

1 **Running title: Higher reproductive performances in recently restored grasslands**

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

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Abstract

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

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

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

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

38

Keywords

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

42

INTRODUCTION

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

52 Calcareous grasslands are local biodiversity hotspots in temperate regions (Prendergast et al.
53 1993; WallisDeVries et al. 2002; Jacquemyn et al. 2003). These habitats have suffered
54 intensive fragmentation due to the abandonment of traditional agropastoral systems and the
55 resulting encroachment, reforestation, urbanization or transformation into arable lands
56 (Poschlod & WallisDeVries 2002; Piqueray et al. 2011a). In order to preserve and enhance
57 the ecological value of those habitats, ecological restoration projects have taken place all over
58 Europe.

59 Criteria used in judging whether a restoration is successful are numerous (Hobbs & Norton
60 1996; Ruiz-Jaen & Aide 2005; Zedler 2007; Cristofoli & Mahy 2010; Piqueray et al. 2015).
61 Most studies evaluating calcareous grasslands' restoration success focused on the recovery of
62 plant species diversity and composition (e.g. Lindborg & Eriksson 2004; Kiehl &
63 Pfadenhauer 2007; Fagan et al. 2008; Maccherini et al. 2009; Piqueray et al. 2011b;
64 Maccherini & Santi 2012; Redhead et al. 2014). Few studies have evaluated calcareous
65 grasslands' restoration success by a population approach (but see Endels et al. 2005). The
66 discipline of population biology provides one perspective on what might be considered a
67 successful restoration, namely that "populations must be restored to a level that allows them
68 to persist over the long term" (Montalvo et al. 1997). Restored populations must therefore
69 possess attributes necessary for dispersal, growth, reproduction and adaptive evolutionary
70 changes (Montalvo et al. 1997).

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

84 After colonization, restored populations must be able to persist over the long term through
85 offspring production (Montalvo et al. 1997). In the case of limited dispersal in space and time,
86 founding populations can be small and represent only a minor portion of the genetic diversity
87 of the source population (Montalvo et al. 1997; Hufford & Mazer 2003). This leads to
88 increased risks of inbreeding depression and decreased adaptive potential of the restored
89 population (Barrett & Kohn 1991; Ellstrand & Elam 1993). Small populations are more

90 exposed to random environmental fluctuations (Menges 1991; Widen 1993; Heschel & Paige
91 1995), may be less attractive to pollinators (Sih & Baltus 1987; Hendrix & Kyhl 2000) and
92 may consequently have lower reproductive success. Loss of genetic variation in founding
93 populations can lead to a lower fitness (Shaffer 1981; Lande 1988; Williams 2001; Reed &
94 Frankham 2003; Matthies et al. 2004). Nevertheless, rapid population growth and expansion
95 can also decrease risks of reducing fitness in newly created populations (Nei et al. 1975;
96 Leimu & Mutikainen 2005; Bizoux et al. 2011).

97 Finally, species performances in restored habitats may be affected by environmental
98 conditions that gently differ from the reference habitat of the species and subsequently affect
99 fitness (Vergeer et al. 2003; Quintana-Ascencio et al. 2007; Adriaens et al. 2009).

100 Colonization of a new habitat by maladapted genotypes can lead to a population sink (Pulliam
101 1988). Conversely, colonization by genotypes able to survive and reproduce in the new
102 created habitat could increase species persistence in the landscape (Blais & Lechowicz 1989).

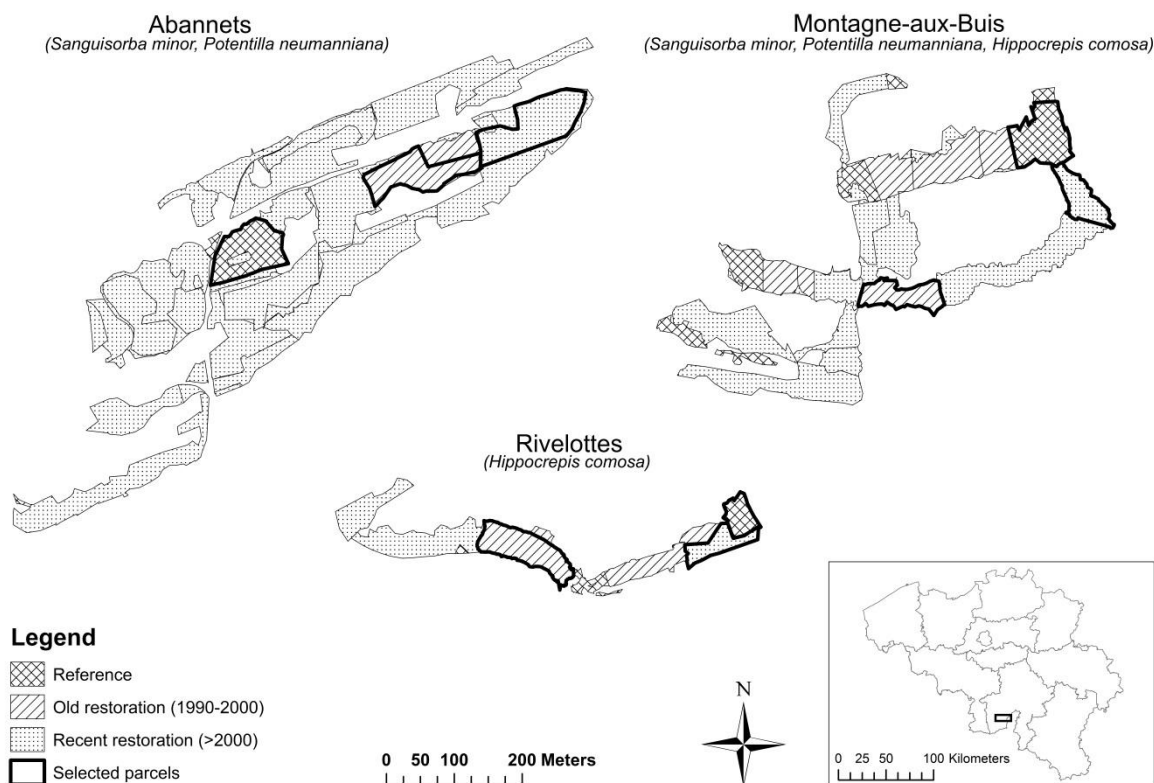
103 In this context, we took advantage of three calcareous grasslands sites in Southern Belgium,
104 where reference parcels coexist with parcels restored in the last twenty years and with more
105 recently restored parcels. Using occurrence data, we evaluated the colonization stage of three
106 specialist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

108 MATERIAL AND METHODS

109 Study area, sites and parcels

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

121 Three sites were selected in the region: “La Montagne-aux-buis” in Dourbes, “Les Abannets”
122 in Nismes and “Les Rivelottes” in Treignes. At each site, three calcareous grasslands parcels
123 were selected (fig. 1): (1) *Reference grassland*, i.e. calcareous grassland known to have
124 existed for more than two centuries and considered the reference ecosystem for the restoration
125 (SER (Society for Ecological Restoration International Science & Policy Working Group)
126 2004), (2) *Old restoration*, i.e. grassland restored between 1990 and 2000, (3) *Recent*
127 *restoration*, i.e. grassland restored between 2004 and 2006. Restored parcels derived from
128 forty to one hundred-year-old forests of oak coppices (Montagne-aux-buis, Abannets) or pine
129 stands (Rivelottes, Abannets) established on former calcareous grasslands. Pine stands were
130 *Pinus nigra* or *Pinus sylvestris* plantations. Dense shrub oak coppices were mainly formed
131 with *Prunus spinosa*, *Crataegus monogyna* and *Corylus avellana*, with intermingled scarce
132 trees of *Quercus robur*. Restoration protocols included trees and shrubs clearing mainly
133 followed by sheep and goat grazing.



134
 135 **Figure 1** -- Study region (Viroin Valley, Southern Belgium) and selected parcels of the three
 136 study sites (Montagne-aux-buis: 50°05'N-4°34'E, Abannets: 50°04'N-4°34'E, Rivelottes:
 137 50°05'N-4°40'E). Occurrence of study species was observed in each site. Fitness components
 138 were measured in only two sites for each study species (as indicated under sites names).

139 **Study species**

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

146 **Data collection**

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

150 The occurrence of species at the parcel scale was evaluated by observing species presence or
 151 absence in 1 m² plots systematically placed in parcels, representing a sampling rate of ca. 6%
 152 of each parcel area (between 123 and 505 observed plots, depending on the parcel for a total
 153 of 2303 plots).

154 For each study species, twenty plots (1 m²) were randomly selected in each parcel (among
 155 plots where the species was present) of two sites (fig. 1), and one individual was randomly
 156 selected (in each plot) for fitness components measurements.

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

164 As competition and environmental variability can directly affect species performances we
 165 evaluated the bare ground cover in 1 m² plots around each selected individual. We estimate
 166 the bare ground cover using van der Maarel (1979) scale (0=0%; 1=<0.1%; 2=0.1-1%; 3=1-
 167 5%; 5=5-25%; 7=25-50%; 8=50-75%; 9=75-100%).

168 **Table 1 -- Fitness traits evaluated on study species.**

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

	<i>Sanguisorba minor</i>	<i>Potentilla neumanniana</i>	<i>Hipocrepis comosa</i>
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)

172

173

174 **Data analysis**

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

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

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

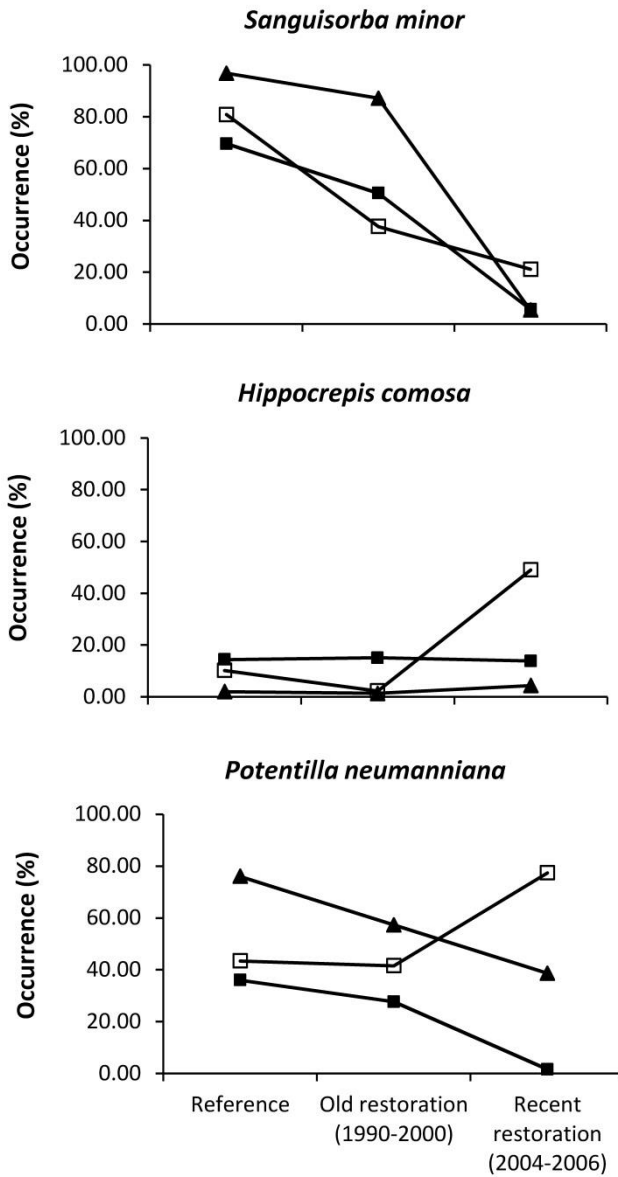
191 Response variables were arcsine-square root- (Pn_seeds, Hc_seeds) or log- (Sm_inflos,
192 Sm_seeds, Pn_flowers, Hc_seeds/fruit, Hc_flowers) transformed when needed to meet the
193 assumptions of statistical analyses. All analyses were performed with R 2.14.0 (The R
194 Foundation for Statistical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

195 **RESULTS**

196 **Colonization of restored parcels**

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

208



210

211 **Figure 2** -- Species occurrence (% of presence in 1m² plots placed systematically in parcels
 212 representing a sampling rate of ~6% of each parcel's area) in selected parcels of the three
 213 study sites (Abannets, Montagne-aux-buis and Rivelottes sites are represented by triangles,
 214 white squares and black squares respectively).

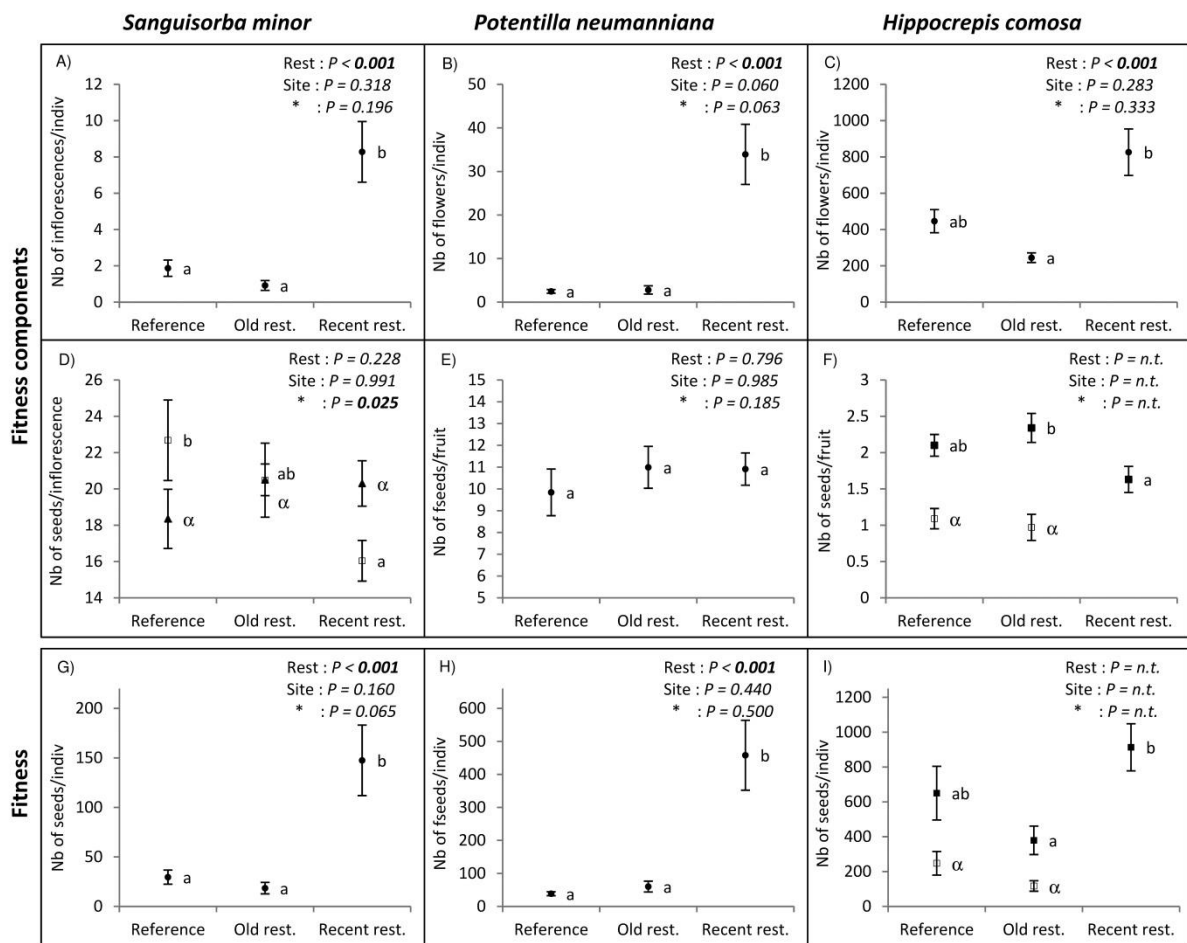
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216 **Reproductive success**

217 **Fitness components** -- Individuals of *S. minor* and *P. neumanniana* produced significantly
218 more inflorescences, or flowers, in recent restorations than others parcels (respectively
219 $df=2,114$; $F=18.49$; $P<0.001$; and $df=2,114$; $F=37.40$; $P<0.001$; fig. 3A and 3B). *S. minor*
220 individuals produced 8.3 ± 1.7 inflorescences in recent restorations, compared to 1.9 ± 0.5
221 inflorescences in reference grasslands and 0.9 ± 0.3 inflorescences in old restorations (fig. 3A).
222 *P. neumanniana* individuals produced 33.9 ± 6.9 flowers in recent restorations, compared to
223 2.5 ± 0.4 flowers in reference grasslands and 2.8 ± 1.0 flowers in old restorations. For *S. minor*,
224 the production of seeds per inflorescence was site dependent. For this species, the seeds
225 production per inflorescence was significantly higher for the reference grassland than for the
226 recent restoration of the Montagne-aux-buis site ($df=2,25$; $F=4.75$; $P=0.018$; fig. 3D). For *P.*
227 *neumanniana*, no differences were found for the number of seeds per fruit between the
228 reference and the restored grasslands (fig. 3E). Concerning *H. comosa*, the number of flowers
229 was significantly higher ($df=2,114$; $F=8.40$; $P<0.001$; fig. 3C) for recent restorations
230 (826.0 ± 128.0 flowers) than for old restorations (244.8 ± 26.5 flowers). For this species,
231 differences of seeds production per fruit were site dependent. Data was missing for one parcel
232 of the Montagne-aux-buis site because the parcel was grazed before measurements. No
233 differences were observed between reference and old restoration parcels in this site. At the
234 Rivelottes site, individuals of the old restoration produced significantly more seeds per fruit
235 ($df=2,56$; $F=4.32$; $P=0.018$; fig. 3F) than individuals of the recent restoration (respectively
236 2.3 ± 0.2 and 1.6 ± 0.2 seeds/fruit).

237 **Final fitness** -- Individuals of *S. minor* and *P. neumanniana* had a significantly higher final
238 fitness (respectively $df=2,114$; $F=13.9$; $P<0.001$; and $df=2,114$; $F=23.1$; $P<0.001$) in recent
239 restorations than other parcels. *S. minor* individuals produced 147.5 ± 35.6 seeds in recent
240 restorations, compared to 29.5 ± 7.1 seeds in reference grasslands and 18.4 ± 5.8 seeds in old
241 restorations. *P. neumanniana* individuals produced 458.0 ± 106.0 seeds in recent restorations,
242 compared to 38.4 ± 5.9 seeds in reference grasslands and 60.1 ± 16.4 seeds in old restorations
243 (fig. 3H). For *H. comosa*, differences in seeds production per individual were site dependent.
244 Individuals of the recent restoration of the Rivelottes site produced significantly more seeds
245 ($df=2,56$; $F=4.39$; $P=0.017$; 913.0 ± 135.0 seeds) than individuals of the old restoration
246 (379.0 ± 81.6 seeds). Data was missing for one recently restored parcel of the Montagne-aux-
247 buis site. At this site, no difference of final fitness was observed between the reference and
248 the old restoration (fig. 3I).

249



251

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

262

263 **Bare ground cover**

264 In the Montagne-aux-buis site, the bare ground cover was significantly higher on the recent
 265 restoration compared to other parcels, whatever the species ($df=2,57$; $F=7.06$; $P=0.002$ for *S.*
 266 *minor*; $df=2,57$; $F=5.55$; $P=0.006$ for *P. neumanniana*; $df=2,57$; $F=7.27$; $P=0.002$ for *H.*
 267 *comosa*; table 2). In the Abannets site, the bare ground cover was not significantly different
 268 around *P. neumanniana* individuals ($df=2,57$; $F=0.12$; $P=0.883$; table 2) but significantly
 269 higher in the recent restoration and the reference around *S. minor* individuals ($df=2,57$;
 270 $F=11.61$; $P<0.001$; table 2). In the Rivelottes site, there was significantly more bare ground
 271 cover in the reference parcel than in the recent restoration ($df=2,57$; $F=3.67$; $P=0.032$; table
 272 2).

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

274 Percentage of bare ground cover was estimated in 1m² plots placed around each individual.
 275 The van der Maarel (1979) scale was used to estimate de bare ground percentage in the plot
 276 (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
 277 and standard errors of bare ground cover are given for reference grasslands, old and recent
 278 restorations of study sites. Significantly different means are followed by different letters.
 279 Significant p-values are in bold.

280

Species	Sites	Reference	Old restorations	Recent restorations	p-value
<i>S. minor</i>	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
<i>P. neumanniana</i>	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
<i>H. comosa</i>	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

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282

283

DISCUSSION

284 **Colonization of restored parcels**

285 The first step to evaluate restoration success using a population approach is to determine if
 286 species have been able to form new populations on restored grasslands. Like other ecological
 287 processes, colonization and species expansion can require long periods of time. Following
 288 biotope restoration, grasslands may exhibit a colonization credit due to a time lag for species
 289 dispersal (Cristofoli et al. 2009). Some species can therefore not yet be observed in the
 290 vegetation but can be expected to colonize restored grasslands in ensuing years. In the
 291 calcareous grassland of our study region, however, Piqueray et al. (2011c) showed that there
 292 was no colonization credit for our three study species. The three species considered in the
 293 present study have been able to colonize restored habitats. This is in agreement with the
 294 observations of Delescaille (2006, 2007) in the Abannets site. However they were globally
 295 less present on restored grasslands than on reference grasslands. There are probably
 296 multifactorial causes explaining patterns of occurrence of each target species on study sites,
 297 including emergence from soil seed bank, multiple dispersal agent (like wind, sheep, goats or
 298 human management), spatial distribution of grasslands patches in the landscape and

299 environmental variability. Although restored sites were afforested forty to one hundred years
300 ago, it is conceivable that *P. neumanniana* and *S. minor* emerged from the soil seed bank, as
301 showed by a study conducted at the Abannets site (Delescaille et al. 2006). Indeed, those two
302 species have a long-term persistent seed bank (over several decades, >25 years) (Poschlod et
303 al. 1998). Moreover *P. neumanniana* and *S. minor* could have colonized restored parcels
304 through dispersal by sheep or goats that grazed parcels of different calcareous grasslands sites
305 during the grazing season. Those species are not well dispersed by wind (Poschlod et al.
306 1998), and sheep play a major role in dispersal across the landscape (Fischer et al. 1996;
307 Poschlod et al. 1998). *H. comosa* seed bank persists only between six and twenty years in the
308 soil (Poschlod et al. 1998), and seeds of this species are not efficiently dispersed by wind
309 (Poschlod et al. 1998). The presence of this species on restored grasslands could be explained
310 by parcels management, as dispersal by goats has been observed for this species (Müller-
311 Schneider 1954). *H. comosa* occurrences on restored and reference grasslands were
312 comparable. However, it has to be noted that reference grasslands are remnant grasslands that
313 have been isolated in the landscape for more than one century. These grasslands surely
314 represent a core area for the calcareous grasslands ecological network, but they may also
315 exhibit an extinction debt that can affect specialist plant species (Piqueray et al. 2011c).

316 Interestingly, in one site (the Montagne-aux-buis), we found the occurrence of *P.*
317 *neumanniana* and *H. comosa* to be higher in the recent restoration than the reference
318 grasslands and the old restoration parcels (fig.2). Species recolonization can be influenced by
319 the distance between the restored parcels and the nearest reference grassland (Helsen et al.
320 2013). In this site, the recent restoration is close to the reference parcel and the old restoration
321 is 120 meters away from the nearest reference habitat. However, study parcels are always
322 close to another calcareous grassland parcel. No parcel is isolated in a forest or agricultural
323 landscape. Environmental variability between sites or parcels could also explain differences in
324 patterns of species occurrence. In a previous work, Piqueray et al. (2011b) pointed no
325 significant differences in soil conditions between parcels or sites of the same study area.
326 However, our results showed that the bare ground cover was higher in this recent restoration
327 of the Montagne-aux-buis site. This could increase availability of microsites for germination
328 (Piqueray et al. 2013) and seedlings emergence (Kotorová & Lepš 1999; Zobel et al. 2000)
329 and modify competition regimes as compare to reference grasslands.

330 **Reproductive success of restored populations**

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

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

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

364 **Implications for the future**

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

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

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601

602

603 **Figure captions**

604 **Figure 2** -- Study region (Viroin Valley, Southern Belgium) and selected parcels of the three
605 study sites (Montagne-aux-buis: 50°05'N-4°34'E, Abannets: 50°04'N-4°34'E, Rivelottes:
606 50°05'N-4°40'E). Occurrence of study species was observed in each site. Fitness components
607 were measured in only two sites for each study species (as indicated under sites names).

608 **Figure 2** -- Species occurrence (% of presence in 1m² plots placed systematically in parcels
609 representing a sampling rate of ~6% of each parcel's area) in selected parcels of the three
610 study sites (Abannets, Montagne-aux-buis and Rivelottes sites are represented by triangles,
611 white squares and black squares respectively).

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

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