Rapid restoration of a species-rich ecosystem assessed from soil and vegetation indicators: the case of calcareous grasslands restored from forest stands.

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Abstract

Calcareous grasslands have long been recognized as biodiversity hotspots in Europe. However, in recent decades these ecosystems have seen rapid decline. In Belgium, more than 100 ha of calcareous grasslands have been restored from oak coppices and pine forests since the 1990s. The aim of the present study was to provide a quantitative assessment of the success of these restoration efforts, using two sets of indicators: one related to soil conditions, the other related to vascular plant communities. Soil conditions were evaluated by comparing soil samples from pre-restoration forest stands, restored grasslands (3 age classes: 2-4 years; 5-8 years, 10-15 years) and reference grasslands. The analysis revealed no significant differences in soil N, P and K contents between pre-restoration forests and restored and reference grasslands. We observed a decrease in the mineralization rate indicators in both pre-restoration forests and recent grassland restorations, which was resorbed in older restorations. Floristic surveys revealed that plant species composition of older restorations was most like reference grasslands. However, some differences in species composition persisted after 15 years. Moreover, a few rare species did not colonise restored grasslands despite a close seed source. Non-recolonization by a set of species expected on calcareous grasslands may be due to dispersal limitation and higher cover by native invasive grasses in restored parcels. These results were discussed in term of implications for management.

Introduction

In the face of destruction and fragmentation of natural and semi-natural habitats, protection and management of the remaining (semi-) natural habitat fragments is no longer sufficient to ensure the long-term viability of all elements contributing to biodiversity. Consequently, habitat restoration has become a necessity to recreate functional ecological networks (Jongman & Pungetti 2004). This approach serves to restore patches of degraded or destroyed habitat by accelerating or skipping successional stages. This is accomplished by directing ecosystem development towards a specific community structure or ecosystem type (Bakker & Berendse 1999; Jordan et al. 1987). However, habitat restoration can be cost prohibitive and efforts to restore natural communities must demonstrate their success in reaching target ecosystems (Fagan et al. 2008). Hence, quantitative indicators are needed to evaluate the efficiency of restoration practices and to improve future restoration projects (e.g. Holl & Cairns 2002; Samu et al. 2008). Ideally, these indicators may address different components of the restored ecosystem.

Habitat restoration is of vital importance in temperate semi-natural calcareous grasslands, and has received considerable attention in recent years. Those ecosystems are considered one of the richest in biodiversity in temperate regions (Prendergast et al. 1993; WallisDeVries et al. 2002) and the most species-rich plant communities in the world at a small scale (<10m²) (Willems 2001). Once widespread over Europe, calcareous grasslands have undergone dramatic fragmentation since the end of the nineteenth century. Following the abandonment of the traditional agro-pastoral systems that were responsible for their extension, these communities have been replaced by arable land, trees plantations, or have undergone spontaneous encroachment and succession to forest communities (Poschlod & WallisDeVries 2002). The maintenance and enhancement of calcareous grassland networks is now recognized as a priority in European biodiversity conservation policies, as reflected in the Habitat Directive 92/43/EEC.

Restoration success depends primarily on two factors, the reformation and maintenance of suitable environmental conditions and the recolonization capacity of target species (Perrow & Davy 2002; Piqueray & Mahy 2009). Soil conditions in calcareous grasslands are characterized by low soil fertility, and nutrient transfer historically occurred through traditional grazing practices (Willems 2001). A negative relationship between soil fertility and calcareous grasslands species richness has been demonstrated (e.g. Al-Mufti et al. 1977; Janssens et al. 1998) as well as the harmful consequences of intentional soil nutrient enrichment (Bobbink et al. 1998; Jacquemyn et al. 2003; Willems et al. 1993). On arable lands, attempts to restore soils to historically low fertility levels was shown to be the major constraint for the recovery of calcareous grassland plant communities (Hutchings & Stewart 2002; Walker et al. 2004). However, to our knowledge, soil nutrient status has never been studied in calcareous grasslands restored from forest stands. Green (1972) stressed that natural succession should lead to global eutrophication of any habitat. Hurst and John (1999) showed nitrogen enrichment in calcareous grassland during the first step of encroachment by the native invasive grass *Brachypodium pinnatum* in England. Data from other grassland types indicate that afforestation can induce different changes in soil conditions. On one hand, it often induces a reallocation of major nutrients (N, P) from mineral soil to litter (Farley & Kelly 2004; Ross et al. 1999), as well as changes in the form of these nutrients (Chen et al. 2008; Farley & Kelly 2004). After clear-cutting, litter decomposition can increase the nutrient release (Ouro et al. 2001). On the other hand, shrub and tree colonization can induce modifications to the soil micro-climate, and subsequently cause changes in soil microbial activity responsible for the mineralization of soil organic matter. This can lead to modifications in soil carbon stock, acidity and C:N ratio (Thuille & Schulze 2006). Therefore, we argue that mineralization indicators (e.g. pH, C:N ratio, and Fe), as well as fertility indicators (e.g. N, P, and K) need to be considered to assess the restoration of soil conditions. Decreasing pH and subsequent increasing Fe availability could be consequences of a lower mineralization rate (Bonneau & Souchier 1979).

In plant community recovery efforts, species richness has been widely used as an indicator of restoration efficiency (e.g. Cristofoli et al. 2009; Lindborg & Eriksson 2004; Willems 2001). However, it is only a crude indicator of successful restoration (Kiehl et al. 2006; Mortimer et al. 1998). An ecosystem may be species-rich, but the species may be non-representative of the native community. Therefore, restoration success should be evaluated against a reference habitat, such as a local reference site (Piqueray & Mahy 2009; Ruiz-Jaen & Aide 2005; Society for Ecological Restoration International Science & Policy Working Group 2004).

In Belgium, over 90% of calcareous grasslands have been lost (Adriaens et al. 2006; Bisteau & Mahy 2005a). A large proportion were afforested with *Pinus sylvestris* and *P. nigra* at the end of the 19th century (Vandermotten & Decroly 1995). Other areas experienced a natural process of succession following grazing abandonment and were progressively replaced by oak woodlands. To stop this decline, approximately 100 ha of calcareous grasslands have been restored in Belgium over the last 15 years. All restored sites derived from pre-forests or 40-100 year old forest established on ancient calcareous grasslands. Restoration protocols included tree and shrub clearing followed by sheep and goat grazing (André & Vandendorpel 2004; Graux 2004). Restoration of species-rich grasslands from secondary forest or pre-forest ecosystems was studied in different parts of Europe (Kiefer & Poschlod 1996; Pärtel et al. 1998; Zobel et al. 1996), but rarely in Western Europe (Hutchings & Stewart 2002).

In this paper we combined soil condition and plant community data with the aim of assessing the success of calcareous grasslands restoration from afforested sites in Belgium. Using indicators computed from these two datasets, we compared restored sites with calcareous grassland reference sites and forest stands equivalent to pre-restoration conditions and addressed the following questions: 1) Do restoration actions lead to the reestablishment of native calcareous grassland plant communities; 2) How does soil of restored sites compare to soil of reference grasslands and pre-restoration sites?

Material and Methods

Study sites

The study area was located in two Belgian regions: the Viroin Valley, and the Lesse and Lomme Valleys (Table 1), both located in Calestienne, a narrow Devonian limestone strip running southwest to northeast. Both regions support large areas of calcareous grasslands, and are considered the core areas for calcareous grassland conservation in Belgium. Although several grassland communities occurred within the study area (see Butaye et al. (2005) and Piqueray et al. (2007) for communities description), we focused on the most widespread community : *Mesobromion* calcareous grasslands. A total of 12 sites (six per region) where grassland restoration had occurred were selected for the study (Table 1). Selected sites were restored half from pine stands and half from oak coppices. Pine stands were up to 100-year-old *Pinus nigra* or *P. sylvestris* plantations. Dense shrub oak coppices were mainly formed with *Prunus spinosa*, *Crataegus monogyna* and *Corylus avellana*, with intermingled scarce trees of *Quercus robur*. Historical maps, aerial pictures and field surveys were used to delimit three parcel types: 1) *reference grasslands*, i.e. calcareous grasslands known to have existed for more than two centuries. They harbour the typical local calcareous grassland vegetation and are considered as the reference ecosystem for restoration. 2) *restored parcels*, i.e. former grasslands that were afforested and were then subject to forest clearings with subsequent management (mainly grazing) with the aim of restoring grasslands. Trees and shrubs were exported from the parcels, but tree stumps remained. 3) *pre-restoration forest stands*, i.e. forests established on former calcareous grasslands. Both reference grasslands and pre-restoration forest stands were adjacent to restored parcels, on similar topographic situations. The time passed since restoration (in years) was known for each restored parcel. Restored parcels were chosen to equally cover three age classes since restoration: 2-4 years, 5-8 years and 10-15 years. These classes were mainly defined due to field constraints, e.a. first year was omitted as many uncertainties remained on the location of last clearcuts location at the beginning of our project. Each site comprised at least one parcel of each type. In the "Tienne des Vignes" and "Montagne au Buis" sites, two restored parcels originated from the same forest stand but were restored at different times, these restored parcels had thus the same reference forest stands and reference grasslands. In addition, at "Les Pairées" and "Abannets" sites, two restored parcels were of the same age but originated from two different forest stands (Pine and Oak). In summary, we sampled 8 pre-restoration forests parcels, 12 restored parcels (four per age class) and 8 reference grassland parcels (Table 1).

Table 1: Sampled parcels in the study region, name of the site and age classes (forests are pre-restoration forests; grasslands are reference grasslands). Forest type and age since restoration are respectively given for pre-restoration forests and restored parcels.

Name	Localisation	Region	Age class	Forest stand	Real age
Tienne des Vignes	50°06'N – 5°10'E	Lesse and Lomme	Forest	Pine	/
Lorinchamps	50°06'N – 5°14'E	Lesse and Lomme	Forest	Oak	/
Tienne d'Aize	50°07'N – 5°09'E	Lesse and Lomme	Forest	Oak	/
Les Pairées	50°06'N – 5°11'E	Lesse and Lomme	Forest	Pine/Oak	/
Niémont	50°06'N – 4°42'E	Viroin	Forest	Pine	/
Montagne-aux-Buis	50°05'N – 4°34'E	Viroin	Forest	Oak	/
Rivelottes	50°05'30''N – 4°40'E	Viroin	Forest	Pine	/
Abannets	50°04'30''N – 4°34'E	Viroin	Forest	Pine/Oak	/
Tienne des Vignes	50°06'N – 5°10'E	Lesse and Lomme	2-4 years	/	2 years
Lorinchamps	50°06'N – 5°14'E	Lesse and Lomme	2-4 years	/	2 years
Tienne des Vignes	50°06'N – 5°10'E	Lesse and Lomme	5-8 years	/	8 years
Tienne d'Aize	50°07'N – 5°09'E	Lesse and Lomme	5-8 years	/	8 years
Les Pairées pine	50°06'N – 5°11'E	Lesse and Lomme	10-15 years	/	10 years
Les Pairées oak	50°06'N – 5°11'E	Lesse and Lomme	10-15 years	/	10 years
Niémont	50°06'N – 4°42'E	Viroin	2-4 years	/	2 years
Montagne-aux-Buis North	50°05'N – 4°34'E	Viroin	2-4 years	/	4 years
Rivelottes	50°05'30''N – 4°40'E	Viroin	5-8 years	/	5 years
Montagne-aux-Buis South	50°05'30''N – 4°34'E	Viroin	5-8 years	/	7 years
Abannets pine	50°04'30''N – 4°34'E	Viroin	10-15 years	/	15 years
Abannets oak	50°04'30''N – 4°34'E	Viroin	10-15 years	/	15 years
Tienne des Vignes	50°06'N – 5°10'E	Lesse and Lomme	Grassland	/	/
Lorinchamps	50°06'N – 5°14'E	Lesse and Lomme	Grassland	/	/
Tienne d'Aize	50°07'N – 5°09'E	Lesse and Lomme	Grassland	/	/
Les Pairées	50°06'N – 5°11'E	Lesse and Lomme	Grassland	/	/
Niémont	50°06'N – 4°42'E	Viroin	Grassland	/	/
Montagne-aux-Buis	50°05'N – 4°34'E	Viroin	Grassland	/	/
Rivelottes	50°05'30''N – 4°40'E	Viroin	Grassland	/	/
Abannets	50°04'30''N – 4°34'E	Viroin	Grassland	/	/

Soil analysis

Five soil samples were collected in each parcel, i.e. pre-restoration forests, restored parcels, and reference grasslands. Each sample consisted of a bulk soil sample collected systematically with a 2 cm diameter auger within a 1 m^2 quadrat. The minimum total soil volume collected was 100 cm^3 per quadrat. The soil was thin at all study sites (circa 10 cm); therefore the entire soil layer, from ground surface to bedrock, was collected. Soil depth was measured at the four corners of the quadrat. Mean soil depth was estimated for each soil sample. In forests and reference grasslands, quadrats were randomly localized. In restored grasslands, samples were collected nearby randomly selected tree stumps. Positioning quadrats in the close vicinity of tree stumps ensured that the sample was not situated in a small remnant of the former grassland. Soil samples were sieved (< 2 mm and one fraction < 0.5 mm) after air-drying.

Total nitrogen content was estimated following the modified protocol of the Kjeldahl method described by Bremner & Mulvaney (1982). Available phosphorus was determined using a modified version of Olsen & Sommers (1982). Phosphorus was extracted by shaking 5 g of soil with 100 ml of 0.5N $NaHCO_3$, at pH of 8.5, for 30 minutes. Carbon black was added to clear filtrates. After filtering, a 5 ml aliquot of extracted phosphorus was diluted in a 100 ml volumetric flask with 10 ml 1.5% $(NH_4)_6Mo_7O_{24}.4H_2O$ and distilled water. The sample was coloured blue by adding 0.5 ml of 1% $SnCl_2$, and phosphorus content was measured by spectrophotometry at 560 nm. Total organic content was measured following the Springer-Klee method, i.e. a hot sample oxidation with $K_2Cr_2O_7$ and titration of oxidant excess with 0.1N $(NH_4)_2Fe(SO_4)_2.6H_2O$. Mobilizable fractions of K and Fe were determined after extraction with 1N CH_3COONH_4-EDTA. The soil: solution ratio was 1:5, and the extraction solution pH was buffered at 4.65. Elements were measured in the remaining extraction solution by ICP-OES (VARIAN Vista MPX). Soil pH was estimated with a Hellige pH-indicator.

A preliminary data analysis (results not shown) indicated variation among sites with significant mean differences for all soil variables. To reduce site effect, and to assess the effect of restoration as a function of the reference ecosystem, i.e. reference grasslands, soil data were transformed as follows:

$X_{ij} = X_{0,ij} - X_{ref,j,}$

where X_{ij} is the transformed value of the considered soil parameter in quadrat *i* in site *j*, $X_{0,ij}$ is the initial value of the soil parameter in quadrat *i* in site *j*, and $X_{ref,j,}$ is the mean value of the soil parameter over all quadrats of the reference grassland in site j. In order to avoid pseudo-replication the average soil values of each parcel were considered for analyses.

We computed MANOVA (Wilks'lambda) in order to test for an overall difference among mean transformed value for the different soil parameters among the different types of parcels: pre-restoration forests, restored parcels of different ages (three classes) and reference grasslands. Thereafter, single response of each soil parameter was tested with a one-way ANOVA followed by post-hoc Fisher pairwise comparisons tests. By definition, the mean value for reference grasslands was zero. A canonical discriminant analysis was computed in order to illustrate patterns of variation in soil conditions among parcel types.

Floristic analysis

Floristic surveys and species cover (%) were recorded in 20 quadrats (1 m^2) in each restored parcel. Quadrats were located on cardinal directions one meter away from five randomly selected tree stumps. In addition, eighty quadrats were sampled in the reference grasslands and eighty other in pre-restoration forests (equally distributed among regions and then among grasslands/forests of each region). Only herbaceous and shrub species (<2m height) were considered. Species cover values were log-transformed for analyses. In order to avoid pseudo-replication the average species cover of each parcel were considered for analyses.

Detrended Correspondence Analysis (DCA) was performed to examine similarities in species composition among parcels (reference grasslands, restored parcels of different age classes, i.e. 2-4 years, 5-8 years and 10-15 years, pre-restoration forests). We computed an Multiple Response Permutation Procedure (MRPP) with 10000 permutations in order to compare Bray-Curtis distances between and within age classes. In case of significant test, pairwise comparison of age classes was peformed. We tested if plant species composition differed among parcel types by identifying indicator species using the indicator value method INDVAL (Dufrêne & Legendre 1997). Significant indicator species were detected for each parcel type following 999 Monte Carlo permutations, at P=0.05. In order to compare plant community structure from a more ecological perspective, species were classified into five ecological groups based on phytosociological alliances (Bisteau & Mahy 2005b) : (1) species of calcareous grasslands (characteristic of the *Festuco-Brometea* or *Sedo-Scleranthetea*), that was divided in two sub-groups : native invasive grass species (NIG) (*Brachypodium pinnatum* and *Bromus erectus*) and other grassland species; (2) ruderal species (i.e. characteristic of the *Artemisietea*, *Plantaginetea* and *Epilobietea*); (3) meadow species (characteristic of the *Molinio-Arrhenatheretea*); (4) edge species (characteristic of the *Trifolio-Geranietea*); and (5) woodlands and forest species (characteristic of the *Querco-Fagetea*). Shrubs were considered separately. Mean values for species richness, species evenness, shrub cover, bare ground cover and total cover of species belonging to each ecological groups (in %) were compared among parcel types with one-way ANOVAs followed by post-hoc Fisher pairwise comparisons tests. Shrub cover, bare ground cover, edge species cover, other grassland species cover and ruderal species cover were log-transformed, and grassland species cover and forest species cover were Asin-transformed to improve normality and homoscedasticity.

Finally, canonical correspondence analysis (CCA) was used to assess the influence of soil parameters on the representation of ecological groups within parcels.

DCA and CCA were performed using CANOCO 4.5 (ter Braak & Smilauer 2002). MRPP was performed using 'Vegan' R-package (Oksanen et al. 2008). MANOVA was conducted unsing the 'Stats' R-package. Canonical discriminant analysis was performed using 'Candisc' R-package (Friendly 2007) ANOVAs were conducted with MINITAB 15 (Minitab Inc. 2007). Nomenclature followed Lambinon et al. (2004).

Results

Soil analysis

Reference grassland soil parameter values are provided in Table 2. Reference grassland pH was, on average, slightly acidic (Table 2), but a high standard deviation reflected large variation for this parameter, with values ranging from 4.5 to 8.5. Pearson correlations revealed that N, P, K, C and pH transformed values exhibited inter-correlations (Table 2). Fe and C:N were independent from each other soil parameters. The first axis of the canonical discriminant analysis explained 79,5% of soils parameters variation. It was mainly linked to mineralization indicators (pH, Fe, C:N) (Fig.1). It discriminated Pre-restoration forests and 2-4 years restorations with positive coordinates, corresponding to lower pH, higher C:N, higher Fe content, from 10-15 years restorations and reference grasslands with negative coordinates.

Table 2: Mean values and standard deviation of soil parameters in reference grasslands and Pearson correlations between transformed soil parameters. Correlation p-levels are given when significant: *:*p*<0.001; **:0.001<*p*<0.01; *:0.01<*p*<0.05.**

	N (%)	P (mg/100g)	K (ppm)	C (%)	Fe (ppm)	C:N ratio	pH	Soil depth (cm)
Mean	0.575	0.963	15.97	7.26	15.15	12.7	5.7	8.19
SD	0.119	0.263	5.22	1.4	2.96	1.4	1.2	2.74
pH	0.566**	0.615***	0.166	0.395*	-0.205	-0.279		n.c.
C:N	-0.039	0.039	-0.053	0.344	0.360			n.c.
Fe	0.102	-0.167	0.198	0.272				n.c.
C	0.913***	0.576**	0.490**					n.c.
K	0.542**	0.198						n.c.
P	0.614***							n.c.

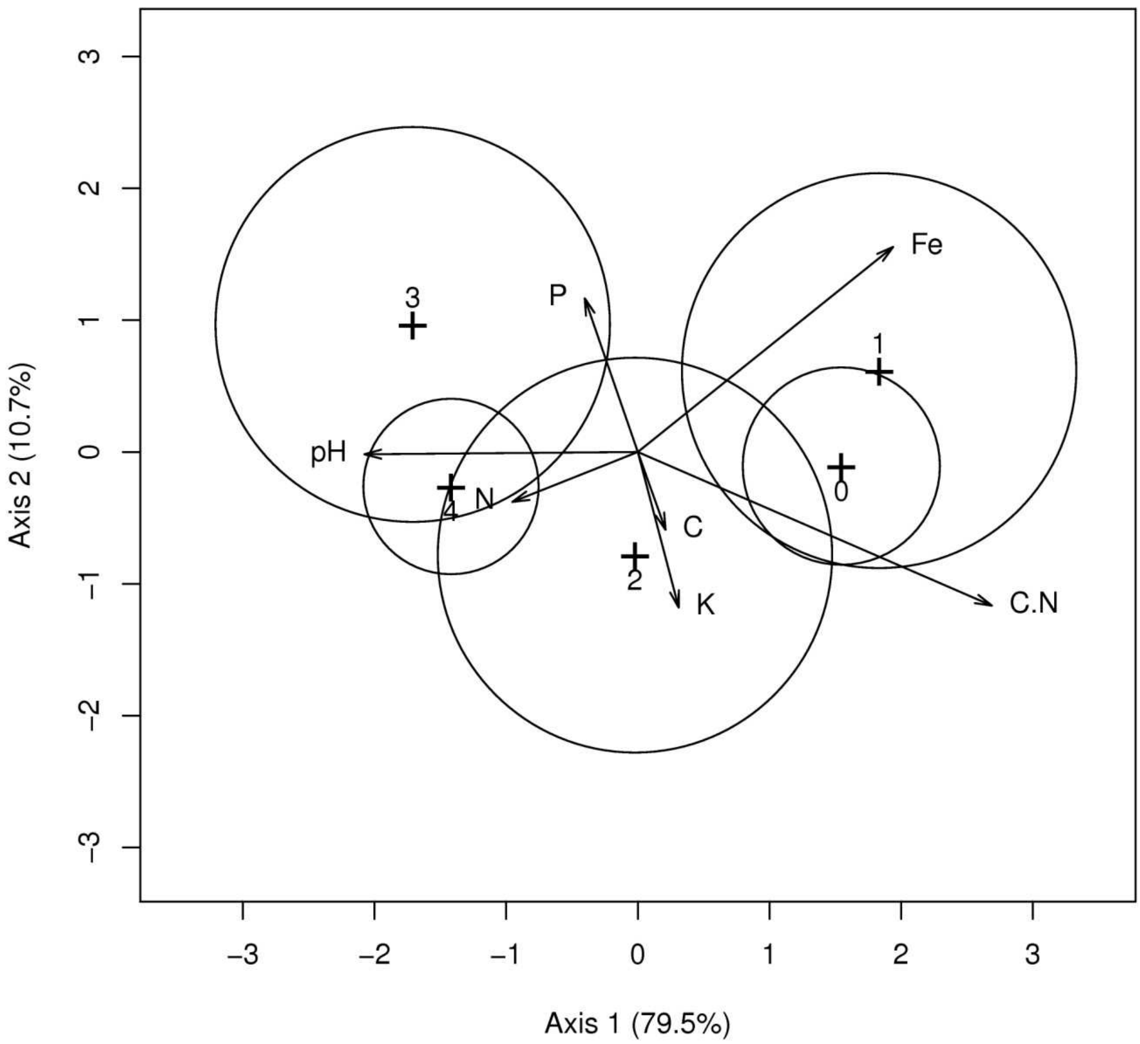

Figure 1: Canonical discriminant analysis ordination plot (axes 1 and 2) of soil parameters in pre-restoration forests (0) , restored parcels (1=2-4 years;2=5-8 years;3=10-15 years) and reference grasslands (4). Mean coordinates and 95% confidence ellipses are plotted.

Comparison of transformed soil parameters revealed marginal differences among parcels types (MANOVA, Wilks' Lambda=0.154, P=0.058). The mean transformed values for N, P, K, C and soil depth did not differ significantly among parcel types (Table 3). However, significant differences for C:N ratio were detected. A significant positive difference was observed for C:N ratio between pre-restoration forest, restored parcels 2-4 and 5-8 years old, and reference grasslands. Older restorations (10-15 years old) did not exhibit a significant difference relative to reference grasslands. Only marginal differences were found for pH, with lower pH in pre-restorations forests and young restorations and the highest values in older restorations.

Althought decreasing Fe content were observed from pre-restoration forests to reference grasslands, this trend was not significant.

Table 3: Mean differences for soil parameters between reference grasslands, pre-restoration forests and restored grasslands of different age classes. (-) indicates a deficiency, (+) indicates a surplus. *p-values* are the result of ANOVA. Different letters indicate significant differences.

	Pre-restoration forests	**Restored grasslands**			**Reference grasslands**	***p-value***
		2-4 years	5-8 years	10-15 years		
N (g/100g)	- 0.050	- 0.026	+ 0.016	+ 0.002	0	0.660
P (mg/100g)	- 0.012	+ 0.032	+ 0.032	+ 0.111	0	0.941
K (ppm)	+ 0.376	- 0,7	+ 0,765	- 0.63	0	0.811
C (g/100g)	- 0.054	+ 0.463	+ 0.909	- 0.04	0	0.882
Fe (ppm)	+ 3.95	+ 5.76	+ 2.84	+ 2.77	0	0.107
C:N	+ 1.32 [a]	+ 1.35 [a]	+ 1.40 [a]	- 0.093 [b]	0 [b]	0.016
pH	- 0.5	- 0.6	+ 0.3	+ 0.5	0	0.053
Soil depth (cm)	+ 0.66	+ 1.78	- 0.91	- 1.46	0	0.153

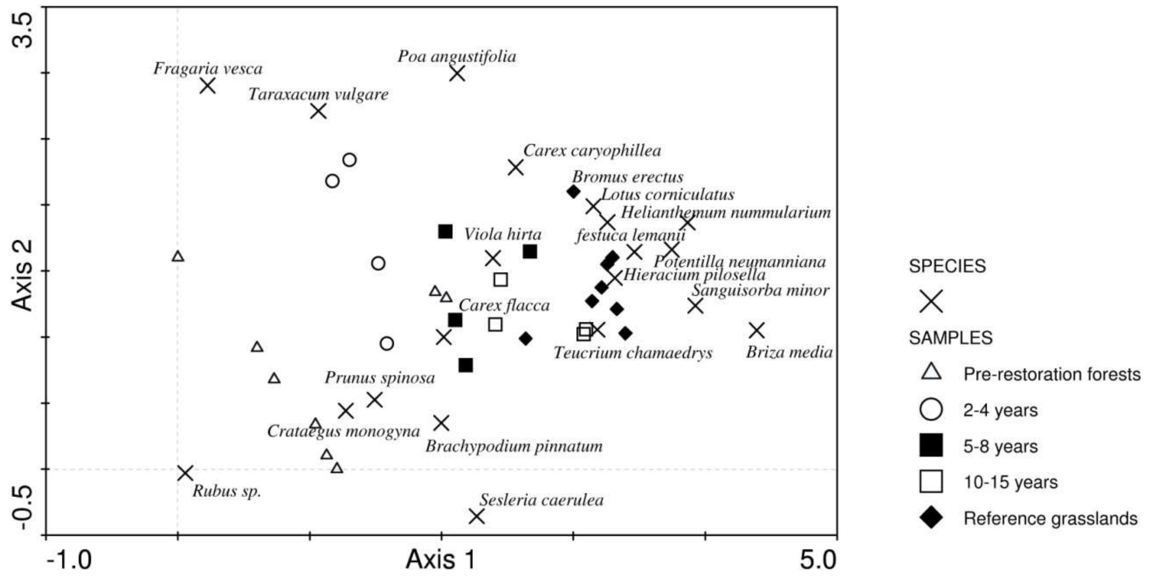

Figure 2: DCA ordination scatter plot (axes 1 and 2) of species composition in pre-restoration forests, reference and restored calcareous grasslands of different age classes. Species with weight >10% were plotted.

Floristic analysis

A total of 210 species was found among our 400 vegetation samples. The first two DCA axes explained respectively 13.6% and 6,9% of the species variation among parcels (Fig. 2). The positions of parcels on axis 1 indicated the clear influence of restoration age with younger restorations (2-4 years) closer to pre-restoration forest and older restorations (10-15 years) closer to reference grasslands. MRPP showed that Bray Curtis distances estimated on species composition among age classes were significant (A=0.139, p<0.001). Pairwise comparisons indicated significant mean Bray Curtis distance among all age class, excepted between 5-8 years and 10-15 years restorations (Table 4). Distance between age classes showed that the older a restoration was, the closer to reference grasslands it was. However, the oldest restoration and the reference grasslands were still significantly different in their species composition. Pre restoration forest samples are rather distant from every other age class. Axes 1 and 2 of the *CCA* respectively explained 21.1% and 13.7% of the ecological group composition variation among parcels. *CCA* opposed shrubs, forest and ruderal species to grassland and edge species on axis 1 (Fig. 3). This axis was correlated with high N content and pH and with low C:N ratio and Fe content. Axis 2 of the *CCA* discriminated meadow species that were linked to the higher K contents.

Table 4: Bray-Curtis mean distance between relevés of the different age classes. MRPP p-levels are given when significant: *:*p*<0.001; **:0.001<*p*<0.01; *:0.01<*p*<0.05.**

		Pre-restoration forests	Restored grasslands		
			2-4 years	5-8 years	10-15 years
Restored grasslands	2-4 years	0.70*			
	5-8 years	0.71**	0.62**		
	10-15 years	0.73**	0.66*	0.51	
Control grasslands		0.81***	0.74**	0.59*	0.52*

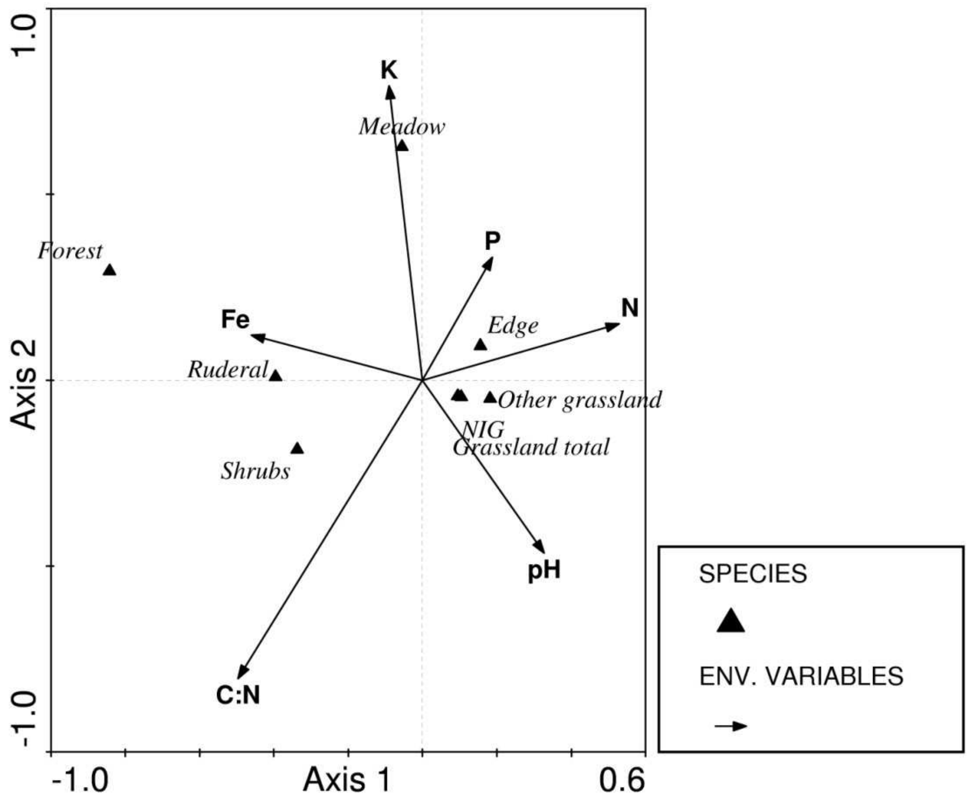

Figure 3: CCA ordination scatter plot (axes 1 and 2) of species ecological groups along soil parameters gradients.

ANOVA showed significant differences for vegetation parameters among age classes, except for the mean cover of meadow species (Table 5). Post hoc comparisons indicated that pre-restoration forests displayed lower species richness than all other parcel types. Species richness tended to increase with increasing age of restored parcels. The highest mean species richness was observed in reference grasslands but was not significantly different from 10-15 years and 5-8 years old restoration. Evenness was significantly higher in reference grasslands than in all other parcel types; while no significant difference was observed among pre-restoration forests and restored parcels. The mean cover of grassland species increased with the age of restored parcels and was not significantly different between 10-15 years old restoration and reference grasslands (Table 5). However, this was mainly due to the overdevelopment of NIG as significant differences was found between reference grasslands and restored parcels for the mean cover of other grassland. NIG cover was the lowest in the forests and in 2-4 years old restored parcels. Older restoration parcels had the highest mean cover of NIG, but the difference with reference grasslands was not significant. Bare ground displayed an inverse pattern, with a minimum cover observed in oldest restorations. In oldest restorations, grassland species tended to replace ruderal species, better represented in recent restoration parcels. Pre-restoration forests were mainly characterized by a relatively high shrub cover.

Shrub cover tended to lower with increasing restoration age, 10-15 years old restored parcels being not significantly different from reference grasslands.

Table 5: Comparisons of vegetation parameters mean values between reference grasslands, pre-restoration forests and restored grasslands of different age classes. *p-values* are the result of ANOVA. Different letters indicate significant differences.

	Pre-restoration forests	Restored grasslands			Reference grasslands	*p-value*
		2-4 years	5-8 years	10-15 years		
Species richness	6.3^{a}	15.0^{b}	16.8^{bc}	18.9^{bc}	20.3^{c}	<0.001
Evenness	0.57^{a}	0.60^{a}	0.58^{a}	0.58^{a}	0.70^{b}	0.021
Grassland species cover	24.6^{a}	37.8^{ab}	62.0^{bc}	72.9^{c}	73.6^{c}	<0.001
NIG cover	20.0^{a}	16.0^{a}	41.7^{b}	39.7^{b}	28.7^{ab}	0.046
Other grassland species cover	4.6^{a}	21.8^{b}	20.3^{b}	33.2^{c}	45.0^{d}	<0.001
Edge species cover	0.9^{a}	5.0^{b}	4.3^{b}	4.1^{b}	6.6^{b}	0.008
Ruderal species cover	3.8^{a}	22.1^{b}	6.5^{ab}	1.2^{a}	1.2^{a}	0.001
Forest species cover	0.4^{b}	0.8^{b}	0.1^{a}	0.1^{a}	0.0^{a}	0.001
Meadow species cover	2.2	1.6	1.9	2.3	54.7	0.547
Shrubs cover	22.0^{c}	12.4^{bc}	8.7^{bc}	5.2^{ab}	2.3^{a}	0.005
Bare ground cover	51.8^{c}	15.9^{b}	2.8^{a}	2.4^{a}	7.0^{ab}	<0.001

Based on the INDVAL analysis, ten species were significant ($P<0.05$) indicators of pre-restoration forests, consisted mainly in shrubs (60%). Thirty-eight species were significant indicators of the 2-4 years class, with 45% ruderal taxa. Ten species were significant indicators of the 5-8 years class. Ruderal species were well represented (33%), but grassland species were also present (27%). Fourteen and 41 species were significant ($P<0.05$) indicators of the 10-15 years and reference grasslands, respectively. In both groups, grassland species were prominent (71 and 76%, respectively). Further information concerning indicator species identity can be found in Appendix A. Nineteen species, mainly characteristic of calcareous grasslands, were present in reference grasslands but not in restored parcels (*Anthericum liliago*, *Carex humilis*, *Cuscuta epithymum*, *Globularia bisnagarica*, *Himanthoglossum hircinum*, *Koeleria macrantha*, *Lathyrus pratensis*, *Melampyrum pratense, Myosotis ramosissimus, Ophrys apifera, Plantago media, Prunella laciniata*, *Pulsatilla vulgaris*, *Ranunculus bulbosus*, *Sorbus torminalis*, *Thlaspi perfoliatum*, *Trifolium montanum, Valerianella dentata*, *Veronica prostrata*). Only few grassland species were found in pre-restoration forests.

Discussion

How does soil of restored sites compare to reference grasslands and pre-restoration sites?

In this study we used fertility and mineralization indicators for comparing soil characteristics among pre-restoration situations, restored ecosystems and reference grasslands. Globally our results pointed to low differences between pre-restoration and reference ecosystems, which is encouraging for calcareous grasslands restoration from afforested sites. However, differences in mineralization indicators were more pronounced than differences in fertility indicators.

Soil fertility level is a major factor for successful restoration, as typical calcareous grasslands are characterized by low nutrient levels (Critchley et al. 2002b; Grime 1979). Soil nutrients contents observed in reference grasslands in the present study were congruent with the literature (Critchley et al. 2002a; Janssens et al. 1998; Wells & Cox 1993). Our results pointed out low trophic conditions for all reference sites, with low concentrations of total nitrogen and available phosphorus concentrations. Neither pre-restoration forest, i.e, parcels afforested < 100 years ago, nor restored grasslands exhibited significantly higher nutrient levels compared to adjacent reference grasslands. Our results contrast with the patterns reported about restoration of formerly arable lands (Walker et al. 2004), and are encouraging for calcareous grasslands restoration from afforested sites. However, some authors stressed the fact that grassland afforestation increased nutrient availability rather than nutrient content (Chen et al. 2008; Farley & Kelly 2004). Further investigations are thus needed to study the different nutrient forms in restored and reference grassland soils. Also, some more synthetic indicators such as standing crop may be used as a complement to this study in order to confirm the equivalent nutrient status between restored and reference grasslands.

Multivariate analysis of soil characteristics pointed to a general pattern of differentiation between pre-restoration forests/young restorations and old restoration/reference grasslands, mainly due to mineralization indicators. However, only C:N ratio showed significant difference among the parcel types and discriminated reference grasslands and old restorations from other parcel types. The lack of significant differences between old restorations and reference parcels suggests that mineralization status of restored grasslands can be restored rapidly despite initial differences. In our study, pH of calcareous grassland was slightly acidic (pH=5.7). This was due to the fact that only the upper soil layer could be analysed due to soil thinness. In this layer, intense root activity and the organic matter decomposition tends to acidify the soil solution (Mengel & Kirkby 1987). pH tended to be lower in pre-restoration forests and young restorations as compared to reference grasslands. The lower pH and higher C:N ratio in pre-restoration forests and recently restored parcels as compared to reference grasslands, may be due to a lower mineralization rate in forests, leading to a pH decrease.

Do restoration actions lead to the reestablishment of native calcareous grassland plant communities?

Species richness has been widely used as a biological indicator of restoration efficiency in calcareous grasslands. We observed increasing species richness with increasing restoration age, which was congruent with similar studies addressing calcareous grassland restoration (Dzwonko & Loster 1998; Lindborg & Eriksson 2004; Zobel et al. 1996). However, species richness cannot be used as a unique measure of successful restoration. Restoration can only be considered a success when referenced against target ecosystems, and sites are effectively colonized by species characteristic of those ecosystems, that establish communities with similar structure. Based on indicators of composition and structure of plant communities, our results indicated a rapid evolution of restored grasslands communities towards reference grasslands characteristics, but still with significant differences.

Floristic similarity between reference and restored grasslands increased with the age of restoration. Observed plant species succession was similar to von Blanckenhagen and Poschlod (2005) description. Firstly, ruderal species developed, and then grassland species increased without however reaching cover exhibited in reference grasslands. Contrary to Dzwonko and Loster (Dzwonko & Loster 2007), who showed high shrub cover in ca. 10 years restored sites, we observed decreasing values of shrub cover in old restorations. This could be explained by the fact that, in our sites, shrubs were regularly mechanically cut in addition to grazing. The proportion of calcareous grassland species increased with the age of the restored patch. It suggests that a large proportion of native calcareous grassland species were able to colonize restored sites within a period of 15 years, which is encouraging for future restoration actions. However, we cannot exclude the possibility that this rapid restoration success was partly due to initial conditions of the oldest restored patches. Parcels restored first may have been less degraded at the onset of the restoration efforts. Therefore, some native grassland species may have been established prior to restoration, surviving in small calcareous grassland refugia. It has been shown by Schrautzer et al. (2009) that vegetation prior to restoration could influence restoration success. Nevertheless, our relevés revealed rather few grassland species in pre-restoration forest stands. Moreover most of them were found only in the site "Les Pairées", that was one of the oldest to be restored.

Despite a general tendency towards plant communities restoration, we noted two important differences between restored and references grasslands. First, we observed a lower evenness in old restored sites. This was likely due to the dominance of native invasive grasses (NIG, indicator of plant community structure) in these areas. Second, nineteen species present in reference grasslands did not recolonize restored sites (indicator of plant composition). A similar situation was described in Central Europe grasslands (Kiefer & Poschlod 1996). Fifteen of them are typical grassland species, which represent 24% of the grassland species found in our study. Among the missing species, ten are listed in the regional Red Data Book (Saintenoy-Simon et al. 2006): *Anthericum liliago*, *Carex humilis*, *Cuscuta epithymum*, *Globularia bisnagarica*, *Himanthoglossum hircinum*, *Ophrys apifera, Pulsatilla vulgaris*, *Trifolium montanum, Veronica prostrata* and *Valerianella dentata*. Except the last, all are typical calcareous grassland species. Furthermore, INDVAL analysis revealed that many other species remained better representated in reference grassland (Appendix 1), especially some species described as typical of ancient grasslands (Karlík & Poschlod 2009).

In restoration from arable fields, abiotic conditions were identified as a major constraint for plant communities restoration (Walker et al. 2004). In our study, differences in soil conditions were only revealed in pre-restoration forests and young restorations compared to reference grasslands. In older restorations, soil parameters were similar to these of reference grasslands. Therefore, soil parameters considered in this study were likely not the cause for the difference observed in species composition between old restorations and reference grasslands.

Plant community structure in oldest restored grasslands may be a constraint for the establishment of some typical grasslands species. High cover of graminoid species, such as *Brachypodium pinnatum*, and low bare ground availability observed on restored sites, particularly old sites, could lower availability of microsites for germination and seedling emergence of most sensitive grassland species (Kotorova & Leps 1999; Zobel et al. 2000). Higher cover of graminoid species in restored sites may also modify competition regimes as compared to reference grasslands. It is generally admitted that graminoid species control in restored calcareous grasslands requires higher grazing pressure than in ancient grasslands (Delescaille 2000; Dutoit & Alard 1996; Verbeke & Lejeune 1996). The increased native invasive grass cover observed in restored patches can be due to insufficient grazing pressure. A possible explanation for such low grazing pressure is that most grazed sites include both restored and ancient grasslands, forming a unique operational grazing unit. Therefore managers often choose to under-graze the restored parcels rather than over-graze the ancient non-degraded grasslands. Additional control of native invasive grasses following restoration may be necessary to promote native calcareous grassland species recolonization. Management measures may include summer mowing of restored parcels in addition to grazing (Bobbink & Willems 1991; Burger 1984; Green 1980).

Dispersal limitation is an alternative, non-exclusive, hypothesis we did not test in the present study to explain that a set of typical species did not recolonize restored grasslands, even after 15 years. Habitat colonization by plant species may result from both spatial and temporal (seed bank) propagule dispersal. Temporal dispersal is limited in calcareous grasslands as most target species present a transient seed bank (Bisteau & Mahy 2005b; Kalamees & Zobel 1997). Moreover, most calcareous grassland species are characterized by low spatial autonomous dispersal capacities (Stampfli & Zeiter 1999). Nevertheless, restored parcels in our study were adjacent to reference grasslands, and most target species occurred within a few meters from restored site. In addition, migrating sheep flocks were used to graze restored sites among calcareous grassland patches in the study regions. Sheep are effective dispersal agents as they can bring large amounts of seeds in their fleece and dung (Couvreur et al. 2004; Fischer et al. 1996; Hellström et al. 2003). Reintroduction of sheep grazing after clear-cutting is of chief importance for the reclamation of the calcareous grassland species pool (Poschlod et al. 1998). Conditions were therefore optimal for dispersal of grassland species into restored patches. Further research should examine if non-recolonizing species and effectively recolonizing species differ in their dispersal traits.

Conclusions and implication for restoration

Assessment of success of calcareous grassland restoration from forest stands, with soil and plant community indicators, point to a rather rapid success. Several indicators revealed that vascular plant communities tended to restore rather rapidly. However, differences still persist between 10-15 years reference grasslands and oldest restored areas. The latter had a lower evenness and were not colonized by a set of typical grassland species. This differences in plant composition persisted despite very similar soil conditions between pre-restoration forests and reference grasslands and full restoration of soil conditions in oldest restored grasslands. Higher native invasive grasses cover and lower bare ground availability in old restored parcels may be factors limiting the establishment of typical grassland species.

To ensure full success of restoration, management strategies, such as additional mowing, should aim at limiting native invasive grasses development and insure sufficient (similar to reference grasslands) bare ground availability in restored areas. Also, directed sowing of target species may help to counteract species difficulties at colonizing restored areas.

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Appendix

Appendix A: Occurrence of species in the five age classes. First value is the number of relevés where it occurred (total number of relevés is 80 in each class). Second value is the mean abundance [%] in relevés where it occurred. Only significant indicators were listed. Age class for which they were indicator is in bold and underlined.

Species	Pre-restoration forests	Restored grasslands 2-4 years	5-8 years	10-15 years	Reference grasslands	Ecological group
Anthericum liliago	0/-	0/-	0/-	0/-	**9/2.1**	Edge
Anthyllis vulneraria	0/-	2/1	4/2	2/0.5	**8/0.9**	Grassland
Arenaria sepyllifolia	0/-	**9/1**	0/-	0/-	2/0.8	Grassland
Avenula pubescens	1/0.5	1/1	5/1.2	**40/0.8**	16/0.8	Meadow
Brachypodium pinnatum	52/26.8	40/32	79/33.4	**80/36.1**	77/21.4	Grassland
Briza media	0/-	0/-	3/2	26/2.1	**45/3**	Grassland
Bromus erectus	11/19.2	0/-	22/31.8	19/15	**30/24.1**	Grassland
Campanula rotundifolia	1/0.5	9/1	27/0.5	**48/0.6**	36/0.6	Grassland
Carex caryophyllea	1/10	13/10.4	15/6.1	35/2.4	**44/2.6**	Grassland
Carex flacca	23/10.1	58/15.8	56/8.6	**78/13**	57/7.1	Grassland
Carex humilis	0/-	0/-	0/-	0/-	**10/12.3**	Edge
Carlina vulgaris	0/-	0/-	1/1	1/0.5	**4/0.8**	Grassland
Carpinus betulus	10/0.9	**12/1.7**	3/0.7	0/-	3/0.7	Shrub
Catapodium rigidum	0/-	**4/0.6**	0/-	0/-	0/-	Grassland
Centaurea scabiosa	0/-	2/0.5	6/0.9	24/1.6	**24/1.9**	Grassland
Centaurea jacea	0/-	2/0.5	4/1.5	38/2.7	**38/3**	Meadow
Cerastium fontanum	0/-	**6/1.3**	0/-	1/0.5	0/-	Meadow
Cerastium pumilum	0/-	0/-	3/1	0/-	**6/0.5**	Grassland
Cirsium acaule	0/-	0/-	3/6.7	9/1.3	**13/4.3**	Grassland
Cirsium arvense	0/-	**19/1.3**	1/1	0/-	0/-	Ruderal
Cirsium vulgare	0/-	**37/2.6**	3/0.8	0/-	0/-	Ruderal
Clematis vitalba	**13/13.2**	16/1.1	10/5.4	1/0.5	1/1	Shrub
Convolvulus arvensis	0/-	1/0.5	**4/5.8**	0/-	0/-	Ruderal
Cornus mas	**6/2**	0/-	0/-	0/-	0/-	Shrub
Cuscuta epithymum	0/-	0/-	0/-	0/-	**5/0.5**	Grassland
Cytisus scoparius	0/-	**9/0.7**	0/-	0/-	0/-	Shrub
Danthonia decumbens	0/-	1/0.5	0/-	3/0.7	**6/2.5**	Grassland
Daucus carota	0/-	**10/0.6**	0/-	0/-	5/0.8	Ruderal
Echium vulgare	0/-	2/1.5	**5/4.5**	0/-	0/-	Ruderal
Eupatorium cannabinum	0/-	**10/5.3**	4/3.1	0/-	0/-	Ruderal
Euphorbia amygdaloides	0/-	2/1.5	**9/0.7**	8/0.6	0/-	Forest
Euphorbia cyparissias	7/1.4	**28/2.7**	24/1	24/1.6	24/1.4	Grassland
Festuca lemanii	0/-	9/32.9	13/5.2	42/11.6	**68/5.2**	Grassland
Festuca rubra	**12/11**	0/-	4/1.6	0/-	2/37.5	Meadow
Fragaria vesca	17/4	**61/16.7**	43/3.9	2/2.5	10/1.3	Ruderal
Fragaria viridis	0/-	0/-	0/-	**32/1**	16/7.1	Grassland
Fraxinus exelsior	**10/2.1**	3/1	2/0.5	0/-	1/1	Shrub
Galium aparine	6/0.7	**19/0.6**	1/0.5	0/-	0/-	Ruderal
Galium mollugo	4/0.6	**13/4.2**	5/0.7	0/-	1/1	Ruderal
Galium pumilum	4/0.5	3/2.8	43/0.9	**66/1.3**	52/0.7	Grassland
Galium verum	0/-	1/0.5	12/3.5	22/1.1	**24/3.6**	Edge

Species	Pre-restoration forests	Restored grasslands 2-4 years	5-8 years	10-15 years	Reference grasslands	Ecological group
Genista tinctoria	0/-	1/0.5	8/0.8	**18/2.6**	13/2.8	Grassland
Genistella sagittalis	0/-	1/0.5	0/-	25/1.2	**22/2.3**	Grassland
Geranium robertianum	3/0.7	**5/1.4**	0/-	0/-	0/-	Forest
Geum urbanum	4/1.8	**11/0.8**	2/0.5	0/-	0/-	Ruderal
Hedera helix	**7/0.6**	3/0.5	0/-	1/0.5	0/-	Forest
Helianthemum nummularium	2/1.3	4/1	38/3.3	43/1.7	**54/8.1**	Grassland
Hieracium pilosella	0/-	5/1.2	17/4.6	0/-	**28/4.7**	Grassland
Hippocrepis comosa	0/-	14/2.2	11/0.6	1/5	**18/4**	Grassland
Hypericum perforatum	0/-	35/1.2	**43/0.7**	32/0.5	35/0.7	Ruderal
Inula conyzae	0/-	**14/1.6**	9/2	0/-	3/2.2	Edge
Knautia arvensis	0/-	1/1	4/1.8	17/1.3	**18/3.1**	Meadow
Koeleria macrantha	0/-	0/-	0/-	0/-	**24/1.6**	Grassland
Lapsana communis	1/1	**16/1.9**	0/-	0/-	0/-	Ruderal
Leontodon hispidus	0/-	1/0.5	10/1.8	**24/0.9**	16/4.2	Grassland
Leucanthemum vulgare	0/-	1/5	3/1.3	0/-	**5/3.2**	Meadow
Ligustrum vulgare	4/2	0/-	**9/5.3**	1/0.5	1/0.5	Shrub
Linum catharticum	0/-	4/0.5	31/0.8	**45/0.6**	29/0.5	Grassland
Lotus corniculatus	0/-	25/1.2	41/1.4	53/1.5	**60/1.6**	Grassland
Medicago lupulina	1/0.5	4/0.8	3/2.8	3/0.5	**12/0.7**	Grassland
Mercurialis perrenis	1/1	**4/7.3**	0/-	0/-	0/-	Forest
Mycelis muralis	3/1	**8/0.5**	4/0.6	0/-	0/-	Forest
Myosotis arvensis	1/0.5	**12/1.4**	5/1	0/-	3/1.5	Ruderal
Myosotis ramosissima	0/-	0/-	0/-	0/-	**5/0.5**	Grassland
Ononis repens	0/-	0/-	1/0.5	2/2	**5/1.1**	Grassland
Origanum vulgare	0/-	**15/4.1**	4/4.5	0/-	6/1.7	Edge
Picris hieracioides	0/-	**10/3.7**	5/3.2	0/-	0/-	Ruderal
Pimpinella saxifraga	0/-	4/0.5	**45/0.7**	39/0.6	34/1	Grassland
Pinus sylvestris	0/-	0/-	1/0.5	0/-	**5/0.9**	Shrub
Plantago lanceolata	0/-	10/1.4	0/-	11/0.6	**28/2**	Meadow
Plantago major	0/-	**7/1.4**	0/-	0/-	0/-	Meadow
Plantago media	0/-	0/-	0/-	0/-	**9/1.8**	Grassland
Plathantera chlorantha	2/0.8	0/-	**18/0.9**	10/0.7	4/1.4	Grassland
Poa nemoralis	**6/2.6**	2/10.3	0/-	0/-	0/-	Forest
Poa pratensis	0/-	22/6.8	**31/3.4**	24/3.8	15/8.4	Edge
Poa trivialis	4/3.1	**6/4.4**	2/0.5	0/-	0/-	Meadow
Polygala comosa	0/-	2/0.5	7/0.6	**16/0.6**	6/0.6	Grassland
Polygala vulgaris	0/-	11/1.5	**13/2.1**	0/-	10/0.8	Grassland
Potentilla neumanniana	1/0.5	12/0.9	27/0.7	33/0.9	**58/2.5**	Grassland
Primula veris	4/1.1	**10/3.6**	6/2	2/10.3	3/0.7	Grassland
Prunella laciniata	0/-	0/-	0/-	0/-	**4/1.1**	Grassland
Prunus avium	**3/0.8**	0/-	0/-	0/-	0/-	Shrub
Prunus spinosa	41/3.5	32/5.8	43/4.8	**61/4.3**	22/4.6	Shrub
Pulsatilla vulgaris	0/-	0/-	0/-	0/-	**5/4.1**	Grassland
Ranunculus bulbosus	0/-	0/-	0/-	0/-	**13/1.8**	Grassland

Species	**Pre-restoration forests**	**Restored grasslands** 2-4 years	5-8 years	10-15 years	**Reference grasslands**	**Ecological group**
Ranunculus repens	0/-	**6/0.7**	0/-	0/-	1/0.5	Meadow
Rosa arvensis	**8/9.2**	0/-	0/-	0/-	0/-	Shrub
Rosa canina	13/4.1	20/1.5	**29/1.1**	19/0.6	7/0.7	Shrub
Rubus idaeus	1/35	**6/2.6**	0/-	0/-	0/-	Shrub
Rubus sp.	38/28.1	**65/7.3**	18/8.1	8/0.9	0/-	Shrub
Sanguisorba minor	0/-	12/0.9	30/1.7	49/4	**68/5.4**	Grassland
Scabiosa columbaria	0/-	0/-	3/2.7	36/0.8	**36/2.6**	Grassland
Senecio jacobaea	0/-	**7/2**	1/3	0/-	2/0.8	Ruderal
Sesleria caerulea	9/10.2	4/5.5	**27/6.6**	17/4.5	24/9	Grassland
Solidago virgaurea	0/-	0/-	1/1	**20/1.7**	1/4	Ruderal
Sonchus arvensis	0/-	11/0.7	**19/0.9**	4/0.5	1/0.5	Ruderal
Sonchus asper	0/-	**14/0.5**	0/-	0/-	0/-	Ruderal
Stachys officinalis	3/0.7	0/-	2/1	**11/3.2**	8/4.5	Grassland
Stellaria media	**4/2.1**	0/-	0/-	0/-	0/-	Ruderal
Taraxacum sect. Ruderalia	18/2	**62/4.4**	37/2.8	25/1.1	16/1	Ruderal
Teucrium chamaedrys	3/3.8	2/0.8	43/3.8	44/1.2	**56/3.7**	Grassland
Thlaspi perfoliatum	0/-	0/-	0/-	0/-	**3/0.5**	Grassland
Thymus preacox	0/-	0/-	0/-	2/0.5	**9/1.3**	Grassland
Thymus pulegioides	0/-	4/21.5	5/2.4	4/1.6	**23/1.5**	Grassland
Torilis japonica	0/-	**7/1.1**	3/0.7	0/-	0/-	Ruderal
Tragopogon pratensis	0/-	0/-	**6/1.2**	0/-	2/0.5	Meadow
Trifolium dubium	0/-	0/-	**10/2**	0/-	0/-	Ruderal
Trifolium montanum	0/-	0/-	0/-	0/-	**6/1.8**	Grassland
Trifolium repens	0/-	**6/1.1**	2/2.8	0/-	1/0.5	Meadow
Trisetum flavencens	0/-	2/0.8	**19/5.1**	0/-	4/5.6	Meadow
Verbascum thapsus	0/-	**5/1**	0/-	0/-	0/-	Ruderal
Veronica arvensis	0/-	6/0.8	0/-	0/-	**8/0.5**	Ruderal
Veronica officinalis	0/-	**10/1.8**	1/1	0/-	0/-	Grassland
Vicia cracca	0/-	**5/0.6**	1/0.5	0/-	0/-	Ruderal
Vicia sativa	0/-	4/0.9	0/-	0/-	**10/0.7**	Ruderal
Vicia sepium	**2/0.8**	0/-	0/-	0/-	0/-	Edge
Viola hirta	12/1	62/2.5	71/1.8	**80/2.3**	55/2	Edge