Running title: Higher reproductive performances in recently restored grasslands

Specialist plant species harbour higher reproductive performances in recently restored calcareous grasslands than in reference habitats

Mélanie Harzé*, Grégory Mahy, Jean-Philippe Bizoux, Julien Piqueray and Arnaud Monty

University of Liege, Gembloux Agro-Bio Tech, Biodiversity and landscape Unit, 2, Passage des Déportés, BE-5030 Gembloux, Belgium.

* Corresponding author.
E-mail address: melanie.harze@ulg.ac.be
Tel: 0032 81 62 25 45

Abstract

Background and aims_ Calcareous grasslands are local biodiversity hotspots in temperate regions that suffered intensive fragmentation. Ecological restoration projects took place all over Europe. Their success has traditionally been assessed using a plant community approach. However, population ecology can also be useful to assess restoration success and to understand underlying mechanisms.

Methods_ We took advantage of three calcareous grassland sites in Southern Belgium, where reference parcels coexist with parcels restored in the late twentieth century and with more recently restored parcels. We evaluated the colonization stage of three specialist species (*Sanguisorba minor*, *Potentilla neumanniana* and *Hippocrepis comosa*) using occurrence data. We also measured the reproductive traits of 120 individuals per species and compared components of fitness between recent restorations, old restorations and reference habitats.

Key results_ We found that the occurrence of *H. comosa* was similar in the different restoration classes, whereas both *P. neumanniana* and *S. minor* occurrences decreased from reference grasslands to recent restorations. In contrast, these two latter species exhibited a much higher reproductive output in recent restorations, thanks to an increased production of flowers and seeds.

Conclusions_ Our results suggest that, during colonization of recently restored calcareous grasslands, favourable environmental conditions, low competition and sufficient genetic mixing may lead to an increased fitness of individuals and a faster population growth than in the reference habitat. These results demonstrate how population processes can increase ecological resilience and highlight the interest of a population-based approach to assess the success of ecological restoration.

Keywords

Plant population, specialist, fitness, reproductive success, ecological restoration, ecological resilience, calcareous grassland.

INTRODUCTION

Recent decades have been dominated by unprecedented rates of habitat perturbations by human activities. Important changes in land use have led to the destruction and fragmentation of (semi)natural habitats, threatening biodiversity worldwide (Saunders et al. 1991; Balmford et al. 2005). As the conservation of remnant habitats is not sufficient to guarantee the long-term survival of many plant species (Rodrigues et al. 2004), active habitat restoration has become a necessity to preserve biodiversity worldwide. However, habitat restoration can be cost prohibitive, and efforts to reach restoration goals must demonstrate their success (Fagan et al. 2008).

Calcareous grasslands are local biodiversity hotspots in temperate regions (Prendergast et al. 1993; WallisDeVries et al. 2002; Jacquemyn et al. 2003). These habitats have suffered intensive fragmentation due to the abandonment of traditional agropastoral systems and the resulting encroachment, reforestation, urbanization or transformation into arable lands (Poschlod & WallisDeVries 2002; Piqueray et al. 2011a). In order to preserve and enhance the ecological value of those habitats, ecological restoration projects have taken place all over Europe.

Criteria used in judging whether a restoration is successful are numerous (Hobbs & Norton 1996; Ruiz-Jaen & Aide 2005; Zedler 2007; Cristofoli & Mahy 2010; Piqueray et al. 2015). Most studies evaluating calcareous grasslands’ restoration success focused on the recovery of plant species diversity and composition (e.g. Lindborg & Eriksson 2004; Kiehl & Pfadenhauer 2007; Fagan et al. 2008; Maccherini et al. 2009; Piqueray et al. 2011b; Maccherini & Santi 2012; Redhead et al. 2014). Few studies have evaluated calcareous grasslands’ restoration success by a population approach (but see Endels et al. 2005). The discipline of population biology provides one perspective on what might be considered a successful restoration, namely that “populations must be restored to a level that allows them to persist over the long term” (Montalvo et al. 1997). Restored populations must therefore possess attributes necessary for dispersal, growth, reproduction and adaptive evolutionary changes (Montalvo et al. 1997).

For a successful habitat restoration, species must first colonize newly created patches and establish new populations. The probability for a species to colonize a new habitat notably depends on its presence in the local species pool, the presence of dispersal agents, the ability of seeds to germinate, the longevity of the soil seed bank and the spatial position of patches in the landscape (Bakker & Berendse 1999; Helsen et al. 2013). Most calcareous grasslands species are badly represented in the persistent seed bank (Kalamees & Zobel 1998; Bisteau & Mahy 2005). However, some species can persist for several decades in the soil seed bank and emerge after restoration by clear cutting (Poschlod et al. 1998). Grazing by sheep and goats plays a major role in species dispersal in the landscape (Poschlod et al. 1998). Accordingly, grasslands management practices imitating traditional shepherding may enhance species colonization on restored grasslands. In the absence of itinerant grazing, long distance dispersal is thought to be limited for most calcareous grasslands species even if some species are potentially wind dispersed (Tackenberg et al. 2003).

After colonization, restored populations must be able to persist over the long term through offspring production (Montalvo et al. 1997). In the case of limited dispersal in space and time, founding populations can be small and represent only a minor portion of the genetic diversity of the source population (Montalvo et al. 1997; Hufford & Mazer 2003). This leads to increased risks of inbreeding depression and decreased adaptive potential of the restored population (Barrett & Kohn 1991; Ellstrand & Elam 1993). Small populations are more exposed to random environmental fluctuations (Menges 1991; Widen 1993; Heschel & Paige 1995), may be less attractive to pollinators (Sih & Baltus 1987; Hendrix & Kyhl 2000) and may consequently have lower reproductive success. Loss of genetic variation in founding populations can lead to a lower fitness (Shaffer 1981; Lande 1988; Williams 2001; Reed & Frankham 2003; Matthies et al. 2004). Nevertheless, rapid population growth and expansion can also decrease risks of reducing fitness in newly created populations (Nei et al. 1975; Leimu & Mutikainen 2005; Bizoux et al. 2011).

Finally, species performances in restored habitats may be affected by environmental conditions that gently differ from the reference habitat of the species and subsequently affect fitness (Vergeer et al. 2003; Quintana-Ascencio et al. 2007; Adriaens et al. 2009). Colonization of a new habitat by maladapted genotypes can lead to a population sink (Pulliam 1988). Conversely, colonization by genotypes able to survive and reproduce in the new created habitat could increase species persistence in the landscape (Blais & Lechowicz 1989).

In this context, we took advantage of three calcareous grasslands sites in Southern Belgium, where reference parcels coexist with parcels restored in the last twenty years and with more recently restored parcels. Using occurrence data, we evaluated the colonization stage of three specialist species in the different parcels. We then compared components of fitness between recent restorations, old restorations and reference habitats.

MATERIAL AND METHODS

Study area, sites and parcels

The study was conducted in the Viroin valley, located in the Calestienne Region (southern Belgium), a 5 km-wide and 100 km-long belt of Devonian limestone hills and plateaus, with a SW-NE orientation (fig. 1). Calcareous grasslands of this area were developed under traditional agropastoral practices. Due to the abandonment of traditional agriculture, urbanization and afforestation in the nineteenth century, calcareous grasslands have declined dramatically in the region (Adriaens et al. 2006; Piqueray et al. 2011a). Thanks to restoration projects, the calcareous grasslands area increased gradually from less than 40 ha in the 1980s to more than 150 ha today. All remaining and restored grasslands are now managed with grazing and mowing in order to keep the habitat open. Despite the huge loss of habitat since the nineteenth century, this region is considered one of the core areas for calcareous grasslands conservation in Belgium.

Three sites were selected in the region: “La Montagne-aux-buis” in Dourbes, “Les Abannets” in Nismes and “Les Rivelottes” in Treignes. At each site, three calcareous grasslands parcels were selected (fig. 1): (1) *Reference grassland*, i.e. calcareous grassland known to have existed for more than two centuries and considered the reference ecosystem for the restoration (SER (Society for Ecological Restoration International Science & Policy Working Group) 2004), (2) *Old restoration*, i.e. grassland restored between 1990 and 2000, (3) *Recent restoration*, i.e. grassland restored between 2004 and 2006. Restored parcels derived from forty to one hundred-year-old forests of oak coppices (Montagne-aux-buis, Abannets) or pine stands (Rivelottes, Abannets) established on former calcareous grasslands. Pine stands were *Pinus nigra* or *Pinus sylvestris* plantations. Dense shrub oak coppices were mainly formed with *Prunus spinosa*, *Crataegus monogyna* and *Corylus avellana*, with intermingled scarce trees of *Quercus robur*. Restoration protocols included trees and shrubs clearing mainly followed by sheep and goat grazing.

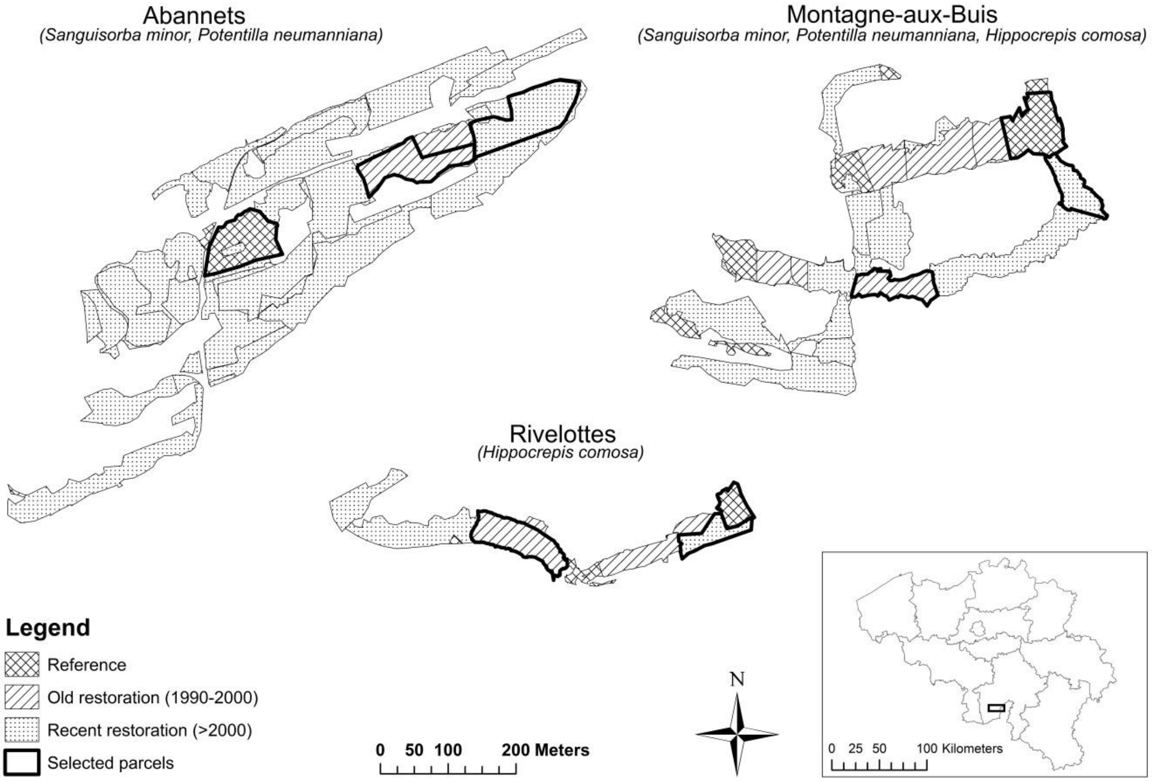

Figure 1 -- Study region (Viroin Valley, Southern Belgium) and selected parcels of the three study sites (Montagne-aux-buis: 50°05’N-4°34’E, Abannets: 50°04’N-4°34’E, Rivelottes: 50°05’N-4°40’E). Occurrence of study species was observed in each site. Fitness components were measured in only two sites for each study species (as indicated under sites names).

Study species

Three species considered calcareous grassland specialists (Piqueray et al. 2007) were selected: *Sanguisorba minor* Scop. (Rosaceae), a polycarpic perennial forbs producing flower heads consisting of fifteen to thirty wind-pollinated flowers; *Potentilla neumanniana* Rchb. (Rosaceae), a creeping perennial forbs producing yellow entomophilous flowers; and *Hippocrepis comosa* L. (Fabaceae), a perennial legume producing yellow entomophilous flowers. All three species are abundant in the study area (Adriaens et al. 2006).

Data collection

In order to evaluate the success of calcareous grasslands restoration, indicators of species colonization and individual fitness were compared between the reference and restored grasslands (old and recent restorations).

The occurrence of species at the parcel scale was evaluated by observing species presence or absence in 1 m^2 plots systematically placed in parcels, representing a sampling rate of ca. 6% of each parcel area (between 123 and 505 observed plots, depending on the parcel for a total of 2303 plots).

For each study species, twenty plots (1 m^2) were randomly selected in each parcel (among plots were the species was present) of two sites (fig. 1), and one individual was randomly selected (in each plot) for fitness components measurements.

The method used to measure fitness components depend on the species (see details of the recording method in table 1). For *S. minor*, the number of inflorescences per individual (Sm_inflos) and the number of seeds per inflorescence (Sm_seeds/inflo) were recorded. For *P. neumanniana* and *H. comosa* the number of flowers per individual (respectively Pn_flowers and Hc_flowers) and the number of seeds per fruit (respectively Pn_seeds/fruit and Hc_seeds/fruit) were recorded. The final fitness was measured as the total seeds produced per individual (see details of the recording method in table 1).

As competition and environmental variability can directly affect species performances we evaluated the bare ground cover in 1 m^2 plots around each selected individual. We estimate the bare ground cover using van der Maarel (1979) scale (0=0%; 1=<0.1%; 2=0.1-1%; 3=1-5%; 5=5-25%; 7=25-50%; 8=50-75%; 9=75-100%).

Table 1 -- Fitness traits evaluated on study species.

Final fitness, i.e. seeds production over one season, was either directly measured (*S. minor*) or estimated from fitness components. Fitness components (in bold) allow for a better understanding of reproductive performance variation.

	Sanguisorba minor	*Potentilla neumanniana*	*Hipocrepis comosa*
Fitness components	**Sm_inflos**: Number of inflorescences per individual	**Pn_flowers**: Number of flowers per individual (Pn_flowers/stem x Pn_stems)	**Hc_flowers**: Number of flowers per individual (Hc_flowers/inflo x Hc_inflos)
		Pn_flowers/stem: Mean number of flowers per stem (measured on 5 randomly selected stems per individual)	Hc_flowers/inflo: Mean number of flowers per inflorescences (measured on 20 randomly selected inflorescences per individual)
		Pn_stems: Number of stems per individual	Hc_inflos: Number of inflorescences per individual
	Sm_seeds/inflo: Mean number of seeds per inflorescence (measured on all the inflorescences of each individual)	**Pn_seeds/fruit**: Mean number of seeds per fruit (measured on 20 randomly selected fruits per individual)	**Hc_seeds/fruit**: Mean number of seeds per fruit (measured on 20 randomly selected fruits per individual)
			Hc_fruits/inflo: Mean number of fruits per inflorescence (measured on 20 randomly selected inflorescences per individual)
Final fitness	**Sm_seeds**: Number of seeds per individual	**Pn_seeds**: Number of seeds per individual (Pn_seeds/fruit x Pn_flowers)	**Hc_seeds**: Number of seeds per individual (Hc_seeds/fruit x Hc_fruits/inflos x Hc_inflos)

Data analysis

In order to evaluate species colonization in restored parcels, a generalized linear model was fitted to the occurrence data (binomial family), followed by an analysis of deviance with restoration classes ((1) reference grassland, (2) old restoration, (3) recent restoration) and site as crossed factors. The analysis was followed by a pairwise comparison of proportions (number of plot where the species was present/total number of plots, using the "pairwise.prop.test" function in R) among restoration classes.

In order to compare fitness components and the final fitness of each study species in restored and reference parcels, two-way analyses of variance were performed (with restoration classes and site as crossed factors) on each variable (fitness components and final fitness, table 1). In the case of a significant interaction with site effect, one-way ANOVAs were performed for each site separately. If a significant difference was observed between restoration classes, a Tukey's comparison test was performed.

To compare bare ground cover between study sites and parcels, one-way ANOVAs were performed for each site with restoration classes as fixed factor ((1) reference grassland, (2) old restoration, (3) recent restoration). If a significant difference was observed between restoration classes, a Tukey's comparison test was performed.

Response variables were arcsine-square root- (Pn_seeds, Hc_seeds) or log- (Sm_inflos, Sm_seeds, Pn_flowers, Hc_seeds/fruit, Hc_flowers) transformed when needed to meet the assumptions of statistical analyses. All analyses were performed with R 2.14.0 (The R Foundation for Statistical Computing 2011).

RESULTS

Colonization of restored parcels

The occurrence of *S. minor* was significantly more important in reference grasslands than in restored grasslands. It was also more important in old restorations than in recent restorations (*df*=2,2300; *deviance*=1177.11; *P*<0.001) (fig.2). For *P. neumanniana*, the occurrence was more important in the reference grasslands and the old restoration than the recent restoration (*df*=2,2300; *deviance*=46.81; *P*<0.001), except in the Montagne-aux-buis site, where the occurrence of *P. neumanniana* was significantly higher in recent restoration than in other parcels (*df*=2,717; *deviance*=74.73; *P*<0.001). *H. comosa* was globally less abundant than the two other species, and no significant differences were observed between reference and restored grasslands except in the Montagne-aux-buis site, where the occurrence of *H. comosa* was significantly higher in recent restoration (*dF*=2,717; *deviance*=163.02; *P*<0.001) than in other parcels (fig. 2).

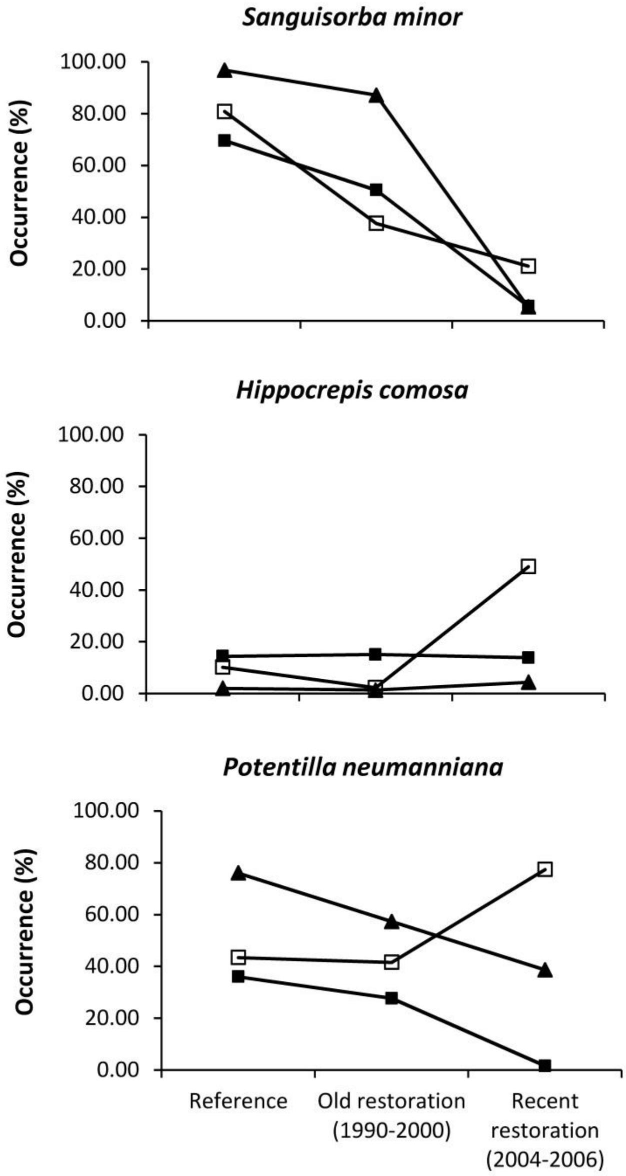

Figure 2 -- Species occurrence (% of presence in 1m^2 plots placed systematically in parcels representing a sampling rate of ~6% of each parcel's area) in selected parcels of the three study sites (Abannets, Montagne-aux-buis and Rivelottes sites are represented by triangles, white squares and black squares respectively).

Reproductive success

Fitness components -- Individuals of *S. minor* and *P. neumanniana* produced significantly more inflorescences, or flowers, in recent restorations than others parcels (respectively *df*=2,114; *F*=18.49; *P*<0.001; and *df*=2,114; *F*=37.40; *P*<0.001; fig. 3A and 3B). *S. minor* individuals produced 8.3±1.7 inflorescences in recent restorations, compared to 1.9±0.5 inflorescences in reference grasslands and 0.9±0.3 inflorescences in old restorations (fig. 3A). *P. neumanniana* individuals produced 33.9±6.9 flowers in recent restorations, compared to 2.5±0.4 flowers in reference grasslands and 2.8±1.0 flowers in old restorations. For *S. minor*, the production of seeds per inflorescence was site dependent. For this species, the seeds production per inflorescence was significantly higher for the reference grassland than for the recent restoration of the Montagne-aux-buis site (*df*=2,25; *F*=4.75; *P*=0.018; fig. 3D). For *P. neumanniana*, no differences were found for the number of seeds per fruit between the reference and the restored grasslands (fig. 3E). Concerning *H. comosa*, the number of flowers was significantly higher (*df*=2,114; *F*=8.40; *P*<0.001; fig. 3C) for recent restorations (826.0±128.0 flowers) than for old restorations (244.8±26.5 flowers). For this species, differences of seeds production per fruit were site dependent. Data was missing for one parcel of the Montagne-aux-buis site because the parcel was grazed before measurements. No differences were observed between reference and old restoration parcels in this site. At the Rivelottes site, individuals of the old restoration produced significantly more seeds per fruit (*df*=2,56; *F*=4.32; *P*=0.018; fig. 3F) than individuals of the recent restoration (respectively 2.3±0.2 and 1.6±0.2 seeds/fruit).

Final fitness -- Individuals of *S. minor* and *P. neumanniana* had a significantly higher final fitness (respectively *df*=2,114; *F*=13.9; *P*<0.001; and *df*=2,114; *F*=23.1; *P*<0.001) in recent restorations than other parcels. *S. minor* individuals produced 147.5±35.6 seeds in recent restorations, compared to 29.5±7.1 seeds in reference grasslands and 18.4±5.8 seeds in old restorations. *P. neumanniana* individuals produced 458.0±106.0 seeds in recent restorations, compared to 38.4±5.9 seeds in reference grasslands and 60.1±16.4 seeds in old restorations (fig. 3H). For *H. comosa*, differences in seeds production per individual were site dependent. Individuals of the recent restoration of the Rivelottes site produced significantly more seeds (*df*=2,56; *F*=4.39; *P*=0.017; 913.0±135.0 seeds) than individuals of the old restoration (379.0±81.6 seeds). Data was missing for one recently restored parcel of the Montagne-aux-buis site. At this site, no difference of final fitness was observed between the reference and the old restoration (fig. 3I).

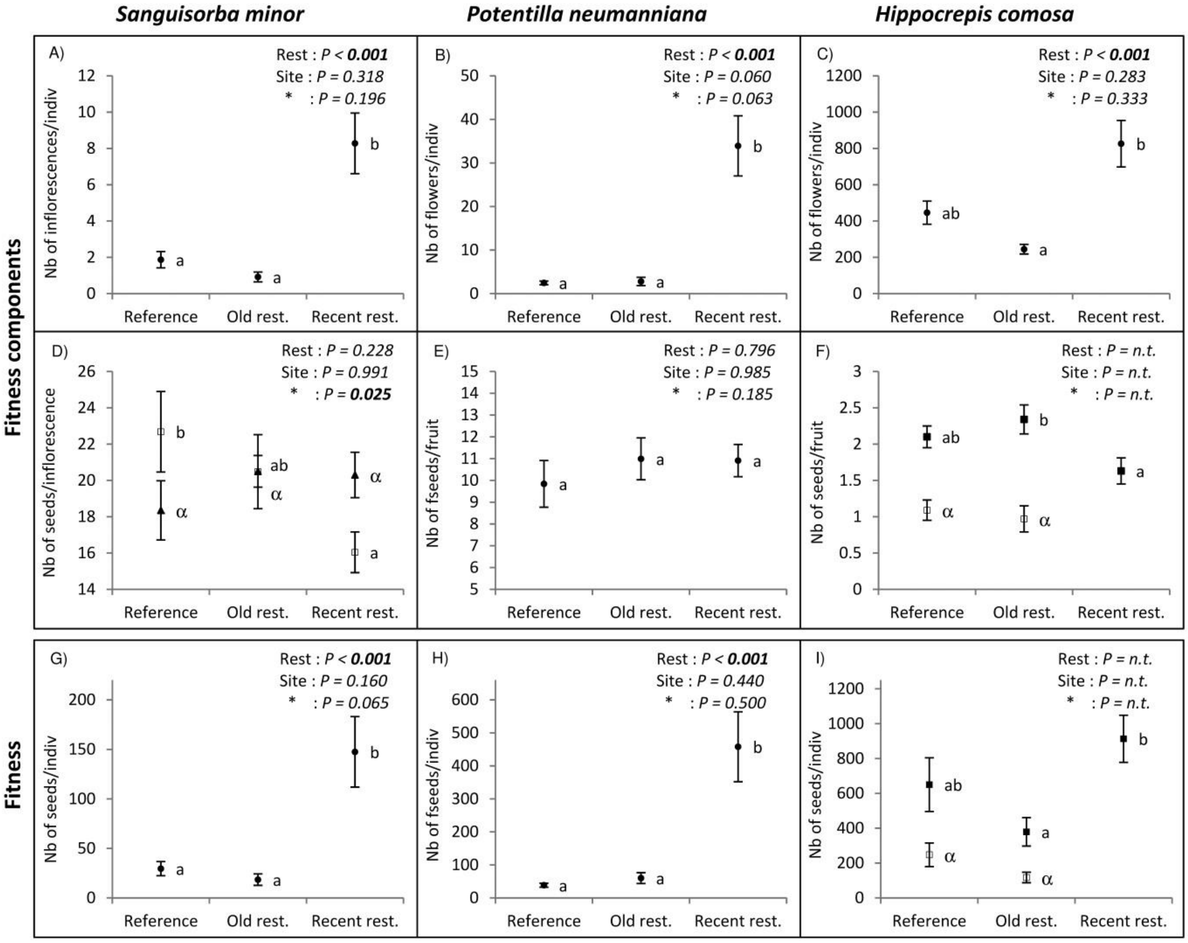

Figure 3 -- Means and standard error of fitness components (Graphs A) to F)) and fitness traits (Graphs G) to I)) of study species for reference grasslands, old and recent restorations. For a same symbol, significantly different means are followed by different letters. P-values of the two ways variance analyses for the different factors (restoration class, site and their interaction) are given up-right on each graph, and significant p-values are in bold (n.t.=not tested). When a significant interaction between the two factors was pointed out, results were drawn for each site separately. Results of AV2 for 2 sites are represented by black points, results of AV1 for the Abannets, Montagne-aux-buis and Rivelottes sites are represented by triangles, empty squares and full squares respectively. Data were missing for *Hippocrepis comosa* in one parcel because the parcel was grazed before fruits and seeds were collected.

Bare ground cover

In the Montagne-aux-buis site, the bare ground cover was significantly higher on the recent restoration compared to other parcels, whatever the species (*df*=2,57; *F*=7.06; *P*=0.002 for *S. minor*; *df*=2,57; *F*=5.55; *P*=0.006 for *P. neumanniana*; *df*=2,57; *F*=7.27; *P*=0.002 for *H. comosa*; table 2). In the Abannets site, the bare ground cover was not significantly different around *P. neumanniana* individuals (*df*=2,57; *F*=0.12; *P*=0.883; table 2) but significantly higher in the recent restoration and the reference around *S. minor* individuals (*df*=2,57; *F*=11.61; *P*<0.001; table 2). In the Rivelottes site, there was significantly more bare ground cover in the reference parcel than in the recent restoration (*df*=2,57; *F*=3.67; *P*=0.032; table 2).

Table 2 -- Differences of bare ground cover between study parcels.

Percentage of bare ground cover was estimated in $1m^2$ plots placed around each individual. The van der Maarel (1979) scale was used to estimate de bare ground percentage in the plot (0=0%; 1=<0.1%; 2=0.1-1%; 3=1-5%; 5=5-25%; 7=25-50%; 8=50-75%; 9=75-100%). Means and standard errors of bare ground cover are given for reference grasslands, old and recent restorations of study sites. Significantly different means are followed by different letters. Significant p-values are in bold.

Species	Sites	Reference	Old restorations	Recent restorations	p-value
S. minor	Mont.-aux-buis	0.15 ± 0.15^{a}	0.15 ± 0.15^{a}	1.50 ± 0.46^{b}	**0.002**
	Abannets	0.85 ± 0.25^{b}	0.00 ± 0.00^{a}	1.50 ± 0.30^{b}	**<0.001**
P. neumanniana	Mont.-aux-buis	0.15 ± 0.15^{a}	0.25 ± 0.25^{a}	1.40 ± 0.42^{b}	**0.006**
	Abannets	0.60±0.28	0.45±0.25	0.45±0.21	0.883
H. comosa	Mont.-aux-buis	0.30 ± 0.21^{a}	0.00 ± 0.00^{a}	1.35 ± 0.41^{b}	**0.002**
	Rivelottes	2.50 ± 0.44^{b}	1.00 ± 0.34^{a}	1.70 ± 0.39^{ab}	**0.032**

DISCUSSION

Colonization of restored parcels

The first step to evaluate restoration success using a population approach is to determine if species have been able to form new populations on restored grasslands. Like other ecological processes, colonization and species expansion can require long periods of time. Following biotope restoration, grasslands may exhibit a colonization credit due to a time lag for species dispersal (Cristofoli et al. 2009). Some species can therefore not yet be observed in the vegetation but can be expected to colonize restored grasslands in ensuing years. In the calcareous grassland of our study region, however, Piqueray et al. (2011c) showed that there was no colonization credit for our three study species. The three species considered in the present study have been able to colonize restored habitats. This is in agreement with the observations of Delescaille (2006, 2007) in the Abannets site. However they were globally less present on restored grasslands than on reference grasslands. There are probably multifactorial causes explaining patterns of occurrence of each target species on study sites, including emergence from soil seed bank, multiple dispersal agent (like wind, sheep, goats or human management), spatial distribution of grasslands patches in the landscape and environmental variability. Although restored sites were afforested forty to one hundred years ago, it is conceivable that *P. neumanniana* and *S. minor* emerged from the soil seed bank, as showed by a study conducted at the Abannets site (Delescaille et al. 2006). Indeed, those two species have a long-term persistent seed bank (over several decades, >25 years) (Poschlod et al. 1998). Moreover *P. neumanniana* and *S. minor* could have colonized restored parcels through dispersal by sheep or goats that grazed parcels of different calcareous grasslands sites during the grazing season. Those species are not well dispersed by wind (Poschlod et al. 1998), and sheep play a major role in dispersal across the landscape (Fischer et al. 1996; Poschlod et al. 1998). *H. comosa* seed bank persists only between six and twenty years in the soil (Poschlod et al. 1998), and seeds of this species are not efficiently dispersed by wind (Poschlod et al. 1998). The presence of this species on restored grasslands could be explained by parcels management, as dispersal by goats has been observed for this species (Müller-Schneider 1954). *H. comosa* occurrences on restored and reference grasslands were comparable. However, it has to be noted that reference grasslands are remnant grasslands that have been isolated in the landscape for more than one century. These grasslands surely represent a core area for the calcareous grasslands ecological network, but they may also exhibit an extinction debt that can affect specialist plant species (Piqueray et al. 2011c).

Interestingly, in one site (the Montagne-aux-buis), we found the occurrence of *P. neumanniana* and *H. comosa* to be higher in the recent restoration than the reference grasslands and the old restoration parcels (fig.2). Species recolonization can be influenced by the distance between the restored parcels and the nearest reference grassland (Helsen et al. 2013). In this site, the recent restoration is close to the reference parcel and the old restoration is 120 meters away from the nearest reference habitat. However, study parcels are always close to another calcareous grassland parcel. No parcel is isolated in a forest or agricultural landscape. Environmental variability between sites or parcels could also explain differences in patters of species occurrence. In a previous work, Piqueray et al. (2011b) pointed no significant differences in soil conditions between parcels or sites of the same study area. However, our results showed that the bare ground cover was higher in this recent restoration of the Montagne-aux-buis site. This could increase availability of microsites for germination (Piqueray et al. 2013) and seedlings emergence (Kotorová & Lepš 1999; Zobel et al. 2000) and modify competition regimes as compare to reference grasslands.

Reproductive success of restored populations

A second step in evaluating the restoration success using a population approach comprises assessing performances, e.g. the fitness of recently established individuals as compared to reference ones. In this study, all three studied species produced more flowering units and more seeds per individual in recent restorations than in the reference grasslands (with a less obvious pattern for *H. comosa*, see fig.3). These results are hopeful concerning population persistence in restored grasslands, as individuals with a higher fitness are prone to increase population dynamics and decrease extinction risks. We must however be careful as we did not test seeds quality and viability. Rosaceae are known to produce a significant proportion of non-viable seeds (ENSCONET 2009). Moreover, seeds could be predated before germination occurs. The observation of a high fitness in recently founded populations can be explained by a high genetic diversity of created populations (Leimu & Mutikainen 2005). Recent populations can indeed have been created from multiple colonization events from several sources populations through grazing, since sheep herds typically graze alternatively in many calcareous sites. In addition, the seed bank may be a source of genetic diversity (Templeton & Levin 1979) that could be restored when habitat conditions are suitable again. A high genetic diversity associated with a rapid demographic extension may have promoted reproductive success in recent populations (Leimu & Mutikainen 2005).

The number of seeds per inflorescences or per fruits was not significantly different between reference and restored grasslands. This shows that the key parameter influencing final fitness is the number of flowers, or inflorescences and not the number of seeds produced by floral unit. This suggests that pollination is not a limiting factor for seed production.

Finally, higher reproductive performances in recent restoration may be explained by environmental conditions. In our study sites, Piqueray et al. (2011b) showed that soil conditions have been restored and do not differ according to previous land use (oak or pine forest). They pointed out low differences between reference and restored grasslands in terms of soil fertility, but the mineralisation rate was higher on restored sites and may explain individual success. In addition, recently restored parcels generally exhibit higher bare ground cover that offers microsites for germination and decreases competition (Piqueray et al. 2013). However, the higher bare ground cover was not the only parameter that can explain fitness variability between parcels. Indeed, in the Abannets site, the fitness of *P. neumanniana* was significantly different between parcels despite no differences of bare ground cover. And for *H. comosa*, the fitness was higher on the recent restoration of the Rivelottes site despite a reduction of bare ground cover compared to reference grasslands (fig. 3 and table 2).

Implications for the future

Our results suggest that, during the colonization of recently restored calcareous grasslands, the fitness of individual is hardly affected by any process that could reduce their reproductive capacity like unfavourable environmental conditions or insufficient genetic mixing. In contrast, the high reproductive output of individuals in restored parcels is expected to enhance population growth, which may finally compensate for the lower initial occurrence. These results therefore demonstrate how population processes can increase ecological resilience (sensus Suding et al. 2004). When the massive colonization is over, both abiotic and biotic conditions in the restored habitat should approach those of the reference habitats (Piqueray et al. 2011b), and the fitness of individuals should be reduced to levels comparable to the reference grasslands. This final situation seems to almost be reached for *H. comosa* that exhibit similar occurrences in all restorations classes. That is the species for which final fitness was comparable between recent restorations and reference habitats (fig.3). *S. minor* and *P. neumanniana*, in contrast, exhibit slower colonization still in progress, with occurrences of respectively 82±8% and 52±12% in reference habitats, 58±15% and 42±9% in old restorations and only 11±5% and 39±22% in recent restorations. For these two species, final fitness was respectively five times and eleven times higher in recent restorations than in reference habitats.

From a management point of view, these processes are encouraging. Seed dispersal seems sufficient to establish satisfactorily diverse populations. One must keep in mind, however, that the patterns observed in this study might not be true for rarer species and/or those whose seed dispersal relies on agents not related to management schemes and/or those relying on declining populations of specific pollinators for successful reproduction. Using a population approach for these species will be necessary to fully assess the success of restoration programmes in calcareous grasslands in Belgium.

Acknowledgement

We would like to thank the Marie-Victorin Centre (director L. Woué) for welcoming us, the Service public de Wallonie – Département de la Nature et des Forêts for field access, Louis-Marie Delescaille for his helpful comments and his help concerning site selection, species knowledge and management practices, Simon Vaneberg for the field work he performed and the two anonymous reviewers for their pertinent comments on the manuscript.

References

Adriaens D., Honnay O., Hermy M. (2006) No evidence of a plant extinction debt in highly fragmented calcareous grasslands in Belgium. Biological Conservation 133: 212--224. http://dx.doi.org/10.1016/j.biocon.2006.06.006

Adriaens D., Jacquemyn H., Honnay O., Hermy M. (2009) Conservation of remnant populations of *Colchicum autumnale* - the relative importance of local habitat quality and habitat fragmentation. Acta Oecologica 35: 69--82. http://dx.doi.org/ 10.1016/j.actao.2008.08.003

Bakker J., Berendse F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. Trends in Ecology and Evolution 14: 63--68. http://dx.doi.org/10.1016/S0169-5347(98)01544-4

Balmford A., Bennun L., Ten Brink B., Cooper D., Côté I.M., Crane P., Dobson A., Dudley N., Dutton I., Green R.E., Gregory R.D., Harrison J., Kennedy E.T., Kremen C., Leader-Williams N., Lovejoy T.E., Mace G., Robert May, Mayaux P., Morling P., Phillips J., Redford K., Ricketts T.H., Rodríguez J.P., Sanjayan M., Schei P.J., Van Jaarsveld A.S., Walther B.A. (2005) The convention on biological diversity's 2010 targets. Science 307: 212--213. http://dx.doi.org/ 10.1126/science.1106281

Barrett S.C.H., Kohn J.K. (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk D.A., Holsinger K.H. (eds) Genetics and conservation of rare plants: 3--30. Oxford (UK), Oxford University Press.

Bisteau E., Mahy G. (2005) Vegetation and seed bank in a calcareous grassland restored from a *Pinus* forest. Applied Vegetation Science 8: 167--174. http://dx.doi.org/10.1111/j.1654-109X.2005.tb00642.x

Bizoux J.P., Cristofoli S., Piqueray J., Mahy G. (2011) Conservation of an endemic metallophyte species: effects of population history and vegetative density on the reproductive success of V*iola calaminaria*. Journal for Nature Conservation 19: 72--78. http://dx.doi.org/ 10.1016/j.jnc.2010.06.002

Blais P.A., Lechowicz M.J. (1989) Variation among populations of *Xanthium strumarium* (Compositae) from natural and ruderal habitats. American Journal of Botany 76: 901--908.

Cristofoli S., Mahy G. (2010) Ecological restoration: Context, constraints, and indicators. Biotechnology, Agronomy and Society and Environment 14: 203--211.

Cristofoli S., Piqueray J., Dufrêne M., Bizoux J.P., Mahy G. (2009) Colonization credit in restored wet heathlands. Restoration Ecology 18: 645--655. http://dx.doi.org/ 10.1111/j.1526-100X.2008.00495.x

Delescaille L.M. (2006) La restauration des pelouses calcicoles en Région wallonne : aspects scientifiques et techniques (première partie). Parcs et Réserves 61: 4--11.

Delescaille L.M. (2007) La restauration des pelouses calcicoles en Région wallonne : aspects scientifiques et techniques (seconde partie). Parcs et Réserves 62: 4--15.

Delescaille L.M., Taupinart E., Jacquemart A.L. (2006) L'apport de la banque de graines du sol dans la restauration des pelouses calcicoles : un exemple dans la vallée du Viroin (prov. de Namur, Belgique). Parcs et Réserves 61: 4--12.

Ellstrand N.C., Elam D.R. (1993) Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24: 217--242.

Endels P., Jacquemyn H., Brys R., Hermy M. (2005) Rapid response to habitat restoration by the perennial *Primula veris* as revealed by demographic monitoring. Plant Ecology 176: 143--156. http://dx.doi.org/ 10.1007/s11258-004-0026-0

ENSCONET. (2009) ENSCONET Seed collecting manual for wild species. European Native Seed Conservation Network [online]. Available from http://ensconet.maich.gr/fr/Decharge.htm [accessed 29 Jan. 2015].

Fagan K.C., Pywell R.F., Bullock J.M., Marrs R.H. (2008) Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. Journal of Applied Ecology 45: 1293--1303. http://dx.doi.org/ 10.1111/j.1365-2664.2008.01492.x

Fischer S.F., Poschlod P., Beinlich B. (1996) Experimental studies on the dispersal of plants and animals on sheep in calcareous grasslands. Journal of Applied Ecology 33: 1206--1222.

Helsen K., Hermy M., Honnay O. (2013) Spatial isolation slows down directional plant functional group assembly in restored semi-natural grasslands. Journal of Applied Ecology 50: 404--413. http://dx.doi.org/ 10.1111/1365-2664.12037

Hendrix S.D., Kyhl J.F. (2000) Population size and reproduction in *Phlox pilosa*. Conservation Biology 14: 304--313. http://dx.doi.org/ 10.1046/j.1523-1739.2000.98253.x

Heschel M.S., Paige K.N. (1995) Inbreeding depression, environmental stress, and population size variation in Scarlet Gilia (*Ipomopsis aggregata*). Conservation Biology 9: 126--133. http://dx.doi.org/ 10.1046/j.1523-1739.1995.09010126.x

Hobbs R.J., Norton D.A. (1996) Towards a conceptual framework for restoration ecology. Restoration Ecology 4: 93--110. http://dx.doi.org/ 10.1111/j.1526-100X.1996.tb00112.x

Hufford K.M., Mazer S.J. (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology and Evolution 18: 147--155. http://dx.doi.org/ 10.1016/S0169-5347(03)00002-8

Jacquemyn H., Brys R., Hermy M. (2003) Short-term effects of different management regimes on the response of calcareous grasslands vegetation to increased nitrogen. Biological Conservation 111: 137--147. http://dx.doi.org/ 10.1016/S0006-3207(02)00256-2

Kalamees R., Zobel M. (1998) Soil seed bank composition in different successional stages of a species rich wooded meadow in Laelatu, Western Estonia. Acta Oecologica 19: 175--180. http://dx.doi.org/ 10.1016/S1146-609X(98)80021-0

Kiehl K., Pfadenhauer J. (2007) Establishment and persistence of target species in newly created calcareous grasslands on former arable fields. Plant Ecology 189: 31--48. http://dx.doi.org/ 10.1007/s11258-006-9164-x

Kotorová I., Lepš J. (1999) Comparative ecology of seedling recruitment in an oligotrophic wet meadow. Journal of Vegetation Science 10: 175--186. http://dx.doi.org/ 10.2307/3237139

Lande R. (1988) Genetics and demography in biological conservation. Science 241: 1455--1460. http://dx.doi.org/ 10.1126/science.3420403

Leimu R., Mutikainen P. (2005) Population history, mating system, and fitness variation in a perennial herb with a fragmented distribution. Conservation Biology 19: 349--356. http://dx.doi.org/ 10.1111/j.1523-1739.2005.00480.x

Lindborg R., Eriksson O. (2004) Effects of restoration on plant species richness and composition in Scandinavian semi-natural grasslands. Restoration Ecology 12: 318--326. http://dx.doi.org/ 10.1111/j.1061-2971.2004.00334.x

Maccherini S., Bacaro G., Favilli L., Piazzini S., Santi E., Marignani M. (2009) Congruence among vascular plants and butterflies in the evaluation of grassland restoration succes. Acta Oecologica 35: 311--317. http://dx.doi.org/ 10.1016/j.actao.2008.12.002

Maccherini S., Santi E. (2012) Long-term experimental restoration in a calcareous grassland: identifying the most effective restoration strategies. Biological Conservation 146: 123--135. http://dx.doi.org/ 10.1016/j.biocon.2011.11.032

Matthies D., Brauer I., Maibom W., Tscharntke T. (2004) Population size and the risk of local extinction: empirical evidence from rare plants. Oikos 105: 481--488. http://dx.doi.org/ 10.1111/j.0030-1299.2004.12800.x

Menges E.S. (1991) Seed germination percentage increases with population size in a fragmented prairie species. Conservation Biology 5: 158--164. http://dx.doi.org/ 10.1111/j.1523-1739.1991.tb00120.x

Montalvo A.M., Williams S.L., Rice K.J., Buchmann S.L., Cory C., Handel S.N., Nabhan G.P., Primack R., Robichaux R.H. (1997) Restoration biology: a population biology perspective. Restoration Ecology 5: 277--290. http://dx.doi.org/ 10.1046/j.1526-100X.1997.00542.x

Müller-Schneider P. (1954) Ueber endozoochore samenverbreitung durch weidende Haustiere. Plant Ecology 5-6: 23--28. http://dx.doi.org/ 10.1007/BF00299548

Nei M., Maruyama T., Chakraborty R. (1975) The bottleneck effect and genetic variability in populations. Evolution 29: 1--10.

Piqueray J., Bisteau E., Bottin G., Mahy G. (2007) Plant communities and species richness of the calcareous grasslands in southeast Belgium. Belgian Journal of Botany 140: 157--173.

Piqueray J., Bisteau E., Cristofoli S., Palm R., Poschlod P., Mahy G. (2011a) Plant species extinction debt in a temperate biodiversity hotspot: community, species and functional traits approaches. Biological Conservation 144: 1619--1629. http://dx.doi.org/ 10.1016/j.biocon.2011.02.013

Piqueray J., Bottin G., Delescaille L.M., Bisteau E., Colinet G., Mahy G. (2011b) Rapid restoration of a species rich ecosystem assessed from soil and vegetation indicators the case of calcareous grasslands restored from forest stands. Ecological Indicators 11: 724--733. http://dx.doi.org/ 10.1016/j.ecolind.2010.06.007

Piqueray J., Cristofoli S., Bisteau E., Palm R., Mahy G. (2011c) Testing coexistence of extinction debt and colonization credit in fragmented calcareous grasslands with complex historical dynamics. Landscape Ecology 26: 823--836. http://dx.doi.org/ 10.1007/s10980-011-9611-5

Piqueray J., Ferroni L., Delescaille L.M., Speranza M., Mahy G., Poschlod P. (2015) Response of plant functional traits during the restoration of calcareous grasslands from forest stands. Ecological Indicators 48: 408--416. http://dx.doi.org/ 10.1016/j.ecolind.2014.08.039

Piqueray J., Saad L., Bizoux J.P., Mahy G. (2013) Why some species cannot colonise restored habitats? The effects of seed and microsite availability. Journal for Nature Conservation 21: 189--197. http://dx.doi.org/ 10.1016/j.jnc.2012.12.005

Poschlod P., Kiefer S., Tranke U., Fischer S.F., Bonn S. (1998) Plant species richness in calcareous grasslands as affected by dispersability in space and time. Applied Vegetation Science 1: 75--90. http://dx.doi.org/ 10.2307/1479087

Poschlod P., WallisDeVries M.F. (2002) The historical and socioeconomic perspective of calcareous grasslands: lessons from the distant and recent past. Biological Conservation 104: 361--376. http://dx.doi.org/ 10.1016/S0006-3207(01)00201-4

Prendergast J.R., Quinn R.M., Lawton J.H., Eversham B.C., Gibbons D.W. (1993) Rare species, the coincidence of diversity hotspots and conservation stategies. Nature 365: 335--337. http://dx.doi.org/ 10.1038/365335a0

Pulliam H.R. (1988) Sources, sinks, and population regulation. American Naturalist 132: 652--661.

Quintana-Ascencio P.F., Weekley C.W., Menges E.S. (2007) Comparative demography of a rare species in Florida scrub and road habitats. Biological Conservation 137: 263--270. http://dx.doi.org/ 10.1016/j.biocon.2007.02.009

Redhead J.W., Sheail J., Bullock J.M., Ferreruela A., Walker K.J., Pywell R.F. (2014) The natural regeneration of calcareous grassland at a landscape scale: 150 years of plant community re-assembly on Salisbury Plain, UK. Applied Vegetation Science 17: 408--418. http://dx.doi.org/ 10.1111/avsc.12076

Reed D.H., Frankham R. (2003) Correlation between fitness and genetic diversity. Conservation Biology 17: 230--237. http://dx.doi.org/ 10.1046/j.1523-1739.2003.01236.x

Rodrigues A.S.L., Andelman S.J., Bakan M.I., Boitani L., Brooks T.M., Cowling R.M., Fishpool L.D.C., Da Fonseca G.A.B., Gaston K.J., Hoffmann M., Long J.S., Marquet P.A., Pilgrim J.D., Pressey R.L., Schipper J., Sechrest W., Stuart S.H., Underhill L.G., Waller R.W., Watts M.E.J., Yan X. (2004) Effectiveness of the global protected area network in representing species diversity. Nature 428: 640--643. http://dx.doi.org/ 10.1038/nature02422

Ruiz-Jaen M.C., Aide T.M. (2005) Restoration success: how is it being measured? Restoration Ecology 13: 569--577. http://dx.doi.org/ 10.1111/j.1526-100X.2005.00072.x

Saunders D.A., Hobbs J.A., Margules C.R. (1991) Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5: 18--32. http://dx.doi.org/ 10.1111/j.1523-1739.1991.tb00384.x

SER (Society for Ecological Restoration International Science & Policy Working Group). (2004) The SER international primer on ecological restoration [online]. Available from http://www.ser.org/ [accessed 29 Jan. 2015].

Shaffer M.L. (1981) Minimum population sizes for species conservation. BioScience 31: 131--134. http://dx.doi.org/ 10.2307/1308256

Sih A., Baltus M.S. (1987) Patch size, pollinator behaviour, and pollinator limitation in catnip. Ecology 68: 1679--1690.

Suding K.N., Gross K.L., Houseman G.R. (2004) Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution 19: 46--53. http://dx.doi.org/ 10.1016/j.tree.2003.10.005

Tackenberg O., Poschlod P., Bonn S. (2003) Assessment of wind dispersal potential in plant species. Ecological Monographs 73: 191--205. http://dx.doi.org/10.1890/0012-9615(2003)073[0191:AOWDPI]2.0.CO;2

Templeton A.R., Levin D.A. (1979) Evolutionary consequences of seed pools. American Naturalist 114: 232--249.

The R Foundation for Statistical Computing. (2011) ISBN 3-900051-07-0, Platform: i386-pc-mingw32/i386 (32-bit).

van der Maarel E. (1979) Transformation of cover-abundance values in phytosociology and its effects on community similarity. Vegetatio 39: 97--114. http://dx.doi.org/ 10.1007/BF00052021

Vergeer P., Rengelink R., Copal a., Ouborg N.J. (2003) The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. Journal of Ecology 91: 18--26. http://dx.doi.org/ 10.1046/j.1365-2745.2003.00736.x

WallisDeVries M.F., Poschlod P., Willems J.H. (2002) Challenges for the conservation of calcareous grassland in northwestern Europe: integrating the requirements of flora and fauna. Biological Conservation 104: 265--273. http://dx.doi.org/ 10.1016/S0006-3207(01)00191-4

Widen B. (1993) Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, *Senecio integrifolius* (Asteraceae). Biological Journal of the Linnean Society 50: 179--195. http://dx.doi.org/ 10.1111/j.1095-8312.1993.tb00925.x

Williams S.L. (2001) Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. Ecological Applications 11: 1472--1488. http://dx.doi.org/10.1890/1051-0761(2001)011[1472:RGDIET]2.0.CO;2

Zedler J.B. (2007) Success: an unclear, subjective descriptor of restoration outcomes. Ecological Restoration 25: 162--168. http://dx.doi.org/ 10.3368/er.25.3.162

Zobel M., Otsus M., Liira J., Moora M., Möls T. (2000) Is small-scale species richness limited by seed availability or microsite availability? Ecology 81: 3274--3282. http://dx.doi.org/10.1890/0012-9658(2000)081[3274:ISSSRL]2.0.CO;2

Figure captions

Figure 2 -- Study region (Viroin Valley, Southern Belgium) and selected parcels of the three study sites (Montagne-aux-buis: 50°05’N-4°34’E, Abannets: 50°04’N-4°34’E, Rivelottes: 50°05’N-4°40’E). Occurrence of study species was observed in each site. Fitness components were measured in only two sites for each study species (as indicated under sites names).

Figure 2 -- Species occurrence (% of presence in 1m^2 plots placed systematically in parcels representing a sampling rate of ~6% of each parcel’s area) in selected parcels of the three study sites (Abannets, Montagne-aux-buis and Rivelottes sites are represented by triangles, white squares and black squares respectively).

Figure 3 -- Means and standard error of fitness components (Graphs A) to F)) and fitness traits (Graphs G) to I)) of study species for reference grasslands, old and recent restorations. For a same symbol, significantly different means are followed by different letters. P-values of the two ways variance analyses for the different factors (restoration class, site and their interaction) are given up-right on each graph, and significant p-values are in bold (n.t.=not tested). When a significant interaction between the two factors was pointed out, results were drawn for each site separately. Results of AV2 for 2 sites are represented by black points, results of AV1 for the Abannets, Montagne-aux-buis and Rivelottes sites are represented by triangles, empty squares and full squares respectively. Data were missing for *Hippocrepis comosa* in one parcel because the parcel was grazed before fruits and seeds were collected.