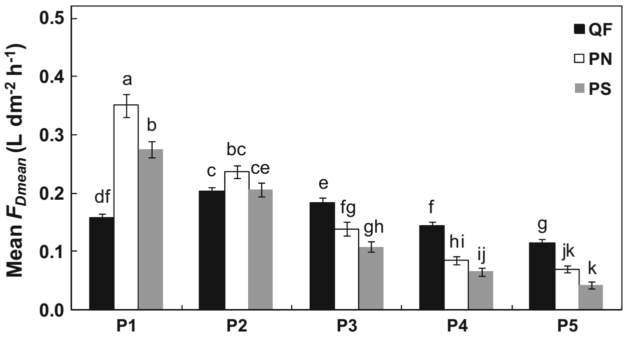

Fig. 3 Mean of the daily mean sap flux density (Mean F_{Dmean}, L dm^{-2} h^{-1}) for *Quercus faginea* (QF), *Pinus nigra* (PN) and *Pinus sylvestris* (PS) for each period of measurements (*P1*, *P2*, *P3*, *P4* and *P5*). *Vertical bars* represent the standard error of the mean for each period of measurement. *Letters* denote significant differences in daily mean sap flux density among all species and periods

trees decreased from P1 to P5 with the less intense decrease for trees interacting with *P. sylvestris* trees (Fig. 4).

For *P. sylvestris*, we found a significant effect of the identity of species in the immediate neighbourhood ($P = 0.025$) and of the period of measurement ($P < 0.001$) on $F_{D\%}$, but no effect of CI and of DBH (Table 4). The interactions between the level of species interaction and the period as well as between CI and the period were also significant ($P < 0.001$, Table 4). $F_{D\%}$ of *P. sylvestris* trees decreased from P1 to P5 with a less intense decrease for trees interacting with conspecific neighbours (Fig. 4).

For *Q. faginea*, we observed a significant effect of the identity of species in the immediate neighbourhood and of the period of measurement ($P < 0.001$) on $F_{D\%}$, but no effect of CI and of DBH (Table 4). Furthermore, the interactions between the level of species interaction and the period ($P < 0.001$) as well as between CI and the period ($P = 0.005$) were also significant (Table 4). $F_{D\%}$ of *Q. faginea* trees increased from P1 to P2 and then slightly decreased until P5 (Fig. 4). Trees interacting *P. sylvestris* trees were able to maintain high $F_{D\%}$ values throughout the summer, while trees interacting with conspecific neighbours showed the highest decrease in $F_{D\%}$ (Fig. 4).

Foliar isotope composition

Mean species foliar carbon isotope composition ($\delta^{13}C$) varied between −25.4 ‰ for *Q. faginea* and −26.4 ‰ for *P. nigra*. Tests revealed a significant effect of the identity of species in the immediate neighbourhood on $\delta^{13}C$ *for P. nigra* ($P = 0.009$) with more negative values for the trees interacting with *Q. faginea* or conspecific neighbours (Fig. 5). A significant effect of the type of species interaction was also found for *Q. faginea* ($P = 0.001$) with more negative values for trees interacting with *P. nigra* or *P. sylvestris* (Fig. 5). For *P. sylvestris*, no effect of the type of species interaction could be found on $\delta^{13}C$ ($P = 0.136$). None of the three species showed a significant effect of CI on $\delta^{13}C$ ($P > 0.05$). There was a significant effect of DBH on $\delta^{13}C$ for *Q. faginea* ($P = 0.045$) and *P. sylvestris* ($P = 0.031$), but not for *P. nigra* ($P = 0.226$).

Discussion

Transpiration under non-limiting soil water conditions

Before any drought occurred, we found higher rates of sap flux density for the two coniferous species than for *Q. faginea* (Fig. 3). This result contrasts with previous studies, which showed that Mediterranean broadleaves generally have higher rates of sap flux density than do conifers (Carnicer et al. 2013). The discrepancy between these previous results and ours might be explained by the fact that needles of conifer species are fully transpiring early in spring, while leaves of ring-porous species such as *Q. faginea* are established later in the season when new xylem tissues are built.

Table 4 Degrees of freedom (*df*), *F*and *P* values from the mixed linear models used to test the fixed effects of the level of species interaction (*Neighbourhood*), the Hegyi competition index (CI), the period of measurement (*Period*), the diameter at breast height (DBH) and the interactions between *Neighbourhood* and *Period* and between CI and *Period*, on mean daily relative sap flux density ($F_{D\%}$) for *Pinus nigra*, *Pinus sylvestris and Quercus faginea*

	Pinus nigra			*Pinus sylvestris*			*Quercus faginea*		
	df	*F* value	*P* value	*df*	*F* value	*P* value	*df*	*F* value	*P* value
Neighbourhood	2	16.5	<0.001***	2	5.6	0.025*	2	14.5	<0.001***
CI	1	0.2	0.671	1	0.5	0.990	1	1.5	0.246
Period	4	460.2	<0.001***	4	652.1	<0.001***	4	227.9	<0.001***
DBH	1	0.8	0.388	1	2.2	0.178	1	0.2	0.642
Neighbourhood × *period*	8	12.3	<0.001***	8	8.5	<0.001***	8	36.2	<0.001***
CI × *period*	4	4.5	0.001**	4	15.9	<0.001***	4	7.7	0.005**

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

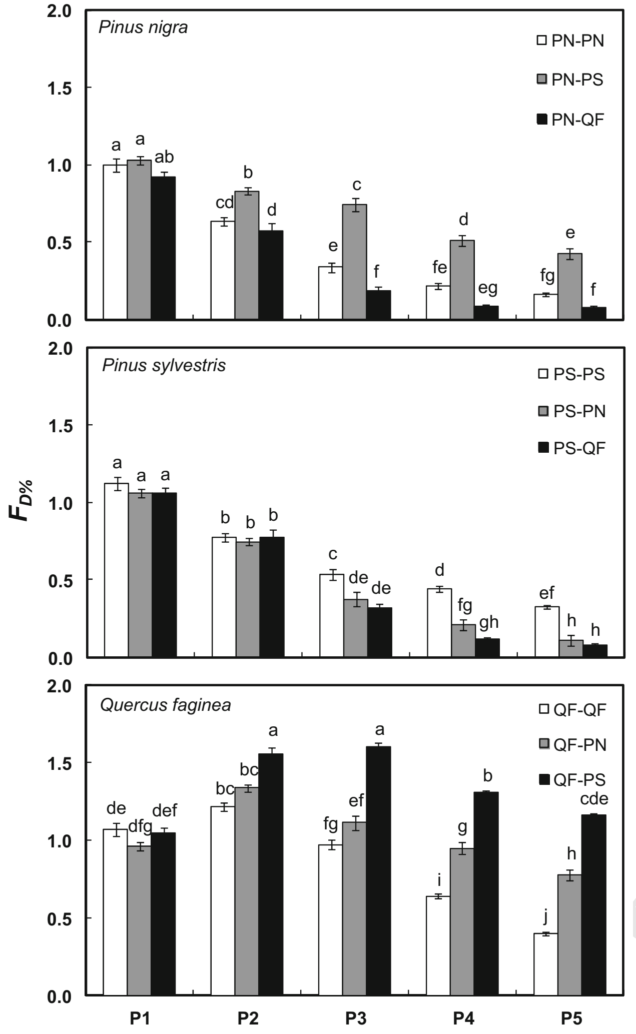

Fig. 4 Mean of the daily mean relative sap flux density ($F_{D\%}$) for each measurement period (*P1*, *P2*, *P3*, *P4* and *P5*) and each level of species interaction for *Quercus faginea* (QF), *Pinus nigra* (PN) and *Pinus sylvestris* (PS). *Vertical bars* represent the standard error of the mean for each period of measurements. For a given species, *letters* denote significant differences in $F_{D\%}$ among all periods and mixture levels

Another explanation for higher rates of sap flux density for the two coniferous species before the drought might be the delayed establishment of the leaves of *Q. faginea* at our study site due to unusually late frosts in May 2013 (Fig. 2). The leaves on *Q. faginea* were still completing their development during the first measurement period, so maximum sap flux density rates were only reached during the second measurement period (P2, Fig. 3).

Influence of drought on transpiration

Both *P. nigra* and *P. sylvestris* showed a strong decrease in transpiration from P1 on, whereas the decrease for *Q. faginea* was less severe and started after P2 (Fig. 3).

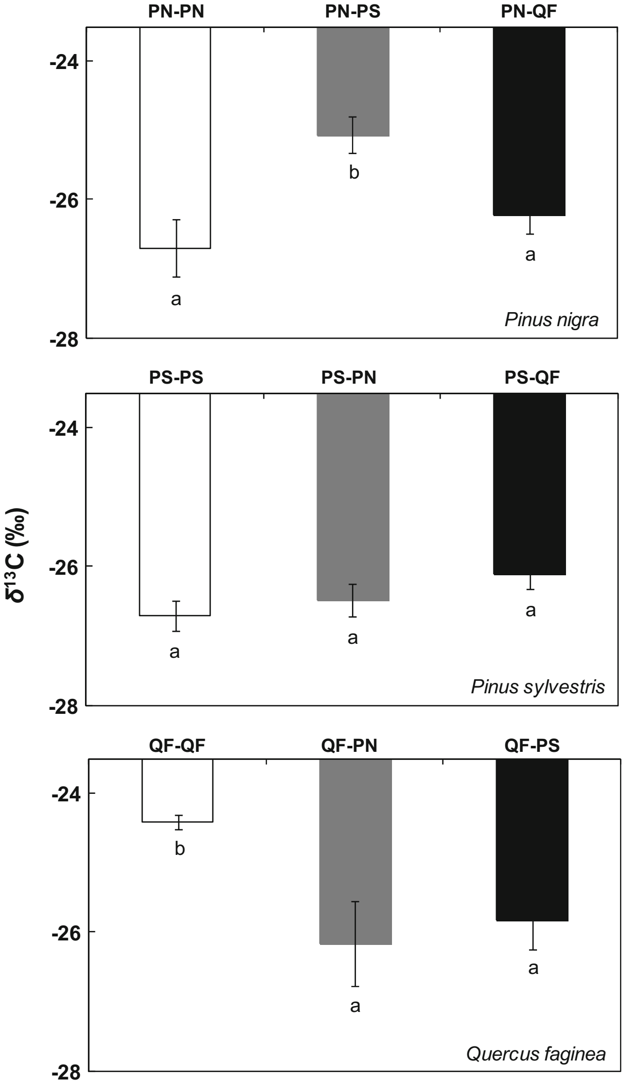

Fig. 5 Species mean carbon isotope composition (δ^{13}C, ‰) of target trees of *Pinus nigra*, *Pinus sylvestris* and *Quercus faginea* when interacting with conspecific (PN–PN, PS–PS, QF–QF) or non-conspecific (PN–PS and PN–QF for *Pinus nigra*; PS–PN and PS–QF for *Pinus sylvestris*; QF–PN and QP–PS for *Quercus faginea*) neighbours. *Vertical bars* represent the standard error of the mean for each neighbourhood. For a given species, *small letters* denote significant differences in δ^{13}C among mixture levels

Drought negatively affected the transpiration of all three species, but at clearly different levels (Fig. 3). When pooling all the trees of a given species, the mean decrease in sap flux density ranged from 30 % for *Q. faginea* to 85 % for *P. sylvestris*. Poyatos et al. (2005) also found a stronger decrease in transpiration for *Pinus* than for *Quercus* trees during a drought stress. This result reflects the higher sensitivity of coniferous species to drought (Carnicer et al. 2013) and is explained by differences among species in their ecophysiological strategies to cope with water deficit. Indeed, *Q. faginea* can be characterized as a drought-adapted species, thanks to its deep-rooting system (Corcuera et al. 2004; Montserrat-Marti et al. 2009) which access higher water resources during droughts and allow the tree to maintain stomata open over longer periods (Cochard et al. 1996). Regulation of water loss for *Quercus* species starts only after a very low value of water potential has been crossed (typically −2.0 MPa; Bréda et al. 1993). Leaf and soil water potential values were not measured here, but our results suggest that such a threshold value was reached late during the summer, probably after P3. Inversely, pines, which have a shallower rooting system than oaks, close their stomata early on during periods of limited water availability in order to avoid very negative leaf water potential values and thus potential risks of vessel cavitation (Irvine et al. 1998). However, it must be noted that the two pine species also present important differences in their strategy to deal with drought stress. Indeed, *P. sylvestris* is less susceptible to xylem embolism than is *P. nigra* (Choat et al. 2012). Furthermore, *P. sylvestris* relies mostly on superficial soil layers to extract water (Kalliokoski et al. 2008), while *P. nigra* seems better adapted to drought-prone climates (Peñuelas and Filella 2003).

Functional and ecological interpretation

Our main findings were: (1) the strong effect of tree species identity around the target trees on their response to drought and (2) the considerable differences in these effects among species. Our results strongly support the hypothesis that local tree species interactions influence the response of these Mediterranean tree species transpiration to drought, but to different extents. Throughout the summer and during the course of the drought, *Q. faginea* trees that were surrounded by *Pinus* species exhibited a much lower decline in $F_{\mathrm{D\%}}$ than *Q. faginea* trees surrounded by conspecific competitors (Fig. 4). Such a response seems to indicate that *Q. faginea* trees experienced a reduced competition for available soil water when surrounded by *Pinus* trees than when surrounded by conspecific trees. Similarly, the interaction with another *Pinus* species resulted in a less pronounced decline in $F_{\mathrm{D\%}}$ of *P. nigra* trees during the drought than when interacting with conspecific neighbours (Fig. 4). However, *P. nigra* trees that were in interaction with *Q. faginea* exhibited the same decline in $F_{\mathrm{D\%}}$ as in the mono-specific neighbourhoods (Fig. 4). In contrast, the presence of *P. nigra* or *Q. faginea* trees in the neighbourhood of *P. sylvestris* individuals resulted in a higher decline in $F_{\mathrm{D\%}}$ with progressing drought as compared to trees mainly surrounded by conspecific competitors (Fig. 4). The differences in the response of *P. sylvestris* transpiration to drought probably indicate lower water availability for the trees in interaction with another species.

The transpiration response observed in the different neighbourhoods suggests the following conclusions: (1) Community assembly in this Mediterranean ecosystem with coexisting species differing in functional characteristics strongly affects soil water content. (2) Interactions among species can have contrasting consequences for water availability levels depending on the species considered. We develop below our interpretation of the functional and ecological response of each species to these interactions.

For *P. nigra* and *Q. faginea*, the results suggest that when these species are interacting with *P. sylvestris*, soil water depletion is reduced throughout a drought period (Fig. 4). We assume that complementarity mechanisms for soil water uptake could partially explain this pattern. Species interactions may improve the availability and/or access to water resources during the dry period of *P. nigra* and *Q. faginea* when competing with *P. sylvestris*. Furthermore, the interaction between *P. nigra* and *Q. faginea* seemed also to increase the water accessibility of *Q. faginea* at the outmost levels of drought stress (P4 and P5; Fig. 4). Comparable positive species interactions were previously observed in tree plantations (Forrester et al. 2010; Kunert et al. 2012) where complementary water-use led to less competition for soil water resources among the interacting species and to higher overall transpiration rates. This interpretation is consistent with $\delta^{13}C$ values of these species in the different neighbourhoods (Fig. 5). Reduced water availability usually parallels increased (i.e. less negative) $\delta^{13}C$ values in organic material (Saurer et al. 2004). As *P. nigra* exhibited more negative $\delta^{13}C$ values when interacting with *P. sylvestris,* and *Q. faginea* had the most negative values when interacting with the two *Pinus* species (Fig. 5), this indicates greater soil water availability in these neighbourhoods for these two species. As leaf samples were taken before drought started to affect tree transpiration, these results reflect a strong, long-term physiological acclimation of these trees to the different soil water deficit conditions encountered every year in the different neighbourhoods.

For *P. sylvestris*, the lower $F_{\mathrm{D\%}}$ rates in the neighbourhoods where *P. nigra* or *Q. faginea* are present (Fig. 4) could be explained by the fact that this species relies mostly on water from shallow soil horizons (Irvine et al. 1998) and, in our case, was interacting with species with higher transpiration rates (Fig. 3). Therefore, the water available to a given *P. sylvestris* tree may have dropped faster in these neighbourhoods than in situations where a tree was competing with the less "water-hungry" individuals of its own species. The fact that any significant effect of species interactions was found on $\delta^{13}C$ (Fig. 5) could suggest that this negative effect of species interactions remains rather small. The differences in local water availability between the neighbourhoods with the two other species and the one with *P. sylvestris* trees solely were

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probably not great enough to cause any notable leaf-level physiological acclimation in *P. sylvestris*.

Finally, it must be noted that even though we only selected dominant and co-dominant trees, differences in the aboveground vertical stratification of the canopy might occur in the different plots. As the canopy architecture of the three study species is highly contrasted (Poorter et al. 2012), differences in light interception and in vapour pressure deficit in the different neighbourhoods could have also contributed to the variability in transpiration rates we observed.

Conclusion

Our study brings new insights into the management of mixed Mediterranean forests. We have shown that managing these forests as mixed-species stands would only alleviate the effect of water stress during summer droughts for the deepest-rooted species like *Q. faginea* and *P. nigra*. When such deep-rooted species interact with shallower-rooted species, they seem to benefit from a reduction in competition for water resources in the deep soil layers which dry out more slowly than superficial ones. In contrast, mixing a shallow-rooted conifer species such as *P. sylvestris* with deeper-rooted species may actually be detrimental to its water acquisition and use. Our results also provide worrisome evidence that mixing *Pinus* and *Quercus* species in Mediterranean forests could accelerate the ongoing conversion from mixed conifer-broadleaved forests to pure broadleaved forests in this region under future drier climatic conditions (Carnicer et al. 2014).

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Paper VI

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ECOSYSTEMS

Does Drought Influence the Relationship Between Biodiversity and Ecosystem Functioning in Boreal Forests?

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ABSTRACT

In mixed forests, interactions among species influence ecosystem functioning but environmental conditions also play an important role in shaping relationships between biodiversity and ecosystem functioning. In the context of climate change, the carbon and water balance in pure versus mixed forest stands may be differentially influenced by changing soil water availability. To test this hypothesis, we compared the influence of biodiversity on stand water use efficiency (WUE_S) in boreal forests between wet and dry years. We assessed the carbon isotope composition ($\delta^{13}C$) of tree rings in *Betula pendula*, *Pinus sylvestris*, and *Picea abies* growing in pure versus mixed stands. In addition, we tested whether differences in WUE_S affected patterns of stand basal area increment (BAI_S). No biodiversity effect was found for stand $\delta^{13}C$ ($\delta^{13}C_S$) during the wet year. However, there was a significant increase in $\delta^{13}C_S$ between the wet and the dry year and a significant effect of biodiversity on $\delta^{13}C_S$ in the dry year. The increase in $\delta^{13}C_S$ in mixed stands was associated with both selection and complementarity effects. Although BAI_S decreased significantly in the dry year, changes in $\delta^{13}C_S$ did not translate into variations in BAI_S along the biodiversity gradient. Our results confirmed that the physiological response of boreal forest ecosystems to changing soil water conditions is influenced by species interactions and that during dry growing seasons, species interactions in mixed stands can lead to lower soil moisture availability. This illustrates that biodiversity effects can also be negative in mixed stands in the sense that soil resources can be more intensively exhausted. Overall, our results confirm that in boreal forests, the biodiversity–ecosystem functioning relationship depends on local environmental conditions.

Key words: biodiversity; boreal forest; drought; $\delta^{13}C$; mixed forest; water use efficiency.

INTRODUCTION

The biodiversity and ecosystem functioning (B–EF) relationship has received growing interest from ecologists throughout the last two decades due to

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the rapid loss of diversity observed during the last century (Symstad and others 2003). Several mechanisms have been put forward to explain how community composition and richness can either negatively or positively influence ecosystem functions. A negative influence on biodiversity can result from direct competition for resources among species in a given ecosystem. Negative interactions commonly arise when ecological niches overlap and/or when species share the same functional characteristics and are thus functionally redundant in the ecosystem (Naeem 2008; Vilà and Sardans 1999). In contrast, positive B–EF relationships are commonly attributed to two other mechanisms: complementarity and selection. Complementary use of resources among species refers both to ecological niche partitioning and facilitation (Loreau and Hector 2001) and implies species coexistence without major interspecific competition for resources. The selection effect recognizes that the probability of occurrence of high-performing species is greater in highly diverse ecosystems (Loreau and Hector 2001). Studies investigating complementarity and selection effects mainly focused on ecosystem productivity (for example, Bradford 2011; Fargione and others 2007; Isbell and others 2009; Morin and others 2011; Zhang and others 2012), whereas only a few were interested in other ecosystem functional traits (for example, Forrester and others 2010; Kunert and others 2012; Meinen and others 2009).

In addition to competition, complementarity and selection effects, local environmental conditions also play an important role in shaping B–EF relationships (Belote and others 2011; Hooper and Dukes 2004). Under the assumptions of the ‘‘stress-gradient’’ hypothesis, which predicts that the net outcome of biotic interactions (competition and facilitation) shifts from negative to positive along gradients of limiting physical conditions (Bertness and Callaway 1994), positive biodiversity effects are expected to be more common in severely resource-limited conditions whereas negative effects should prevail in richer and milder environments. This general conceptual model has recently been refined (Maestre and others 2009) and is widely supported in the literature (Herbert and others 2004; Jucker and Coomes 2012; Steudel and others 2012; He and others 2013; Wang and others 2013). In the context of climatic change, most regions around the world are expected to encounter more extreme environmental conditions (IPCC 2007). The ‘‘stress-gradient’’ hypothesis is therefore of great interest because B–EF relationships are likely to change in the future. Whether or not more diverse ecosystems might be better adapted and/or more resilient to these changes is an important issue to investigate.

In the boreal climate zone, simulations predict a general shift from short, cool summers toward longer, warmer summers (IPCC 2007; Jylhä and others 2010). Thus, boreal forest ecosystems are expected to encounter more frequent and intense reduced soil water availability in summer. Contrasted responses of carbon and water fluxes in forest ecosystems to warmer and drier climate have already been observed (reviewed in Boisvenue and Running 2006). Few studies so far were conducted on the response of boreal forests to these conditions. Nevertheless, Dulamsuren and others (2010) showed that increasing summer temperatures accompanied by decreasing precipitation lead to reduced productivity in taiga forests in Mongolia.

Plants adapt to reduced soil water conditions through numerous physiological and/or morphological processes (review in Kozlowski and Pallardy 2002). At the leaf level, under drought conditions, they must manage the trade-off between optimum carbon gain for growth and loss of water through transpiration (Farquhar and others 1982) which usually results in an increase in intrinsic water use efficiency ($\mathrm{WUE_{int}}$), defined as the ratio between $\mathrm{CO_2}$ assimilation during photosynthesis and stomatal conductance for water vapor (for example, Zhang and Marshall 1994). At the ecosystem level, carbon and water fluxes are influenced by species-specific functional responses to environmental conditions and by intra- and inter-specific interactions both below- and aboveground. Both positive and negative interactions among species may occur in mixed stands and this may lead to differing spatial and temporal resource availability and physiological and morphological adaptations within species. These complex interactions mean that general predictions on stand-level water use efficiency ($\mathrm{WUE_s}$) under dry conditions cannot solely be based on individual species responses to these conditions.

In this context, little information is available concerning the relationship between tree biodiversity and the regulation of carbon and water fluxes in boreal forest ecosystems. Gamfeldt and others (2013) found positive relationships between tree species richness and multiple ecosystem services (tree biomass, soil carbon storage, berry production, and game production potential) in production forests in Sweden and explained the observed positive B–EF relationship by facilitation processes among tree species. In contrast, Grossiord and others (2013a) found no complementary effect for biomass production and $\mathrm{WUE_S}$ in a young boreal plantation, though they did find a weak selection effect. These studies, however, did not compare the B–EF relationship in contrasted environmental conditions.

In this study, we tested the following assumptions: (i) in boreal forest ecosystems, the stand-level carbon and water balance under non-limiting soil water conditions (wet year) should not necessarily depend on biodiversity effects, and (ii) under limiting soil water conditions (dry year), species identity, species combinations, and richness levels should influence ecosystem functioning. We analyzed the influence of species richness on time-integrated WUE_S estimated from the carbon isotope composition ($\delta^{13}C$) (Farquhar and others 1982) of ring whole wood measured in tree rings from 2 years with contrasting soil water conditions (wet year in 2004 and dry year in 2006). Samples were taken from 26 mature stands in Eastern Finland, which were either pure or mixed with varying percentages of *Betula pendula*, *Pinus sylvestris*, and *Picea abies*. We also tested whether changes in WUE_S would influence stand basal area increment (BAI_S).

MATERIALS AND METHODS

Site Description

The study was conducted in August 2012 in 26 stands of boreal forest that are distributed over a 2,000 km^2 area around Joensuu, Finland (62.60°N, 29.76°E). The stands were 30 m × 30 m and included pure, 2-species mixture, or 3-species mixture of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and Silver birch (*Betula pendula*). Four replicates of each combination were selected except for pure birch stands that were sampled only twice. Mean tree age within the stands was 33.9 ± 5.8 years. Tree species characteristics are given in Table 1.

The stands were selected according to the following specifications: no change in management had occurred in the last 5 years; confounding factors like altitude, topography, or soil type were kept to a minimum between selected stands; in a given forest patch, the stands were more than 500 m apart to avoid spatial autocorrelation; stands were mostly even-aged and single-layered. More detailed information on the selection procedure of the study stands can be found at http://www.fundiveurope.eu. The selected stands were situated between 80 and 200 m a.s.l. on Podzols soils (FAO classification) above mica schist bedrock. Mean annual rainfall in the region is around 700 mm and mean annual temperature is 2.1°C.

Table 1. Overall Mean Species Diameter at Breast Height (cm) and Mean Height (m) with Standard Errors for *Betula pendula*, *Pinus sylvestris*, and *Picea abies*

Species	Mean diameter at breast height (cm)	Mean height (m)
Betula pendula	16.4 ± 0.2	17.9 ± 0.2
Pinus sylvestris	19.2 ± 0.1	17.6 ± 0.1
Picea abies	17.7 ± 0.2	16.1 ± 0.1

Selection of the Target Years

The daily water balance model ''BILJOU'' (Granier and others 1999) was implemented to quantify soil water availability at a daily time-scale in the study region and then to select the driest and the wettest year over the past 15 years. We used above-canopy measurements of daily meteorological conditions (rainfall, global radiation, air temperature and humidity, wind speed) from the Hyytiälä forestry field station (61.84°N, 24.28°E, 153 m a.s.l.) to run the model. Although this meteorological station is situated 300 km south-west of Joensuu, it is the nearest source from which data could be obtained to estimate daily relative extractable soil water (REW) over the 1997–2011 period (Figure 1). The model was initialized assuming that soil was at field capacity on 1st January. We used a leaf area index of 5.0 m^2 m^{-2} and a soil holding capacity of 125 mm as input data for model simulations. We discarded the years 1998 and 2010 because of too many missing data. Soil water deficit was assumed to occur when the REW dropped below 0.4, a threshold value that has been shown to induce stomatal closure and interrupt radial growth in forest trees (Granier and others 1999). For each remaining year from 1997 to 2011, we characterized annual water stress using the starting day and the duration of the period when REW dropped below the threshold limit coupled with a water stress index which cumulates the difference between simulated REW and the 0.4 threshold (Granier and others 1999). These calculations revealed that in 2006 water stress was the most severe and in 2004 it was the least severe (Figure 1). Drought started on June 16 in 2006 whereas there was no drought in 2004. Total precipitation during the growing season (May–September) was 157 and 303 mm in 2006 and 2004, respectively.

Wood Carbon Isotope Composition

Six trees per species in each pure stand and three trees per species in mixed stands were randomly selected among the ten trees with the largest

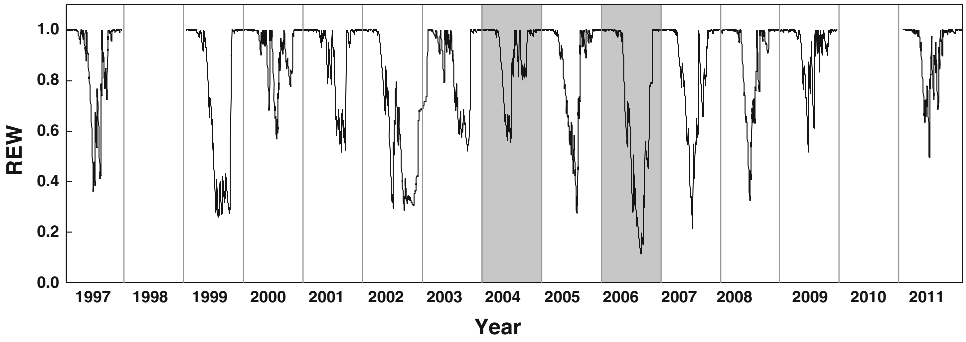

Figure 1. Time-course of relative extractable water (REW) for the years 1997–2012 simulated with the ''BILJOU'' model. The two selected years are highlighted in *gray* (wet for 2004 and dry for 2006).

diameter at breast height. For each selected tree, we extracted one 5-mm-diameter wood core at breast height (1.3 m) from the south side of the trunk. The fresh samples were shipped to INRA Nancy and oven-dried at 50°C for 72 h to reach constant weight before being filed with a scalpel for tree-ring dating. For both target years, we carefully extracted the late wood with a scalpel. The wood fragments from a given species in a given stand were then pooled and ground into a fine powder. We combined the wood fragments from individuals of the same species in the same stand to obtain enough material for carbon isotope analyses, to ensure adequate sample homogeneity, and to reduce experimentation costs. Although we sampled only a few trees per species in each stand, the level of replication was adequate given the imprint of environmental signals on tree-ring carbon isotope composition (for example, Peñuelas and other 2008). From 0.4 to 1.5 mg of the ground wood samples were weighed on a high-precision scale (MX5, Mettler Toledo, Viroflay, France) and placed into tin capsules (Elemental Microanalysis Limited, Devon, UK) for δ^{13}C analyses. Samples were analyzed with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility of UC Davis, California, USA. All isotopic measurements are reported in the standard delta notation (δ, ‰):

$$\delta(\text{‰}) = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1, \tag{1}$$

where R_{sample} and R_{standard} are the ^{13}C/^{12}C ratios of the samples and the Vienna Pee Dee Belemnite (VPDB) standard.

Wood Basal Area Increment

To quantify productivity, we randomly selected five trees per species from each pure stand, and three trees per species in mixed stands, among the twelve (pure stands) or eight (mixed stands) trees with the largest diameter at breast height. For each tree, one 5-mm-diameter wood core was extracted at breast height from the south side of the trunk. These cores were different from the ones taken for δ^{13}C analysis. Once air dried, the core samples were mounted on wooden boards and then sanded with paper of progressively finer grit sizes. A high resolution (2,400 dpi) flatbed scanner was then used to image the cores, following which diameter growth measurements and crossdating were performed with the CDendro software suite (Cybis Elektronik & Data, Saltsjöbaden, Sweden). For each of the studied years, as well as the year following the dry year, diameter growth measurements of the individual trees were converted to basal area increment, then standardized basal area increment (BAI_i, cm^2 cm^{-2} y^{-1}) was obtained by dividing basal area increment by the basal area of each tree.

Stand-Level Carbon Isotope Composition and Basal Area Increment

Based on species-specific δ^{13}C values ($\delta^{13}\text{C}_i$) for each stand, we calculated stand-level carbon isotope composition ($\delta^{13}\text{C}_\text{S}$). The contribution of a given species to $\delta^{13}\text{C}_\text{S}$ depends on its species-specific contribution to stand CO$_2$ assimilation rates (Lloyd and Farquhar 1994). Consequently, when $\delta^{13}\text{C}_i$ values are scaled up from species level to stand level, $\delta^{13}\text{C}_i$ values should be weighted by species assimilation rates. However, because species-specific CO$_2$ assimilation rates were not available in this study, we used species total basal area (BA_i, cm^2) in each stand as a proxy, following Bonal and others (2000). $\delta^{13}\text{C}_\text{S}$ was thus calculated as:

$$\delta^{13}\text{C}_\text{S} = \frac{\sum_{i=1}^{N}(\delta^{13}\text{C}_i \times \text{BA}_i)}{\sum_{i=1}^{N}\text{BA}_i}, \tag{2}$$

where N is the number of species in the mixture.

δ^{13}C in C$_3$ plants constitutes a record of the intercellular/atmospheric CO$_2$ concentration ratio during the period in which the carbon was fixed and is strongly positively correlated to leaf intrinsic water use efficiency ($\mathrm{WUE_{int}}$) (Farquhar and others 1982) as shown in the following equation:

$$\mathrm{WUE_{int}} = \frac{C_a}{1.6} \times \left\{ 1 - \left[\frac{(\delta_a - \delta^{13}\mathrm{C} \times 1{,}000)}{1{,}000 + \delta^{13}\mathrm{C}} - a \right] \times \frac{1}{(b-a)} \right\}, \tag{3}$$

where C_a is the $\mathrm{CO_2}$ concentration in the atmosphere, δ_a and $\delta^{13}\mathrm{C}$ are, respectively, the carbon isotope compositions of the atmosphere and of the sample, a represents the fractionation occurring due to diffusion in air (4.4 ‰), and b is the fractionation caused by carboxylation (27 ‰).

To estimate stand basal area increment ($\mathrm{BAI_S}$, $\mathrm{cm^2\ cm^{-2}\ y^{-1}}$) of the two studied years (2004 and 2006) and of the year following the driest year (2007), we considered the relative importance of each species in terms of contribution to total basal area. Thus, $\mathrm{BAI_S}$ was calculated as:

$$\mathrm{BAI_S} = \frac{\sum_{i=1}^{N} (\mathrm{BAI}_i \times \mathrm{BA}_i)}{\sum_{i=1}^{N} \mathrm{BA}_i}. \tag{4}$$

Data Analysis

All analyses were performed using the statistical software R 2.14.1 (R Development Core Team 2011). We tested three effects that could impact $\delta^{13}\mathrm{C}_i$, $\delta^{13}\mathrm{C_S}$, BAI_i, and $\mathrm{BAI_S}$. First, tree biodiversity was characterized for each stand with the Shannon biodiversity index. This index expresses the uncertainty of predicting the proportion of stand basal area a given tree species represents in binary digits (ranging from zero for pure stands to the logarithm in base two of the total basal area of the species in the stand). Second, competition intensity in each stand was expressed as the total basal area of the stand. The third effect was year. We applied a series of mixed-effect models to analyze the impact of the three effects and their interactions on $\delta^{13}\mathrm{C}_i$, $\delta^{13}\mathrm{C_S}$, BAI_i, and $\mathrm{BAI_S}$ with "stand" as a random effect. To test for any biodiversity effect during the recovery phase to drought (year 2007), we applied the same mixed-effect model on $\mathrm{BAI_S}$. Contrasts were used to test for relevant differences between fixed effects when the overall model was significant. We calculated the difference in $\delta^{13}\mathrm{C_S}$ and $\mathrm{BAI_S}$ between the dry and wet year ($\Delta\delta^{13}\mathrm{C_S}$ and $\Delta\mathrm{BAI_S}$, respectively) and used Spearman regressions to test the relationship between the Shannon biodiversity index and $\Delta\delta^{13}\mathrm{C_S}$ or $\Delta\mathrm{BAI_S}$ (Table 2).

When a significant effect of biodiversity on $\delta^{13}\mathrm{C_S}$ or $\mathrm{BAI_S}$ was observed, we evaluated the net biodiversity effect, the complementarity effect, and the selection effect on $\delta^{13}\mathrm{C_S}$ or $\mathrm{BAI_S}$ with the method developed by Grossiord and others (2013b). We used one-way ANOVA and post-hoc Tukey tests to check for differences in these biodiversity effects among species richness levels. We used t tests to check all three biodiversity effects for differences from 0 for each mixture level and for each mixture type (identity of the mixture).

RESULTS

Wood Carbon Isotope Composition

We observed considerable variability in $\delta^{13}\mathrm{C}_i$ across species, species mixtures, and years, ranging from −28.7 ‰ for birch in a 2-species mixture with spruce to −26.2 ‰ for spruce in a 2-species mixture with pine during the wet year, and from −27.4 ‰ for birch in a mixture with spruce to −24.2 ‰ for pine in a 3-species mixture during the dry year. Mixed-effect models revealed a significant species effect ($P < 0.001$), year effect ($P < 0.001$), and an interaction between Shannon index and year ($P = 0.019$), but no effect of the Shannon index alone, the interactions of the Shannon index and the species, or the interactions of the year and the species ($P > 0.050$). The significant effect of the interaction between Shannon index and year was only observed for pine and spruce ($P = 0.012$ and $P = 0.020$, respectively), indicating for these species a differential response of $\delta^{13}\mathrm{C}_i$ to the mixture in the 2 years.

For each species, whatever the richness level, we found significantly higher values of $\delta^{13}\mathrm{C}_i$ during the dry year than during the wet year ($P = 0.003$ for birch, $P = 0.017$ for pine, and $P = 0.037$ for spruce). For the wet year, we did not observe any effect of the Shannon index or of competition intensity on $\delta^{13}\mathrm{C}_i$ for any species ($P > 0.050$). In contrast, during the dry year, pine and spruce showed a significant positive Shannon index effect ($P = 0.013$ and $P = 0.027$, respectively) and a competition intensity effect ($P < 0.001$ and $P = 0.010$, respectively), whereas birch did not display any significant patterns ($P > 0.050$).

At the plot level, $\delta^{13}\mathrm{C_S}$ values ranged from −28.1 ‰ for a pine–spruce mixture to −26.6 ‰ for a pure stand of spruce during the wet year and from −26.9 ‰ in a pure stand of pine to −24.6 ‰ for a 3-species mixture during the dry year (Figure 2). We observed a significant effect of year on $\delta^{13}\mathrm{C_S}$ ($P < 0.001$), with higher values of $\delta^{13}\mathrm{C_S}$ during the dry year, as well as a significant interaction between Shannon index and year ($P = 0.012$). Specifically, although there was no

Table 2. Mean Species-Specific Shannon Biodiversity Index, Wood Carbon Isotope Composition ($\delta^{13}C_i$, ‰), and Size-standardized Basal Area Increment (BAI_i, $cm^2\ cm^{-2}\ y^{-1}$) for the Dry and Wet Years, with Standard Errors for *Betula pendula*, *Pinus sylvestris*, and *Picea abies*, and for Each Mixture Level

Species	Mixture	Shannon	$\delta^{13}C_i$ wet (‰)	$\delta^{13}C_i$ dry (‰)	BAI_i wet ($cm^2\ cm^{-2}\ y^{-1}$)	BAI_i dry ($cm^2\ cm^{-2}\ y^{-1}$)
Betula pendula	1	0.2 ± 0.1	−27.1 ± 0.3	−25.6 ± 0.1	$17.1 \times 10^{-3} \pm 0.8 \times 10^{-3}$	$18.2 \times 10^{-3} \pm 0.9 \times 10^{-3}$
	2	0.9 ± 0.1	−27.8 ± 0.2	−25.8 ± 0.3	$19.2 \times 10^{-3} \pm 0.9 \times 10^{-3}$	$19.2 \times 10^{-3} \pm 0.9 \times 10^{-3}$
	3	1.4 ± 0.1	−27.8 ± 0.2	−25.9 ± 0.5	$21.3 \times 10^{-3} \pm 1.5 \times 10^{-3}$	$19.8 \times 10^{-3} \pm 1.4 \times 10^{-3}$
Pinus sylvestris	1	0.1 ± 0.1	−27.3 ± 0.2	−26.2 ± 0.2	$11.9 \times 10^{-3} \pm 0.7 \times 10^{-3}$	$11.4 \times 10^{-3} \pm 0.7 \times 10^{-3}$
	2	0.9 ± 0.0	−27.2 ± 0.1	−25.7 ± 0.3	$16.4 \times 10^{-3} \pm 0.9 \times 10^{-3}$	$15.1 \times 10^{-3} \pm 0.7 \times 10^{-3}$
	3	1.4 ± 0.1	−26.9 ± 0.2	−25.4 ± 0.6	$20.6 \times 10^{-3} \pm 1.2 \times 10^{-3}$	$16.5 \times 10^{-3} \pm 0.8 \times 10^{-3}$
Picea abies	1	0.1 ± 0.1	−26.9 ± 0.1	−26.2 ± 0.2	$22.9 \times 10^{-3} \pm 1.0 \times 10^{-3}$	$17.9 \times 10^{-3} \pm 0.8 \times 10^{-3}$
	2	0.9 ± 0.0	−27.2 ± 0.2	−25.4 ± 0.3	$21.3 \times 10^{-3} \pm 1.3 \times 10^{-3}$	$18.4 \times 10^{-3} \pm 1.1 \times 10^{-3}$
	3	1.4 ± 0.1	−27.2 ± 0.2	−25.3 ± 0.2	$26.6 \times 10^{-3} \pm 1.5 \times 10^{-3}$	$21.9 \times 10^{-3} \pm 1.3 \times 10^{-3}$

significant relationship between the Shannon index or the competition index and $\delta^{13}C_S$ during the wet year ($P > 0.050$), a highly significant and positive Shannon index effect was apparent ($P = 0.008$) during the dry year, along with a marginal influence of competition intensity ($P = 0.067$). Differences in $\delta^{13}C_S$ between the dry and the wet year ($\Delta\delta^{13}C_S$) for each stand were positively correlated with the Shannon index ($P = 0.038$).

Wood Basal Area Increment

The basal area increment (BAI_i) of individual species ranged from $0.3 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ for pine mixed with birch to $52.9 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ for spruce mixed with pine during the wet year, and from $0.7 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ for pine mixed with birch to $41.7 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ for birch mixed with spruce during the dry year. Mixed-effect models revealed a significant species effect ($P < 0.001$), year effect ($P < 0.001$), and an interaction between the species and the year ($P < 0.001$), but no effect of the Shannon index alone, the interactions between the Shannon index and species, or the interactions between the Shannon index and the year ($P > 0.050$). For spruce, we found significantly lower values of BAI_i during the dry year than during the wet year ($P < 0.001$), whereas for birch and pine, no significant changes in BAI_i were found. For the wet year, we did not observe any effect of the Shannon index or competition intensity on BAI_i for any species ($P > 0.05$). For the dry year, pine showed a significant positive Shannon index effect ($P = 0.035$) and no competition intensity effect ($P = 0.813$). Birch and spruce did not display any significant Shannon or competition effects during the dry year ($P > 0.050$).

BAI_S values ranged from $14.9 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ in a birch–pine mixture to $61.3 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ in a pure stand of spruce for the wet year and from $16.9 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ in a birch–pine mixture to $59.8 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ in a birch–spruce mixture for the dry year (Figure 2). In 2007, the year following the driest year, BAI_S values ranged from $16.8 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ in a pure pine stand to $59.4 \times 10^{-3}\ cm^2\ cm^{-2}\ y^{-1}$ in a pine–spruce mixture (Figure 2). The interaction between the Shannon index and the year was not significant ($P = 0.976$). However, we observed a significant effect of the year on BAI_S ($P = 0.001$), with higher values during the wet year in comparison to the dry and the recovery years (Figure 2). We did not detect any significant effect of the Shannon index or the competition index on BAI_S in either year ($P > 0.050$). We found that differences in BAI_S between the dry and the wet year (ΔBAI_S) were not correlated with the Shannon index ($P = 0.143$).

Biodiversity Effects on Stand Carbon Isotope Composition

Overall, there was no significant change in either net, complementarity or selection effect on $\delta^{13}C_S$ with increasing species richness during the dry year ($P > 0.050$) (Figure 3). However, when each mixture level was considered independently, complementarity, selection, and net effects were significantly higher than zero in the 2-species mixtures ($P < 0.050$), and mainly positive—although not significantly different from 0—in the 3-species mixtures ($P > 0.050$). When we tested each different species mixture for significance of the biodiversity effects, we found that none of the three effects were significantly different from zero ($P > 0.050$) in the pine–spruce mixtures. However, for birch–pine

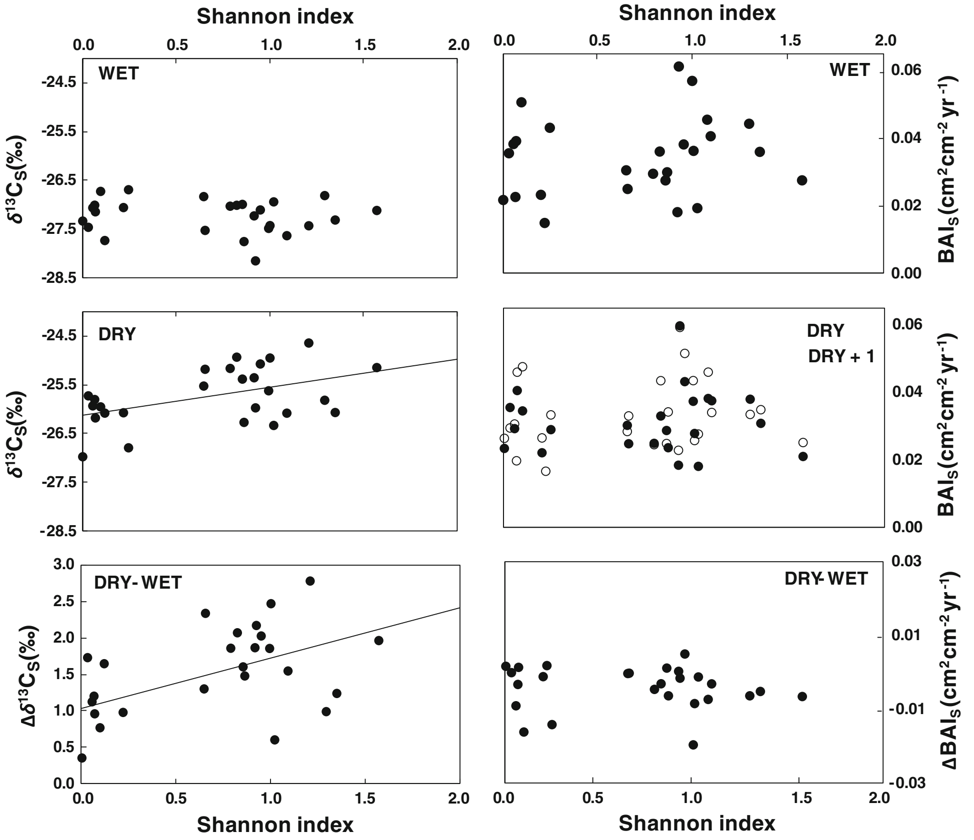

Figure 2. Stand carbon isotope composition ($\delta^{13}C_S$, ‰), stand size-standardized basal area increment (BAI_S, $cm^2\ cm^{-2}\ y^{-1}$) for the wet, the dry, and the recovery year (DRY + 1, *white circles*), and the differences in $\delta^{13}C_S$ and BAI_S between the wet and the dry year ($\Delta\delta^{13}C_S$ and ΔBAI_S, respectively) as a function of the Shannon biodiversity index. The *black lines* represent the fitted regression model when the model was significant ($R^2 = 0.22$ and 0.23 for $\delta^{13}C_S$ and $\Delta\delta^{13}C_S$, respectively).

and birch–spruce mixtures, net and complementarity effects were significantly higher than zero ($P < 0.050$), whereas no selection effects emerged ($P > 0.050$).

DISCUSSION

Climate Effect

Our stable isotope analysis revealed a strong physiological response in boreal trees to changing soil water conditions and helped clarify the mechanisms responsible for observed changes both at the leaf and stand level. The increase in both individual species' $\delta^{13}C_i$ and stand-level $\delta^{13}C_S$ from wet to dry years (Figure 2) reflects an active regulation of carbon and water fluxes in this boreal ecosystem and the strong sensitivity of the three species to limiting soil water conditions. Assuming that the difference in δ_a between the two selected years was negligible, the magnitude of the increase in $\delta^{13}C_S$ reflected a mean increase in WUE_S of 17.0 ± 1.8 μmol mol^{-1} (that is, +19.2 ± 1.5%). This increase is consistent with the extensive body of literature documenting the functional response of boreal trees to drought conditions (for example, Vaganov and others 2009; Zhang and Marshall 1994; Saurer and others 2004). This pattern indicates that in the context of climate change, when more frequent periods of water shortage in north-European regions can be expected (IPCC 2007), the three locally adapted species studied here will be more efficient in using available water resources for carbon acquisition. However, carbon sequestration and storage rates in wood may well decline; indeed, we found that the increase in stand $\delta^{13}C_S$ during the dry year was associated with a decrease in BAI_S. This is unsurprising as numerous studies conducted in boreal forests have shown lower productivity under water limiting conditions (for example, Kljun and others 2007).

Biodiversity Effects Under Wet Conditions

Our results confirmed our first hypothesis that under non-limiting soil water conditions there would be little or no effect of biodiversity on $\delta^{13}C_S$ and BAI_S (Figure 2). Interactions among species under non-limiting soil water conditions did not lead to any clear B–EF relationships in our boreal study forests, which is consistent with the expectations of the "stress-gradient" hypothesis (Bertness and Callaway 1994). This result is also

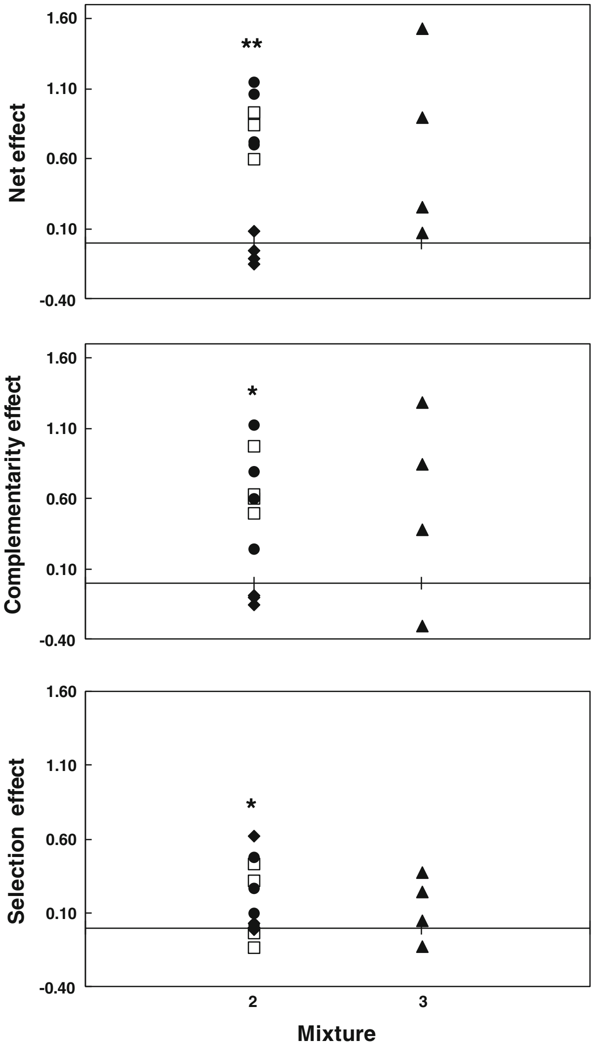

Figure 3. Net, complementarity, and selection effects on stand carbon isotope composition ($\delta^{13}C_S$, ‰) for the dry year as a function of species richness. *Black circles* indicate birch and pine mixtures, white *squares* indicate birch and spruce mixtures, *black diamonds* indicate pine and spruce mixtures, and *black triangles* indicate 3-species mixtures. *Asterisks* denote significant differences from zero in the biodiversity effects for each species richness level (*t* test, $^*P < 0.05$; $^{**}P < 0.01$).

consistent with a recent study we conducted over a wet summer on the influence of biodiversity on species- and stand-level carbon and water balance in a young boreal plantation in Finland (Grossiord and others 2013a). Therein, we argued that in addition to non-limiting soil water conditions, the young ontogenic stage of the plantation likely caused the absence of any biodiversity effect. However, the present study does not support this assumption. Similar observations made in other ecosystem types also reported the absence and/or the decrease of biodiversity effects under non-limiting environmental conditions (for example, Hughes and Stachowicz 2004; Steudel and others 2012).

Contrasting Biodiversity Effects Under Wet and Dry Conditions

The major result of this study was the positive relationship between biodiversity and the difference in $\delta^{13}C_S$ between dry and wet years ($\Delta\delta^{13}C_S$) (Figure 2). Drier conditions globally enhanced $\delta^{13}C_S$, and thus WUE_S, but this influence depended on species interactions. Even though the relationship was rather dispersed, stands with greater diversity showed the greatest increase in $\delta^{13}C_S$, pointing to a greater compromise between carbon accumulation and water consumption under limiting soil water conditions. However, those changes in ecosystem-scale physiological acclimatization to drought failed to minimize the decrease in BAI_S during the dry year and did not influence the productivity of these plots the following year (Figure 2). The literature actually mentions this apparent inconsistency (for example, Vaganov and others 2009). The compromise between carbon acquisition and water loss at leaf or plant level usually has a moderate effect on how much assimilated carbon is invested in secondary growth (Flanagan and Johnsen 1995). Carbon allocation to above- or belowground components in response to soil water conditions strongly varies among species (for example, Axelsson and Axelsson 1986) and could partially explain the absence of enhanced BAI_s in our study.

Origin of the Biodiversity Effects

Partitioning the influence of biodiversity on $\delta^{13}C_S$ into selection and complementarity effects revealed that during the dry year the proportionally greater increase in WUE_S observed in the 2-species mixtures was a result of both mechanisms (Figure 3). Selection effects refer to a higher performance of one or several species in mixtures on the cost of others. Therefore, the pattern of increasing $\delta^{13}C_S$ we observed was most likely driven by the two conifers rather than by birch, because both conifers showed higher $\delta^{13}C_i$ in 2-species mixtures whereas birch did not (Table 1). Higher $\delta^{13}C_S$ values in the 2-species mixtures with birch were also partially explained by a complementarity effect, probably as a result of different rooting strategies among the species. Birch is known to have deeper rooting systems that explore larger volumes than those of pine and spruce, thus enabling birch to access both shallow and deep soil layers (Kalliokoski and others 2008). The fact that birch $\delta^{13}C_i$ values did not respond to increasing diversity during the dry year supports this assumption and suggests that the effects of intra- and inter-specific interactions for water acquisition for this species are similar. In contrast, the complementarity effect for $\delta^{13}C_S$ was not significant in stands where the two conifers cohabited. *Pinus sylvestris* and *Picea abies* are known to present similar rooting system development when cohabiting in mixed stands (Kalliokoski and others 2008). It therefore appears that the two conifers were in part functionally redundant in terms of carbon and water acquisition and use in these mixed stands.

Although in the 3-species mixtures none of the three biodiversity effects were significantly different from zero, overall they were generally consistent with results for the 2-species mixed stands (Figure 3). Numerous B–EF studies have revealed such saturating or even hump-shaped relationships (for example, Gamfeld and others 2013; Vilà and others 2013). One explanation for this type of pattern is increasing functional redundancy at higher levels of species richness (as observed for the two conifers here), which leads to faster and more complete exploitation of available resources in stands with greater richness.

Other Potential Origins of the Biodiversity Effects

Variations in $\delta^{13}C$ in tree rings reflect physiological processes of acclimatization to environmental conditions and physical archives of short- or long-term changes in soil moisture and/or air humidity in forests (Saurer and others 2004; Andreu and others 2008; Mölder and others 2011). Therefore, the significantly higher $\delta^{13}C_S$ values we observed along the diversity gradient in the dry year could be viewed not only as a consequence of species interactions, but also as a result of lower soil water availability in mixed stands as compared to pure ones. High soil moisture exhaustion in mixed stands during dry years was already reported in other hydrological investigations in mixed species forests (Gebauer and others 2012). Such a feedback effect could arise because of the particularly high competitiveness of birch for water resources and its high water consumption (Kalliokoski and others 2008). This would lead to a higher drought exposure for the conifers in the mixed stands and thus reduced transpiration through stomatal regulation. This increased drought exposure may have been also the cause for the absence of any increase in BAIs in the mixed stands. Nevertheless, differences in canopy structure inducing different atmospheric humidity and light conditions between pure and mixed stands (Kelty 2006) as well as belowground competition among these species for nutrients (nitrogen, phosphorus, etc.) may also have contributed to the mixture effects we detected; however, this is beyond the scope of the present study.

CONCLUSION

In our boreal study region, we demonstrated a clear interaction between the biodiversity/ecosystem carbon and water flux relationship and environmental conditions, consistent with the ''stress gradient'' hypothesis. The increase in $\delta^{13}C_S$, and thus in ecosystem-level water use efficiency, with drier soil conditions was much higher in mixed stands than in pure ones, but this did not prevent a decrease in aboveground growth rates with drought. At the species level, functional similarities between the two conifers lead to a redundant contribution of these two species to ecosystem-level carbon and water balance. In contrast, the presence of birch in mixed stands strongly impacted the water use efficiency of the conifers under limiting water conditions. Our results suggest that species interactions in mixed stands during dry growing seasons can lead to lower soil moisture availability as compared to pure ones. This illustrates that biodiversity effects can also be negative in mixed stands in the sense that soil resources can be more intensively exhausted. Our study highlights the need to take into account not only species growth characteristics but also species functional traits when managing boreal-mixed forest to bolster-mixed forests in a context of climate change.

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Paper VII

Tree diversity does not always improve resistance of forest ecosystems to drought

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Climate models predict an increase in the intensity and frequency of drought episodes in the Northern Hemisphere. Among terrestrial ecosystems, forests will be profoundly impacted by drier climatic conditions, with drastic consequences for the functions and services they supply. Simultaneously, biodiversity is known to support a wide range of forest ecosystem functions and services. However, whether biodiversity also improves the resistance of these ecosystems to drought remains unclear. We compared soil drought exposure levels in a total of 160 forest stands within five major forest types across Europe along a gradient of tree species diversity. We assessed soil drought exposure in each forest stand by calculating the stand-level increase in carbon isotope composition of late wood from a wet to a dry year ($\Delta\delta^{13}C_S$). $\Delta\delta^{13}C_S$ exhibited a negative linear relationship with tree species diversity in two forest types, suggesting that species interactions in these forests diminished the drought exposure of the ecosystem. However, the other three forest types were unaffected by tree species diversity. We conclude that higher diversity enhances resistance to drought events only in drought-prone environments. Managing forest ecosystems for high tree species diversity does not necessarily assure improved adaptability to the more severe and frequent drought events predicted for the future.

climate change | ecology | species interaction |
carbon isotope composition | FundivEUROPE

Biodiversity plays an important role in ecosystem functioning by promoting a wide range of functions and services (1–3). This beneficial effect of biodiversity is determined by mechanistic processes directly under the influence of species interactions: complementarity among species for resource use through ecological niche partitioning and/or facilitation processes increase ecosystem performance because resources are better shared among neighboring species and are thus potentially more available (4). Previous studies have demonstrated that, apart from enhancing performance, diverse terrestrial ecosystems may also be more resilient and more resistant to biotic stresses such as insect pests or diseases (5, 6). However, it remains unclear whether higher biodiversity also leads to improved resistance of terrestrial ecosystems to the more frequent droughts expected in temperate regions (7). The rare case studies published thus far have shown contrasting results. Two reported that species in more diverse ecosystems could be more resistant to drought stress (8, 9), whereas another suggested that enhanced biodiversity could trigger higher exposure to drought (10). Improving our understanding of how species diversity influences the resistance of terrestrial ecosystems to a fluctuating climate is crucial.

More frequent and intense droughts will greatly affect the carbon and water cycles of the terrestrial biosphere (11), in particular in forested ecosystems (12). Many societies around the world rely on forests for essential services such as wood production, hunting, or watershed protection. We therefore urgently need to improve our knowledge of the physiological response of these ecosystems to drier climatic conditions to propose new climate-smart management options. Forests, although influenced by local environmental conditions, play a major role in the global carbon and water balance as they release into and assimilate from the atmosphere huge amounts of CO_2 while losing water vapor through transpiration. Tree species are known to vary widely in the ecological strategies they use to deal with drought stress. It could therefore be expected that in highly diverse forests composed of tree species with contrasting functional traits, limited water resources could be better partitioned among the neighboring species as a result of complementarity and facilitation processes (4). Such forests should be more resistant to deal with drought stress because the trees should be able to maintain better access to diminishing water resources as the drought

Significance

In the context of climate change, expected drier and warmer environmental conditions will have drastic consequences on forest functions and services and may bring about important drought-induced die-off events. Biodiversity promotes forest ecosystem performance and resistance to insect pests and diseases, but whether or not diverse forests are also better adapted to deal with drought stress remains unknown. Within our study network of 160 forest stands across Europe, we found that mixed species forests are less exposed to drought stress in some regions only. Therefore, managing forest ecosystems for high tree species diversity does not necessarily assure improved resistance to the more severe and frequent drought events predicted for the future.

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progresses. In contrast, if the interacting species in a diverse forest have similar functional traits (i.e., functional redundancy), ecological niche overlap (13) may lead to more stressful conditions during drought than in pure stands due to lower water availability for each species.

Carbon isotope composition in C_3 plant tissues ($\delta^{13}C$) provides an integrated record of the ratio of intercellular to atmospheric CO_2 concentrations during the period when the carbon was fixed and thus reflects the balance between net CO_2 assimilation and stomatal conductance (14). Plants typically react toward drought stress by closing their stomata and reducing carbon assimilation rates. However, leaf stomatal conductance is affected to a greater extent than assimilation, causing a concomitant increase in $\delta^{13}C$ (14, 15). Therefore, under soil drought conditions, $\delta^{13}C$ from organic material has been widely accepted as an indicator of the intensity of drought exposure in plants (16, 17) (*SI Text*). If complementarity for water use is occurring among species, $\delta^{13}C$ values should increase less between wet and dry soil conditions with increasing tree species diversity (i.e., a negative relationship). Inversely, if tree species occupy redundant ecological niches, $\delta^{13}C$ values should either have a similar or higher increase between wet and dry conditions with increasing tree species diversity (i.e., a null or positive relationship).

In a previous study, we analyzed the influence of drought on the relationship between tree species diversity and the increase in stand-level carbon isotope composition between a wet and dry year ($\Delta\delta^{13}C_S$) in boreal forests (10). Species diverse forests were shown to be more affected by drought stress than less diverse ones (i.e., a positive relationship between $\Delta\delta^{13}C_S$ and tree species diversity). In the present study, we extend our research to five major forest types across Europe, which extends from northern hemiboreal forests to southern Mediterranean forests (Table S1). Our objective was to test whether the relationship between $\Delta\delta^{13}C_S$ and tree species diversity would be consistent across a large range of climatic and edaphic conditions. At each of the five study sites, we selected a set of representative canopy trees (Table S2) in 21–42 forest stands varying in tree species diversity. For each site, we used a water balance modeling approach to select 1 y within the last 14 y with high drought stress and 1 reference y when no drought occurred (Figs. S1 and S2). We measured the $\delta^{13}C$ in the tree rings of the selected canopy trees and calculated $\Delta\delta^{13}C_S$ for each stand.

Results

Within a given forest type, there was a large variability among species in $\Delta\delta^{13}C$ values (Fig. S3), pointing out that drought response highly varied across tree species. Nevertheless, whatever the forest type, $\Delta\delta^{13}C$ always displayed positive values (Fig. S3), which is consistent with the considerable literature on the impact of drought on tree- or species-level $\delta^{13}C$ (16, 17). This result confirmed that the tree rings selected for the targeted dry year coincided with years when all tree species clearly suffered from a severe and long-lasting water shortage.

Mean site $\Delta\delta^{13}C_S$ values were highly variable across the different forest types (Fig. 1). This variability can be partly explained by the large differences in the intensity of drought stress during the selected dry years across forest types (Table S3). We found a wide variability in $\Delta\delta^{13}C_S$ among forest stands within a given forest type (Fig. 2). The observed range of values (typically around 2‰) suggested highly contrasting ecosystem-level carbon and water tradeoffs among stands indicating highly contrasting soil water availability during the dry year. Among the confounding factors that were included in the $\Delta\delta^{13}C_S$ statistical model (competition intensity, light interception levels, microclimate, and soil nutrient availability), in the hemiboreal and mountainous beech forest types only competition intensity explained part of the large variability (Fig. S4), the other factors being nonsignificant (Table S4). In the temperate beech and thermophilous deciduous forest types, variations in $\Delta\delta^{13}C_S$ were only significantly correlated with tree species diversity (Fig. 2 and Table S4). Variations observed in the Mediterranean forest were not explained by tree diversity nor by any of the confounding factors (Table S4).

Discussion

The positive relationship between $\Delta\delta^{13}C_S$ and tree species diversity that had been previously observed for boreal forests (10) indicated a higher drought exposure in the more diverse stands. This previous study suggested that drought stress could exacerbate competition for water among neighboring tree species. However, in the present study, we found evidence that such a detrimental effect of tree species diversity cannot be generalized to European forest types. Indeed, we observed either no relationship (hemiboreal, mountainous beech, Mediterranean forests) or negative relationships (temperate beech, thermophilous deciduous forests) between $\Delta\delta^{13}C_S$ and tree species diversity (Fig. 2).

The negative relationship between $\Delta\delta^{13}C_S$ and tree species diversity that we observed for temperate beech and thermophilous deciduous forests implies that water availability was higher in diverse stands than in pure ones under drought conditions. These observations are consistent with other studies in which tree species diversity was found to reduce drought stress (8, 9). Higher water availability in more diverse stands suggests niche partitioning and/or facilitation processes among the interacting species. Below-ground partitioning may occur when species that extend their root systems toward deeper soil layers coexist with

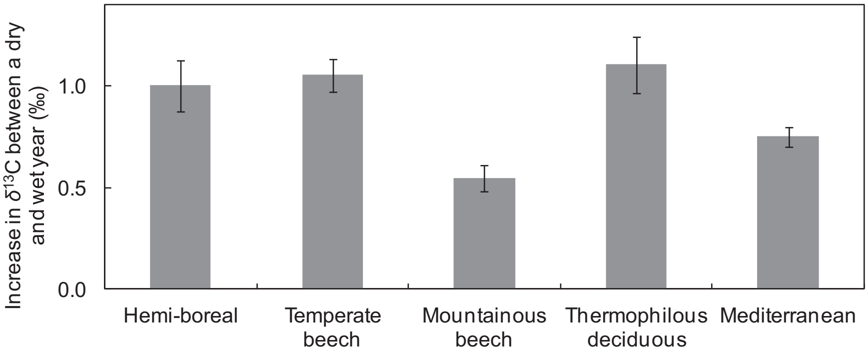

Fig. 1. Intensity of the physiological response to drought stress during the targeted dry year. Mean ± SE of the increase in stand-level carbon isotope composition ($\Delta\delta^{13}C_S$, ‰) between the dry year and the year without water stress are shown for all stands with all tree species diversity levels for a given forest type. The increase in $\Delta\delta^{13}C_S$ between dry and wet conditions characterizes the intensity of drought stress to which the ecosystems were subjected.

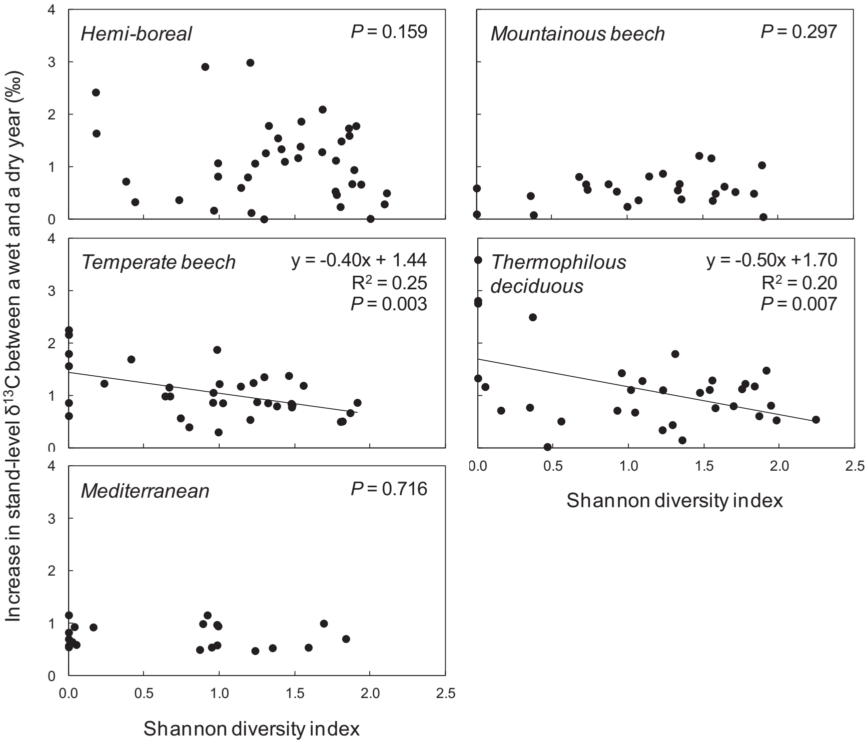

Fig. 2. Increase in stand-level carbon isotope composition in relation to tree species diversity for each forest type. Relationship between the increase in stand-level carbon isotope composition ($\Delta\delta^{13}C_S$, ‰) and the Shannon diversity index for the hemiboreal, mountainous beech, temperate beech, thermophilous deciduous, and Mediterranean forest types. Solid lines show the statistically significant relationships ($P < 0.05$). Corresponding equations, R^2, and P values are given for each significant relationship.

others that occupy the superficial layers. Facilitation mechanisms such as hydraulic lift whereby deeper rooting tree species take up water and redistribute it via their root system to drier superficial soil layers are also known to take place in mixed forest ecosystems (18). Higher functional diversity of the fungal community could also partially improve water availability in more diverse stands (19). These underlying processes nevertheless remain speculative and future research is needed to shed light on the relative contribution of these potential processes (20).

For the three other forest types (hemiboreal, mountainous beech, and Mediterranean), the relationship between $\Delta\delta^{13}C_S$ and tree species diversity was nonsignificant (Fig. 2), suggesting that no net resource partitioning or facilitation processes were occurring. For these three forest types, tree species diversity did not play an important role in modulating ecosystem-level response to drought stress, despite strong functional differences among species in their response to drought (Fig. S3). Intraspecific and interspecific interactions therefore seem to have an equal influence on the level of drought exposure in these ecosystems. The tree species that compose these forests may be "ecological equivalents" (21) that use similar competitive strategies to deal with drought stress. However, for both the hemiboreal and mountainous beech forests, the variability among stands in $\Delta\delta^{13}C_S$ was partly explained by competition intensity (i.e., basal area; Fig. S4). Stands with higher basal area showed the greatest increase in $\Delta\delta^{13}C_S$ and thus the strongest soil water limitation. In these forest types, silvicultural practices controlling stand basal area may therefore be one management option to improve resistance to drought.

Environmental conditions are highly variable along the north-south gradient considered in this study and could account for the inconsistency of the response among the different forest types. Negative relationships occurred in the temperate beech and thermophilous deciduous forests, the two sites that showed the highest mean drought stress intensity and the highest drought stress frequency over the last 14 y (Table S3). In contrast, drought events in the boreal and hemiboreal forests were moderate and rather rare (Table S3), and these are the sites where we observed positive relationships (10) or no effect of species interaction. Thus, overall, the global pattern we found seems consistent with the "stress-gradient hypothesis" (22, 23), although our study was not designed to test this hypothesis. This hypothesis indeed suggests that facilitation and complementarity processes should occur more frequently and should be more important under drier conditions, whereas competition should dominate under favorable ones. The Mediterranean forest was also characterized by high drought stress intensity and frequency (Table S3), but we found no effect of species interactions. We suspect that the very shallow soils found at this site prevented any chance for the establishment of below-ground complementarity processes such as root stratification.

We conclude that higher tree species diversity offers a greater resistance to drought events in some forest types but that this pattern cannot be generalized to all forest ecosystems. Forest response is likely to be context dependent. Local tree species associations are probably interacting with local environmental conditions, and this would explain the complexity of the relationship between biodiversity and forest resistance to drought stress we observed across Europe. Managing forest ecosystems for high tree species diversity does not necessarily assure improved resistance to the more severe and frequent drought events predicted for the future.

Materials and Methods

Experimental Sites. The sites used in this study are included in a permanent network of stands established in 2011–2012 in existing mature forests in five European countries (Germany, Poland, Romania, Italy, and Spain) within the framework of the FP7-FunDivEurope project (24). The study areas include highly variable climatic conditions and host Europe's most important broadleaved and coniferous tree species (*SI Text*). In each site, 21–42 forest stands (30 × 30 m) were selected for a total of 160 stands covering a gradient of tree species diversity, from pure to the local maximum of between three and five species per stand (*SI Text*). Each level of tree species diversity was replicated approximately 10 times, but with different species compositions: the experimental setup was not designed to separate the effect of the presence of particular species or particular combinations of species from the effect of tree species diversity. To minimize confounding factors among stands within a given site, stands were selected for similar developmental stage, soil characteristics, slope, altitude, past management practices, and canopy structure characteristics (24). This selection focuses the study on rather average site conditions and is not suited to assess the influence of species diversity across a wider range of environmental factors. With this design we aimed, however, to eliminate other local factors that could have affected ecosystem functioning as much as tree species diversity. Nevertheless, some variability among stands within a given site could not be avoided, and stand characteristics were included in the statistical analyses as confounding factors. A detailed description of stand selection and characteristics can be found in *SI Text* and ref. 24. To characterize each stand's tree species diversity, we calculated the Shannon diversity index of each stand with species basal area as a surrogate for abundance in the equation (*SI Text*).

Selection of Target Years. To select a year with nonlimiting soil water conditions during the growing season and a year with severe soil drought during the growing season, we used the water balance model BILJOU (25) to estimate the daily REW (unitless) for each site during the period from 1997 to 2010 (Fig. S1). Based on REW, a water stress index (25) was calculated and used to select the 2 y (Fig. S2).

Wood Sample Preparation and Analyses. We selected a subsample of dominant and codominant trees per species and per stand to avoid confounding factors related to light interception (*SI Text*). For each tree, we extracted one wood core at breast height and carefully extracted the latewood for each selected year. Latewood sections from a given species, a given year, and a given stand were bulked and analyzed for δ^{13}C with a mass spectrometer. By selecting only the latewood, whose δ^{13}C mainly characterizes the functioning of the trees during the second part of the growing season, we avoided potential effects related to the remobilization of stored photosynthates from the previous growing season (26) or to a favorable spring climate.

Stand-Level Carbon Isotope Composition. We calculated the stand-level δ^{13}C for each year using species-level δ^{13}C, with the sum of basal area of each species in each stand as the weighting factor for the contribution of each species (*SI Text*). $\Delta\delta^{13}C_S$ was then defined as the increase in stand-level δ^{13}C between the dry and the wet conditions and therefore characterizes the ecosystem-level physiological response to soil drought.

Characterization of Confounding Factors. The following stand characteristics were measured to take them into account in the statistical model: leaf area index (i.e., light regimes), soil C/N (i.e., nutrient availability), stand basal area (competition intensity), and altitude (local climatic conditions).

Data Analyses. For each site, linear mixed models were used to determine the fixed effects of the Shannon diversity index and the confounding factors on $\Delta\delta^{13}C_S$ (Table S4). Interactions between the Shannon index and the confounding factors were initially integrated into the model but then were removed because none were significant.

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Paper VIII

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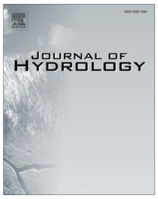

Impact of interspecific interactions on the soil water uptake depth in a young temperate mixed species plantation

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SUMMARY

Interactions between tree species in forests can be beneficial to ecosystem functions and services related to the carbon and water cycles by improving for example transpiration and productivity. However, little is known on below- and above-ground processes leading to these positive effects. We tested whether stratification in soil water uptake depth occurred between four tree species in a 10-year-old temperate mixed species plantation during a dry summer. We selected dominant and co-dominant trees of European beech, Sessile oak, Douglas fir and Norway spruce in areas with varying species diversity, competition intensity, and where different plant functional types (broadleaf vs. conifer) were present. We applied a deuterium labelling approach that consisted of spraying labelled water to the soil surface to create a strong vertical gradient of the deuterium isotope composition in the soil water. The deuterium isotope composition of both the xylem sap and the soil water was measured before labelling, and then again three days after labelling, to estimate the soil water uptake depth using a simple modelling approach. We also sampled leaves and needles from selected trees to measure their carbon isotope composition (a proxy for water use efficiency) and total nitrogen content. At the end of the summer, we found differences in the soil water uptake depth between plant functional types but not within types: on average, coniferous species extracted water from deeper layers than did broadleaved species. Neither species diversity nor competition intensity had a detectable influence on soil water uptake depth, foliar water use efficiency or foliar nitrogen concentration in the species studied. However, when coexisting with an increasing proportion of conifers, beech extracted water from progressively deeper soil layers. We conclude that complementarity for water uptake could occur in this 10-year-old plantation because of inherent differences among functional groups (conifers and broadleaves). Furthermore, water uptake depth of beech was already influenced at this young development stage by interspecific interactions whereas no clear niche differentiation occurred for the other species. This finding does not preclude that plasticity-mediated responses to species interactions could increase as the plantation ages, leading to the coexistence of these species in adult forest stands.

1. Introduction

Current climate change models predict an increase in the intensity and frequency of drought events in central Europe in the coming decades (Christensen et al., 2007). Since one of the key drivers of wood production and sustainability of forest ecosystems is water availability (Breckle, 2002), the expected drier climatic conditions represent a serious threat to their productivity and resilience. To maintain high wood production rates in forest ecosystems in the future, we must elaborate new climate-smart management strategies that are able to counteract the effects of a drier climate on water availability for forest trees.

During the last few decades, accumulated evidence has brought to light beneficial effects of tree diversity on forest ecosystem

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Nomenclature

δ^2H_S	deuterium isotope composition of soil water (‰)
δ^2H_X	deuterium isotope composition of xylem water (‰)
$\delta^{13}C$	foliar carbon isotope composition (‰)
ψ_{pd}	predawn leaf water potential (MPa)
BA	cumulative basal area of the surrounding trees (cm^2)
D_S	mean soil water uptake depth (cm)
H	Shannon biodiversity index (unitless)
LMA	leaf mass per unit area ($g\ m^{-2}$)
N%	foliar nitrogen content (%)
P	total daily precipitation ($mm\ day^{-1}$)
PFT_C	percentage of conifer trees of the surrounding trees (%)
REW	daily relative extractable soil water (unitless)
R_G	total daily global radiation ($MJ\ m^{-2}$)

functions and services such as productivity (for a review see Scherer-Lorenzen, 2014). The degree to which resources are available for productivity depends on the capacity of a plant to exploit the resources available in the ecosystem – a capacity that can be enhanced by complementarity mechanisms. Indeed, while coexisting species compete with each other for resources, thereby influencing the structure and dynamics of the whole community. Complementarity in mixed species forests implies that co-occurring species exploit distinct ecological niches and can then use the available resources at different places or at different times (Loreau and Hector, 2001). The partitioning of ecological niches in mixed-species forests results either from inherent differences among species or from a differentiation that occurs in species ecological niches due to plasticity-mediated responses (Valverde-Barrantes et al., 2013). The consequence of this so-called niche stratification in mixed-species ecosystems is that during resource-limiting events or periods, competition for resources among species is lessened, and vital functions like transpiration and photosynthesis can be maintained at higher levels than in mono-specific situations.

Complementarity refers to above- and below-ground niche partitioning among species. Above-ground niche stratification in mixed-species forests is achieved because tree species have differing canopy structures; this leads to more efficient light interception in mixed stands than in monocultures (Kelty, 2006). This effect can promote plant photosynthesis and is therefore often cited as one of the main positive mechanisms underlying the higher productivity of mixed-species forested ecosystems (Forrester, 2014). However, higher photosynthesis rates necessarily induce higher transpiration rates in mixed-species communities. This might result in faster exhaustion of soil water levels, which in turn would be detrimental to community productivity. This negative effect can, however, be compensated for by below-ground niche stratification among species. Indeed, tree species also inherently differ in their rooting depth strategies. Some species typically restrict their roots to shallow soil layers while others develop a deep rooting system (e.g. Zapater et al., 2011). Furthermore, despite genetic constraints, plants display plasticity-mediated responses to neighbourhood interactions at below-ground level (e.g. Valverde-Barrantes et al., 2013); these diverging capacities of response to biotic interactions among species also contribute to partitioning in the below-ground space occupancy.

Many studies on rooting depth or root biomass distributions in mixed temperate forests have found evidence of below-ground niche stratification among species (Bolte and Villanuena, 2006; Hendriks and Bianchi, 1995). However, these vertical profiles do not precisely reflect the depth at which a given tree species is able to extract water under limiting soil water conditions. We must know the precise soil water uptake depth for the species coexisting in a given mixed stand to be able to clearly determine whether complementarity for water acquisition is actually occurring.

Assessing the stable isotope (deuterium or oxygen) composition of water in soil and plants is a powerful – and non-destructive – tool to help determine plant water acquisition patterns and complementarity in water use (Ehleringer and Dawson, 1992). Since roots do not fractionate against the heavier isotope (^2H or ^{18}O vs. ^1H and ^{16}O, respectively) during water uptake (Dawson and Ehleringer, 1993), the instantaneous isotope signature of water in the xylem reflects the mean isotope composition of water extracted from the soil. When a clear vertical gradient in soil water isotope composition exists, the comparison of plant (i.e. xylem) water isotope composition with that of soil water from different soil depths reveals the actual mean soil water uptake depth by a plant. Evaporation at the soil surface and precipitation with different isotopic composition both result in vertical isotope profiles that vary with soil depth (Ehleringer and Dawson, 1992). However, these processes do not always result in simple log-shaped vertical gradients (e.g. Bonal et al., 2000) and this can make interpreting the water uptake depth impossible. However, by applying highly isotopic enriched water onto the soil surface, we can create an artificial vertical gradient of soil water isotope signatures which allows us to accurately identify the depth of mean soil water uptake.

In this study, we followed such an approach. At the end of the 2013 summer in a 10-year-old plantation in central Germany with varying degrees of species mixture, we labelled the superficial soil layers with deuterium enriched water and then tested whether interspecific interactions influenced the soil water uptake depth for four temperate tree species – European beech, Sessile oak, Norway spruce and Douglas fir. We compared how the soil water uptake depth for these species varied according to species diversity, competition intensity and the presence of different plant functional types in their direct neighbourhood (i.e. surrounding trees). We further tested whether variation in soil water uptake depth were related to other functional traits that reflect both the competitive strength of a tree species and its access to soil resources. To do this, we analysed the effect of species interactions on foliar carbon isotope composition (i.e. a proxy for water use efficiency) and total nitrogen content.

2. Materials and methods

2.1. Site description

This study was conducted within the framework of the European FunDivEUROPE project, in a 10-year-old stand originally planted for a biodiversity experiment (BIOTREE-SIMPLEX site) (Scherer-Lorenzen et al., 2007) located near Kaltenborn in Thuringia, Germany (50.24°N, 07.00°E, 325 m a.s.l). The climate is Sub-Atlantic, with mean minimum temperatures of −2 °C and 16 °C during the winter and the summer and mean maximum values of 5 °C and 19 °C during the winter and summer (1997–2013 period). Mean annual precipitation is of 651 mm, rather equally distributed throughout the year. The bedrock, belonging to the lower Buntsandstein formation, consists of sandstone disrupted by some schist/clay layers. More detailed information about the

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experimental site has been provided by Scherer-Lorenzen et al. (2007).

For the biodiversity experiment (Scherer-Lorenzen et al., 2007), thirty-six 16 m × 16 m plots were planted during the winter of 2003/2004 following a species replacement series with a simplex design (Cornell, 1990; Ramseier et al., 2005) of European beech (*Fagus sylvatica* L.), Sessile oak (*Quercus petraea* Liebl.), Norway spruce (*Picea abies* (L.) Karst.) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) plants of regional provenances.

These four species are known to present important differences in root topology, particularly between the two broadleaved species and the two coniferous species. When growing on similar soils, beech and oak tend to develop root systems with a few dominant large woody roots that rapidly branch into roots of lesser diameters called "heartroot" (Drexhage et al., 1999; Mauer and Palátová, 2011). Inversely, adult trees of Douglas fir and spruce have roots that are usually found in the humus-reach and superficial layers of the soil and develop very dense roots calls "sinker" (Eis, 1974; Kalliokoski et al., 2008).

At the time of planting, the seedlings that were initially grown in a seedbed were 3–4 years old and 20–60 cm high. All possible mixtures were applied with two plantation densities (1 × 1.5 m and 2 × 1.5 m) and three planting designs: centroids (equal proportions of each species, i.e. 25:25:25:25), intermediate points (three co-dominant species and one subordinate one, i.e. 30:30:30:10) and corner points (a high proportion of one dominant species, i.e. 70:10:10:10). The position of each tree within the plots was randomly assigned. For this study, we selected five plots (one centroid and four corner point plots) in the high-density treatment with 1.5 m between planting rows and a within-row tree-to-tree distance of 1 m (Fig. 1).

To select the study trees, we applied the following criteria. After excluding all trees in the border rows of the selected plots, we first calculated the Shannon biodiversity index (*H*) of each remaining tree by taking into account the basal area of each of the eight trees directly surrounding the focal tree (Fig. 1). We then calculated the cumulative basal area of these surrounding trees (*BA*) as an indicator of the competition intensity each focal tree was subjected to. To estimate the effect of the interaction between different plant functional groups, we calculated the percentage of conifer trees (PFT_C, %) of the eight surrounding trees and established three classes of PFT_C: 0–33%, 33–66% and 66–100%. We then selected 35 trees per species distributed along an *H* index gradient from 0.0 to 1.7 and a *BA* gradient of 3–60 cm^2, and including the three PFT_C classes (Table 1). In order to avoid any confounding effects caused by differing light interception levels, we excluded all suppressed trees (i.e. which were not dominant or co-dominant). Twenty-three oak, 28 beech, 30 Norway spruce and 33 Douglas fir trees were selected for the final analyses (Table 1).

2.2. Soil water balance

In order to characterize the soil water availability during the summer of 2013, we calculated the daily relative extractable water in the soil (*REW*, unitless) from April 2013 to September 2013 (Fig. 2). *REW* varies by definition between 1.0 (field capacity) and 0.0 (permanent wilting point). When *REW* remains between 1.0 and 0.4, it does not limit tree transpiration (Granier et al., 1999). Tree transpiration in this case depends only on air humidity, irradiance and leaf area index. When *REW* falls below 0.4, the resulting water shortage induces a drop in stomatal conductance and thus in leaf gas exchange for most tree species (Granier et al., 1999).

In order to calculate *REW*, we used the "BILJOU©" generic model for forest water balance (Granier et al., 1999, see https://app-geodb.nancy.inra.fr/biljou/). The model calculates the different water fluxes in the ecosystem (tree transpiration, understory evapotranspiration, rainfall interception and drainage) at a daily time scale based on daily rainfall (*P*), global radiation (R_G), air temperature and humidity, and wind speed. Data were obtained from a meteorological station (50.48°N, 10.13°E, 300 m a.s.l.) located 3 km from the site. In order to parameterize the model, a mean leaf area index of 7.0 m^2 m^{-2} was used (S. Mueller, pers. com). Maximum extractable soil water (i.e. the soil water holding capacity) was calculated based on measurements of the texture and thickness of each horizon of the soil down to 135 cm depth (see Scherer-Lorenzen et al., 2007 for more details) and was equal to 150 mm.

2.3. Labelling experiment

2.3.1. Calculation of soil area to label

For the labelling experiment, we considered that the majority of roots of a given tree in the plantation was confined within a 2-m

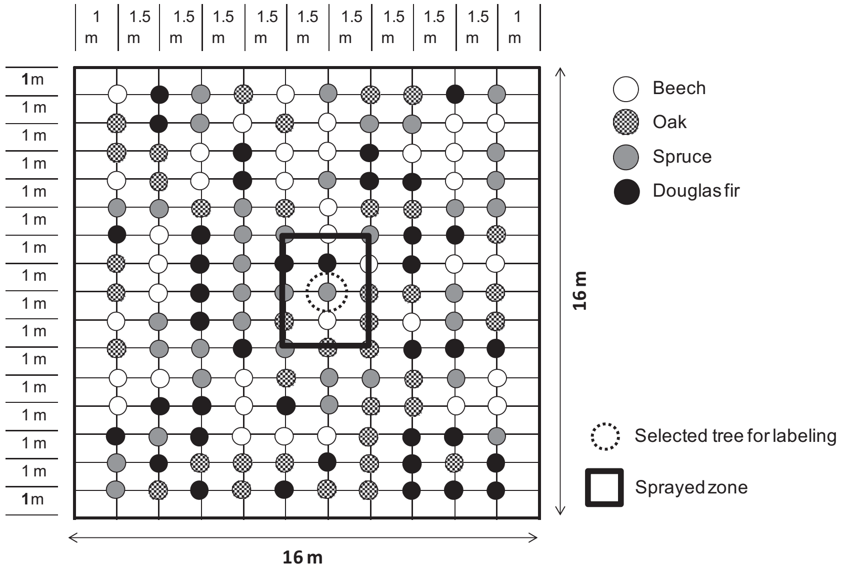

Fig. 1. Schematic representation of a study plot, with area sprayed with highly enriched water around a selected tree. The example illustrated here is in the centroid plot. The bold black rectangle represents the minimum area sprayed around the selected trees (a spruce tree in this example; circled with a black dashed circle).

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Table 1
Number of sampled trees (N), mean diameter at breast height ($DBH_{1.3}$, cm), mean Shannon index in the neighbourhood (H), mean sum of basal area in the neighbourhood (BA, cm^2), number of trees for each percentage class of conifers in the neighbourhood ($\#PFT_C$), mean soil water uptake depth (D_S, cm), mean carbon isotope composition ($\delta^{13}C$, ‰), mean foliar nitrogen concentration (N%, %), mean leaf mass area (LMA, g m^{-2}) and mean leaf predawn water potential (ψ_{pd}, MPa) with standard errors of the mean for each species. Letters indicate significant differences among species for a given measurement.

	Beech	Oak	Douglas fir	Spruce
N	28	23	33	30
$DBH_{1.3}$ (cm)	5.3 ± 0.4	4.4 ± 0.3	10.1 ± 0.5	8.4 ± 0.5
H	0.8 ± 0.4	1.0 ± 0.5	0.9 ± 0.3	0.9 ± 0.5
BA (cm^2)	31.9 ± 11.9	29.3 ± 10.2	27.6 ± 13.8	29.2 ± 11.1
$\#PFT_{C\ (0–33\%)}$	7	10	11	10
$\#PFT_{C\ (33–66\%)}$	9	6	8	5
$\#PFT_{C\ (66–100\%)}$	12	7	14	15
D_S (cm)	29.1 ± 5.3^a	25.6 ± 4.6^a	52.5 ± 5.7^b	43.4 ± 5.5^b
$\delta^{13}C$ (‰)	−29.7 ± 0.3^a	−29.4 ± 0.2^a	−27.8 ± 0.2^c	−28.6 ± 0.2^b
N% (%)	2.2 ± 0.1^a	2.3 ± 0.1^a	1.6 ± 0.1^b	1.6 ± 0.1^b
LMA (g m^{-2})	83.0 ± 3.8^a	83.8 ± 2.7^a	107.3 ± 2.4^b	116.2 ± 4.5^b
ψ_{pd} (MPa)	−0.49 ± 0.4^{ac}	−0.37 ± 0.3^a	−0.68 ± 0.3^c	−0.53 ± 0.2^b

radius around the tree (Gasson and Cutler, 1990). The rectangle formed by four trees in a given plot represents a 1.5-m^2 area (Fig. 1). For each selected tree, we determined that the area to label was represented by the eight surrounding rectangles (12 m^2; Fig. 1). Given that some selected trees were close to each other, several trees shared parts of the same labelled zone. Although this might have introduced some auto-correlation among our results, it was impossible to select trees that were completely independent from each other due to the selection criteria we applied. Finally, when summing up all area surrounding the selected trees, a total labelled area of 660 m^2 was obtained.

2.3.2. Deuterium labelling

Based on Lei et al. (2012) who had studied the fine root length and surface area in the same plots, we expected that most of the active fine roots of the four tree species would be distributed within the first 50 cm of soil. We therefore aimed to create a strong vertical gradient in soil water deuterium isotope composition (δ^2H_S) in the uppermost soil layers (0–30 cm) in order to distinguish potential differences in the mean soil water uptake depth (D_S) by the fine roots between species and between neighbourhood conditions. To obtain this gradient, we sprayed the soil surface uniformly with a volume of highly deuterium-enriched water equivalent to 3 mm of rain: i.e. 4.5 l of solution for each of the eight rectangles surrounding every selected tree. For the total labelled area of 660 m^2, this equated to 1.98 m^3 of solution.

Preliminary analyses of δ^2H_S at the site had shown that the mean value in the upper soil layers was −52.0‰. Our objective was to obtain δ^2H_S values of around 2000‰. We prepared the labelled water in two 1-m^3 plastic tanks in which we mixed a highly concentrated deuterium solution (99.85 atom%, Eurisotop, FR) with tap water provided by the fire department from Bad Salzungen, a nearby town. The deuterium isotope composition of the prepared solution was 22,500‰. Before spraying the highly deuterium enriched water, coarse litter was removed from the ground in order to ensure rapid percolation; the litter was raked back into place after spraying.

2.3.3. Soil sampling

To determine δ^2H_S, we used a Cobra TT pneumatic soil corer (Eijkelkamp Agrisearch Equipment, NL) to take two soil core samples in the centre of each plot down to a depth of approximately 150 cm: the first core was taken one day before spraying, and the second core was taken three days after the labelled water was sprayed (Fig. 2). This time lapse allowed the labelled water to drain and a vertical gradient of δ^2H to form. Unfortunately, soil sampling after labelling could only be performed in three out of the five study plots due to the mechanical failure of the soil corer. Each core extracted was split into eight 20-cm sections and only the central part of the extracted soil was kept and placed into sealed plastic bags. Great care was taken to rinse the soil corer and the operators' hands with tap water and to carefully dry them after each sample had been collected.

2.3.4. Branch sampling

To determine the deuterium isotope composition of the xylem water (δ^2H_X), a 30–40-cm long branch from each study tree was cut out of the upper third of the tree crown with extension loppers one day before and three days after the deuterium labelling (see dashed arrows in Fig. 2). Bark tissue was immediately removed with a knife in order to prevent phloem sap from contaminating the sample. The samples (5 cm long) were then placed into closed glass airtight vials and stored in cool conditions. Great care was taken to rinse the clippers and the operators' hands with tap water after handling each sample.

2.3.5. Isotope analyses

Soil and branch samples were taken to the INRA Technical Platform of Functional Ecology (OC 081) in Nancy, France, and stored at 2 °C to reduce the risk of evaporation. Water from the soil and branch samples was extracted through cold trapping with a custom-made cryogenic vacuum distillation system. The deuterium isotope composition of each water sample was determined

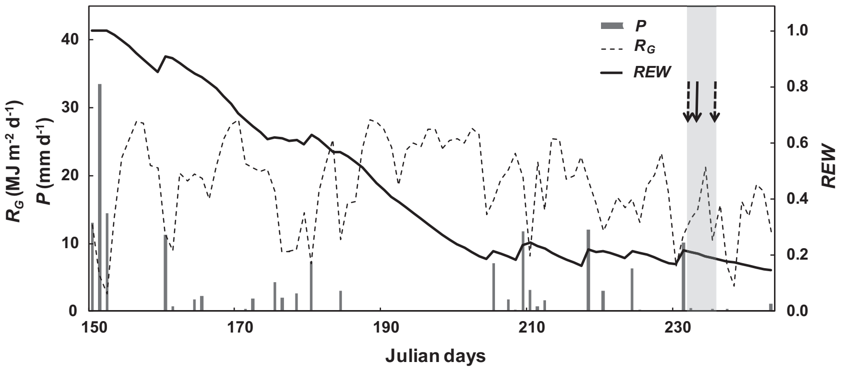

Fig. 2. Seasonal pattern of modelled daily relative soil extractable water (REW, solid line), daily sum of precipitation (P, mm d^{-1}, grey bars) and daily sum of global radiation (R_G, MJ m^{-2} d^{-1}, dashed line) during the summer 2013 (Julian days 150–243). The period indicated in grey represents the duration of the experiment. The vertical bold arrow represents the date of isotope labelling. The vertical dashed arrows represent the date of soil and branch sampling one day before and three days after labelling.

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on two subsamples of 0.4 µl of the extracted water with an IsoPrime isotope ratio mass spectrometer (GV Instruments, Manchester, standard error for repeated measurements of standards ± 0.7‰) coupled to a Pyr-OH liquid auto-sampler (Eurovector, Milan). Two measurements were taken for each water sample but only the second one was retained to avoid any bias related to potential contamination from the previous sample analysed ("memory effect"). The isotopic ratios are expressed relative to the international standard Vienna-standard mean ocean water (V-SMOW) as:

$$\delta = 1000 \times \frac{(R_S - R_{std})}{R_{std}}, \tag{1}$$

where R_S is the ratio of the heavy to the light isotope in the sample and R_{std} refers to the V-SMOW standard.

2.3.6. Estimating mean soil water uptake depth

To estimate mean soil water uptake (D_S) from the δ^2H_X and δ^2H_S values, we used Stahl et al.'s (2013) adapted version of the original model developed by Romero-Saltos et al. (2005). For more detail on this model, please refer to these references. The original model assumes that, for a given time period, trees can extract water from a 50-cm vertical segment of the soil and that the amount of water extracted within this segment follows a normal distribution. Romero-Saltos et al. (2005) selected a length of soil segment of 50 cm based on data from the literature. We conducted several simulations with lengths of 20–60 cm, with a step of 10 cm. These simulations led to a maximum difference in D_S of 5.5 cm for a given tree. The selection of a given length has thus only a minor effect on D_S and we therefore also selected a length of 50 cm.

2.4. Foliar analyses

One day prior to labelling, we used extension loppers to sample 20–30 fully expanded sunlit leaves from the selected broadleaf trees, and three to five 30–40-cm long branches supporting fully mature, sunlit needles from the selected conifers. Each sample was divided into two subsamples which were then placed into sealed plastic bags to avoid any weight loss or in paper bags.

The foliar samples in the sealed plastic bags were stored at 2 °C until total leaf and needle area was determined. Leaf and needle area was determined with an area metre system (Delta-T Devices, UK). The samples were then dried for 48 h at 60 °C and their dry mass was determined with a precision balance (Sartorius, GE). Leaf mass per unit area (LMA; g m^{-2}) (leaf dry mass/fresh leaf area) was then calculated (Table 1).

The foliar samples that were stored in paper bags were oven dried at 60 °C for 48 h at INRA Nancy and then finely ground. About 1.0 mg of the powdered material from each sample was placed into tin capsules to analyse leaf carbon isotope composition ($\delta^{13}C$, ‰) and total nitrogen (N%, %). Analyses were performed with an EA-GC/IRMS (Delta S, Finnigan MAT, Bremen, Germany; standard error of $\delta^{13}C$ for repeated measurements of standards ± 0.2‰) at the INRA Nancy Technical Facility of Functional Ecology (OC 081). Isotopic measurements are reported in the delta notation relative to the Vienna Pee Dee Belemnite (VPDB) standard.

We measured LMA on the foliar samples in order to test any bias related to the potential effect of interspecific interactions on light interception levels. We wanted to avoid such a bias as $\delta^{13}C$ is directly influenced by light interception levels among other environmental conditions (Ferrio et al., 2003).

2.5. Predawn leaf water potential

We used a Scholander-type pressure chamber (PMS Instruments, Corvalis, USA and UP Analytics, Cottbus, Germany) to measure predawn leaf water potential (ψ_{pd}, MPa) on one leaf or one twig per selected tree in the morning prior to labelling. The leaves were sampled between 5:00 and 7:00 a.m. from the upper part of the crown with extension loppers.

2.6. Data analyses

Statistical analyses were performed with the statistical software R 2.14.1 (R Development Core Team, 2011). The variability in δ^2H_X among trees and in δ^2H_S among soil depths obtained before labelling was used to define the threshold at which values of δ^2H_X after labelling would be considered to differ significantly from δ^2H_X values before labelling. Variation in δ^2H_X for a given tree were attributed to deuterium labelling when (1) the difference in δ^2H_X before and after labelling was higher than 30‰ (natural range of variation for a given tree species), and (2) δ^2H_X was higher than – 30‰ after labelling.

A *t*-test was used to compare δ^2H_S values before and after labelling for each soil depth. General linear models were used to test for an effect of species, *H*, *BA* and PFT_C classes on D_S, LMA, $\delta^{13}C$, N% and ψ_{pd}. For each species separately, we then repeated the test by analysing the effects of *H*, *BA* and PFT_C classes on D_S, LMA, $\delta^{13}C$, N% and ψ_{pd}.

3. Results

3.1. Climatic and soil water conditions

REW slowly dropped from the beginning of April and reach the value of 0.4 at the beginning of July (Julian day 192) (Fig. 2). *REW* continued to drop until it stabilized at the end of July with a mean value of 0.2 (Fig. 2). Small rain episodes occurred during August but were insufficient to recharge *REW* above the 0.4 threshold. Although two days prior the labelling 10.1 mm of rain were recorded in one day, *REW* was maintained around 0.2 during the experiment.

3.2. Water uptake depth

Before labelling, δ^2H_S showed a significant ($P < 0.001$) decrease from −46.4‰ to −67.3‰ between the soil surface and the 30 cm depth (Fig. 3a). Below 30 cm, there were no significant differences ($P > 0.050$) in δ^2H_S between soil layers (Fig. 3a).

Labelling induced a strong increase in δ^2H_S in the 10-cm depth superficial layers with a mean value of 677.3‰ (Fig. 3b). δ^2H_S values in the soil profile significantly decreased with depth ($P < 0.001$) to a mean value of −46.6‰ at a depth of 30 cm (Fig. 3b). Below 30 cm in depth, values of δ^2H_S remained highly negative and did not differ between soil layers ($P > 0.050$).

For beech, before labelling, δ^2H_X ranged between −84.1‰ and −56.1‰ (Fig. 4a). After labelling, a total of 25 trees out of the 28 beech trees studied showed a strong increase in δ^2H_X. For oak, the natural δ^2H_X varied between −98.8‰ and −42.3‰ (Fig. 4a) and only one tree showed no increase in δ^2H_X after labelling. For Douglas fir, natural δ^2H_X varied between −85.7‰ and −53.4‰ (Fig. 4a). Among the 33 Douglas fir trees studied, 13 showed a strong increase in δ^2H_X after labelling. For spruce, the natural δ^2H_X ranged between −64.8‰ and −38.1‰ (Fig. 4a) and after the labelling, eight trees out of the 30 studied showed a strong increase in δ^2H_X.

Mean species D_S obtained with the modelling approach ranged from 25.6 ± 4.6 cm in depth for oak to 52.5 ± 5.7 cm in depth for Douglas fir (Table 1). Statistical tests revealed significant differences in D_S among species. Douglas fir and spruce displayed deeper values than oak and beech (Table 1). Statistical tests also revealed significant differences in D_S between plant functional groups ($P < 0.001$), with the lowest values for the conifers and

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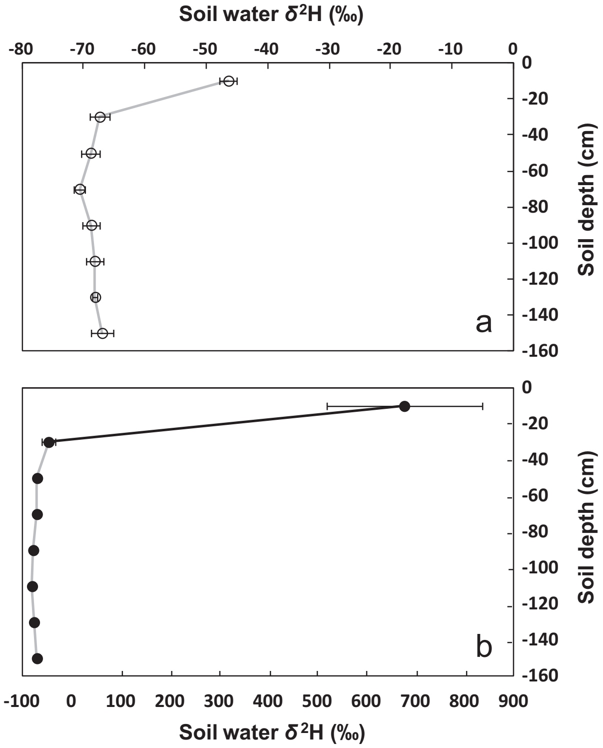

Fig. 3. Mean vertical profile of soil water deuterium isotope composition (δ^2H, ‰), (a) prior to labelling (i.e. natural abundance, open circles) and (b) three days after labelling (black circles). Horizontal bars are standard errors of the mean of five plots prior to labelling and three plots after labelling.

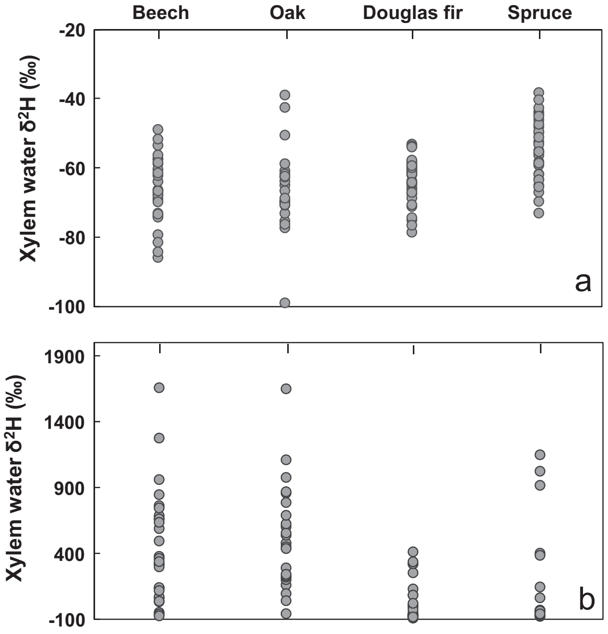

Fig. 4. Xylem water deuterium isotope composition (δ^2H, ‰) for beech, oak, Douglas fir and spruce (a) prior to labelling and (b) three days after labelling. Note that the two *y*-axes have different scales.

the highest for the broadleaved species (Table 1). The global test on all species showed a significant effect of PFT_C classes on D_S ($P < 0.001$), but no effect of H ($P = 0.817$) or BA ($P = 0.061$).

When considering each species independently, we found that there was a significant PFT_C effect ($P = 0.034$) on beech D_S, with deeper depths occurring when the percentage of conifers in the vicinity was high (Fig. 5). There was no effect of H ($P = 0.082$) or BA ($P = 0.739$) on D_S for beech. We found no significant effect of H, BA or PFT_C on D_S for oak, Douglas fir or spruce ($P > 0.050$, Fig. 5).

3.3. Foliar analyses

Mean LMA ranged from 83.0 ± 3.8 g m^{-2} for beech to 116.2 ± 4.5 g m^{-2} for spruce (Table 1). We found significant differences among functional groups for LMA ($P < 0.001$) with the lowest values occurring for broadleaved species and the highest for conifers (Table 1). No effect of H, BA or PFT_C was found either when all species were considered together or when they were considered separately ($P > 0.050$, data not shown). We can therefore exclude the impact of any confounding factors related to light interception on foliar δ^{13}C and N%.

Mean species δ^{13}C ranged from −29.7 ± 0.3‰ for beech to −27.8 ± 0.2‰ for Douglas fir (Table 1). The global test on all the study trees revealed a significant species effect ($P < 0.001$) on δ^{13}C, with the lowest values for beech and oak, and the highest for Douglas fir (Table 1). This test also revealed a significant effect of PFT_C on δ^{13}C ($P = 0.029$), with significantly lower δ^{13}C in the intermediate level (33–66% of conifers present) when compared with the lowest and highest percentages of conifers. We found no effect of H ($P = 0.281$) or BA ($P = 0.234$) on foliar δ^{13}C. Tests on individual species revealed no significant effect of H, BA and PFT_C for the species studied ($P > 0.050$).

Mean species foliar N% ranged from 1.6 ± 0.1% for Douglas fir and spruce to 2.3 ± 0.1% for oak (Table 1). When considering all species, we found a significant species effect ($P < 0.001$) with the lowest values for the two conifers and the highest values for the two broadleaved species (Table 1). N% was not affected by H ($P = 0.445$), BA ($P = 0.623$) or PFT_C ($P = 0.622$). When repeating the test for each species separately, we again found no effect of any of the three variables on N% ($P > 0.050$).

3.4. Predawn leaf water potential

Mean species predawn leaf water potential (ψ_{pd}) ranged from −0.37 ± 0.3 MPa for oak to −0.68 ± 0.3 MPa for Douglas fir (Table 1). The global test on all the study trees revealed a significant species effect ($P < 0.001$) on ψ_{pd} with the lowest (i.e. the most negative) values for Douglas-fir, intermediate values for spruce, and the highest values for beech and oak (Table 1). However, we found no significant effect of H ($P = 0.650$), BA ($P = 0.138$) or PFT_C ($P = 0.174$) on ψ_{pd}. Tests on each individual species did not reveal any significant effect of H, BA or PFT_C on ψ_{pd} ($P > 0.050$).

4. Discussion

4.1. Interspecific differences in soil water uptake depth

At the end of the summer, under limiting soil water conditions (Fig. 2), our results showed clear differences in D_S between individual coniferous and broadleaved species, but no detectable differences within each functional group. The coniferous species generally extracted water from a mean depth of 40–50 cm, whereas the mean depth for beech and oak was generally 20–30 cm (Table 1). Even though the differences in D_S between functional groups were small, they were highly significant. However, it must be pointed out that some variability in δ^2H_X between individual trees within a given species was observed (Fig. 4). Some beech or oak trees did not extract any labelled water at all, and were likely relying on deeper rooting systems. Conversely, a few coniferous trees clearly did extract labelled water; this suggests

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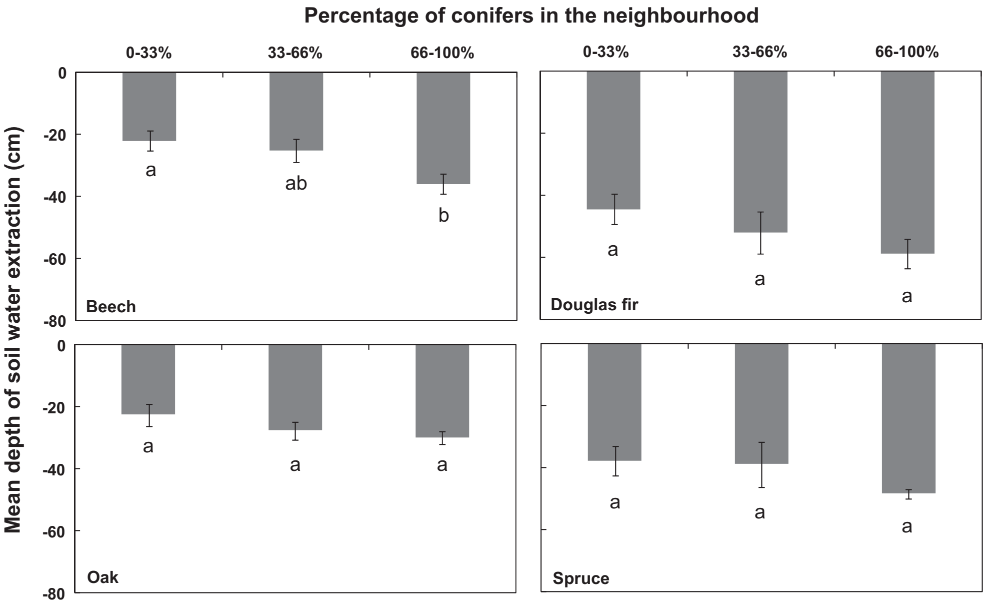

Fig. 5. Mean soil water uptake depth (in cm) for each percentage range of conifers in the direct neighbourhood (0–33%, 33–66% and 66–100%) for beech, oak, Douglas fir and spruce. Vertical bars are standard errors of the mean for each species and each percentage range. Letters denote significant differences in depth among classes of percentage of conifers for each species.

the presence of very superficial fine roots as well as a deeper system. It should be recalled that these data do not reflect the actual rooting depth of these species: the applied isotope approach gives an instantaneous picture of D_S for specific trees at a precise sampling time.

Differences in D_S between plant functional groups were associated with differences in their water status (ψ_{pd}) (Table 1), which reflects the average soil moisture tension at the depth accessed by the roots (Sellin, 1999). Douglas fir and spruce had more negative ψ_{pd} values than the two broadleaved species, which indicates lower water availability for the roots of the coniferous species (Scholander et al., 1965). We did not measure the seasonal change in the vertical profile of soil water content at this site. Nevertheless, considering these differences in ψ_{pd} values, we can hypothesize that the superficial soil layers, where the broadleaved species were mainly extracting water, had higher water availability than the deeper soil layers where the coniferous species were taking up water. This hypothesis is consistent with the fact that *REW* at the site during the measurements was below the limiting threshold for temperate tree water transpiration (Granier et al., 1999) (Fig. 2). However, 10.1 mm of rain fell one day before the experiment (Fig. 2) and probably increased water content in the uppermost soil layers from which the broadleaf trees were mainly extracting water; this could explain the less negative ψ_{pd} values we found for broadleaves. It must be noted that this experiment was conducted only once during the summer. However, the mean depth of water uptake of these trees may vary during the growing season when soil water availability and evaporative demand change (Bréda et al., 2002).

The main finding from the observed differences in D_S between species is that complementarity for water acquisition is already possible in this 10-year old plantation, due to below-ground niche separation. However, such a mechanism seems to occur only when different plant functional groups co-exist as broadleaf and coniferous tree species extract soil water from distinct soil depth. Our results confirm previous observations of complementarity patterns for water use in European conifer/broadleaved mixed forest stands. Indeed, Bolte and Villanueva (2006) found evidence of complementarity by studying the morphology and distribution of fine roots of beech and spruce growing in mixed adult forest stands.

However, our results also highlight the complexity of characterizing the general pattern of water uptake depth of tree species in natural ecosystems. As an example, Büttner and Leuschner (1994) found evidence for some degree of below-ground niche partitioning between oak and beech in a 100–220 year old mixed stand: the oak roots were more superficially distributed than the beech roots. This result contrasts with other studies that found oak to be able to explore deeper horizons than beech trees (Zapater et al., 2011). The inconsistency between these two studies might be related to differences in soil characteristics. In our case, we did not detect any differences between oak and beech. Differences in vertical root occupation of the soil can be found by comparing the rooting depths or densities of broadleaf and coniferous species. Some studies have found that oak and beech have deeper rooting systems than Douglas fir and spruce (e.g. Hendriks and Bianchi, 1995), though this was not corroborated in our study.

The discrepancy among all these studies might be explained by local soil conditions and differences in developmental stage (age) of the forest stands studied. With regard to age, most studies on the vertical root occupation of roots and water uptake of these four species were conducted in mature stands. However, in a previous study conducted at our study site, Lei et al. (2012) studied the fine root length and surface area of the four tree species in the first 30 cm of the soil. They found that coniferous species allocate more carbon to the roots at the young developmental stage, thereby permitting an earlier and thus potentially deeper colonization of the soil space. Consistently with our own results, they hypothesized that the broadleaved species allocated more carbon to aboveground compartments to compete more effectively for light and therefore established their rooting systems later than the coniferous species.

4.2. Impact of interspecific interactions on soil water uptake depth

Our second main finding was the absence of a clear effect of species diversity and competition intensity on the water uptake depth of the species studied. Even though complementarity for water acquisition potentially exists among these species in this study site, our results demonstrate that species diversity did not induce a differentiation of their water uptake depth during a

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drought episode. For the two conifers and oak, the absence of a clear differentiation in vertical root water uptake under interspecific interactions is consistent with previous observations (Hendriks and Bianchi, 1995; Bolte and Villanueva, 2006). All these studies demonstrated the low below-ground competitive ability of these three species, which is commonly interpreted as a low plasticity response of their roots to the interaction with other tree species. However, most studies were based on coarse root distribution, fine-root length or fine root surface area. Our results confirm these studies by showing that biotic interactions equally have no influence on soil water uptake depth for Norway spruce, Douglas fir and oak.

Although species diversity (*H*) or competition intensity (*BA*) did not generally influence the depth at which trees extracted water, this depth varied among individual beech trees according to the percentage of conifers in their neighbourhood (Fig. 5). The other species showed a similar trend, but this effect of the percentage of conifers in the neighbourhood was statistically significant for beech only ($P = 0.034$). In central European forests, beech has been shown to be a highly competitive species with more efficient below-ground spatial occupation than other species, regardless of tree age (Grams et al., 2002). We characterize beech rooting strategy as both more plastic and more efficient than the other three species studied. Our results are also consistent with previous studies which showed a shift of beech fine roots down to deeper soil horizons when competing with coniferous tree species (Hendriks and Bianchi, 1995; Bolte and Villanueva, 2006).

Several underlying mechanisms can explain the deeper foraging of beech roots when competing with coniferous species. First, limiting soil water and nitrogen resources in the upper layers could explain the deeper foraging of beech during this summer. However, in our plantation, the differences in D_S did not seem to be caused by differences in water or nitrogen availability. Indeed, variations in $\delta^{13}C$ reflect physiological acclimatization processes to environmental conditions (Ferrio et al., 2003) and can be used as physical archives of changes in forest soil moisture or air humidity (Saurer et al., 2004; Keitel et al., 2006). No effect of interspecific interactions on $\delta^{13}C$ or N% was found; this shows that no long-term acclimatization to different soil water and nutrient concentrations occurred. Secondly, changes in other soil conditions due to the presence of conifers might impact the rooting strategy of beech. Indeed, besides nitrogen and water, the concentrations of other soil nutrients such as calcium, magnesium and phosphorus can be decreased by the presence of coniferous species (Augusto et al., 2002). An increase in soil acidification in the uppermost soil layers under coniferous species could also partially explain the beech response (Augusto et al., 2002). Furthermore, the presence of conifers is known to influence forest soil microbial community composition and activity (Mardulyn et al., 1993), which play a strong role in root colonization capacity. Finally, beech roots have been found to benefit from the mechanical modification of soil properties by the root systems of coniferous species. Indeed, Hendriks and Bianchi (1995) suggested that in mixed Douglas fir and beech stands, Douglas fir may extend its roots first, thus allowing beech to expand its own roots deeper and more easily because the soil profile has already been "opened". Although the above mechanisms might contribute to rooting depth differentiation for beech observed in our study, future research on rooting strategies in mixed species forests is required and should focus on the underlying processes that govern species-specific responses to interspecific interactions.

5. Conclusions

We revealed significantly deeper water uptake by the two coniferous species than by the two broadleaved species. This result indicates that, at a young stage, complementarity for water uptake can occur when these functional groups coexist, but that within-group complementarity may not occur. Coniferous species have been found to establish roots more rapidly than broadleaved species, and our results further demonstrate that this characteristic allows them to access water from deeper horizons at early developmental stages. Interestingly, during the 2013 summer drought, this ability did not necessarily prove to be useful for the water status of the conifers, as the shallow-rooted broadleaved species seemed to be able to quickly take up water from the upper soil layer soon after rain.

Contrary to our expectations, we did not detect any differentiation in the water uptake depth for three out of the four species studied. This indicates that there was no strong plasticity effect in their response to interspecific interactions at this developmental stage. Only beech displayed a change in soil water uptake depth when competing with a higher proportion of coniferous trees. Our results support previous studies stating that beech is a species with strong below-ground competitive abilities at both adult and early stages. An interesting perspective would be to determine the underlying processes that govern beech root foraging response to competition with coniferous species in European forests.

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Impact of tree species diversity on water and carbon relations in European forests

Abstract: Biodiversity is known to support and boost a wide range of forest ecosystem functions and services like productivity and resistance against insect pests and diseases. However, whether tree species diversity also promotes water and carbon acquisition and use in forest ecosystems is still unclear. Furthermore, in the current context of global warming, information on how tree species diversity can influence the response of forest ecosystems to extreme climatic events such as drought are urgently needed. In this framework, the objective of my PhD thesis was to determine how tree species diversity influences important functions of the water and carbon cycle including transpiration, carbon isotope composition and water extraction depth at the tree- and ecosystem-scale under contrasting soil water conditions. My work was conducted within the FunDivEUROPE project in a network of permanent forest stands and tree plantations across a North-South gradient in Europe covering a wide range of climatic conditions. I found considerable variability among species or forest types in the response of transpiration and carbon isotope composition at the tree- and ecosystem-scale across Europe. Species diversity did not affect the water and carbon relations of tree species and forest ecosystems under non-limiting soil water conditions. However, a strong effect of species diversity was observed under drought conditions in some forest types. Based on these data, I discuss the potential mechanisms of species interactions that may explain the observed patterns. I also point out that the influence of species diversity is highly context-dependent, and changes with local environmental and climatic conditions. In terms of forest management applications, I suggest that, at least in some regions, controlling for tree species diversity along with stand density and total basal area could be recommended to help forests adapt to drier conditions.

Keywords: biodiversity, climate change, complementarity, drought, isotopes, mixed forest, productivity, sap flow, species interaction, transpiration, water use efficiency

Impact de la diversité en espèces d'arbres sur les relations hydriques et carbonés dans les forêts Européennes

Résumé: La biodiversité favorise un grand nombre de fonctions et services écosystémiques des écosystèmes forestiers tels que la production de bois ou la résistance aux attaques d'insectes et aux maladies. Cependant l'impact de la diversité sur l'acquisition et l'utilisation de l'eau et du carbone reste largement méconnu dans ces écosystèmes. De plus, dans le contexte actuel de changement climatique, l'influence de la diversité sur la réponse des écosystèmes forestiers à des événements climatiques extrêmes tels que la sécheresse reste à étudier. L'objectif de ce travail est donc de déterminer l'impact de la diversité en espèces d'arbre sur d'importantes fonctions du cycle de l'eau et du carbone telles que la transpiration, la composition isotopique du carbone ou la profondeur d'extraction de l'eau à l'échelle de l'arbre et de l'écosystème sous des conditions contrastées de disponibilité en eau du sol. Ce travail a été réalisé dans le cadre du projet FunDivEUROPE sur un réseau de parcelles forestières ainsi que dans des plantations expérimentales le long d'un gradient Nord-Sud en Europe afin de couvrir une importante gamme de conditions climatiques. Nos travaux ont montré une importante variabilité de la réponse à la diversité à l'échelle de l'arbre et de l'écosystème en termes de relations hydriques et carbonées à travers l'Europe. La diversité en espèces ne semble pas influencer les relations hydriques et carbonées des espèces et des écosystèmes forestiers dans des conditions non limitantes de disponibilité en eau. Cependant, un fort effet de la diversité a été observé en conditions de sécheresse pour certains types forestiers. A partir de ces résultats, je discute des mécanismes d'interaction entre espèces qui peuvent expliquer les effets observés. Nos données ont montré que l'influence de la diversité en espèces est fortement dépendante du contexte et peut être modifiée par les conditions environnementales locales et les conditions climatiques. En terme de gestion forestière, je suggère que pour certaines régions en Europe, promouvoir la diversité en espèces ainsi que contrôler la densité des parcelles doit être recommandé afin d'adapter les écosystèmes forestiers aux futures conditions climatiques.

Mot clefs: biodiversité, changement climatique, complémentarité, sécheresse, isotopes, forêt mélangée, productivité, flux de sève, interaction entre espèces, transpiration, efficience d'utilisation de l'eau