

Response of plant functional traits during the restoration of calcareous grasslands from forest stands

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Abstract

In this survey, we studied the response of plant functional traits to calcareous grassland restoration in the Calestienne region, Southern Belgium (restoration protocol: forest clear-cutting followed by grazing at all sites). We considered traits related to dispersal, establishment, and persistence that integrate the main challenges of plants to re-establish and survive in restored areas. Functional traits were compiled from databases and compared among (i) pre-restoration and young restoration forests; (ii) restoration areas of different ages; and (iii) old restorations and reference grasslands. The following questions were addressed: (i) What is the early response (2-4 years) in terms of plant functional trait following one restorative clear-cut event? (ii) What plants functional trait responses occur from restorative management (*i.e.* sheep and goat grazing)? (iii) Which differences still persist between the oldest restored parcels (10-15 years), and the historical reference grasslands? Forest clear-cuts induced several changes among functional traits, including decreased mean seed mass and certain vegetative traits (*i.e.* decreased phanerophytes, branching species; and increased short lifespan species *i.e.* annuals and biennials). During restorative management, clonal, epizoochorous and autumn germinating species were favored. Despite numerous other changes during this phase, many differences remained compared to reference grasslands. In particular, geophytes, mycorrhizal and evergreen species abundance were not approaching reference grassland values. The observed pattern helped to draw inferences on the possible mechanisms operating under vegetation recovery following restorative forest clear-cut and subsequent management were identified and described in this study. Results indicated grazing was an important factor, which increased epizoochorous species, and autumn germinating taxa that filled niches in vegetation opened by summer grazing animals. Finally, differences between old restoration and reference grasslands emphasized that management should focus on reduction in soil fertility, and geophyte rhizomatous grasses. Long-term monitoring is vital to assess if management plans are effective in the complete restoration of species functional trait assemblages.

Keywords: Belgium; Calcareous grasslands; Forest clear-cut; Functional traits; Restoration monitoring.

Running head: Calcareous grasslands restoration

Introduction

Semi-natural calcareous grasslands historically developed across Europe from sheep-grazing practices, resulting in unique, species-rich habitats (Korneck et al. 1998; WallisDeVries et al. 2002). They had their maximum extensions during the Roman period, the Medieval age and the early Modern Times (Poschlod et al. 2008; Poschlod & Baumann 2010), but locally also starting from the Neolithic age on (Dutoit et al. 2009). Since the end of the 19th century, semi-natural calcareous grasslands have undergone dramatic decline and fragmentation in European countries (WallisDeVries et al. 2002; Krauss et al. 2010; Piqueray et al. 2011a). Following abandonment of traditional agro-pastoral practices that were responsible for their maintenance, these unique communities were replaced by arable land, afforestations, or spontaneous encroachment and succession from adjacent forest communities (Poschlod & WallisDeVries 2002). In Belgium, over 90% of calcareous grasslands have been lost since the 19th century (Bisteau & Mahy 2005; Adriaens et al. 2006; Piqueray et al. 2011c). A large proportion were afforested at the end of the 19th century with *Pinus sylvestris* L. and *Pinus nigra* Arnold. (Vandermotten & Decroly 1995). Other areas experienced natural successional processes following grazing abandonment, and were progressively replaced by oak woodlands. The maintenance and enhancement of calcareous grassland networks is now recognized as a priority in European biodiversity conservation policies, as cited in the Habitat Directive 92/43/EEC (habitat type 6210). Therefore, since the 1990s restoration practices were applied to redevelop the grasslands, including clear-cutting of trees and shrubs, top-soil removal or cutting regimes to impoverish nutrients as well as sowing or hay spreading (e.g. Kiefer & Poschlod 1996; Poschlod et al. 1998; Hutchings & Stewart 2002; Kiehl et al. 2006; Edwards et al. 2007; Dzwonko & Loster 2008; Fagan et al. 2008; Piqueray et al. 2011b).

In many cases calcareous grassland restoration has been assessed using plant community responses such as diversity and/or species composition (e.g. Kiefer & Poschlod 1996; Ruiz-Jaen & Aide 2005; Karlík & Poschlod 2009; Piqueray & Mahy 2010). These responses were in some cases related to local environmental conditions, but did not explicitly relate species recovery to ecological processes, and/or morphological and physiological mechanisms. Functional traits are a reasonable tool in this respect (van Noordwijk et al. 2012; Lewis et al. 2014). They can either be useful to determine effects of plants on ecosystem functions (effect traits) or to understand the response of plants to environmental changes such as disturbances (response traits) (Lavorel & Garnier 2002). Among response traits, those reflecting dispersal, establishment, and persistence mechanisms integrate the main challenges of plants to survive and re-establish and may therefore play a considerable role in understanding and predicting changes in calcareous grassland composition and structure (Weiher et al. 1999; Poschlod et al. 2000; Lavorel & Garnier 2002). They are also recognized as tools for the monitoring of grassland communities (Ansquer et al. 2009). Therefore, functional traits may be used to evaluate vegetation recovery during and

following restoration (Poschlod et al. 1998; Weiher et al. 1999; Poschlod et al. 2000). By understanding how plant functional traits respond to ecological processes (e.g. disturbance, natural succession following restoration), it becomes possible to predict what species assemblages might naturally occur or be successfully restored (Poschlod et al. 1998).

Plant community dynamics following species-rich grassland restoration from secondary forest or ecosystems by clear-cutting has seldom been studied from a functional point of view (but see, Dzwonko & Loster 2007; Helsen et al. 2013). Dzwonko and Loster (2007) showed that traits related to establishment were a major driver of the plant success in restored grasslands. Also, dispersal traits both in space and time may be relevant for species success in restored habitats (Poschlod et al. 1998; Helsen et al. 2013). After several years, differences between restored and reference grasslands may still remain (see e.g. Zobel et al. 1996; Piqueray et al. 2011b). However, this may depend on management practices following restoration, affecting trait composition of the successional stages after clear-cut (Poschlod et al. 2000; Kahmen & Poschlod 2008b).

In this study, we analyzed the functional response of vascular plants to calcareous grassland restoration by woodland clear-cutting in the following set of questions: (i) What is the early response (2-4 years) in terms of plant functional trait following one restorative clear-cut event? (ii) What plants functional trait responses occur from restorative management (*i.e.* sheep and goat grazing)? (iii) Have all functional traits been re-established in the oldest restored parcels (10-15 years), or do the traits still differ from historical reference grasslands?

Methods

Study sites and field surveys

The study area included two Belgian regions, the Viroin Valley, and the Lesse and Lomme Valleys, both located in Calestienne, a narrow Devonian limestone strip traversing Southwest to Northeast. Both regions support large expanses of grasslands, and are considered the most important regions for calcareous grassland conservation in Belgium. Different grassland communities occur within the two regions, with *Mesobromion* communities the most widespread. *Mesobromion* grasslands are semi-natural calcareous grasslands developing on gentle slopes or on plateaus, dominated by grasses such as *Bromus erectus* and *Brachypodium pinnatum*. They host the highest species richness among the calcareous grasslands communities occurring in Belgium (Piqueray et al. 2007). Both regions have similar species pools (Butaye et al. 2005; Piqueray et al. 2007). They are separated by a distance of only 40km and have similar land-use histories (Adriaens et al. 2006; Piqueray et al. 2011a). Due to the large-scale loss of these ecosystems throughout Belgium, more than 100 ha of calcareous grasslands (ca. equivalent to the remaining

surface) have been restored over the last 25 years in the study area. All restored sites were derived from 40-100 year old forests established on former calcareous grasslands. Restoration protocols included tree and shrub clearing, followed by sheep and goat grazing (André & Vandendorpel 2004; Graux 2004; Delescaille 2006).

Table 1: Description of the 28 experimental parcels. Site is the name of the site where the parcel is located, with its geographical coordinates (Localization) and its Region (L=Lesse and Lomme; V=Viroin). Condition is the parcel type (Forest=Pre-restoration forest; Grassland=Reference grasslands). For Forest and restored parcels, the type of forest stand (Pine=*P.sylvestris*/*P.nigra* plantations; Oak=shrub coppices intermingled with *Q.robur* trees) is provided. For restored parcels, age classes, as well as real age since restoration is given.

Parcel	Site	Localization	Region	Condition	Forest stand	Age class (years)	Real age (years)
1	Tienne des Vignes	50°06'N - 5°10'E	L	Forest	Pine	/	/
2	Lorinchamps	50°06'N - 5°14'E	L	Forest	Oak	/	/
3	Tienne d' Aize	50°07'N - 5°09'E	L	Forest	Oak	/	/
4	Les Pairées	50°06'N - 5°11'E	L	Forest	Pine/Oak	/	/
5	Niémont	50°06'N - 4°42'E	V	Forest	Pine	/	/
6	Montagne-aux-Buis	50°05'N - 4°34'E	V	Forest	Oak	/	/
7	Rivelottes	50°05'30"N - 4°40'E	V	Forest	Pine	/	/
8	Abannets	50°04'30"N - 4°34'E	V	Forest	Pine/Oak	/	/
9	Tienne des Vignes	50°06'N - 5°10'E	L	Restored	Pine	2-4	2
10	Lorinchamps	50°06'N - 5°14'E	L	Restored	Oak	2-4	2
11	Tienne des Vignes	50°06'N - 5°10'E	L	Restored	Pine	5-8	8
12	Tienne d' Aize	50°07'N - 5°09'E	L	Restored	Oak	5-8	8
13	Les Pairées	50°06'N - 5°11'E	L	Restored	Oak	10-15	10
14	Les Pairées	50°06'N - 5°11'E	L	Restored	Pine	10-15	10
15	Niémont	50°06'N - 4°42'E	V	Restored	Pine	2-4	2
16	Montagne-aux-Buis	50°05'N - 4°34'E	V	Restored	Oak	2-4	4
17	Rivelottes	50°05'30"N - 4°40'E	V	Restored	Pine	5-8	5
18	Montagne-aux-Buis	50°05'30"N - 4°34'E	V	Restored	Oak	5-8	7
19	Abannets	50°04'30"N - 4°34'E	V	Restored	Pine	10-15	15
20	Abannets	50°04'30"N - 4°34'E	V	Restored	Oak	10-15	15
21	Tienne des Vignes	50°06'N - 5°10'E	L	Grassland	/	/	/
22	Lorinchamps	50°06'N - 5°14'E	L	Grassland	/	/	/
23	Tienne d' Aize	50°07'N - 5°09'E	L	Grassland	/	/	/
24	Les Pairées	50°06'N - 5°11'E	L	Grassland	/	/	/
25	Montagne-aux-Buis	50°05'30"N - 4°34'E	V	Grassland	/	/	/
26	Montagne-aux-Buis	50°05'N - 4°34'E	V	Grassland	/	/	/
27	Rivelottes	50°05'30"N - 4°40'E	V	Grassland	/	/	/
28	Abannets	50°04'30"N - 4°34'E	V	Grassland	/	/	/

Eight sites, four in the Viroin Valley and four in the Lesse and Lomme Valleys were selected for the present study (Table 1). The eight study sites support old grasslands, as well as afforested abandoned grasslands. At each site, some afforested parcels were clear-cut in grassland restoration efforts.

Since 2003, all sites have been managed by grazing using migrating sheep flocks (duration: 2-3 weeks/year, resulting to a grazing intensity of 1-2 sheep/ha*year; restored parcels are grazed each year, reference parcels every 2 or 3 years depending on site) (Piqueray et al. 2013).

We conducted thorough analyses of historical maps, aerial photographs, and ground field surveys and identified 28 parcels (2 to 5 per site) representing a range of grassland conditions (Table 1). In particular we selected the following:

1) Reference parcels (n = 8, four per region). Calcareous grasslands reported as undisturbed historic sites, in existence for more than two centuries. They harbor *Mesobromion* plant community. This is considered the reference ecosystem for restoration purposes.

2) Restored parcels (n = 12, six per region). Afforested abandoned grasslands, recently (*i.e.* in the last 15 years) clear-cut forests managed primarily through grazing for grassland restoration. The time elapsed since restorative clear-cut (in years) was known for each restored parcel. The parcels were chosen to be representative of three age classes since restoration: 2-4 years, 5-8 years, and 10-15 years; 4 parcels per age class, 2 per region. Six of the parcels were restored from pine stands, and six from oak coppices. *P. nigra* or *P. sylvestris* plantation stands were aged up to 100 years. The species composition of dense shrub oak coppices (> 40 years old) primarily included *Prunus spinosa*, *Crataegus monogyna* and *Corylus avellana*, with sparsely intermingled *Quercus robur* trees. Clear-cut included elimination of trees and shrubs, but tree stumps remained.

3) Forest parcels (n = 8, four per region). Forests established at least 40 years ago on former grasslands.

Mesobromion grasslands (reference parcels) and forest parcels were adjacent to restored parcels under similar topographic conditions. Floristic surveys and species cover (%) were recorded in 20 1-m² quadrats in each restored parcel, *i.e.* total number of quadrats was 80 per age class. Quadrats were located in cardinal directions 1 m from five randomly selected tree stumps. The same parameters were recorded in eighty quadrats, distributed in the *Mesobromion* grasslands, and eighty in forests for herbaceous and shrub species only (< 2 m height). Grasslands and forest sampling quadrats were randomly placed and equally distributed among regions. Nomenclature follows Lambinon et al. (2004).

Functional traits

We investigated a set of functional traits with different outcomes for colonization of new habitats, *i.e.* dispersal, establishment, and persistence (Weiher et al. 1999; Poschlod et al. 2000)(Appendix 1). Temporal and spatial dispersal were assessed through traits relative to seed bank (dispersal in time), and traits relative to spatial dispersal capacity (seed dispersal mode, seed releasing height). Traits evaluated to assess establishment strategies included dormancy, light requirements for germination, and seedling emergence time. In addition, species ability to establish in a competitive or environmentally stressful habitat was tested through the inclusion of establishment-related traits. Persistence traits were relative to plant persistence once established. These traits included vegetative characters that provide species adaptations to successfully remain in a community, *i.e.* life form, clonal growth, branching, and canopy height, among other traits; or reproduce in fragmented habitats, and/or within small populations (autofertility, autogamy). The traits were classified based on their main function, following Weiher et al. (1999) and Poschlod et al. (2000). It is worth noting that a classification among dispersal, establishment, and persistence is not absolute. Furthermore, some traits may be involved in several processes. Trait values were derived from the BIOPOP database (Poschlod et al. 2003; Jackel et al. 2006) (Appendix 1).

Data analysis

In all quadrats, we proceeded to the following data computation. (1) For binary traits, we calculated trait abundance (e.g. abundance of clonal species) as the proportion of quadrat cover occupied by species exhibiting the trait. (2) For numeric traits, we computed the community weighted mean (CWM, Díaz et al. 2007) of trait values (weight was species cover). (3) Nominal traits were dummy-transformed to as many binary traits as there were values for the trait, and subsequently treated as in (1). Species cover data were log-transformed prior to analyses to improve normality.

Correlations among trait abundances/CWM were computed using a Principal Components Analysis (PCA) in the R package "ade4" (Chessel et al. 2004). Phanerophyte abundance was not considered in this analysis in order to remove the influence of restoration action itself (clear-cut).

Calcareous grassland restoration was analyzed based on functional traits in three different stages assessed independently. We first analyzed the functional response to clear-cutting by comparing the characteristics of functional traits between forests and young restored grasslands (2-4 years). Second, functional change in restored grasslands was evaluated by testing the effects of restoration age (fixed continuous effect: real age, Table1) on specific

traits represented in restored sites. Third, we compared 10-15 year old restored grasslands with *Mesobromion* reference grasslands. This component of the study served to identify the remaining functional differences between restored and reference grasslands, and therefore, predictions for the future of calcareous grasslands. Comparisons were computed on arcsin-transformed species representations for nominal and binary traits. Using the R-package "nlme" (Pinheiro et al. 2010), Linear Mixed Effects (LME) was applied for quantitative analyses with parcel as a random grouping effect. This random effect was introduced in order to take into account the non-independence of quadrats from a same parcel. The lmmfit package (Maj 2011) was used to calculate R^2 values for the mixed effects models.

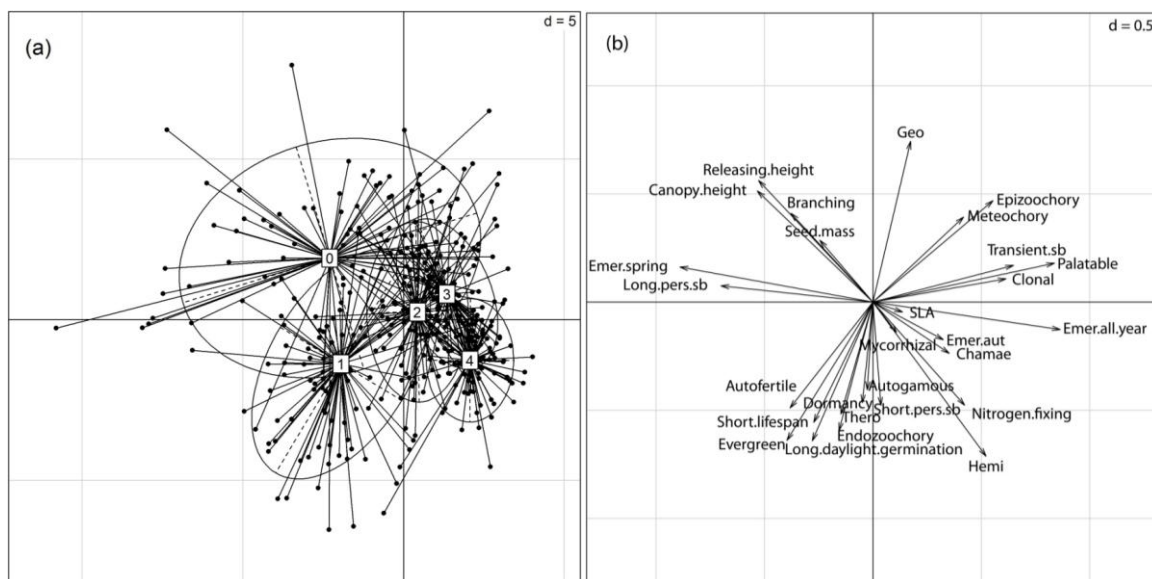


Figure 1: Trait variation distribution along PCA axes 1 (horizontal, 24.2% explained), and 2 (vertical, 17.4% explained) (a) quadrats of the following types: 0 = pre-restoration forests; 1 = 2-4 years restoration; 2 = 5-8 years restoration; 3 = 10-15 years restoration; 4 = reference grasslands; 95% confidence ellipses, and (b) measured traits (see appendix 1 for abbreviations).

Results

The first two PCA axes respectively explained 23.3% and 18.6% of the total trait variation (Fig. 1). The first axis distinguished pre-restoration forests, characterized by a higher canopy height, and plants with germination in spring and reference grasslands, typified by hemicryptophytes, and species with nitrogen fixation ability. The second axis differentiated restored parcels of different ages. Young restorations were characterized by evergreen species, requiring long daylight periods to germinate. Old restorations exhibited inverse results, distinguished by geophytes, epizoochorous, and palatable species. Graphically, the older the restoration the closer it was placed to reference grasslands. All values (mean trait representation) and LME test results are provided in Table 2.

Dispersal

Following the clear-cut, no increase of species with long-term seed banks was observed ($R^2=0.03$; $P = 0.472$). These species showed however the highest representation in recent restorations. Abundance of species with short-term seed banks significantly increased following clearcut (from 6% to 13%, $R^2=0.22$; $P < 0.05$). Thereafter, it showed a significant decrease with increasing time since restoration ($R^2=0.18$; $P < 0.05$); and reached the lowest abundance in old restorations (4%), significantly lower than in reference grasslands (9%, $R^2=0.15$; $P < 0.05$). Abundance of species with transient seed banks progressively and significantly increased during the restoration period ($R^2=0.20$; $P < 0.05$), and was equally represented in old restorations and reference grasslands (respectively, 80% and 79%, $R^2 < 0.01$; $P = 0.562$). Spatial dispersal was important in the restoration phase that included sheep and goat grazing, where a significant increase in epizoochorous species abundance was detected (from 61% (2-4 year old) to 79% (10-15 year old), $R^2=0.15$; $P < 0.05$).

Table 2: Trait CWM (numeric traits) or trait abundances (binomial traits) in forest parcels, 2-4 years/5-8 years/10-15 years following clear-cut, and reference grassland parcels (values in brackets are the between parcel SD). R^2 indicates the R^2 of the LME models for differences between forests, and 2-4 year clear-cut parcels (R^2 -clear-cut), effect of time following the clear-cut (R^2 -restoration), and difference between 10-15 year clear-cut parcels, and reference grassland parcels (R^2 -reference). Significant values are in bold: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Trait	Pre-restoration forest	Young restorations (2-4 years)	Middle-aged restorations (5-8 years)	Old restorations (10-15 years)	Reference grasslands	R^2 (clearcut)	R^2 (restoration)	R^2 (reference)
Traits related to dispersal in time and space								
Seed bank								
Species with transient seed bank	0.72 (0.13)	0.64 (0.06)	0.75 (0.09)	0.80 (0.02)	0.79 (0.05)	0.09	0.20*	<0.01
Species with short-term seed bank	0.06 (0.08)	0.13 (0.03)	0.09 (0.04)	0.04 (0.02)	0.09 (0.04)	0.22*	0.18*	0.15*
Species with long-term seed bank	0.24 (0.18)	0.26 (0.06)	0.19 (0.09)	0.17 (0.03)	0.14 (0.05)	0.03	0.06	0.04
Dispersal type								
Meteorochorous species	0.45 (0.15)	0.44 (0.08)	0.53 (0.10)	0.49 (0.02)	0.50 (0.06)	<0.01	0.02	<0.01
Endozochorous species	0.58 (0.17)	0.67 (0.15)	0.61 (0.05)	0.62 (0.08)	0.69 (0.06)	0.04	<0.01	0.12
Epizochorous species	0.68 (0.11)	0.61 (0.11)	0.73 (0.07)	0.79 (0.04)	0.75 (0.06)	0.03	0.15*	0.06
Mean releasing height (m)	3.51 (2.73)	1.29 (0.40)	1.20 (0.39)	0.99 (0.34)	0.72 (0.29)	0.18	0.06	0.04

Table 2: continued

Trait	Pre- restoration forest	Young restorations (2-4 years)	Middle-aged restorations (5-8 years)	Old restorations (10-15 years)	Reference grasslands	R ² (clearcut)	R ² (restoration)	R ² (reference)
Traits related to establishment								
Mean seed mass (mg)	66.07 (39.66)	16.45 (13.32)	20.38 (9.62)	24.29 (13.01)	14.58 (10.53)	0.13*	<0.01	0.03
Species with seed exhibiting dormancy	0.36 (0.23)	0.45 (0.12)	0.43 (0.06)	0.39 (0.06)	0.41 (0.06)	0.03	0.01	0.01
Species with seed requiring Light>Dark for germination	0.20 (0.18)	0.54 (0.13)	0.31 (0.10)	0.22 (0.04)	0.33 (0.07)	0.33*	0.19*	0.22*
Seedling emergence								
Seedling emergence in spring	0.66 (0.21)	0.61 (0.09)	0.42 (0.02)	0.41 (0.04)	0.24 (0.05)	0.04	0.23**	0.39***
Seedling emergence in autumn	0.02 (0.03)	0.02 (0.02)	0.04 (0.02)	0.05 (0.03)	0.06 (0.02)	0.02	0.11	0.01
Seedling emergence all year	0.32 (0.20)	0.37 (0.11)	0.54 (0.04)	0.54 (0.03)	0.70 (0.06)	0.04	0.16*	0.39***
Mean SLA (mm ² /mg)	22.27 (2.75)	22.65 (1.66)	22.78 (1.28)	22.53 (0.88)	22.20 (0.57)	<0.01	0.01	0.02
Mycorrhizal species	0.84 (0.12)	0.84 (0.03)	0.85 (0.05)	0.80 (0.02)	0.88 (0.05)	0.01	0.02	0.21*
Species with nitrogen fixation ability	0.00 (0.00)	0.04 (0.03)	0.04 (0.03)	0.05 (0.02)	0.08 (0.04)	0.31**	0.04	0.09
Evergreen species	0.31 (0.07)	0.40 (0.15)	0.28 (0.06)	0.19 (0.02)	0.35 (0.09)	0.07	0.22*	0.38**
Palatable species	0.62 (0.25)	0.62 (0.08)	0.82 (0.07)	0.90 (0.02)	0.91 (0.04)	<0.01	0.33**	<0.01

Table 2: continued

Trait	Pre-restoration forest	Young restorations (2-4 years)	Middle-aged restorations (5-8 years)	Old restorations (10-15 years)	Reference grasslands	R ² (clearcut)	R ² (restoration)	R ² (reference)
Traits related to persistence								
Branching species	0.97 (0.04)	0.88 (0.04)	0.91 (0.04)	0.88 (0.06)	0.85 (0.04)	0.36**	0.02	0.04
Mean canopy height (m)	3.74 (2.73)	1.34 (0.37)	1.16 (0.40)	0.96 (0.34)	0.68 (0.32)	0.19	0.07*	0.04
Clonal species	0.68 (0.28)	0.77 (0.04)	0.83 (0.01)	0.88 (0.02)	0.87 (0.05)	0.02	0.13**	<0.01
Autofertile species	0.42 (0.18)	0.47 (0.06)	0.46 (0.07)	0.40 (0.07)	0.48 (0.09)	<0.01	<0.01	0.13
Autogamous species	0.02 (0.05)	0.03 (0.02)	0.01 (0.01)	0.01 (0.01)	0.04 (0.04)	0.04	0.02	0.07
Life-form								
Chamaephytes	0.03 (0.05)	0.03 (0.02)	0.07 (0.01)	0.07 (0.02)	0.12 (0.05)	<0.01	0.17**	0.13
Geophytes	0.25 (0.16)	0.23 (0.08)	0.30 (0.13)	0.32 (0.03)	0.21 (0.06)	<0.01	0.03	0.27*
Hemicryptophytes	0.18 (0.16)	0.48 (0.11)	0.44 (0.11)	0.48 (0.06)	0.61 (0.08)	0.41**	0.02	0.19*
Phanerophytes	0.52 (0.27)	0.22 (0.05)	0.15 (0.03)	0.11 (0.04)	0.04 (0.03)	0.28	0.20***	0.24**
Therophytes	0.02 (0.03)	0.04 (0.02)	0.02 (0.02)	0.02 (0.01)	0.02 (0.03)	0.15*	0.03	<0.01
Short lifespan species	0.02 (0.03)	0.09 (0.05)	0.03 (0.02)	0.02 (0.01)	0.02 (0.03)	0.34**	0.16*	<0.01

Establishment

Clear-cut forests favored the establishment of species with light seeds. Pre-restoration forests and young restoration species showed a significant difference in mean respective seed weight CWM at 66.07 mg and 16.45 mg ($R^2=0.13$; $P < 0.05$). Clear-cut parcels also supported increased abundance of species requiring long light periods to germinate ($R^2=0.33$; $P < 0.05$), and species exhibiting symbiotic relationships with nitrogen fixing bacteria ($R^2=0.31$; $P < 0.01$). During the restoration phase, we observed a decreasing abundance of plants with germination in spring (from 61% to 41%, $R^2=0.23$; $P < 0.01$), and the reference grassland level (24%) was not reached in old restorations ($R^2=0.39$; $P < 0.001$). In addition, species requiring increased light periods to germinate decreased in abundance (from 54% to 22%, $R^2=0.19$; $P < 0.05$), and were measured at levels below the reference grasslands (33%) in old restorations ($R^2=0.22$; $P < 0.05$). Concurrently, evergreen species decreased, and palatable (to grazing animals) species increased (from 40% to 19%, $R^2=0.22$; $P < 0.05$, and from 62% to 90%, $R^2=0.33$; $P < 0.01$, respectively). Mycorrhizal and evergreen species were more abundant in reference grasslands than in old restorations ($R^2=0.21$; $P < 0.05$ and $R^2=0.38$; $P < 0.01$ respectively).

Persistence

Changes in life-form proportions were observed at the different restoration phases. Forests parcels were dominated by phanerophytes (52%), which significantly decreased with increasing restoration age ($R^2=0.20$; $P < 0.001$), but remained more abundant in old restorations (11%) than in reference grasslands (4%, $R^2=0.24$; $P < 0.01$). Hemicryptophytes abundance showed a significant increase following the clear-cut restoration plan (from 18% to 48%, $R^2=0.41$; $P < 0.01$). Subsequently, it stabilized and remained significantly lower than the reference grassland hemicryptophytes abundance level (61%, $R^2=0.19$; $P < 0.05$). Geophyte abundance was 32% in old restoration, which was significantly different from reference grasslands (21%, $R^2=0.27$; $P < 0.05$). Chamaephytes significantly increased from young restoration stages to old restorations (3% to 7%, $R^2=0.17$; $P < 0.01$). Reference grasslands supported an increased abundance of chamaephyte species (12%), but the difference was not significant ($R^2=0.13$; $P = 0.093$). Short lifespan species (annuals and biennials) exhibited the highest abundance in young restorations (9%). Other changes in persistence traits included a significant decrease in branching species abundance following the clear-cut (from 97% to 88%, $R^2=0.36$; $P < 0.01$), significant decrease in canopy height CWM, and a significant increase in clonal species abundance with restoration age ($R^2=0.07$; $P < 0.05$ and $R^2=0.13$; $P < 0.01$, respectively).

Discussion

Global evolution of plant traits during the different restoration phases

Our data showed that plant traits means and abundances were strongly influenced by the different restoration phases (clear-cut, grazing). The observed evolution was far from a linear progression from forest to grassland values. Indeed the clear-cut action induced strong changes that sometimes drove trait abundances/means away of the target values (see e.g. short lifespan species). In the subsequent years, under grazing management, values approached reference grassland values, but these were not always reached and not all values tended to approach the target, suggesting that management could be improved. However, the comparison with the results of Dzwonko and Loster (2007), where no grazing was introduced after clear-cut resulting in a rapid re-encroachment, highlights the importance of management following restoration by clear-cut.

Functional trait shifts immediately following the clear-cut

Trait variation observed among forests and post 2-4 years clear-cut parcels was clearly related to the change from closed woodland to open herbaceous. There was a decrease in canopy height, and phanerophyte abundance, which correspond to the direct effect of restoration actions itself (clear-cut). These were replaced primarily by hemicryptophytes.

Additional changes in trait abundances or CWM were the consequence of another substantial modification of the environmental conditions resulting from the clear-cut, *i.e.* bare ground cover. Bare ground is common in the first years following any clear-cut event (Piqueray et al. 2011b), and may explain some observed effects. In recent clear-cut parcels, Piqueray et al. (2011b) reported a high occurrence of ruderal species, many with short life spans (therophytes and biennial hemicryptophytes). Therophytes and biennial hemicryptophytes are dependent on sexual reproduction, and likely favored the bare ground, which provided abundant and suitable sites for germination (Rusch 1988; Hillier 1990). These conditions may also be advantageous for species with small seeds, that may be restricted in more competitive environments (Turnbull et al. 1999), and for species requiring light for germination.

Poschlod et al. (1998) discussed the important role of a seed bank in the early phases of the restoration process. In the present study, the relationship between seed bank viability and species presence at young restoration sites was not perfectly clear. Abundance of species with long-term seed banks did not increase significantly following the clear-cut. However, these species were well represented in young restorations, and 17 of the 21 species with long-term

seed banks in our species pool were recorded in young restorations (for comparison 6 were in forests, and 12 in reference grasslands; results not shown). Species with long-term seed banks, thought they were present, did not occupy a substantial area; therefore species abundance increase was not significant. Other observed trends supported that some species emerged from seed banks. We found a significant decrease in seed mass CWM following the clear-cut, and increased abundance of species requiring long day light periods to germinate. Seed banks may influence both trends. Indeed, species producing small seeds exhibit longer soil viability (Thompson et al. 1993; Bekker et al. 1998). A requirement for long day light periods may also be an advantage for soil persistence by preventing germination under unfavorable light conditions (Poschlod et al. 2000). Therefore, the role of persistent seed banks for the plant species composition of young restoration stages was only weakly confirmed by our results.

Wind dispersal of seeds can also serve an integral role in rapid colonization of new sites (Poschlod et al. 1998). Lower seed mass CWM in young restoration quadrats confirmed this hypothesis. Poschlod et al. (2000) demonstrated that small-seeded species were more likely to be wind dispersed. However, we did not detect a significant increase in meteorochorous species abundance following clear-cutting. Tackenberg et al. (2003) showed that the wind dispersal potential of meteorochorous species can be very low, therefore this result is not surprising.

Trait change trends under grazing management

Following the clear-cut, the temporal sequence exhibited a continuous trend from woodland vegetation to grassland vegetation, which is in accordance with Helsen et al. (2013). A significant decrease in phanerophytes and increase in chamaephytes toward the reference grassland level was observed. These results were however in sharp contrast to Dzwonko and Loster (2007) where grazing was not reintroduced following clear-cutting.

One of the most important trends observed during the restoration period was an increase in clonal species abundance. Clonality may be an advantageous competitive strategy as clonal species increase in frequency and subsequently cover, under both sexual and asexual reproduction (Weiher et al. 1999; Poschlod et al. 2000). The higher persistence ability of clonal species once established (Fischer & Stöcklin 1997; Weiher et al. 1999; Poschlod et al. 2000) was likely determinant in the success of these species at colonizing restored sites. Römermann *et al.* (2009) showed clonal species were often better adapted to grazing, which was the management protocol introduced following the clear-cut.

Grazing is also likely to influence other functional traits, including changes in seedling emergence during restoration. Autumn germination may be promoted in the event of environmental perturbations such as summer grazing (Eriksson & Eriksson 1997; Kahmen et al. 2002). In our study, we found that species exhibiting autumn (or year round) germination

increased, congruent with results of Kahmen et al. (2002). In another study, Kahmen and Poschlod (2008a) revealed an inverse pattern. However, grazing in this study primarily occurred during the winter months. Therefore plant cover was most strongly reduced in spring, which promoted spring germination. At our study sites, grazing occurred during the vegetative season (*i.e.* spring to late summer). Therefore, plant cover was the most reduced in autumn. A notable unexpected result was the increased abundance of palatable (to sheep and goats) species under grazing management, where we anticipated unpalatable species would have the advantage, and increase in abundance. Results indicated *Mesobromion* grasslands were characterized by an abundance of palatable species (abundance > 90%). Clearly, these results indicate the current management practices are showing success in calcareous grassland restoration. Grazing was also expected to increase species dispersal through epi- and endozoochory (Poschlod et al. 1998). In our study, a significant increase in epizoochorous species abundance was detected. This confirms the important role of sheep in seed dispersal (Fischer et al. 1996; Poschlod et al. 1996; Couvreur et al. 2004).

Differences between 10-15 year clear-cut parcels and reference grasslands

Other interesting differences in traits abundances or CWM were observed between 10-15 year clear-cut parcels, and *Mesobromion* reference grasslands. During restoration, some traits abundances were approaching the reference grassland values, but had not reached it yet. Phanerophytes remained more abundant in the 10-15 year restored parcels relative to the reference *Mesobromion* parcels. However, hemicryptophytes and species with year round seedling emergence were less abundant in the 10-15 year restored parcels compared with the reference *Mesobromion* parcels. The restoration processes are far from complete (10-15 years following the clear-cuts), and the grassland community structure can pre-date the species meeting the functional trait abundances of the reference grassland. If management of the restored parcels remains adequate, it is expected the functional trait abundances will continue to approach *Mesobromion* grassland values; however the grasslands must undergo continued monitoring.

The incomplete grassland re-establishment of the 10-15 year clear-cut parcels was also indicated by the minor abundance of mycorrhizal species, which indicates successful integration among different ecosystem components. Mycorrhizal fungal communities are known to exhibit sharp differences between grasslands and forests (Öpik et al. 2006). Trees notably differ from herbaceous species as ectomycorrhizal taxa (Haris 2009). Mycorrhizal fungi are known to determine plant diversity (Van der Heijden et al. 1998). Richter and Stutz (2002) provided evidence that restoration of mycorrhizal fungal communities is a prerequisite for target plant

community establishment. However, Haris (2009) reported that microbial arrival follows plant establishment and the debate is therefore ongoing.

Finally, some trait abundances were not approaching vegetation values of the reference grasslands during the restoration process. First, we observed a decrease in evergreen species, which were underrepresented in 10-15 year old restorations. Aerts (1995) reported the evergreen habit is primarily an adaptation to nutrient-poor environments. In the years following clear-cuts, litter decomposition may increase nutrient availability (Ouro et al. 2001), and therefore be detrimental to evergreen species. Evidence of an increase in soil nutrient content was not detected by Piqueray et al. (2011b) in the same study site. However, they did not completely exclude that such a fertility increase may exist, and decreased evergreen species in restored parcels could support this hypothesis. Second, results found geophytes overrepresented in 5-8 and 10-15 year restoration parcels. This was probably due to the increased cover/frequency of *Brachypodium pinnatum*, as the proportion of geophyte species did not highly increase in restored parcels (in average, 13% and 15% of species were geophytes, respectively in 2-4 year and 10-15 years restored parcels, result not shown). In a former study, Piqueray et al. (2011b) showed *B. pinnatum* gained dominance 10-15 years following restoration in the study sites. This species is able to persist even in afforestations and therefore has an advantage to spread after clear-cutting. Furthermore, the species is known to be competitive through decreasing light penetration at ground level due to litter accumulation (Hurst & John 1999), but also through its dense root system (Kutschera & Lichtenegger 1982) restricting establishment rates (Poschlod et al. 2011). This can explain the low occurrence of species requiring light to germinate in old restorations parcels or being low-competitive in the establishment stage, such as light seeded species.

These last results showed trait abundances that obviously progress away from the desired status (i.e. reference grasslands values). They are therefore useful to draw recommendations for the management, in order to reverse this tendency. Geophytes dominance in old restorations suggests management should pay particular attention to *B. pinnatum* control. In our study, geophytes dominance in restored grasslands was not only due to this species, but also due to *Carex flacca* and an addition of species with lower cover. However, *B. pinnatum* is known to be a problem for the conservation of calcareous grasslands (Bobbink & Willems 1987), although the case of *C. flacca* would be worth studying too. Mowing, in addition to grazing, could be implemented. Mowing may be efficient in controlling *B. pinnatum*, and assist in reducing soil nutrient content (Bobbink & Willems 1991; Bakker & Berendse 1999). However, mowing induces different trait selection than grazing. It could therefore lead to the establishment of different plant assemblages, although possibly interesting from a conservation point of view (Kahmen et al. 2002). Another solution could be an increase in grazing intensity during the *B. pinnatum* growing period, although the regeneration capacity of *Brachypodium* may be due to the buds in the underground. This last solution also seems adequate in order to re-target the progresses of

evergreen species and species requiring light to germinate. Indeed, Pakeman (2004) showed high grazing intensity may be favorable to evergreen species in temperate grasslands. This would also permit increased light to reach the soil, and promote germination in species requiring light to germinate. A special attention should however be paid not to fall into overgrazing, that is also known to be detrimental to grassland conservation (Reitalu et al. 2010). Finally, this study provided quantitative evidence that after more than a decade, functional differences remain between restored and reference calcareous grasslands. Therefore, restoration measures must be followed over long time periods to determine efficacy in different spatial and temporal methodologies.

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- 1 **Appendix 1: Trait selection for analyses. Source BIOPOP database (Jackel et al. 2006).**
 2 **The Abbreviations column gives the abbreviations used in Figure 1**

Trait	Values	Variable type	Abbreviations
Traits related to dispersal in time and space			
Seed bank	1:transient, 2:short-term, 3:long-term	Nominal	1:Transient.sb, 2:Short.term.sb, 3:Long.term.sb
Dispersal type	1:Epizoochory, 2:Endozoochory, 3:Meteorochory	Nominal, non- exclusive	1:Epizoochory, 2:Endozoochory, 3:Meteorochory
Releasing height	Value [m]	Numeric	Releasing.height
Traits related to establishment			
Seed mass	Value [mg]	Numeric	Seed.mass
Dormancy	1:True, 2:False	Binary	Dormancy
Germination requirement: Light > Dark	1:True, 2:False	Binary	Long.daylight.ge rmination
Seedling emergence	1:spring, 2:autumn, 3:all year	Nominal	1:Emer.spring, 2:Emer.aut, 3:Emer.all.year
SLA	Value [mm ² /mg]	Numeric	SLA
Mycorrhizal species	1:True, 2:False	Binary	Mycorrhizal
Nitrogen fixation ability	1:True, 2:False	Binary	Nitrogen.fixing
Evergreen	1:True, 2:False	Binary	Evergreen
Palatable species	1:True, 2:False	Binary	Palatable
Traits related to persistence			
Branching species	1:True, 2:False	Binary	Branching
Canopy height	Value [m]	Numeric	Canopy.height
Clonal growth	1:True, 2:False	Binary	Clonal
Autofertility	1:True, 2:False	Binary	Autofertile
Strict autogamy	1:True, 2:False	Binary	Autogamous
Life-form	1:Phanerophyte, 2:Chamaephyte, 3:Hemicryptophyte, 4:Geophyte, 5:Therophyte	Nominal	1:Phanero, 2:Chamae, 3:Hemi, 4:Geo, 5:Thero
Short lifespan species (annual or biennial)	1:True, 2:False	Binary	Short.lifespan

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