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MASTER FAGE
Biologie et Ecologie pour la Forêt, l'Agronomie et
l'Environnement

Spécialité fonctionnement et gestion des écosystèmes

La fragmentation historique des habitats et ses effets sur
la distribution des plantes dans le paysage

Historical habitat fragmentation and its effects on plants
distribution in the landscape

Floriane Colas
Mémoire de stage, soutenu à Nancy le 04/09/2012

Tuteurs de stage : Sara Cousins professeur
Jan Plue chercheur post doctorat

Responsable universitaire : Bernard Amiaud, maître de conférences

Structure d'accueil :
Dep of Physical Geography
and Quaternary Geology
Stockholms university 106 91
Stockholm, Sweden



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I. Introduction

The department of Physical Geography and Quaternary Geology of Stockholm University conducts research on climate change and landscape development. It is composed of research groups working on:

- land and water resources,
- glacier and polar environments,
- landscape analysis.

Prospects of landscape structures and dynamics of ecological process and patterns are focused on by the Landscape Ecology Group. My master thesis was carried out in this research team.

1. Landscape fragmentation

Biodiversity, i.e. species richness, is in serious danger. Indeed, species numbers are in continuous decline, with many being lost. That is why, in order to understand this phenomenon, looking at landscape fragmentation is a solution, as it is one of the main drivers that threaten biodiversity. Fragmentation consists in reducing once continuous habitats into multiple fragments or patches. This implies habitat loss, the decrease of patch size and increase of patch isolation (**Erreur ! Source du renvoi introuvable.**). Fahrig (2003) stated that fragmentation is a secondary threat after habitat loss, but habitat loss is part of fragmentation. The more a habitat is lost, the more likely it will be fragmented. A landscape includes different ecosystems within an area of a few km² of land.

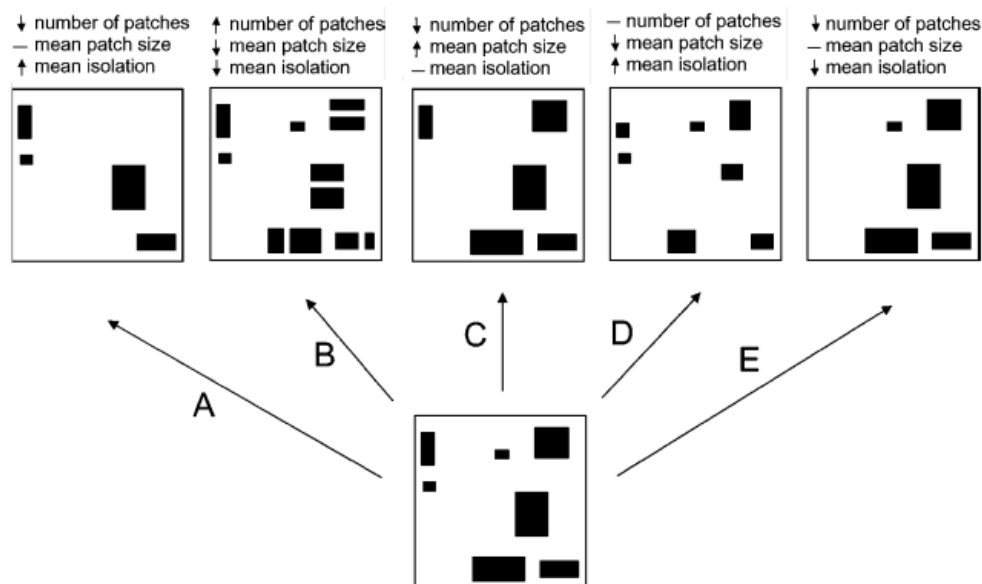


Figure 1 Illustration of habitat loss resulting in some, but not all, of the other three expected effects of habitat fragmentation on landscape pattern. Expected effects are (a) an increase in the number of patches, (b) a decrease in mean patch size, and (c) an increase in mean patch isolation (nearest neighbor distance). Actual changes are indicated by arrows. (Fahrig, 2003)

Nowadays, landscapes with high species diversity, such as semi-natural grasslands (SNG) (Wilson et al., 2012) are threatened by urbanization, abandonment of grazing and fragmentation of the remaining habitat. Many have disappeared because of forest invasion or because they were transformed into arable fields. Nevertheless, besides the few semi-natural grasslands which are left, the landscape still holds some remains of these semi-natural grasslands in midfield islets (MI), which are tiny patches of uncultivated soil on rocky outcrops in arable fields. Together with road verges and forest edges areas, they make up small habitat islands where grassland species can survive in an otherwise unsuitable agriculture-forest matrix (Tikka et al., 2000; Cousins & Eriksson, 2002).

These landscape formations are thus of great importance, due to their role as rescue site for species (Cousins & Eriksson, 2002), and because they could be a valuable source of grasslands species when restoring semi-natural grasslands (Cousins & Lindborg, 2008). Moreover, road and railway verges have been demonstrated to act as corridors which allow seed dispersal (Tikka et al. 2001). But their role in seed dispersal is still ambiguous because others studies do not show improved seed dispersal due to these remnant habitats (Harrison & Bruna, 1999).

Nonetheless, with the limited dispersal of seeds between patches in these fragmented landscapes (Turnbull et al., 2000; Soons et al., 2005; Ozinga et al., 2009) the importance of plants already present in fragments is emphasized. If plants cannot disperse easily, then the established populations play a crucial role for the local persistence of species, in the above-ground vegetation as well as in the seed bank.

2. Seed bank

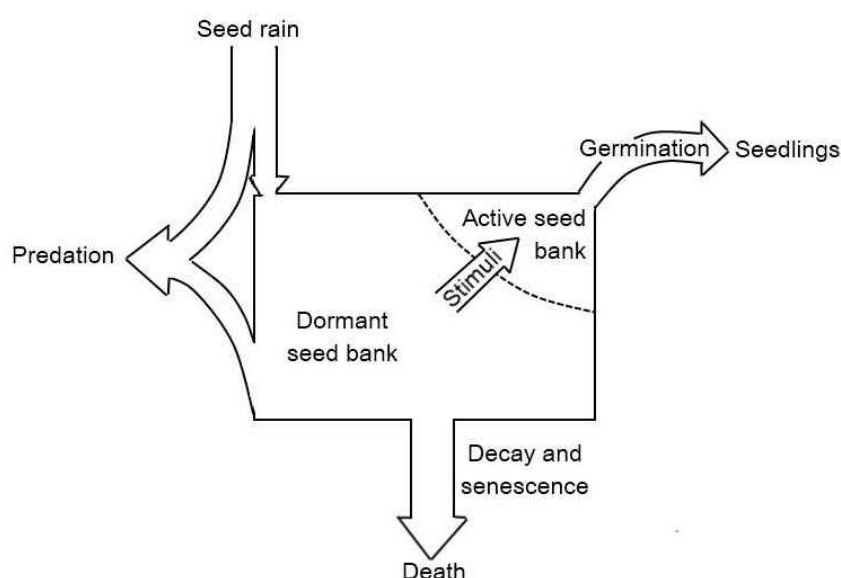


Figure 2 Dynamics of the soil seed bank (Harper, 1977)

A seed is an embryonic plant produced by the sexual reproduction. They develop on a mother plant until maturity, and then they can fall on the ground close to the parent plant or be dispersed by multiple agents such as wind, animals or water. The phenomenon of seeds

coming from surrounding plant is called the seed rain. Some of these seeds can germinate immediately after dispersal if conditions are adequate; others will remain on the ground in a dormant state and become buried, building a seed bank. It can be dormant, with seeds in a physiological dormancy, or active with seeds in an ecological dormancy, i.e. only environmental conditions are limiting for the germination (Figure 2). Transient seed bank are seeds which remains in the soil for less than one year. Others can remain longer in the seed bank and then form the persistent seed bank. A seed bank is built by successive layers of seeds produced in different years. The later seeds are deposited, the more likely they are to contribute to vegetation dynamics, as they are more likely to be exposed to conditions suitable for germination. Or, the older seeds are, the less likely they will germinate (Pakeman & Small, 2005).

The above-ground vegetation is composed of vegetative parts from older plants and their new sprouts, but also by seedling of the year, coming from the seed rain and the seed bank. The contribution of seed bank in above-ground vegetation following recovery after a disturbance has been shown to be up to 44% of the seedlings which appear (Kalamees & Zobel, 2002).

Moreover, seed banks may support the vegetation by increasing the effective population size of a species, enabling a species better chance at long term survival in a habitat patch (Lindborg, 2007). The seed bank is also a reservoir of genetic diversity for a species. On top of that, they may counteract genetic drift or population genetic differentiation, which is an additional inherent threat of fragmentation (Honnay et al., 2008). The seed bank forms an ecological memory of above-ground vegetation, for 50 years at least (Bossuyt & Hermy, 2001). In forests it can even be longer. According to Plue et al. (2010), non-forest species such as ruderals from arable fields, can be found in the seed bank, even after 150 years of forest cover. Therefore, it is important to follow long-term seed bank dynamics, as they can be of interest for ecosystems monitoring.

3. Extinction debt

Time is an important part of many ecological processes. Some plant species can live for a very long time and not just woody species, for example among numerous other species (Ehrlén and Lehtilä, 2002) *Primula veris* can live for 40 years (Kuussaari et al., 2009) and on 71 species half had a life span of more than 35 years. Therefore, before really seeing impacts of environmental change on a *Primula veris* population, a long time often needs to pass. Indeed, effects of habitat fragmentation can take a long period of time before they can be observed in the established vegetation. The time that plants takes to respond to a disturbance and disappear is called extinction debt. Most plants remain immediately after a disturbance, but only for a limited time, after which they can go locally extinct. Even high species richness can be found where vegetation is threatened by extinction because of this time lag.

The evidence for extinction debt were found thanks to the stronger relationship that exists between plant distribution and old management or landscape patterns (Tilman et al., 1994, Helm et al., 2006; Kuussaari et al., 2009). Nevertheless, some studies did not found any

relationship between historical land use and plant distributional patterns, although it has been suggested that the debt in some landscapes might have already been paid off (Adriaens et al., 2006). The historical timescale is therefore to take into account, specifically within landscape ecology.

But, vegetation dynamics are not the only element to take into account: seeds also take part in this problem. In fact, seeds change the temporal persistence of species in an ecosystem; they remain in the soil, unaltered, during some time, whereas above-ground vegetation may experience substantial changes. As a consequence, species may appear extinct from the vegetation, while they still reside in the seed bank. This has recently been called the “pseudoextinction debt” (Figure 3). Actual extinction from a patch thus only takes place when the last seeds from a plant species have died.

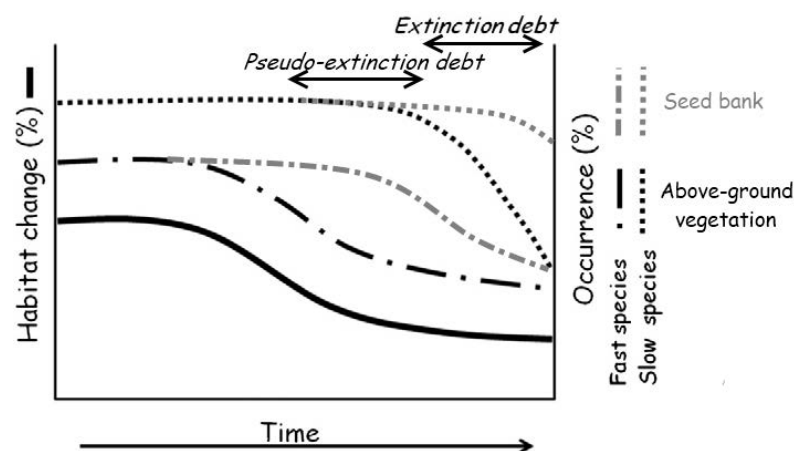


Figure 3 A model of extinction debt and pseudo-extinction debt. Both fast species (or short-lived species) and slow species (or long-lived species) have a seed bank that can elongate extinction debt. Adapted from Cousins & Vanhoenacker (2010) and Plue & Cousins (in review).

4. Semi-natural grasslands

Semi-natural grassland is a habitat that has been continuously managed since a long time by grazing or mowing, without fertilizers addition. It is also one of the two very species rich habitats in the world, together with lowland rainforest (Wilson et al., 2012). Indeed, they have often more than 40 plant species per m² (Eriksson & Eriksson, 1997) and sometimes more than 60 plant species per m² (Kull & Zobel, 1991). The long management history allows this assembly of such exceptional species richness (Kull & Zobel, 1991; Pärtel et al., 1999). On top of that, the richer in plant species the grassland is, the more animal species can be found, showing the importance of these areas for biodiversity and the subsequent need to conserve them.

Semi-natural grasslands are mostly open areas, but they sometimes can be encroached by trees and bushes, until these reach 50 % of cover. But even with the subsequent diminution in light availability, these habitats can still be very rich: the record of 63 plant species per m² was found in Estonian wooded meadow by (Kull & Zobel, 1991). Beyond 50 % of cover, the

light availability begins to be limiting and the grassland communities start to deteriorate (Aavik et al., 2008).

Besides, grasslands are vulnerable. When management stops, they disappear, leaving a forest significantly less rich in species (Cousins & Eriksson, 2002). It has been found that the more species there are, the more stable an ecosystem is (Tilman & Downing, 1994). This is the diversity-stability theory which postulates that keeping as much species as possible is important for the stability and resilience of an ecosystem. But high diversity is not necessarily an advantage for an ecosystem as Pfisterer & Schmid (2002) found that the ecosystem resilience decreased with higher diversity under perturbed condition.

These kinds of grasslands, in addition of being rich in species, are also of a patrimonial interest for their grazing history. They are protected by many conservation measures, as preservation of biodiversity is a society concern. This is linked to the fact that biodiversity is essential for humanity, as the Millennium Ecosystem Assessment 2005 had pointed out the ecosystems services like food provisioning (quality of the meat), health and welfare.

5. Objectives

The main aim of this work was to determine how historical habitat fragmentation impacts plants species distribution and the seed bank. For this purpose, the research questions of this project are:

- Is there an effect of past land use on current plant distribution patterns?
- Does the seed bank of semi-natural grasslands and midfield islet differ in terms of quantity and quality of species?
- Does the seed bank act as a reservoir of species?
- And if so, can it contribute to post-disturbance recovery?

II. Material and methods

1. Study area

a. Description and climate

Selaön (59°24' N, 17°10' E) is the largest island of the Mälaren Lake (Figure 4), situated at 50 km west from Stockholm, in Mid-Sweden. These 91 km² of land are a traditional rural landscape with a patchwork of agricultural fields, forests and semi-natural grasslands. The climate is temperate with a mean temperature for January of -3° C and for July of 18° C. Annual precipitation is 600 mm (Dahlström et al., 2006).

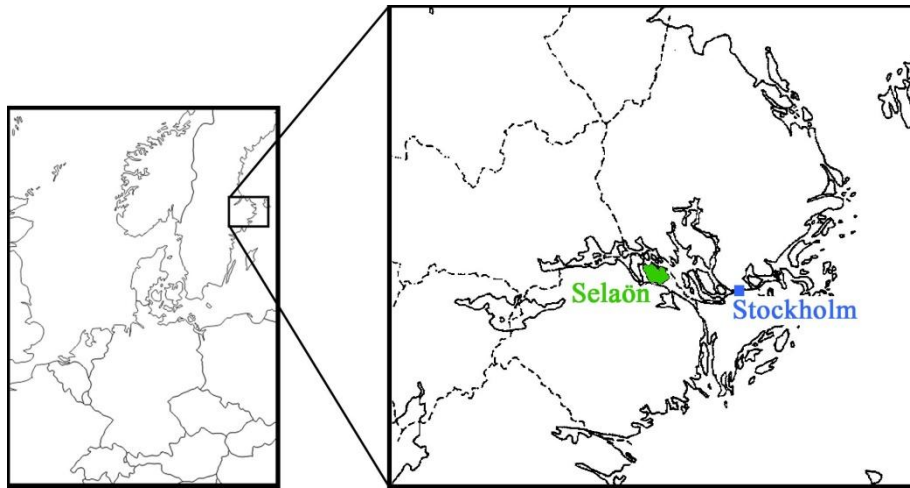


Figure 4 Localization of Selaön

b. Topology and geology

During the last glaciation, the weight of ice pressed down on Scandinavia, which is now experiencing isostatic uplift at a rate of 1 mm per year. Glaciation also shaped Selaön's landscape forming a 25 m high esker, a long ridge of sand and gravel in the south east. There is also some moraine, fragments of rocks left behind after a glacier. The bed rock consists of gneiss and granite, but the former lake brought alluvial deposits to fill the broad valleys.

c. History of the landscape

Several parts of Selaön were under water between the 8th and 11th century, evidenced by Vikings archaeological discoveries of a city close to the water. The island has a strategic position between two important cities during the Viking period: Birka and Sigtuna. This island has a long history of continuous grazing management: some archeological remnants of prehistoric settlements, close to semi-natural grassland that must have been grazed, are dating back almost 4000 years (Dahlström et al., 2006; Dahlström, 2008).

A common practice was to graze the forests, but it was banned in the 1930s, leading to a clear distinction between grassland and forest. Many semi-natural grasslands were abandoned (and thus developed into forest) or planted with trees when commercial forestry became common in the 1940s (Cousins & Eriksson, 2008). The surface area of semi-natural grassland has ever since decreased, whereas forests, inhabited areas and arable fields are increasing (Figure 5 and annexes I to III).

2. Study design

a. Time series and land use

A time series of land cover with three maps of three years: 1854, 1950 and 2011 of the island Selaön were used (Figure 5). The one of 1854 was made from a parish map, which was digitalized and rectified. The map of 1950 was made from black and white aerial photographs.

The last one was digitized from infrared colored aerial photographs (Cousins, 2001; Cousins & Eriksson, 2008), the landscape did not change between 2010 and 2012.

For each map, land use was specified. Between 1854 and the two others maps, legends of land use changed, that is why, and also in order to enable simpler analysis, the different land use types were restricted to only: “arable field”, “semi-natural grassland”, “midfield islets”, “forest”, “inhabited” and “others”.

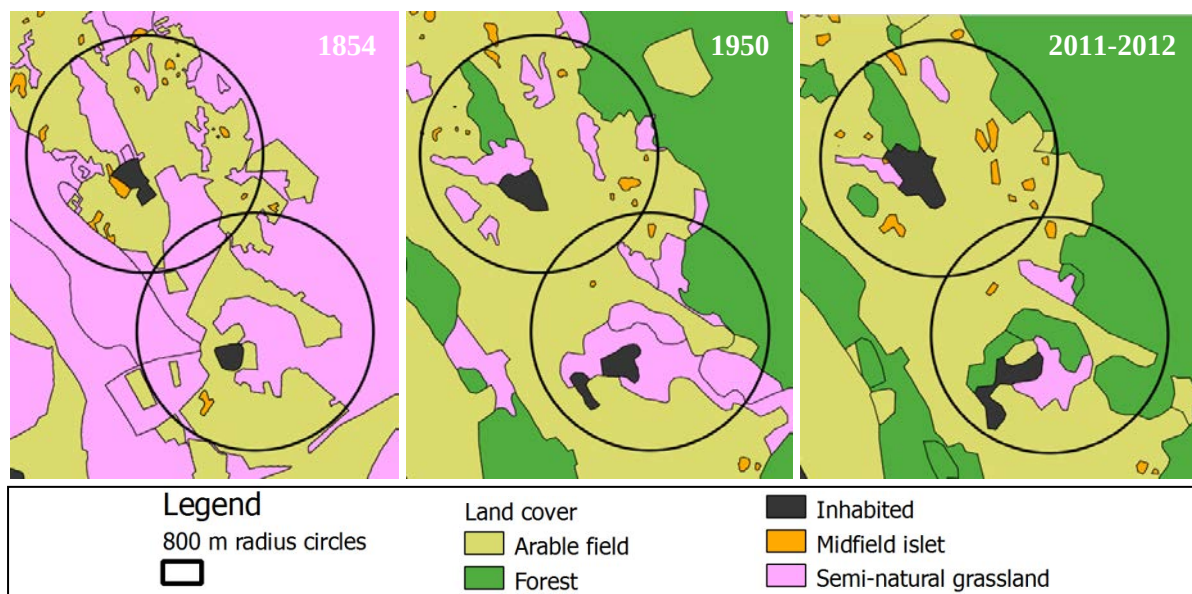


Figure 5 Land cover maps of a section of Selaön at three different times 1854, 1950 and 2012.

Table 1 The different classes of the historical land cover, their code, their description and their quantity.

Code	Description	Quantity
MI1854	Midfield islet since 1854; old midfield islet	4
MI1950	Midfield islet since 1950 but was not a semi-natural grassland in 1854	5
MISNGMI	Midfield islet in 1854 that was a semi-natural grassland in 1950 but then a midfield islet in 2011	2
SNG1854	Semi-natural grassland since 1854; old semi-natural grassland	21
SNG1950MI	Semi-natural grassland that became a midfield islet between 1854 and 1950	7
SNG2011MI	Semi-natural grassland that became a midfield islet between 1950 and 2011	2
SNGAFMI	Semi-natural grassland that was an arable field in 1950 and that is now a midfield islet	1
SNGFSNG	Grazed forest in 1854, forest in 1950 and semi-natural grassland in 2011	2
SNGs1950	Semi-natural grassland since 1950	2
SNGs2011	Semi-natural grassland since 2011; new semi-natural grassland	2

Arable field also includes “cultivated grasslands”. These cultivated grasslands are fertilized in order to produce more and are very species poor. 1854 category of “grazed forest” was considered as semi-natural grassland, since trees were very sparse. It is the high species richness of these environments, only grazed by cattle, which are of interest. It is in a similar landscape; an Estonian wooden meadow, that the number of 60 species per m² was found by Kull and Zobel (1991). In the present day map, similar environments can still be found; they are semi-natural grasslands with more than 50% of tree cover. Inhabited consists in village and isolated farms with their garden. Others are land use like wetlands and reeds.

To access the time and the land use with a single variable we used a code giving information about the history as semi-natural grassland since 1854 and midfield islets since 1950 with the meaning that they previously were not semi-natural grassland, for the details see Table 1.

b. Plot selection

Ten 800 m radius circles have been selected in the landscape, representing a gradient in semi-natural grasslands habitat decrease and isolation. Then, 134 points of $2 \times 2 \text{ m}^2$ have been located in two land use types: midfield islet and semi-natural grassland.

c. Field experiment

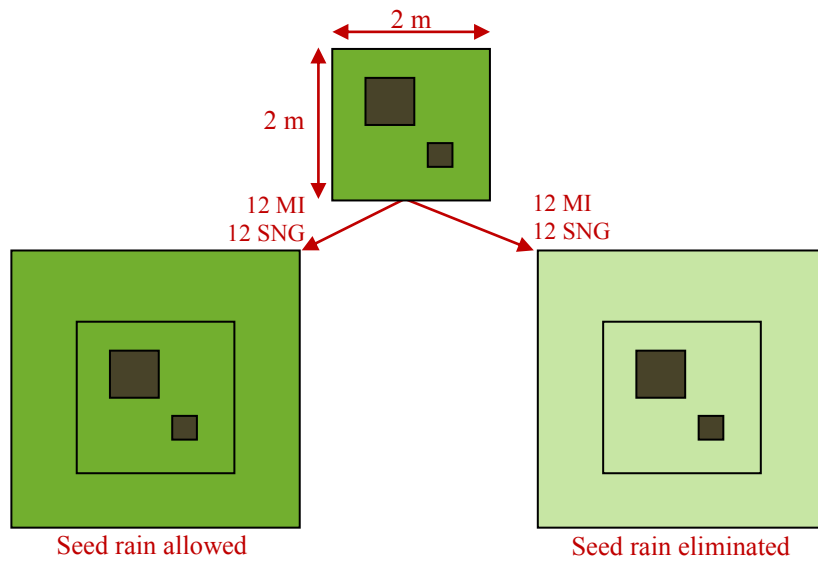


Figure 6 Field experiment design. The brown squares ($50 \times 50 \text{ cm}^2$ and $25 \times 25 \text{ cm}^2$ respectively) represent the portion of soil where the vegetation and more or less 2 cm of topsoil was removed. The light green indicates that the vegetation was clipped; the dark green, no treatment used. MI is for midfield islets and SNG for semi-natural grassland.

In order to characterize the potential of the seed bank during post-disturbance recovery, a field experiment was set-up. From the 134 plots of the previous experiment, 48 plots were selected: 24 in midfields islets and 24 in semi-natural grasslands. In each plot of $2 \times 2 \text{ m}^2$, one subplot of $50 \times 50 \text{ cm}^2$ and one subplot of $25 \times 25 \text{ cm}^2$, had their above-ground vegetation removed before the growing season, at the end of March. Then, for both land use types, half of the plots were randomly selected to be mowed; inflorescences were cut, in the area of $2 \times 2 \text{ m}^2$ (Figure 6), but also within 2 m around the plot in order to eliminate local seed rain as much as possible (Kalamees and Zobel, 2002).

3. Data collection

a. Seed bank

- Ex situ experiment: 2010

Seed bank samples were collected in September 2010. For this purpose, 25 core samples of 3.5 cm diameter and 5 cm depth were taken from each plot. The litter was removed, all

samples of the same plot were mixed and concentrated following Heerdt et al. (1996), forming one pooled sample for each plot. Then, they were spread out on a sterilized potting soil and left to germinate under conditions of 16 h day and 8 h night and of daytime temperature from 25 to 30°C. After 14 weeks the experiment was stopped, when seedling that could have been identified were counted and removed. A cold stratification was performed for 10 weeks, to simulate winter conditions in order to end the dormancy of dormant seeds. Temperatures ranged from 0 to 2°C. This was followed by a second germination period for 24 weeks.

- ***In situ* experiment: 2012**

Surveys were conducted in June and July. Large plots were divided into 4 sub-plots, to yield some information on the position of species and the survival rates, after follow-up the study over the coming years. Every plant and species was counted, and the presence of seed leaves was checked to determine if it was a seedling or a vegetative sprout.

b. Vegetation

Above-ground vegetation surveys were conducted in June-July 2011; for all the 134 plots a record of the presence of plant species in the 2×2 m² plot was realized. Moreover, all the midfield islets were fully surveyed to check if the species recorded into the plot were a good representation of the full islet. As being completely surrounded by arable fields, long distance dispersal between these islets and remaining semi-natural grasslands is expected to be limited. In 2012, the vegetation around each subplot in the semi-natural grasslands was fully inventoried (presence/absence data).

4. Data analysis

The fragmentation and community analysis were realized with the data collected in 2010 and 2011. For the isolation between patches, the distance was measured at the three different times 1854, 1950 and 2011, between the patches suitable for a grassland species, i.e. semi-natural grasslands and midfield islets. We always measured the shortest distance between the edges of the patches. It was log-transformed, and the resulting values were analyzed via a Kruskal-Wallis test. Then, a multiple comparison test following Siegel and Castellan (1988) was done using the *pgirmess* package in R (Giraudeau, 2011).

In order to see the decrease of the patch size, the mean area of semi-natural grasslands was calculated among the landscape at each time step for each circle of the fragmentation gradient. An ANOVA was then realized followed by a Tukey HSD test in case of significant differences.

To study the community response to the fragmentation, a nestedness analysis was performed, following the NODF method. It calculates nestedness between every row and every column and then gives an average value for rows, columns and both. When a matrix is highly nested the larger fragments of the matrix contains more species than the smaller ones. This means

also that in the smaller fragments the most common species, which are most resistant to the negative consequences of fragmentation remain. To this purpose, the functions *nestednodf* in the *vegan* package (Oksanen et al., 2011) was used. Then, *oecosimu* was used to perform 99 monte-carlo randomizations to enable hypothesis testing. A correlation between ranks obtained from the nested matrix and the logarithm of patch area was checked by using the Spearman's rank correlation. The ranks were subsequently analyzed in function of the categories of historical land use type (Table 1) using Kruskal-Wallis tests followed by multiple comparison tests to establish which land use types were significantly different.

In order to establish the importance of the seed bank as a reservoir of species lost from the vegetation, the proportion of plots with species only present as a seed in the seed bank was calculated among all the plots where species were present, either seed or vegetative. The same was done with seedlings. Because midfields islet are often quite isolated, we used a full vegetation inventory of the midfield islet to improve the confidence about which species are only present in either the seed bank or vegetation. The proportion values of species belonging to seeds against the ones belonging to vegetation were then compared with an independent samples t-test.

A previous test showed that the number of species was related to the categories of land cover, and also because we could have a measure index not so sensible of the rare species, we used a Shannon index (H) for the data of 2012. As H was not normally distributed, non-parametric tests were used for the analysis. For analysis historical land type on H, a Kruskal-Wallis test was applied, when it turned out significant multiple comparison tests. For the comparison between seedlings and vegetative sprouts, a Mann-Whitney test was employed.

All the statistics were done in the R software package (R Development Core Team, 2011).

III. Results

1. Fragmentation of the landscape

a. Habitat loss

During these 150 years, semi-natural grasslands have decreased to let arable fields become the dominant land use type. Arable field rose from 36% to 60% of occupancy in the landscape. Simultaneously, semi-natural grassland decrease from 63% to 10% (Figure 7).

b. Isolation

The isolation can be represented as the distance between patches where the grasslands species can survive. The further a patch will be from any other patch where a plant can disperse and establish, the more isolated this patch will be. In 1845, the patches were less isolated than in the other years (Figure 8), evidenced by a median of 83 m for 1845, 90 m for 1950 and 152 m

for 2011. Even though the variation of the distance is important in 1845, the values are nonetheless statistically significant ($p\text{-value} < 0.001$), with 2011 significantly different from the other two times. We can thus say that there – on average – is an increase of the distance between suitable patches for grasslands species. That is to say, there is an increase of patch isolation in the landscape since 1845.

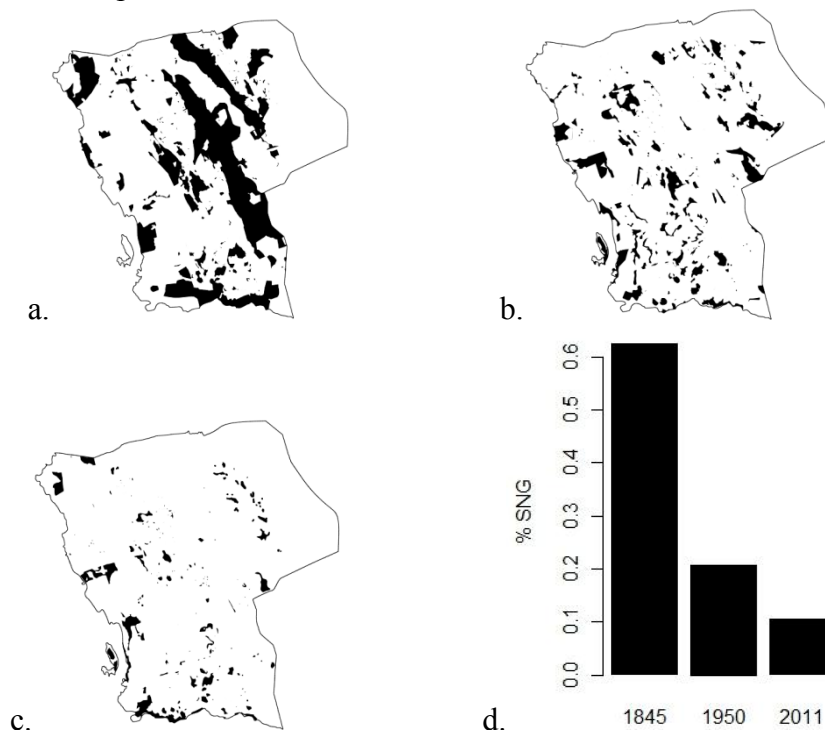


Figure 7 Surface of semi-natural grasslands in 1854 (a), 1950 (b) and 2011(c) with their proportion among all other land types (d).

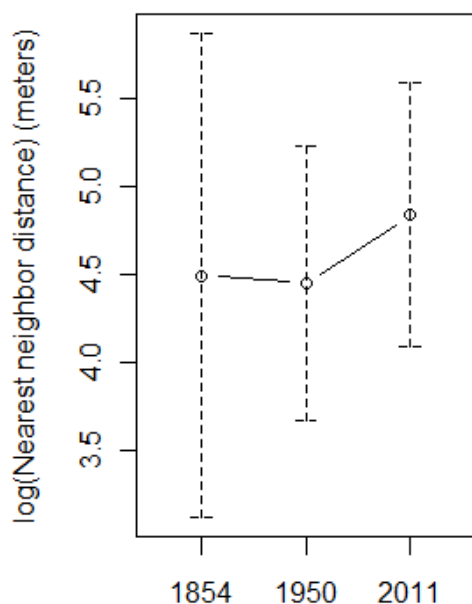


Figure 8 Logarithm of nearest neighbor distance in meters in function of the time. Segments represent the standard deviation

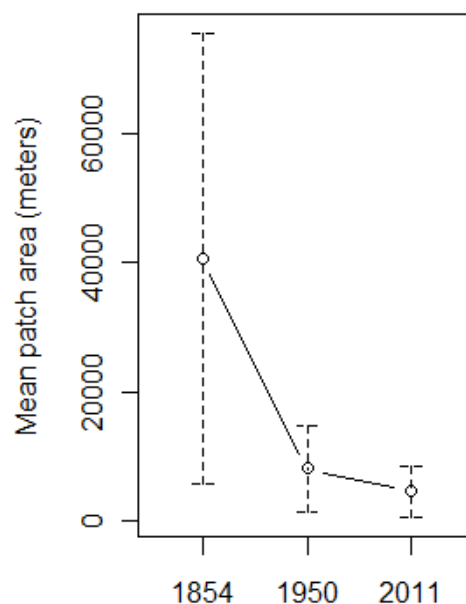


Figure 9 Mean area of semi-natural grasslands patches in function of the time. Segments represent the standard deviation.

c. Decrease of patch size

The mean area of all semi-natural grassland patches in the landscape in 1845 was 4.06 ± 3.48 ha, 0.81 ± 0.67 ha in 1950 and now is 0.46 ± 0.40 ha. Despite the important deviation in 1854 there is a significant statistical difference between the three time periods (p-value = 0.003) (Figure 9) and the multiple comparison show that both 2011 and 1854 are significantly different (p-value = 0.002) as well as 1950 and 1854 (p-value = 0.038), but not 2011 and 1950 (p-value = 0.388). Between 1854 and 1950 the mean size of the patches of semi-natural grassland decreased, leading to smaller patches.

2. Fragmentation effects on community

a. Nestedness

The higher the value of the NODF nestedness metric is, the more likely the observed community data are nested. For the vegetation, nestedness of sites is 30.61 and of species, it is 58.08. For all the data it is 53.78. As for the seed bank, site nestedness is 41.99 and species nestedness is 62.45. For the all matrix it is 59.25. All those values are significant with p-value < 0.01. There is a significant pattern in the overlap for both of them. One notice that the sites are less nested than species.

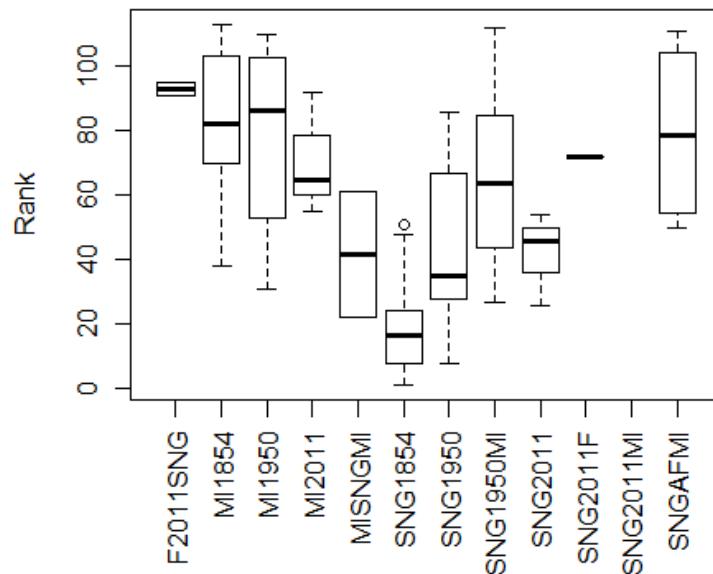


Figure 10 Nestedness rank of the vegetation (Rank) in function of the historical land type (for details see Table 1)

b. Patch area

There is no significant relation between patch size and the seed bank nestedness rank (Table 2). The surface area of a patch thus seems unrelated to the nestedness observed in the seed bank community data. On the other hand, nested structure of the vegetation records seems clearly related to the surface area of a patch in 2011 and 1854, but peculiarly not of 1950.

Table 2 Spearman's rank correlation of the nestedness' rank and the surface area of the sampled patches.

Rank in function of	Vegetation			Seed bank		
	S	p-value	rho	S	p-value	rho
Area 2011	275563	≤ 0.001	-0.60	178166	0.71	-0.04
Area 1950	179410	0.66	-0.04	165752	0.73	0.03
Area 1854	108090	≤ 0.001	-0.48	77544	0.61	-0.06

c. Land cover type

The Kruskal-Wallis test on the nested rank related to the land use showed a significant difference (p-value ≤ 0.001) for the vegetation (Figure 10), but not for the seed bank (p-value = 0.08). The post hoc tests on vegetation revealed a significant difference between the following classes: MI1854 – SNG1854; MI1950 – SNG1854; SNG1950MI – SNG1854 and SNGAFMI – SNG1854. Old semi-naturals grasslands always have smaller rank than midfield islets. In other words, the patches of semi-natural grasslands since 1854 contain the most species and likely the most grazing dependent ones. And grasslands that were transformed in midfield islets in 1950 contain less species and mainly the ones widely spread in the landscape, that resist the most to the fragmentation.

3. Seed bank as a reservoir

By inventorying both the vegetation and seed bank, we can estimate which species are only preserved in the seed bank. The average proportion of species only found in the seed bank of the *ex situ* experiment is 30 ± 40 %. When the entire vegetation of the islet is inventoried, this proportion is then of 29 ± 4 %.

As for 2012, the *in situ* experiment, the proportion of species found only as seedling in the plot is of 59 ± 44 %, but when the surrounding vegetation is taken into account, this proportion is then only of 21 ± 33 %.

4. Colonization dynamics

Usually, plant cover increased greatly from June over July, signaling substantial colonization of the disturbed plots. One plot on a midfield islet (M11) responded differently showing a more abundant vegetation in June (Figure 11 a and b). This plot was mainly filled with ruderal species like *Fumaria officinalis*, *Chenopodium album* and *Artemisia vulgaris*. Due to their short life-cycle (both are annuals) *Fumaria officinalis* and *Chenopodium album* had almost disappeared in July. For some plots, the edges of the square had already disappeared in July due to vegetative colonization from outside the plot (Figure 11 c and d). The plants mainly responsible for this colonization were *Fragaria vesca* and *Hieracium pilosella*, both species producing stolons.

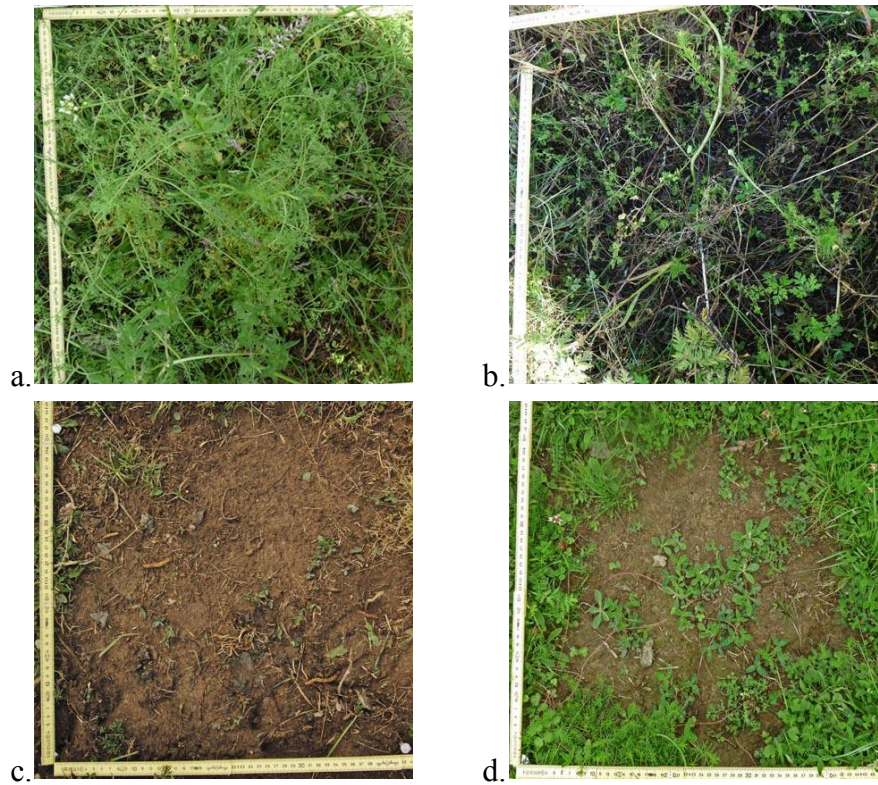


Figure 11 Pictures showing the dynamics of plant colonization in the large square of two plots, M11 (a and b) and S8 (c and d) at two times June (a and c) and July (b and d).

Table 3 The top 20 of the most common species in midfield islets (MI) and semi-natural grasslands (SNG); among seedlings (Seed) and vegetative sprout (Veg). The number is the number of plots where the species was found; between brackets is the total number of plots.

Species	MI	SNG	Seed	Veg
<i>Achillea millefolium</i>	15 (24)	19 (24)	21 (48)	29 (48)
<i>Agrostis capillaris</i>	14 (24)	19 (24)	25 (48)	30 (48)
<i>Anthoxanthum odoratum</i>	5 (24)	11 (24)	9 (48)	14 (48)
<i>Betula pendula</i>	4 (24)	14 (24)	17 (48)	1 (48)
<i>Campanula rotundifolia</i>	9 (24)	6 (24)	15 (48)	5 (48)
<i>Festuca ovina</i>	3 (24)	11 (24)	2 (48)	13 (48)
<i>Filipendula vulgaris</i>	7 (24)	13 (24)	1 (48)	20 (48)
<i>Galium boreale</i>	7 (24)	15 (24)	12 (48)	20 (48)
<i>Galium verum</i>	17 (24)	10 (24)	14 (48)	25 (48)
<i>Hypericum maculatum</i>	5 (24)	10 (24)	15 (48)	3 (48)
<i>Hypericum perforatum</i>	16 (24)	7 (24)	22 (48)	4 (48)
<i>Luzula campestris</i>	3 (24)	14 (24)	12 (48)	15 (48)
<i>Rubus idaeus</i>	9 (24)	7 (24)	16 (48)	2 (48)
<i>Stellaria graminea</i>	10 (24)	15 (24)	23 (48)	2 (48)
<i>Taraxacum officinalis</i> agg.	8 (24)	18 (24)	24 (48)	9 (48)
<i>Trifolium pratense</i>	5 (24)	16 (24)	19 (48)	18 (48)
<i>Trifolium repens</i>	5 (24)	23 (24)	27 (48)	12 (48)
<i>Veronica chamaedrys</i>	9 (24)	15 (24)	22 (48)	14 (48)
<i>Veronica officinalis</i>	1 (24)	15 (24)	16 (48)	1 (48)
<i>Viola canina</i>	10 (24)	18 (24)	22 (48)	18 (48)

The seedlings are an important share of the plants recolonizing the disturbed plots. In terms of the number of individuals, seedlings on average represent 60 ± 19 % of all the plant

individuals in a plot. They are significantly different from the vegetatively resprouting individuals which represent 20 ± 0.19 % (t-test p-value ≤ 0.001). With a mean proportion of seedlings in the small plots at 33 ± 18 %, and at 43 ± 20 % for the large plots, the large plots are richer in seedling than the larger ones (p-value = 0.018), but when midfield islets and semi-natural grasslands are separate, there is no longer a significant difference (t-test p-value = 0.06 for midfield-islets and p-value = 0.12 for semi-natural grasslands).

5. Species abundances

In midfields islets, *Hypericum perforatum*, *H. maculatum* and *Potentilla argentea* are the more common species among the seedlings, whereas *Galium verum* and *Agrostis capillaris* are the most common species which vegetatively resprout. In semi-natural grasslands, *Alchemilla glaucescens* and *Taraxacum officinalis* are the more abundant species among the seedlings. Vegetative sprouts were most often recorded in *Plantago lanceolata* and *Filipendula vulgaris* (Table 3).

6. Shannon index

a. Historical land use

Only the data recorded in July were used for this analysis because it is the most comprehensive in terms of species.

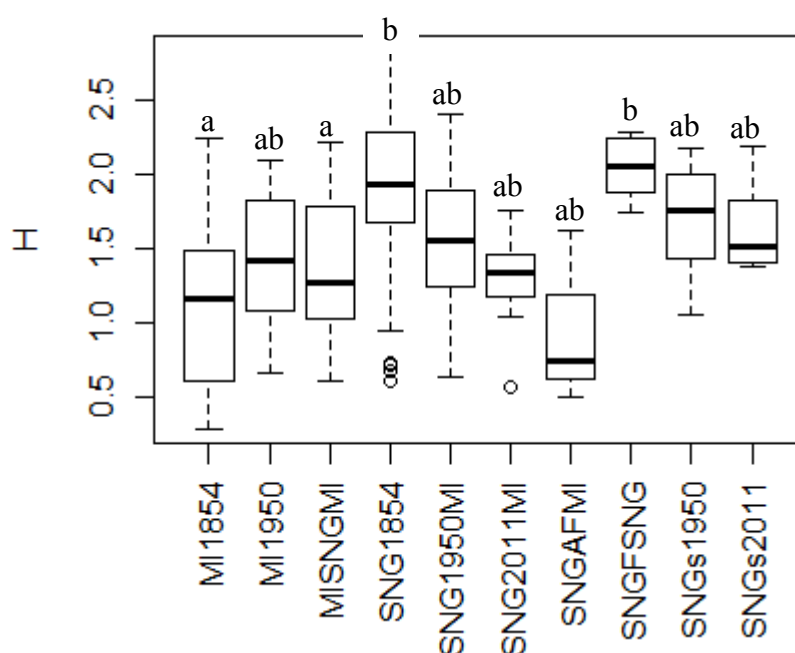


Figure 12 Shannon index (H) in function of the historical land type (for details see Table 1). Letters show differences found with a multiple comparison test, boxes with a same letter are not different.

The higher the Shannon index, the more diverse the community. In July, the Shannon index is statistically different between the different classes of the historical land use (p-value ≤ 0.001) and multiple comparisons found differences between MI1854 – SNG1854, MI1854 – SNGFSNG and SNG1854 – SNG2011MI. The old semi-natural grasslands (SNG1854 and

SNGFSNG) tend to be different from the old midfield islets (MI1854) and the recent ones that were until recently semi-natural grassland (SNG2011MI).

a. Comparison of seedlings and vegetative sprouts

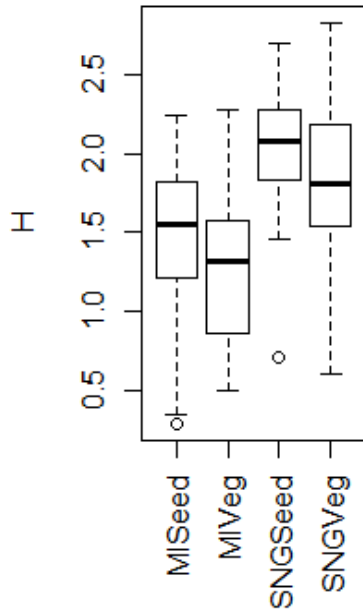


Figure 12 Shannon index (H) in function of the actual land type: either semi-natural grasslands (SNG) or midfield islets (MI); and in function also of the type of the plant recorded: either seedling (Seed) or vegetative sprouts (Veg).

Independently of the land use type, the Shannon index based on the seedling flora on average attained 1.78 ± 0.52 , which is significantly different using a Mann-Whitney test (p-value = 0.006) compared to the Shannon index based on the vegetatively resprouting flora (1.57 ± 0.57 , Figure 13). The seedling flora is thus more diverse than vegetative resprouting flora. However, when the actual land use is added there is still a significant difference using a Kruskal-Wallis test (p-value ≤ 0.001), but multiple comparison testing showed that in the midfield islets there is no significant difference between the species diversity of seedlings and vegetative sprouts. The same trend was observed in the semi-natural grasslands. On the other hand, there was a significant difference between the remaining pair wise comparisons. In other words, the seedling flora of semi-natural grassland plots is more diverse than the seedling flora on the midfield islet plots.

IV. Discussion

1. Landscape changes

The history of landscape fragmentation has persistent effects on the current plant communities, both on the above-ground vegetation, as well as on the post-disturbance seedlings originating from the seed bank. Even if the seed bank is not directly influenced by the historical land use, the emergent seedlings clearly were. This demonstrates that *in situ* studies are essential to assess impacts of fragmentation on the seed bank, although it might only present incomplete information if no *ex situ* seed bank assessment is done.

The temporal changes in the different land cover types were used to reconstruct the historical fragmentation of semi-natural grasslands on Selaön, in order to see the impact of historical fragmentation on plant diversity. The difference between semi-natural grasslands and forests in 1854's map is not obvious, because of the classification as either grazed forest or meadow

with trees. Both of them are managed as semi-natural grasslands, it is only the percentage of tree cover that changes. Moreover, they are more likely to resemble the current day semi-natural grasslands than the current forest characterized by high forest cover and intense forest management. That said, the amount of semi-natural grasslands in 1845 is likely to be slightly biased, because a grazed forest it is not exactly the same as an open semi-natural grassland. Even though Kull & Zobel (1991) found wooded meadows to be very species rich, this richness was attributed to a large number of forest species like *Anemone nemorosa* or *Luzula pilosa*, altogether with grassland specialists such as *Briza media* or *Antennaria dioica* (Ekstam et al., 1992). Besides, the oldest semi-natural grasslands are the most diverse, pointing out the importance of these long managed habitats for conservation measures.

The 1950s seem to be a period different from 1854 and 2011; the area of semi-natural grassland is unrelated to current-day species richness patterns unlike both other times and it is before 1950 that most of the landscapes changes occurred. Actually, it is between 1854 and 1950 that grazed forest changed either into semi-natural grassland or into forest. As grazing history translates into high species diversity in the semi-natural grasslands, Selaön had undergone a drastic decrease and fragmentation of pasture areas before 1950. Moreover, the landscape had changed not only in the respective areas of the different land covers, but also in the different types of grasslands (Cousins & Eriksson, 2002). For example in 1854, there were very dry open grasslands on bedrock as well as wet meadow with trees. Both grasslands type were grazed. In 1950 and at present, there is only some distinction for wet grasslands and the amount of trees.

2. Fragmentation estimation

It is in the areas the most preserved from fragmentation, the old semi-natural grassland, that more species can be found. Therefore, fragmentation has an effect on where species are distributed. This can be seen through the nestedness results; midfield islets that were once semi-natural grasslands in 1854 only contain the most common and fragmentation resistant species. Conversely, old grasslands contain the most of the species, including the grazing dependent ones. Both historical land use and patch area, are linked; they are elements of the historical fragmentation showing that it has impacts of plants communities. Moreover, they are intimately connected; indeed a midfield islet is more often smaller than a semi-natural grassland. If midfield islets were large enough to sustain sheep or cow grazing, then they would probably be used as pasture, therefore becoming semi-natural grasslands.

As for patch isolation, the variation in isolation is quite significant. This is especially the case in 1845 where some values are extreme compared the main body of the data. This may appear strange, particularly when 1845 is supposed to be the least fragmented. But, when looking into the distance values and the 1845 map, the points most distant from their neighbor (i.e. exceeding 600 m) are actually the least isolated points. In reality, they are surrounded by extremely large semi-natural grasslands, illustrating the importance of the distance to the next grassland as the isolation distance. Thus, since the grasslands which are supposedly isolated are so large, the isolation distances can easily be neglected. This is maybe why adding these

variables to the model was not relevant because it did not reflect the actual isolation of the plots.

Isolation is a difficult measure to grasp. The distance between patches is obviously important, but the size of the patch where a plot lies, is also important. Using the formula originally developed by Hanski (1999), Moilanen & Nieminen (2002) provide a formula including patch area, the inter-patch distance and α , a constant to scale the effect of the distance to migration, i.e. $1/\alpha$ is the average migration distance. But the problem, even if this indicator is better than just the distance between the patches, it is more complex to calculate, and an α -value is needed. For plants this is difficult to estimate. Franzén & Eriksson (2003) used three different values (1, 3 and 5) for four species and had statistically significant results only with 3 and 5. Helm et al. (2006) used an arbitrary value for calcareous grasslands of 0.34 corresponding to an average migration distance of 3 km. This variety of α -values shows that it is a delicate value to estimate. However, Moilanen & Nieminen (2002) stated that the connectivity formula might not be too sensitive in errors of α . Moreover, not only distance and area, but patch shape has also been shown to be important to patch connectivity (Hanski, 1998).

Nevertheless, spatial connectivity is rarely found significant (Lindborg & Eriksson, 2004; Helm et al., 2006) or only marginally (Adriaens, 2007), which allow us to consider only the nearest neighbor distance to quickly estimate the importance of connectivity and its opposite isolation. With the data at hand, it soon became clear that isolation increased significantly over the last century and a half.

3. Post-disturbance recovery

The proportion of seedlings compared to the proportion of vegetative sprouts is 60 %, this follows the 59 % found by Bullock et al. (1994), even though their seedlings are only coming from the seed rain, maybe because their seed bank was depleted. Our data are still significantly less than the 82 % found by Kalamees & Zobel (2002) or the 98 % by Vandvik and Goldberg (2006). Bullock et al. (1994) showed that small gaps are more likely to be colonized by vegetative sprouts than by seedlings, hence the role of the seed bank would be expected to be more important in the larger gaps. But by small gap they meant very small gaps of less than 5 cm² and by large they meant gaps larger than 7cm². This complies with our data as the smaller subplots contained more seedlings than the larger subplots. However, we are unsure why more vegetative recolonization was recorded in the large subplots. Perhaps most logically, this is because removal of the vegetation was easier in the small subplots.

In fact, the soil disturbance was not easy to perform. In some plots, the grassland sward was so dense that it was difficult to remove the aboveground vegetation. This can explain why in some plots the vegetative growth was more important than in others. In comparison, the disturbances created by boars which we saw in the field were much more cleared of vegetative sprouts and almost only contained seedlings. This is because they dig deeper – more than 10 cm for eating roots (Kalamees & Zobel, 2002; Pakeman & Small, 2005).

The part of the seed bank contributing to post-disturbance recolonization has been shown to be less important than the seed rain (Bullock et al., 1994; Kalamees & Zobel, 2002). Our data are inconclusive on that respect, as we know seedling recorded this far are solely originating from the seed bank. Effects of fragmentation may only be detected in the seed rain since it is mostly derived from the immediate surrounding vegetation, which is strongly altered due to the historical fragmentation. In addition, we observed no differences between the clipped and non-clipped plots (p-value = 0.97 in July), yet effects on the seed rain and consequently next years seedlings are not to be expected until the following years. It will then be essential to clearly separate the seed rain from the seed bank.

Studies over time are really important in seed bank experiments because if a species can germinate, we do not know whether it will survive or not (Turnbull et al., 2000), especially because the seedling stage is a critical period deterministic of a seedlings' survival chances (Harper, 1977). In some plots for both species of *Hypericum maculatum* and *H. perforatum*, numerous seedlings were found: 240 and even 306 of both species in the large subplot (for respectively 361 and 476 plants in total). Subsequent years will determine whether these plants all recruit or if only a few individuals manage to establish. The latter is more likely since none of these species were found in large population in the established vegetation.

4. Fragmentation seed bank and seedlings

With regards to the effects of fragmentation on the *in situ* and *ex situ* seed bank, they do not respond in the same way. Where we see no clear effect from the *ex situ* seed bank, there is more pronounced effect on the *in situ* seed bank. The *ex situ* seed bank is different from what germinates *in situ*. This shows that to appreciate the whole range of species in the seed bank both types of studies are necessary: *ex situ* greenhouse experiment allows showing most of the species present in the seed bank, but the artificial conditions do not allow every seed to germinate. For example, some species found in the seed bank in the *ex situ* experiment in 2010 were not found in the seedlings like *Chelidonium majus* or *Solanum dulcamara*. But it can be biased because less plots were sampled, therefore decreasing the probability to find the same species. On the other hand, the *in situ* experiment had permitted to see some species usually absent in greenhouse experiments, as *Primula veris*, *Polygala vulgaris* or *Veronica verna*. These plants were present in the vegetation and recorded among the seedlings which germinated in the gaps, but not found in the seed bank.

The results show that the seed bank is not related to the historical land use of its patch area, compared to the vegetation. This difference can be due to the pseudo-extinction debt effect. The seed bank may display a postponed response to detrimental effects of fragmentation. To be present in the vegetation, a species has to pass through different filters: environmental ones or competitive ones, when for the seed bank filters are different. By being dormant, there is for example no competition between the seeds, this is when they will germinate, that the same filters as vegetation will occur. This can be why, for seedlings, there is an effect of the historical land use; seedlings are already confronted to their environment and have to survive.

And because the historical land use is affecting the landscape, seedlings are consequently affected also.

In other words, the seed bank shows the potential pool of species which may once establish in a specific location, whereas the germinated seedlings show the actual contribution of the seed bank to post-disturbance recovery. Therefore, *in situ* experiments to assess the seed bank contribution to vegetation dynamics in fragmented landscapes are essential to evaluate how strongly vegetation dynamics are altered by historical habitat fragmentation.

V. Conclusion

With biodiversity threatened worldwide, species rich ecosystems are important to preserve. In order to find best measures to conserve these habitats, fundamental research on driving factors of species extinctions are essential. One of the strongest threats, next to global climate change, is habitat fragmentation. Indeed, next to the severe loss of habitat in our human modified landscapes, remaining habitat patches become increasingly smaller and more isolated. Moreover, the history of how a habitat patch experiences habitat fragmentation may impact the distribution of plants as observed today.

The aboveground vegetation in a fragmented landscape is significantly influenced by this history. For example, the semi-natural grasslands characterized by their long history of continuous grazing are the most species rich habitats, indicating that conservation efforts should be aimed at the habitats with a long management history. Historical fragmentation also alters the seedling flora which emerges following a disturbance, although a similar effect was not recorded in the seed bank flora. It can be a strong effect of pseudo-extinction debt on the seed bank.

But to thoroughly understand seed bank dynamics it is essential to discriminate seedlings which are seed bank or seed rain derived. Moreover, knowing exactly how the seed bank may drive colonization dynamics will permit a better understanding of how seeds could be useful within conservation and management schemes.

With the need to conserve the species rich semi-natural grasslands, determining whether the seed bank can be used as a reservoir of species of high conservation value or not, is essential to the potential use of the seed bank in conservation and management schemes. Understanding whether the species inside this reservoir can spread out in the favorable patches of the fragmented landscape, will permit to decide where to create new (future) semi-natural grasslands.

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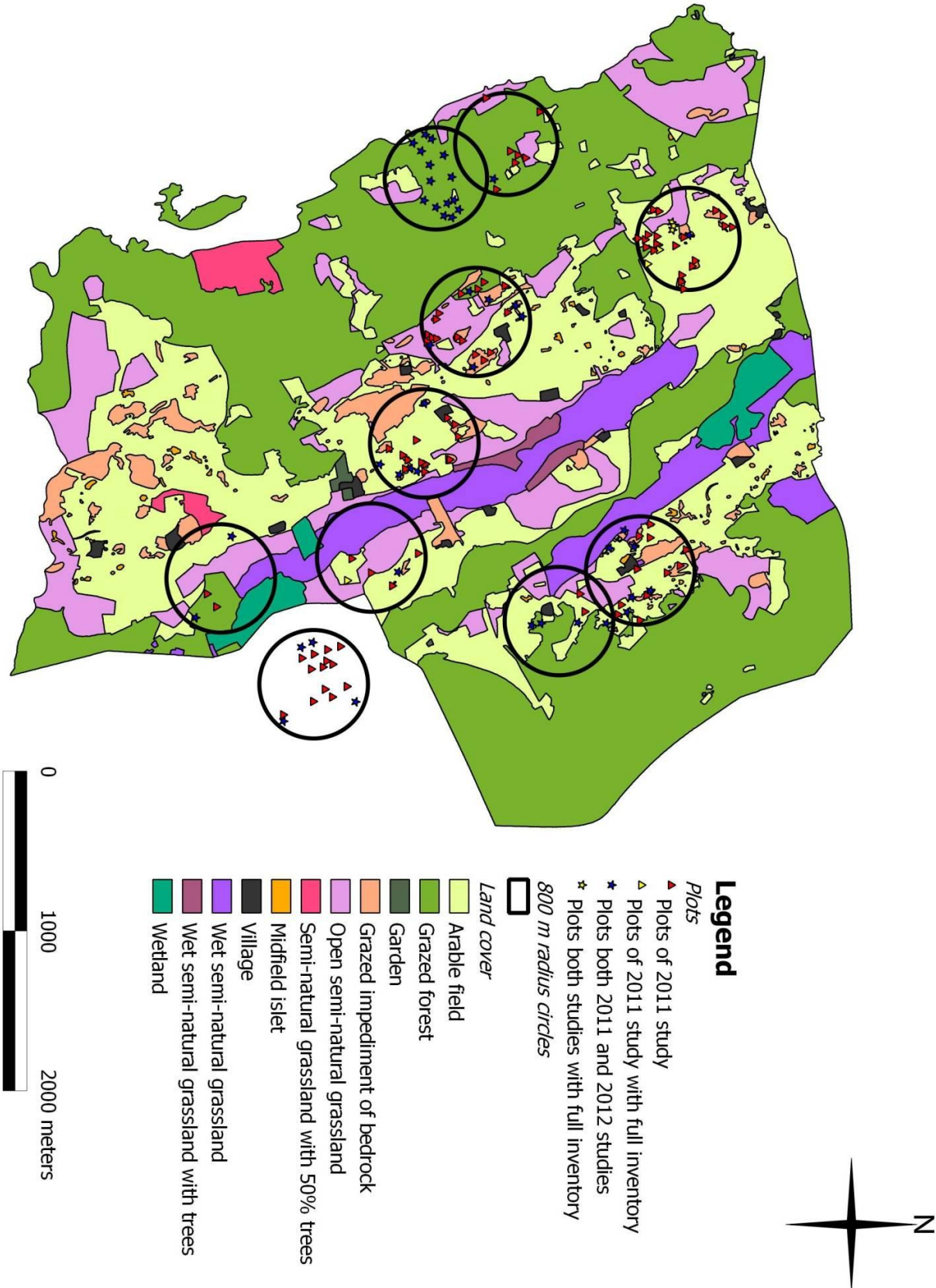
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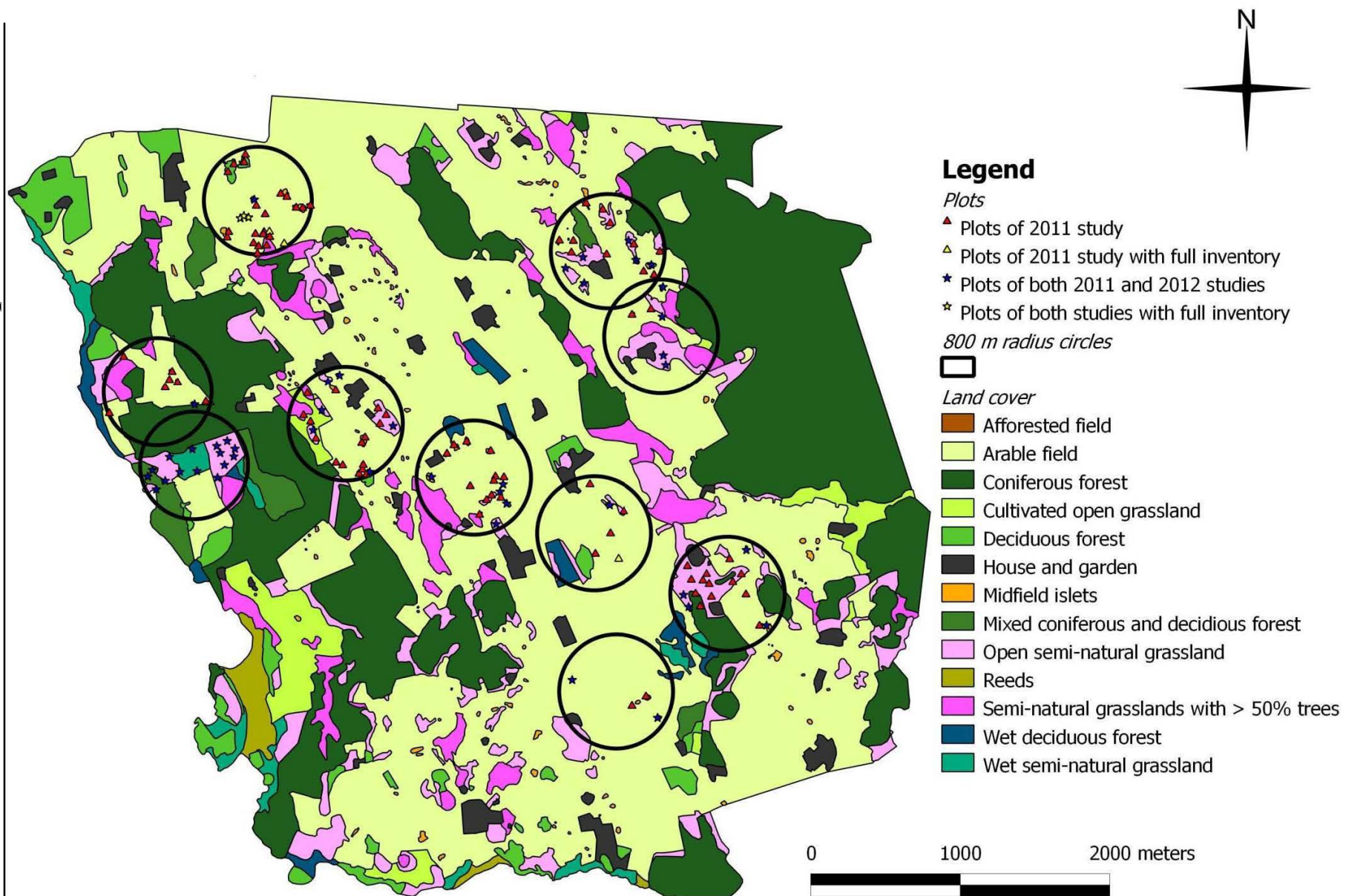
ANNEXES

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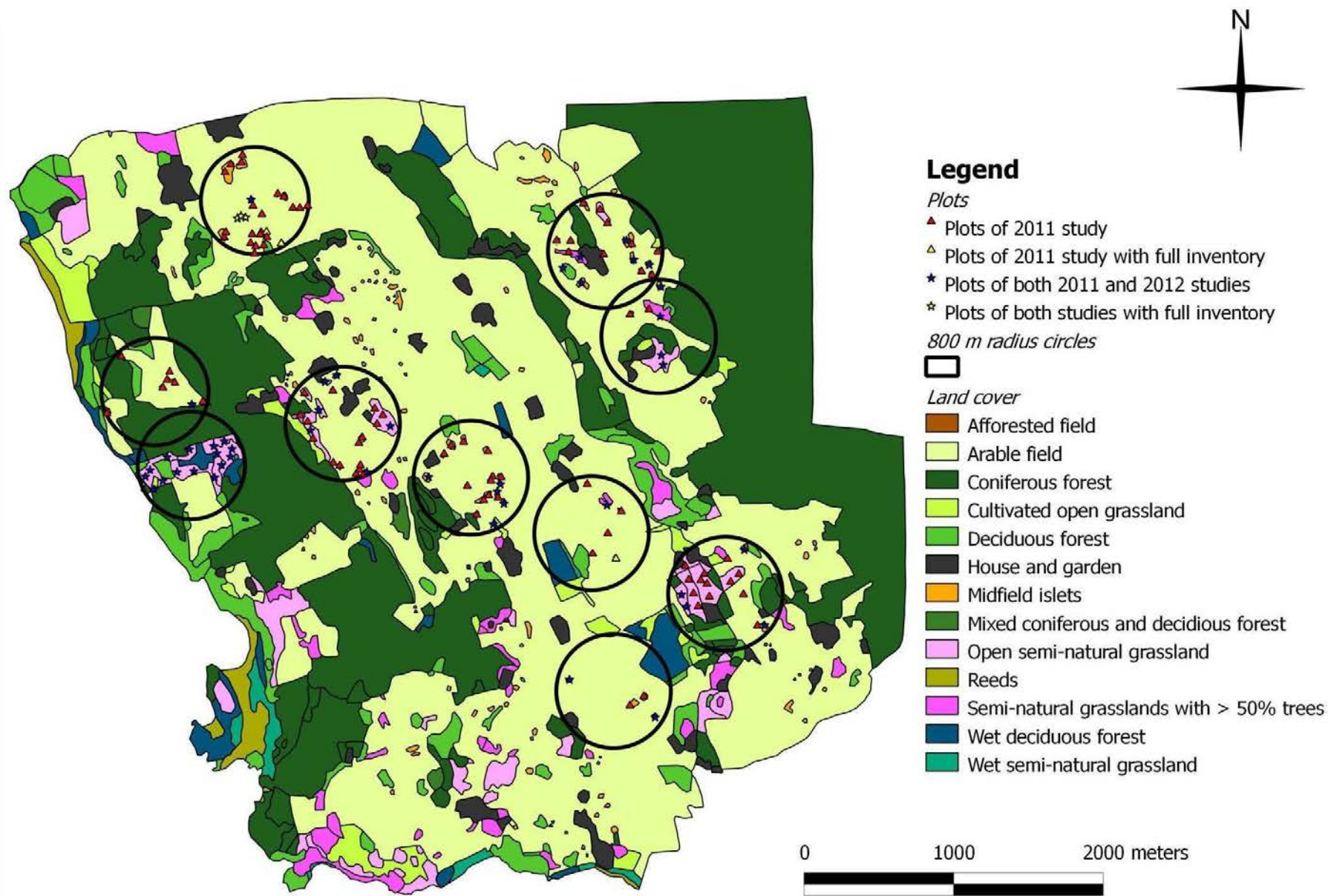
ANNEX I – Land cover in 1854 and plots localization



ANNEX II – Land cover in 1950 and plots localization



ANNEX III – Land cover in 2011 and plots localization



ABSTRACT

Habitat fragmentation is a major threat for global biodiversity. One of the most species rich communities worldwide, i.e. semi-natural grasslands, have significantly decreased in surface area in Europe, with remnant patches being ever more isolated. To assess how historical fragmentation affects plant diversity, we first reconstructed historical grassland fragmentation using land cover maps from a Swedish agricultural landscape from three distinct time periods: 1854, 1950 and 2011. We then defined land cover types on which we inventoried the above-ground vegetation and seed bank. In a subset of 48 plot locations, equally distributed among semi-natural grasslands and remnant grassland fragments, the vegetation was removed to monitor *in situ* vegetation recovery from the seed bank. These combined data were analyzed to evaluate the impact of historical fragmentation on plant species richness patterns in the landscape. Species distributions in the above-ground vegetation, as well as among seedlings from *in situ* seed bank experiment are affected by historical fragmentation. But the seed bank from *ex situ* experiment is not. Therefore *in situ* experiments are essentials to access the impacts of the fragmentation on the seed bank. Our most important finding is that seeds may hold the key for the future biodiversity in fragmented landscapes.

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La fragmentation des habitats est une menace majeure pour la biodiversité mondiale. Les prairies semi-naturelles, une des communautés les plus riches en espèce dans le monde, ont vu de façon significative leur surface diminuer en Europe, avec les fragments subsistants étant toujours plus isolés. Pour apprécier les effets de la fragmentation historique sur la diversité des plantes, nous avons tout d'abord reconstruit l'histoire de la fragmentation des prairies en utilisant des cartes de couverture de sol d'un paysage agricole suédois, à trois périodes distinctes : 1854, 1950 et 2011. Ensuite nous avons défini les types de couverture de sol sur lesquelles nous avons inventorié les parties aériennes de la végétation et la banque de graines. Dans un sous-ensemble de 48 parcelles, distribuées de façon équilibrée entre les prairies semi-naturelles et les fragments de prairie restant, la végétation a été retirée pour suivre *in situ* le recouvrement par la végétation provenant de la banque de graines. Ces données combinées ont été analysées pour évaluer l'impact de la fragmentation historique sur la répartition de la richesse spécifique des plantes dans le paysage. La distribution des plantes – parties aériennes – ainsi que celle des plantules provenant de l'expérience *in situ* de colonisation à la suite d'une perturbation, sont affectées par l'histoire de la fragmentation. Mais les plantes issues de la banque de graine de l'expérience *ex situ* ne le sont pas. En conséquence les *expériences in situ* sont essentielles pour accéder aux impacts de la fragmentation sur la banque de graine. Notre plus grande découverte est que les graines peuvent jouer un rôle clef pour le future de la biodiversité dans les paysages fragmentés.