Effects of adding an arbuscular mycorrhizal fungi inoculum and of distance to donor sites on plant species recolonization following topsoil removal

Effects of adding an arbuscular mycorrhizal fungi inoculum and of distance to donor sites on plant species recolonization following topsoil removal

Vania Torrez, (corresponding author torflorvania@gmail.com)[1]
Tobias Ceulemans (tobias.ceulemans@bio.kuleuven.be)[1]
Joachim Mergeay (joachim.mergeay@inbo.be)[3]
Luc de Meester (Luc.demeester@bio.kuleuven.be)[2]
Olivier Honnay (olivier.honnay@bio.kuleuven.be)[1]

[1]Plant conservation and population biology, Department of Biology, University of Leuven, Kasteelpark Arenberg 31, 3001 Heverlee, Belgium
[2]Laboratory of aquatic ecology, evolution and conservation, Department of Biology, University of Leuven, Charles Deberiotstraat 32, 3000 Leuven, Belgium
[3]Research Institute for Nature and Forest (INBO), Gaverstraat 4, 9500 Geraardsbergen, Belgium

Questions: Does addition of an arbuscular mycorrhizal fungi (AMF) inoculum increase the short-term restoration success of a nutrient-poor grassland (NPG) after topsoil removal? Does distance to intact remnant grassland (IRG) patches affect the restoration success, and does the effect of inoculum addition depend on the distance to IRGs?
Location: Meerdaal forest, Oud-Heverlee, Belgium.
Methods: In a topsoil-removed site of 8.5ha, where 24 IRG patches (c. 10% of the area) were kept, 48 plots (1m^2) were established at three distances (5, 10 and 20m) from the edge of IRG patches. Half of the plots at each distance class were inoculated with a custom-made AMF-inoculum, whereas the remaining were used as controls. We recorded the plant species abundance in the plots, just before the addition of the AMF-inoculum, and one year after. We used repeated measures ANOVAs to test for effects of inoculum addition, distance to the IRG patches, and their interaction, on plant species richness, diversity, and on the plant community similarity between IRG patches and plots. We also

evaluated the response of AMF-dependent plant species, specialist plant species of NPG, and plant species with specific seed dispersal adaptations.

Results: Adding the inoculum positively affected the species richness and/or diversity of all plant species, AMF-dependent plant species and specialist plant species. It increased plant community similarity to the IRG patches. Increasing distance from the IRG patches had a negative effect on the richness and/or diversity of all plant species and specialist plant species. The positive effect of inoculum addition on richness and/or diversity of all plant species, AMF-dependent plant species and specialist plant species decreased with increasing distance from the IRG patches to the plots, likely indicating priority effects.

Conclusions: The application of a custom-made AMF-inoculum increased the short-term restoration success of NPG after topsoil removal. Dispersal limitation of specialist plant species of NPG, however, likely negatively affected the effect of inoculum addition. Apart from the AMF-effect, the reported strongly positive short-time effect of the inoculation was likely due to the high density of IRG patches at the site, and to the presence of organic-matter and other micro-organisms in the inoculum.

Keywords: AMF inoculum; dispersal limitation; dispersal syndromes; ecological restoration; nutrient-poor grasslands; priority effects.

Nomenclature: van der Meijden (2005).

Introduction

Owing to a widespread decline of species-rich semi-natural grasslands in Europe (Poschlod & Wallis De Vries 2002), ecological restoration of these habitats has been a priority since the 1970ies (Bakker 1989; Walker et al. 2004). These activities have also been strongly supported by the European Habitat directive (92/43/EEC), which aims at protecting and conserving habitats and wild fauna and flora. By restoring degraded ecosystems to their previous state, both species

diversity and ecosystem functioning can recover as well (Brudvig 2011). Whether or not successful restoration is achieved, largely depends on a range of biotic and abiotic constraints (Walker et al. 2004; Cramer et al. 2008), especially regarding soil properties and the capacity of target plant species to recolonize. In this respect, it has been shown that high soil nutrient levels constrain the successful restoration of nutrient-poor ecosystems (Fagan et al. 2008, 2010), whereas dispersal limitation (Standish et al. 2007; Öster et al. 2009) and the absence of soil biota (Kardol et al. 2006; Vergeer et al. 2006) may limit the arrival and establishment of seedlings of the target species (Öster et al. 2009).

Soil nutrient enrichment is largely caused by anthropogenic activities such as fertilization and atmospheric deposition from combustion processes (Peñuelas et al. 2012), and has been shown to strongly affect the occurrence and community composition of plant species. For instance, several studies have reported lowered species richness and shifts to dominance of a few competitive species in grasslands under increased nitrogen (N) input (Bobbink et al. 2010) and under phosphorus (P) enrichment (Ceulemans et al. 2013, 2014). In order to reduce nutrient levels at ecological restoration sites, high intensity interventions such as topsoil removal are often the only solution, especially for removing the immobile soil-P (Weijtmans et al. 2009; Pedley et al. 2013). However, Geissen et al. (2013) has recommended against using topsoil removal as a nature management technique due to its negative effects on soil quality and soil biota. It has indeed been reported that after topsoil removal only a small subset of the target plant species was able to recolonize the restoration sites (Verhagen 2007; Bekker 2008), even when target plant species were occurring in adjacent communities (Dobson et al. 1997; De Graaf et al. 1998; Bakker & Berendse 1999; Vergeer et al. 2006). Failure of target plant species to establish, despite low seed dispersal constraints may result from a lack of soil biota such as arbuscular mycorrhizal fungi (AMF) (van der Heijden 2004; Verhagen 2007), which are generally removed with the topsoil (Vergeer et al. 2006).

Arbuscular mycorrhizal fungi form a symbiosis with >80% of the land plant species (Smith & Read 2008). The symbiosis is based on a reciprocal exchange

of resources: the host plant provides photosynthates to the fungus, and in return it receives vital inorganic nutrients (Smith & Read 2008). AMF may also contribute to seedling establishment by integrating the seedlings into an existing mycorrhizal network connected with already established adult individuals (Simard & Durall 2004; van der Heijden & Horton 2009). Furthermore, the symbiosis induces metabolic changes in the hosts through impact on defense hormone production (Pozo & Azcón-Aguilar 2007; Jung et al. 2012). As a consequence, plant resistance against soil pathogens (Whipps 2004), nematodes (De la Peña et al. 2006), and abiotic stress, such as drought, salinity and heavy metals (Miransari 2010; Smith et al. 2010) is increased.

Clearly, AMF are vital to plant communities. Yet once absent, spontaneous re-colonization of AMF occurs very slowly (Allen and Allen 1992), as dispersal happens through colonization of roots from plant to plant (Read et al. 1976) and through transport of spores by wind (Egan et al. 2014), small mammals (Fracchia et al. 2011), water (Walker 1988), or soil fauna (Klironomos & Moutiglis 1999). The natural restoration of the entire fungal community may take decades (Baar et al. 2008). Vergeer et al. (2006) reported that two and a half years after topsoil removal, the abundance of AMF spores corresponded to 55-70% of the AMF spore numbers found in natural grasslands. Addition of AMF inoculum may therefore be crucial to accelerate the development of target plant communities at restoration sites that have been topsoil stripped.

In addition to local biotic and abiotic conditions, plant assembly also strongly depends on the landscape context of the restoration site. Evidence shows differential effects of distance to seed sources on plant species recolonization, including strong effects (Bischoff et al. 2009; Pottier et al. 2009), and weak or non-existing effects (Cole et al. 2010; Matthews & Endress 2010). Furthermore, Helsen et al. (2013) found that spatial isolation filters plant species based on their dispersal capacity, slowing down the community assembly process towards the target community. Priority effects occur when earlier arriving species affect the establishment, growth or reproduction of later arriving species (Harper 1961; Chase 2003). These effects can lead to long-lasting differences in

species composition between the target community and the restored community. Therefore, the spatial context of the restoration site should be incorporated into the restoration design (Matthews et al. 2009).

The aim of this study was to test the effectiveness of the application of a custom-made AMF inoculum in increasing the short-term restoration success of nutrient-poor grasslands where the topsoil had been removed. We hypothesized that the addition of AMF inoculum would enhance establishment success of AMF-dependent plant species. Additionally, we aimed at testing how the spatial context of the restoration site, in relation to intact remnant grassland (IRG) communities that can act as a plant species source, affect the restoration success. We tested the hypothesis that the establishment success of specialist plant species of nutrient-poor grasslands, and of plant species with poor seed dispersal capacities, would decrease with distance from the IRG. Finally, we evaluated whether the effect of the addition of the AMF inoculum depended on the distance to the IRG patches. Here, we hypothesized that colonization by AMF-dependent plant species will decrease with increasing distance from the IRG patches, because well-dispersed plant species will monopolize distant restoration sites, thus negatively affecting the effectiveness of the AMF inoculum in distant plots.

Methods

Site description

The study was carried out in a large clearing in Meerdaal forest in central Belgium, south of Leuven (50° 48’ 32.15’’ N, 4° 40’ 34.42’’ E, 78 m a.s.l.). The study site was a former military domain (c. 8.5 ha) with ammunition storage facilities. In the context of the European habitat directive (92/43/EEC), a nature conservation project was carried out to restore nutrient-poor grasslands and grassy heathlands. After the removal of the remaining military buildings and roads, the topsoil was removed in January 2011, to a depth of 10 cm, to remove the nutrient-rich topsoil layer, down to the mineral soil. At the restoration site, 24 scattered patches of well-developed grassland communities were present around

155 the former ammunition storage locations. These IRG patches (sized between
156 0.03 and 0.04 ha, in total c. 10% of the area) occur in a regular grid pattern, and
157 contain the original species-rich vegetation with characteristic and regionally
158 endangered species of nutrient-poor grasslands such as *Polygala serpyllifolia*,
159 *Thymus pulegioides* and *Campanula rotundifolia*.
160
161 ***Experimental design***
162 In mid-August 2012, a total of 48 permanent plots of 1 m x 1 m were randomly
163 established at three distances (16 replicates at each distance of 5, 10 and 20 m)
164 from the edge to the closest IRG patch. Eight out of the 16 plots at each distance
165 class were inoculated with a custom-made AMF inoculum (see further), whereas
166 the remaining eight plots were not inoculated and served as controls (Fig. 1). To
167 apply the inoculum, we removed the upper 1 cm layer of soil with a hand rake
168 without affecting the sparse already established plants in the plot. We applied
169 900 ml of the custom-made liquid AMF inoculum per plot. After the application of
170 the inoculum, we covered the plots with the removed soil. The plots that did not
171 receive the inoculum addition were submitted to the same soil disturbance with
172 the hand rake and de-mineralized water was applied.
173
174 ***Preparation and* ex situ *testing of the AMF inoculum***
175 AMF-colonized roots can be used as a source of AMF inoculum (Tommerup
176 1984; Klironomos & Hart 2002). Because the origin of the AMF may strongly
177 influence their effectiveness (White et al. 2008; Pellegrino et al. 2011), we used
178 the roots of AMF-dependent plant species collected in October 2011 in the wide
179 surroundings of the study area. These plant species are indicator species of
180 nutrient-poor grasslands that are still relatively common, and included *Achillea*
181 *millefolium*, *Centaurea jacea*, *Danthonia decumbens*, *Hieracium pilosella*,
182 *Hypochaeris radicata*, *Potentilla erecta*, *Stachys officinalis*, and *Succisa*
183 *pratensis*. Roots were soaked and washed out of soil with tap water, but still
184 some soil remained attached. Afterwards, roots were blended separately per
185 species in de-mineralized water with a commercial Philips HR7625 food

processor to chop them in small pieces, between 0.1 and 3 cm. The resulting muddy mixture of each species was equally distributed across 24 containers of 300 ml.

A greenhouse experiment was then ran to test the effectiveness of this custom-made inoculum through assessments of AMF root colonization and spore density, and to test if it contained pathogens and seeds that may confound the spontaneous colonization process. The experiment ran during four months. Three known AMF-dependent plant species (*Campanula rotundifolia*, *Nardus stricta*, and *Festuca filiformis*) were seeded as monocultures in 2 L pots, each pot containing 15 seeds. We used a substrate composed by potting soil and sand (3:1, respectively), both were autoclaved at 120 °C for one hour. The plant species were sown on sterilized substrate (control) and on sterilized substrate inoculated with the inoculum. Each treatment was replicated eight times, resulting in a total of 48 pots. We applied 300 ml of the AMF inoculum to the pots and de-mineralized water to the control pots. Root colonization and spore density were surveyed to test the effectiveness of the inoculum. After four months, roots were washed and stained following Grace & Stribley (1991), with 1:1 1% Methyl blue solution and 85% Lactic acid. Root samples were observed under a dissecting microscope at 32x magnification. The grid line intersect method (Giovannetti & Mosse 1980) was used to measure the degree of AMF colonization. In addition, 100 g of soil per pot was collected to extract the AMF spores, following the sucrose centrifugation method of Brundrett et al. (1994). Spores were counted using a dissecting microscope at 32x magnification. We found that the roots of all individuals that were inoculated were colonized by AMF. The mean percentage of colonization was 57.4% for *C. rotundifolia*, 50.4% for *N. stricta* and 25.1% for *F. filiformis*. In the inoculated pots, we counted between 49-207 spores g^{-1} soil; none of the plants developed any type of disease. There were no AMF spores, and there was no AMF root colonization in the non-inoculated control pots. In addition, only two pots out of the 24 inoculated pots had non-sown *Juncus effusus* growing, which demonstrates a very low probability of seed introduction with the inoculum. In July 2012, the same

procedure was then followed to prepare the AMF inoculum to be used at the study site.

Vegetation surveys

In early August 2012, just before adding the AMF inoculum, we recorded the plant species abundance in the established 1 m x 1 m plots. A grid, consisting of 100 10 cm x 10 cm squares was placed on top of the plots and we counted the number of individuals of plant species that occurred in each square. The same procedure was repeated in July 2013. Additionally, we recorded all plant species occurring in each of the 24 IGR patches (Table S1. Supplementary material). In both surveys, 18% of the individuals were identified at family or genus level due to their early growth stage.

Data analyses

For both vegetation surveys and for each plot, we calculated both plant species richness and the Shannon diversity index to the power of *e* (Jost 2006) for all plant species, for AMF-dependent plant species (following Fitter & Peat (1994)), and for specialist plant species of nutrient-poor grasslands (following Decleer 2007; see Table S1, Supplementary material). In addition, we calculated the plant community similarity between the IRG patches and the experimental plots, using the Jaccard similarity index. Non-metric multidimensional scaling (NMDS) was used to analyze the similarity in plant community composition among plots. The NMDS analysis was based on the plant species abundance x plot matrix of the two surveyed years together, and on Bray-Curtis dissimilarities, with several starting points and a maximum of 1000 iterations. These analyses were done separately for (i) all plant species, (ii) AMF-dependent plant species, and (iii) specialist plant species. Furthermore, we determined the plant species richness according to plant dispersal syndromes. We distinguished between endozoochores, myrmecochores and anemochores (Kleyer et al. 2008; hereafter referred to as plant species dispersed by dung, ants and wind, respectively). The plant species were assigned to the prevailing dispersal syndrome. The nine most

widely distributed plant species in the plots were assigned to the category of well-dispersed plant species. Plant species richness, Jaccard similarity index, and NMDS were calculated using the R program 3.0.2 (R Foundation for Statistical Computing, Vienna, AT), with the package *Vegan* (Oksanen et al. 2011).

Prior to further analyses, F-tests were performed on the vegetation data of the initial survey (2012) to confirm that there were no *a priori* differences between plots at different distances from the IRG patches. To meet assumptions of normality, several response variables were log or square root-transformed.

We then conducted repeated measures ANOVAs to test for effects of inoculum addition (inoculated *vs.* non-inoculated), distance to the closest IRG patch (5, 10 or 20 m), and their interaction, on the differences in plant species richness, Shannon diversity, and Jaccard index between the surveys in 2012 and 2013. ANOVAs were run using the R package *nmle* (Pinheiro et al. 2009). Backwards model selection was used. When a significant effect was found, multiple comparisons were performed with the R package *phia* (De Rosario-Martinez 2012). Repeated measures ANOVAs were done separately for all plant species, AMF-dependent plant species, and specialist plant species. Similar analyses were done using species richness of the three plant dispersal syndrome categories and the well-dispersed plant species, as the dependent variables.

Finally, the NMDS scores (on the first two axes) of the plots in 2012 and 2013 were used as measures of plant community similarity among plots. These values were used as dependent variables in a repeated measures ANOVA, as described above. Again, these analyses were done using the NMDS scores of all plant species, AMF-dependent plant species, and specialist plant species.

Results

In total, we recorded 80 plant species in the IRG patches and 78 plant species in the plots, belonging to 28 families; 52 of them were AMF-dependent plant species, and 11 were nutrient-poor grasslands specialist plant species (Table S1). In 2012, we recorded 55 plant species; 37 were AMF-dependent plant species, and 9 were specialist plant species. In 2013, we registered 66 plant

279 species; 43 were AMF-dependent plant species, and 11 were specialist plant
280 species. Of the nine well-dispersed plant species, five were AMF-dependent
281 (*Holcus lanatus, Hypericum perforatum, Juncus effusus, Molinia caerulea, Rubus*
282 *fruticosus*), and four were not (*Calluna vulgaris, Carex pilulifera, Luzula*
283 *multiflora, Pinus sylvestris*). In 2012, the number of plant species recorded per
284 plot ranged from 1 to 28 (mean = 12, SD = 6.4), and in 2013 it ranged from 4 to
285 32 (mean = 15, SD=7). Prior to inoculation, no significant differences were found
286 among the three distance classes for plant species richness ($F_{2,45}$ = 2.6, P >
287 0.05), Shannon diversity ($F_{2,45}$ = 2.96, P > 0.05), Jaccard index ($F_{2,45}$ = 5.2, P >
288 0.05), NMDS1 ($F_{2,45}$ = 4.2, P > 0.05), and NMDS2 ($F_{2,45}$ = 4.5, P > 0.05).
289
290 **Plant species response to treatments**
291 Inoculation significantly increased total plant species richness, the Jaccard
292 species similarity index between the plots and IRG patches, and the plant
293 community similarities among inoculated plots (Table 1, 2). The effect of the
294 distance treatment on Shannon diversity was significantly higher in plots at 5 m
295 than in plots at 20 m (Table 1, 2). The effect of inoculum addition on plant
296 species richness, Jaccard similarity index, and plant community similarities
297 among plots varied with distance to the IRG patch (Table 1). These variables
298 were significantly higher in inoculated plots at 5 and 10 m than in inoculated plots
299 at 20 m, whereas no significant differences were found between inoculated plots
300 at 5 and 10 m (Fig. 2A, B, C). No significant differences were found among the
301 three distances in the non-inoculated plots for these response variables. Plant
302 species richness, Jaccard index between plots and IRG patches, and plant
303 community similarities among plots were significantly higher in inoculated plots at
304 5 and 10 m, as compared to non-inoculated plots at the same distances.
305 However, there was no difference among inoculated and non-inoculated plots at
306 20 m.
307 For the AMF-dependent plant species, inoculation significantly increased
308 richness, Shannon diversity and Jaccard species similarity with IRG patches
309 (Table 1, 2). The effect of the distance treatment was significant for community

similarities among all plots (Table 1). These similarities were higher in plots at 5 and 10 m than in plots at 20 m (Table 2). The effect of inoculum addition on plant species richness, Shannon diversity, and the Jaccard index varied with distance to the IRG patch (Table 1). Richness was higher in inoculated plots at 5 m than in inoculated plots at 20 m (Fig. 3A), whereas no significant differences were found among inoculated plots at 10 and 20 m, and among 5 and 10 m. Richness in non-inoculated plots did not differ significantly among the distance classes. Shannon diversity and the Jaccard index was significantly lower in inoculated plots at 20 m, as compared to plots at 5 and 10 m, whereas no significant differences were found between inoculated plots at 5 and 10 m (Table 2, Fig. 3B, C). These response variables in non-inoculated plots did not significantly vary with distance. Richness, Shannon diversity and the Jaccard similarity index with IRG patches were significantly higher in inoculated plots at 5 and 10 m, as compared to non-inoculated plots at same distances. However, inoculated and non-inoculated plots at 20 m did not significantly differ.

For the specialist plant species of nutrient-poor grasslands, the inoculum addition significantly increased the Shannon diversity and Jaccard similarity index (Table 1, 2). The effect of the distance treatment on specialist plant species richness and community similarities of specialist plant species among all plots was significant (Table 1), both variables had higher values in plots at 5 and 10 m than in plots at 20 m (Table 2). The effects of the inoculum addition on the Jaccard index varied with distance from the IRG patches (Table 1). The Jaccard index was significantly higher in inoculated plots at 5 or 10 m than in plots at 20 m (Fig. 4), while no significant difference was found between plots at 5 and 10 m. The Jaccard index between non-inoculated plots and IRG patches was not significantly different among the different distance classes. The Jaccard index was significantly higher in inoculated plots at 5 and 10 m, as compared to non-inoculated plots at same distances. However, these similarities were not significantly different in inoculated vs. non-inoculated plots at 20 m.

The inoculum addition significantly increased the richness of plant species dispersed by wind, ants or dung (Table 1, 2). The effect of inoculum addition

significantly varied with the distance treatment (Table 1); plant species richness of the three groups was significantly higher in inoculated plots at 5 and 10 m than in inoculated plots at 20 m (Fig. 5A, B, C). In non-inoculated plots the plant species richness did not vary significantly among the different distance classes. Plant species richness of these three groups was significantly higher in inoculated plots at 5 and 10 m, as compared to non-inoculated plots at same distances. However, plant species richness in inoculated vs. non-inoculated plots at 20 m was not significantly different.

Finally, the effects of inoculum addition, distance and their interaction on NMDS2 scores were not significant for any of the studied groups of plant species (results not shown). Similarly, there were no effects of the two treatments and their interaction on the abundance of the well-dispersed plant species (results not shown).

Discussion

Effects of AMF-inoculum addition

The aim of this study was to test whether the application of an AMF inoculum could improve the short-term restoration success of a nutrient-poor grassland where the topsoil was removed. We predicted that the addition of the inoculum would enhance the establishment success of AMF-dependent plant species. Our results demonstrated that one year after topsoil removal, the plots that were inoculated showed a higher Shannon diversity and richness of AMF-dependent plant species, at least in the plots closest to the IRG patches that could act as plant species sources. Zhang et al. (2012) found similar results, one year after inoculating degraded grasslands in China with a lab propagated AMF inoculum.

The type of inoculum that we used is less expensive than commercial inoculum, and it is less time consuming to produce than inoculum that is produced by isolation of AMF spores and further propagation in plant roots. In addition, it is likely unfeasible to generate a pure inoculum consisting of many different and naturally occurring AMF taxa, because many of these are impossible or very difficult to cultivate and propagate. Although we have no

372 information on the specific AMF taxa that were present in the inoculum, we can
373 assume that they were very similar to the ones naturally occurring in nutrient-
374 poor grasslands in the study region. AMF species composition is indeed known
375 to affect plant diversity and community composition, especially when a majority of
376 the plants in the community are AMF-dependent (Vogelsang et al. 2006). In
377 addition, White et al. (2008) and Pellegrino et al. (2011) experimentally
378 demonstrated that the origin of the inoculated AMF taxa has an important effect
379 on plant performance. These authors reported that inoculum produced through
380 propagation of AMF species from the local ecosystem was more effective in
381 improving plant performance than a commercially available AMF inoculum. The
382 use of native AMF from roots of plant species from undisturbed grasslands may
383 therefore be a convenient alternative to a commercial AMF inoculum, and may
384 offer important ecological and economic advantages.
385 It is important to note that our results cannot be exclusively attributed to
386 AMF in the inoculum, but also to the presence of organic matter and other soil
387 micro-organisms. Soil amendments have been proven to be beneficial in
388 ecological restoration (de Deyn et al. 2003; Carbajo et al. 2011), and to play a
389 facilitating role in establishing the soil microbial-plant association. Organic matter
390 amendments have also been suggested to stimulate and improve plant
391 mycorrhization (Douds et al. 2006, Jaison et al. 2011). In degraded alpine areas
392 in Switzerland, Schmid et al. (2008) found that through applying an (commercial)
393 AMF inoculum only, 65% of the area recovered with vegetation, in comparison to
394 87% when commercial AMF inoculum was applied along with organic nutrients
395 and P-solubilizing Penicillium spp. Nevertheless, the strong root colonization and
396 high density of AMF spores in our greenhouse experiment support the role of
397 AMF on our results. It was also the explicit objective of this study to evaluate a
398 practical and feasible ecological restoration approach, through adding a custom-
399 made inoculum. It is currently indeed not possible to generate a ‘pure’ AMF
400 inoculum consisting of many different, naturally occurring AMF species only,
401 because many of these are impossible or very difficult to cultivate and propagate.

Such a pure inoculum would never mimic the AMF composition of our custom-made inoculum.

Effects of distance to the donor sites

We also tested how the distance to IRG that can act as a plant species sources affected the restoration success. We predicted that the establishment success of nutrient-poor grassland specialist plant species would decrease with distance from the IRG. Our results show that increasing distance from IRG patches had a negative effect on specialist plant species richness. Such step-wise dispersal is consistent with the findings that many grassland specialists are poor dispersers (Martin & Wisley 2006; Cousins & Lindborg 2008; Helsen et al. 2013). We did, however, not find a distance effect on the Jaccard similarity index between specialist plant species in the IRG patches and the study plots. This might be due to the relatively low number of specialist plant species in the area (12 species in total, range in the plots between 0 and 6 species). We also found no effects of distance on the total plant species richness. Similarly, Krauss et al. (2004) found no effects of distance on generalist plant species richness colonization in calcareous grasslands in Germany. In contrast, Öster et al. (2009) reported a decline of generalist plant species richness with increasing distance from species-rich semi-natural grasslands to former arable fields, and Diacon-Bolli et al. (2013) found that the amount of captured diaspores decreased abruptly at 20 m, in a seed rain study in a calcareous grassland. Also the Jaccard similarity index between the IRG patches and the plots did not change with distance in our study, suggesting that generalist plant species disperse and establish well across the studied distances.

We also predicted that establishment success of ant-dispersed plant species, but not of wind- and dung-dispersed plant species, would decrease with distance from the IRG patches. We found that distance between the IRG patches and the study plots had no effect on the richness of plant species dispersed by wind, ant or dung. These results are consistent with the findings of Öster et al. (2009), who reported that wind- and animal-dispersed seeds were able to

successfully disperse up to 10 m from grasslands into former arable fields. That we did not find a negative effect of distance on ant-dispersed plant species is likely due to the very limited occurrence of such species (range in the plots between 2 and 3 species) at our study site.

Effects of AMF inoculum addition depend on the distance to the donor site

Finally, we evaluated whether the effect of the addition of AMF inoculum on plant community composition depended on the distance of the plots to the IRG. We hypothesized that AMF-dependent plant species colonization would decrease with increasing distance from the IRG patches because well-dispersed plant species would monopolize the distant plots, obscuring the effectiveness of the inoculation. We indeed found that in the inoculated plots, the richness of AMF-dependent plant species and the Jaccard similarity index between the IRG patches and plots was relatively constant up to a distance of 10 m, whereas it decreased at 20 m. On the other hand, in the non-inoculated plots, no significant differences were found along the studied distance classes. This pattern of community similarities demonstrates that the positive effect of inoculum addition is reduced in distant plots. We also found that the abundance of well-dispersed plant species did not vary across the studied distances, additionally suggesting that the effectiveness of the inoculation at 20 m distances is obscured by dispersal limitation of AMF-dependent plant species, in combination with priority effects exerted by well-dispersed generalist plant species. Interestingly, the latter was composed by AMF-dependent and non AMF-dependent plant species, then the priority effects exerted over the other AMF-dependent plant species were independent to AMF-dependency. This suggests that well-dispersed generalist plant species created soil legacies, and these legacies contributed to strong priority effects on AMF-dependent plant species (Grman & Suding 2010).

Our findings suggest the high ecological significance of inoculation in early stages of restoration. Even though the positive effect of the addition of AMF inoculum decreases in distant areas due to dispersal limitation, the addition of AMF inoculum should be a desirable part of ecological restoration and can be

considered as a best practice in ecological restoration, likely along with seed addition. Further work should focus on elucidating AMF species composition of roots of plants from inoculated and non-inoculated plots, for example using amplicon-sequencing approaches (e.g. Van Geel et al. 2015), in order to disentangle the role of AMF addition and the addition of organic matter and other soil micro-organisms.

Acknowledgements

VT benefited from a DBOF fellowship from the KU Leuven Research Fund. We acknowledge financing from the KU Leuven Research Fund project PF/2010/07 and Belspo IAP project P7/04. We thank the Nature and Forest Agency (ANB) for permission to perform the experiments in Meerdaal. We thank Dr. Helios de Rosario-Martinez for his statistical help, Veronika Martinová for her help in the greenhouse experiment and Kasper van Acker and David Sanín for their fieldwork assistance.

References

Allen, M.F. & Allen, E.B. 1992. Development of mycorrhizal patches in a successional arid ecosystem. In: Read, D.J., Lewis, D.H., Fitter, A.H. & Alexander, I.J. (eds.) *Mycorrhizas in ecosystems*, pp. 164–170. CAB International, Oxford.

Baar, J., Bergsma, H. & Steffen, F. 2008. The potential role of arbuscular mycorrhizal fungi for transition of highly fertilized grasslands into natural high biodiversity fields. In: Feldman F., Kapulnik Y. & Baar J. (eds.) *Mycorrhiza works*, pp. 217–228. Spectrum Phytomedizin, DPG-Publisher Braunschweig, Germany.

Bakker, J.P. 1989. *Nature management by grazing and cutting: on the ecological significance of grazing and cutting regimes applied to restore former species-rich grassland communities in The Netherlands*. Kluwer, Dordrecht, The Netherlands.

493 Bakker, J.P. & Berendse, F. 1999. Constraints in the restoration of ecological
494 diversity in grassland and heathland communities. *Trends in Ecology &*
495 *Evolution* 14: 63–68.

496 Bekker, R. 2008. *20 Years topsoil removal for nature at sandy soils*. Report
497 Rijksuniversiteit Groningen, The Netherlands.

498 Bischoff, A., Warthemann, G. & Klotz, S. 2009. Succession of floodplain
499 grasslands following reduction in land use intensity: the importance of
500 environmental conditions, management and dispersal. *Journal of Applied*
501 *Ecology* 46: 241–249.

502 Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M.,
503 Bustamante, M., Cinderby, S., Davidson, E., (…) & De Vries, W. 2010.
504 Global assessment of nitrogen deposition effects on terrestrial plant
505 diversity: a synthesis. *Ecological Applications* 20: 30–59.

506 Brudvig, L.A. 2011. The restoration of biodiversity: where has research been and
507 where does it need to go? *American Journal of Botany* 98: 549–558.

508 Brundrett, M.C, Melville, L. & Peterson, L. 1994. *Practical methods in mycorrhizal*
509 *research*. Mycologue publications, Waterloo.

510 Carbajo, V., den Braber, B., van der Putten W.H. & de Deyn, G.B. 2011.
511 Enhancement of late successional plants on ex-arable land by soil
512 inoculants. *PLoS ONE* 6: e21943

513 Ceulemans T, Merckx R, Hens M & Honnay O. 2013. Plant species loss from
514 European semi-natural grasslands following nutrient enrichment – Is it
515 nitrogen or is it phosphorus?. *Global Ecology and Biogeography* 22: 73–
516 82.

517 Ceulemans, T., Stevens, C.J., Duchateau, L., Jacquemyn, H., Gowing, D.J.G.,
518 Merckx, R., Wallace, H., van Rooijen, N., Goethem, T., (…) & Honnay, O.
519 2014. Soil phosphorus constrains biodiversity across European

grasslands. *Global Change Biology* 20: 3814–3822.

Chase, J.M. 2003. Community assembly: when should history matter? *Oecologia* 136: 489–498.

Cole, R.J., Holl, K.D. & Zahawi, R.A. 2010. Seed rain under tree islands planted to restore degraded lands in a tropical agricultural landscape. *Ecological Applications* 20: 1255–1269.

Cousins, S.A.O. & Lindborg, R. 2008. Remnant grassland habitats as source communities for plant diversification in agricultural landscapes. *Biological Conservation* 141: 233–240.

Cramer, V.A., Hobbs, R.J. & Standish, R.J. 2008. What's new about old fields? Land abandonment and ecosystem assembly. *Trends in Ecology & Evolution* 23: 104–112.

Decleer, K. 2007. *Europees beschermde natuur in Vlaanderen en het Belgisch deel van de Noordzee: habitattypen : dier- en plantensoorten*. *Mededelingen van het Instituut voor natuur- en bosonderzoek*. Instituut voor Natuur- en Bosonderzoek: Brussel.

De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., de Ruiter, P.C., Verhoef, H.A., Bezemer, T.M. & van der Putten, W.H. 2003. Soil invertebrate fauna enhances grassland succession and diversity. *Nature* 422: 711–713.

De Graaf, M.C.C., Verbeek P.J.M., Bobbink R. & Roelofs J.G.M. 1998. Restoration of species rich dry heaths: the importance of appropriate soil conditions. *Acta Botanica Neerlandica* 47: 86–111.

De la Peña, E. Echeverría, S.R. van der Putten, W.H., Freitas, H & Moens, M. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytologist* 169: 829–840.

547 De Rosario-Martinez, H. 2012. Analysing interactions of fitted models. R package
548 version 0.2-0. Available from: http://cran.r-
549 project.org/web/packages/phia/phia.pdf<cran.r-
550 project.org/web/packages/phia>.

551 Diacon-Bolli, J.C., Edwards, P.J., Bugmann, H., Scheidegger, C. & Wagner, H.H.
552 2013. Quantification of plant dispersal ability within and beyond a
553 calcareous grassland. *Journal of Vegetation Science* 24: 1010–1019.

554 Dobson, A.P., Bradshaw, A.D. & Baker, A.J. 1997. Hopes for the future:
555 restoration ecology and conservation biology. *Science* 277: 515–522.

556 Douds, D.D., Nagahashi, G., Pfeffer, P. E., Reider, C. & Kayser, W. M. 2006. On-
557 farm production of AM fungus inoculum in mixtures of compost and
558 vermiculite. *Bioresource Technology* 97: 809–818.

559 Dupré, C., Stevens, C.J., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing,
560 D.J.G., David, J., Dise, N.B., Dorland, E., Bobbink, R. & Diekmann, M.
561 2010. Changes in species richness and composition in European acidic
562 grasslands over the past 70 years: the contribution of cumulative
563 atmospheric nitrogen deposition. *Global Change Biology* 16: 344–357.

564 Egan, C., Li, D. & Klironomos, J. 2014. Detection of arbuscular mycorrhizal
565 fungal spores in the air across different biomes and ecoregions. *Fungal*
566 *Ecology* 12: 26–31.

567 Fagan, K.C., Pywell, R.F., Bullock, J.M. & Marrs, R.H. 2008. Do restored
568 calcareous grasslands on former arable fields resemble ancient targets?
569 The effect of time, methods and environment on outcomes. *Journal of*
570 *Applied Ecology* 45: 1293–1303.

571 Fagan, K.C., Pywell, R.F., Bullock, J.M. & Marrs, R.H. 2010. The seed banks of
572 English lowland calcareous grasslands along a restoration
573 chronosequence. *Plant Ecology* 208: 199–211.

574 Fitter, A. H. & Peat, H. J. 1994. The ecological flora database. *Journal of Ecology*
575 82: 415–425.

576 Fracchia, S., Krapovickas, L., Aranda-Rickert, A. & Valentinuz, V.S. 2011.
577 Dispersal of arbuscular mycorrhizal fungi and dark septate endophytes by
578 *Ctenomys cf. knighti* (Rodentia) in the northern Monte Desert of Argentina.
579 *Journal of Arid Environments* 75: 1016–1023.

580 Geissen, V., Wang, S., Oostindie, K., Huerta, E., Zwart, K.B., Smit, A., Ritsema,
581 C.J. & Moore, D. 2013. Effects of topsoil removal as a nature
582 management technique on soil functions. *Catena* 101: 50–55.

583 Giovannetti M. & Mosse B. 1980. An evaluation of techniques for measuring
584 vesicular-arbuscular infection in roots. *New Phytologist* 84:489–500.

585 Grace, C. & Stribley, D.P. 1991. A safer procedure for routine staining of
586 vesicular-arbuscular mycorrhizal fungi. *Mycological Research* 95: 1160–
587 1162.

588 Grman, E & Suding, K.N. 2010. Within-year soil legacies contribute to strong
589 priority effects of exotics on native California grassland communities.
590 *Restoration ecology* 18: 664–670.

591 Harper, J.L. 1961. Approaches to the study of plant competition. In: Milthorpe
592 F.L. (ed.) *Mechanisms in biology competition*, pp. 1–39. Cambridge
593 University Press, Cambridge, United Kingdom.

594 Helsen, K., Hermy, M. & Honnay, O. 2013. Spatial isolation slows down
595 directional plant functional group assembly in restored semi-natural
596 grasslands. *Journal of Applied Ecology* 50: 404–413.

597 Jaison, S., Uma, E. & Muthukumar, T. 2011. Role of organic amendments on
598 arbuscular mycorrhizal formation and function. In: Miransari M. (ed.) *Soil*
599 *microbes and environmental health*. pp. 217–237. Nova Science
600 Publisher, Hauppauge, New York, USA.

601 Jost, L. 2006. Entropy and diversity. *Oikos* 113: 363–375.

602 Jung, S.C., Martinez-Medina, A., Lopez-Raez, J.A. & Pozo, M.J. 2012.
603 Mycorrhiza-induced resistance and priming of plant defenses. *Journal of*
604 *Chemical Ecology* 38: 651–664.

605 Kardol, P., Bezemer, T.M. & van der Putten, W.H. 2006. Temporal variation in
606 plant-soil feedback controls succession. *Ecology Letters* 9: 1080–1088.

607 Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P, Thompson, K.,
608 Sonnenschein, M., Poschlod, P., Van Groenendael, J.M., Klimes, L., (…)
609 & Peco, B. 2008. The LEDA traitbase: A database of life-history traits of
610 Northwest European flora. *Journal of Ecology* 96: 1266–1274.

611 Klironomos, J.N. & Hart, M.M. 2002. Colonization of roots by arbuscular
612 mycorrhizal fungi using different sources of inoculum. *Mycorrhiza* 12, 181–
613 184.

614 Klironomos, J.N. & Moutoglis, P. 1999. Colonization of nonmycorrhizal plants by
615 mycorrhizal neighbors as influenced by the collembolan, *Folsomia*
616 *candida*. *Biology and Fertility Soils* 29: 277–281.

617 Krauss, J., Klein, A.M., Dewenter, I.S. & Tscharntke, T. 2004. Effects of habitat
618 area, isolation and landscape diversity on plant species richness of
619 calcareous grasslands. *Biodiversity and Conservation* 13: 1427–1439.

620 Martin, L.M. & Wilsey, B.J. 2006. Assessing grassland restoration success:
621 relative roles of seed additions and native ungulate activities. *Journal of*
622 *Applied Ecology* 43: 1098–1110.

623 Matthews, J.W. & Endress, A.G. 2010. Rate of succession in restored wetlands
624 and the role of site context. *Applied Vegetation Science* 13: 346–355.

625 Matthews, J.W. & Spyreas, G. 2010. Convergence and divergence in plant
626 community trajectories as a framework for monitoring wetland restoration
627 progress. *Journal of Applied Ecology* 47: 1128–1136.

628 Matthews, J.W., Peralta, A.L., Flanagan, D.N., Baldwin, P.M., Soni, A., Kent,
629 A.D. & Endress, A.G. 2009. Relative influence of landscape vs. local
630 factors on plant community assembly in restored wetlands. *Ecological*
631 *Applications* 19: 2108–2123.

632 Miransari, M. 2010. Contribution of arbuscular mycorrhizal symbiosis to plant
633 growth under different types of soil stress. *Plant Biology* 12: 563–569.

634 Oksanen J., Blanchet, F.G, Kindt, R. Legendre, P., Minchin, P.R., O'Hara, R.B.,
635 Simpson, G.L, Solymos, P., Stevens, M.H.H. & Wagner, H. 2011. Vegan:
636 community ecology package. R package version 2.2-1. Available from:
637 http://cran.r-project.org/web/packages/vegan/index.html

638 Öster, M., Ask, K., Cousins, S.A.O & Eriksson O. 2009. Dispersal and
639 establishment limitation reduces the potential for successful restoration of
640 semi-natural grassland communities on former arable fields. *Journal of*
641 *Applied Ecology* 46: 1266–1274.

642 Pedley, S.M., Franco, A.M.A, Pankhurst, T. & Dolman, P.M. 2013. Physical
643 disturbances enhances ecological networks for heathland biota: A multiple
644 taxa experiment. *Biological Conservation* 160: 173–182.

645 Pellegrino, E., Bedini, S., Avio, L., Bonari, E. & Giovannetti, M. 2011. Field
646 inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi
647 in a Mediterranean agricultural soil. *Soil Biology and Biochemistry* 43:
648 367–376.

649 Peñuelas, J., Sardans, J., Rivasubachm, A. & Janssens, I.A. 2012. The human-
650 induced imbalance between C, N and P in Earth's life system. *Global*
651 *Change Biology* 18: 3–6.

652 Pinheiro, J., Bates, D., DebRoy, S., Sakar, D. & R Core team. 2009. *nlme*: Linear
653 and nonlinear mixed effects models. R package version 3.1-119. Available
654 from: http://cran.r-project.org/web/packages/nlme/index.html

655 Pottier, J., Bédécarrats, A. & Marrs, R.H. 2009. Analyzing the spatial
656 heterogeneity of emergent groups to assess ecological restoration.
657 *Journal of Applied Ecology* 46: 1248–1257.

658 Poschlod, P. & Wallis De Vries, M.F. 2002. The historical and socioeconomic
659 perspective of calcareous grasslands—lessons from the distant and
660 recent past. *Biological Conservation* 104: 361–376.

661 Pozo, M.J. & Azcón-Aguilar, C. 2007. Unraveling mycorrhiza-induced resistance.
662 *Current Opinion in Plant Biology* 10: 393–398.

663 Read, D.J., Koucheki, H.K. & Hodgson J. 1976. Vesicular-arbuscular mycorrhiza
664 in natural vegetation systems. I. The occurrence of infection. *New*
665 *Phytologist* 77: 641–653.

666 Schmid, T., Meyer, J. & Oehl, F. 2008. Integration of mycorrhizal inoculum in
667 high alpine revegetation. In: Feldmann F, Kapulnik Y, Baar J (eds.)
668 *Mycorrhiza works*, pp. 278–288. Spectrum Phytomedizin, DPG-Publisher
669 Braunschweig, Germany.

670 Simard, S.W. & Durall D.M. 2004. Mycorrhizal networks: a review of their extent,
671 function, and importance. *Canadian Journal of Botany* 82: 1140–1165.

672 Smith, S., Facelli, E., Pope, S. & Smith, A.F. 2010. Plant performance in stressful
673 environments: interpreting new and established knowledge of the roles of
674 arbuscular mycorrhizas. *Plant Soil* 326: 3–20.

675 Smith, S.E. & Read D.J. 2008. *Mycorrhizal symbiosis*. 3rd ed. Academic Press,
676 USA.

677 Standish, R.J., Cramer, V.A., Wild, S.L. & Hobbs, R.J. 2007. Seed dispersal and
678 recruitment limitation are barriers to native recolonization of old-fields in
679 western Australia. *Journal of Applied Ecology* 44: 435–445.

680 Tommerup, I.C. 1984. Development of infection by a vesicular–arbuscular

mycorrhizal fungus in *Brassica napus* L. and *Trifolium subterraneum* L. *New Phytologist* 98: 487– 495.

Van der Heijden, M.G.A. 2004. Arbuscular mycorrhizal fungi as support systems for seedling establishment in grasslands. *Ecology Letters* 7: 293–303.

Van der Heijden, M.G.A & Horton T.R. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology* 97: 1139–1150.

Van der Meijden, R. 2005. Heukel's Flora van Nederland. Noordhoff Uitgevers B. V. Groningen, The Netherlands.

Van Geel, M., Ceustermans, A., Van Hemelrijck, W., Lievens, B. & Honnay, O. 2015. Decrease in diversity and changes in community composition of arbuscular mycorrhizal fungi in roots of apple trees with increasing orchard management intensity across a regional scale. *Molecular Ecology* 24: 941–952.

Vergeer, P., van den Berg, L.J.L., Baar, J., Ouborg, N.J. & Roelofs, J.G.M. 2006. The effect of turf cutting on plant and arbuscular mycorrhizal spore recolonisation: implications for heathland restoration. *Biological Conservation* 129: 226235.

Verhagen, H.M.C. 2007. *Changing land use: restoration perspectives of low production communities on agricultural fields after top soil removal*. Ph.D. thesis, University of Groningen, Groningen, The Netherlands.

Vogelsang, K.M., Reynolds, H.L. & Bever, J.D. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist* 172: 554–562.

Walker, C. 1988. Formation and dispersal of propagules of endogonaceous

707 fungi. In: Pegg G.F. & Ayes P.C. (eds.), *Fungal infection in plants,* pp.
708 269-284. Cambridge University Press, Cambridge.

709 Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J. &
710 Pywell, R.F. 2004. The restoration and re-creation of species-rich
711 lowlands grassland on land formerly managed for intensive agriculture in
712 the UK. *Biological Conservation* 119: 1–18.

713 Weijtmans, K., Jongejans, E. & van Ruijven, J. 2009. Sod cutting and soil biota
714 effects on seedling performance. *Acta Oecologica* 35: 651-656.

715 Whipps, J.M. 2004. Prospects and limitations for mycorrhizas in biocontrol of root
716 pathogens. *Canadian Journal of Botany* 82: 1198–1227.

717 White, J.A., Tallaksen, J. & Charvat, I. 2008. The effects of arbuscular
718 mycorrhizal fungal inoculation at a roadside prairie restoration site.
719 *Mycologia* 100: 6–11.

720 Zhang, T., Sun, Y., Shi, Z. & Feng, G. 2012. Arbuscular mycorrhizal fungi can
721 accelerate the restoration of degraded spring grassland in central Asia.
722 *Rangeland Ecology & Management* 65: 426–432.

723

724 **Table 1.** Inoculation strongly positively affects plant species colonization of
725 topsoil removed sites, but the effect of inoculation is generally highly dependent
726 on the distance from the donor site. *t*-values from repeated measures ANOVAs
727 evaluating the effects of inoculum addition, distance to remnant grassland
728 patches, and their interaction on responses of different plant species sets.
729 Significance: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.
730

Species set	Variables	Inoculum (Ino)	Distance (Dist)	Ino x Dist
All plant species	Species richness	2.7***		-3.3***
	Shannon diversity		-2.8**	
	Jaccard index	4.8***		-5.7***
	NMDS1	3.2**		-3.6***
AMF-dependent plant species	'Species richness	2.5**		-2.8**
	Shannon diversity	2.3*		-2.8**
	Jaccard index	4.1***		-4.9***
	NMDS1		3.1**	
Specialist plant species	Species richness		-2.6**	
	Shannon diversity	2.2*		
	Jaccard index	3.4***		-3.5***
	NMDS1		-3.7***	
Richness of plant species dispersed by:	Wind	2.6**		-2.9**
	Ant	2.9**		-3.1**
	Dung	3.1**		-3.3***

731

Table 2. General increase of plant species richness and diversity following inoculation of topsoil removed sites, with the exception of sites at the furthest distances (20 m) from intact remnant grassland patches. Mean (± 1 SE) plant species richness, Shannon diversity and Jaccard index between plots and intact remnant grassland patches, in inoculated and non-inoculated plots, at three distances from the remnant patches. Data for 2012 (prior to inoculation, and 1 year after top soil removal), and for 2013 (one year after the inoculation).

		Inoculum addition				Distance (m)					
		Inoculated		Non-inoculated		5		10		20	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
All plant species	Species richness	10.9 ± 1.4	16.2 ± 2.1	11 ± 1.6	12.5 ± 1.3	11.2 ± 1.6	15.3 ± 2	11 ± 1.5	15.2 ± 1.9	10.5 ± 1.4	12.5 ± 1.2
	Shannon diversity	1.5 ± 0.1	1.7 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	1.7 ± 0.2	1.4 ± 0.1	1.5 ± 0.1
	Jaccard index	0.4 ± 0.04	0.5 ± 0.04	0.3 ± 0.05	0.4 ± 0.05	0.4 ± 0.04	0.5 ± 0.05	0.4 ± 0.1	0.5 ± 0.05	0.3 ± 0.03	0.3 ± 0.1
AMF-dependent plant species	Species richness	6.4 ± 0.9	8.4 ± 1.3	6.1 ± 0.8	6.8 ± 0.8	6.5 ± 0.9	8.4 ± 1	6.1 ± 0.9	7.6 ± 0.9	6.2 ± 0.7	6.8 ± 1
	Shannon diversity	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
	Jaccard index	0.3 ± 0.03	0.4 ± 0.05	0.3 ± 0.04	0.3 ± 0.03	0.3 ± 0.03	0.4 ± 0.05	0.3 ± 0.03	0.4 ± 0.05	0.3 ± 0.03	0.3 ± 0.02
Specialist plant species	Species richness	2.9 ± 0.4	3.6 ± 0.5	2.8 ± 0.5	3 ± 0.5	3.1 ± 0.4	4 ± 0.5	2.8 ± 0.5	3.9 ± 0.5	2.8 ± 0.5	2.9 ± 0.5
	Shannon diversity	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
	Jaccard index	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.07	0.4 ± 0.05	0.5 ± 0.1
Richness of plant species dispersed by:	Wind	5.3 ± 0.5	7.2 ± 0.4	5.3 ± 0.6	5.8 ± 0.5	5.3 ± 0.6	6.9 ± 0.5	5.2 ± 0.5	6.7 ± 0.5	5.3 ± 0.5	5.7 ± 0.4
	Ant	2.2 ± 0.5	3.4 ± 0.4	1.9 ± 0.4	2.2 ± 0.5	2.1 ± 0.4	3.2 ± 0.5	2.1 ± 0.4	3 ± 0.4	1.9 ± 0.4	2.2 ± 0.4
	Dung	3.5 ± 0.4	4.8 ± 0.3	3.4 ± 0.4	3.9 ± 0.5	3.3 ± 0.3	4.7 ± 0.4	3.6 ± 0.3	4.6 ± 0.3	3.5 ± 0.4	3.9 ± 0.3

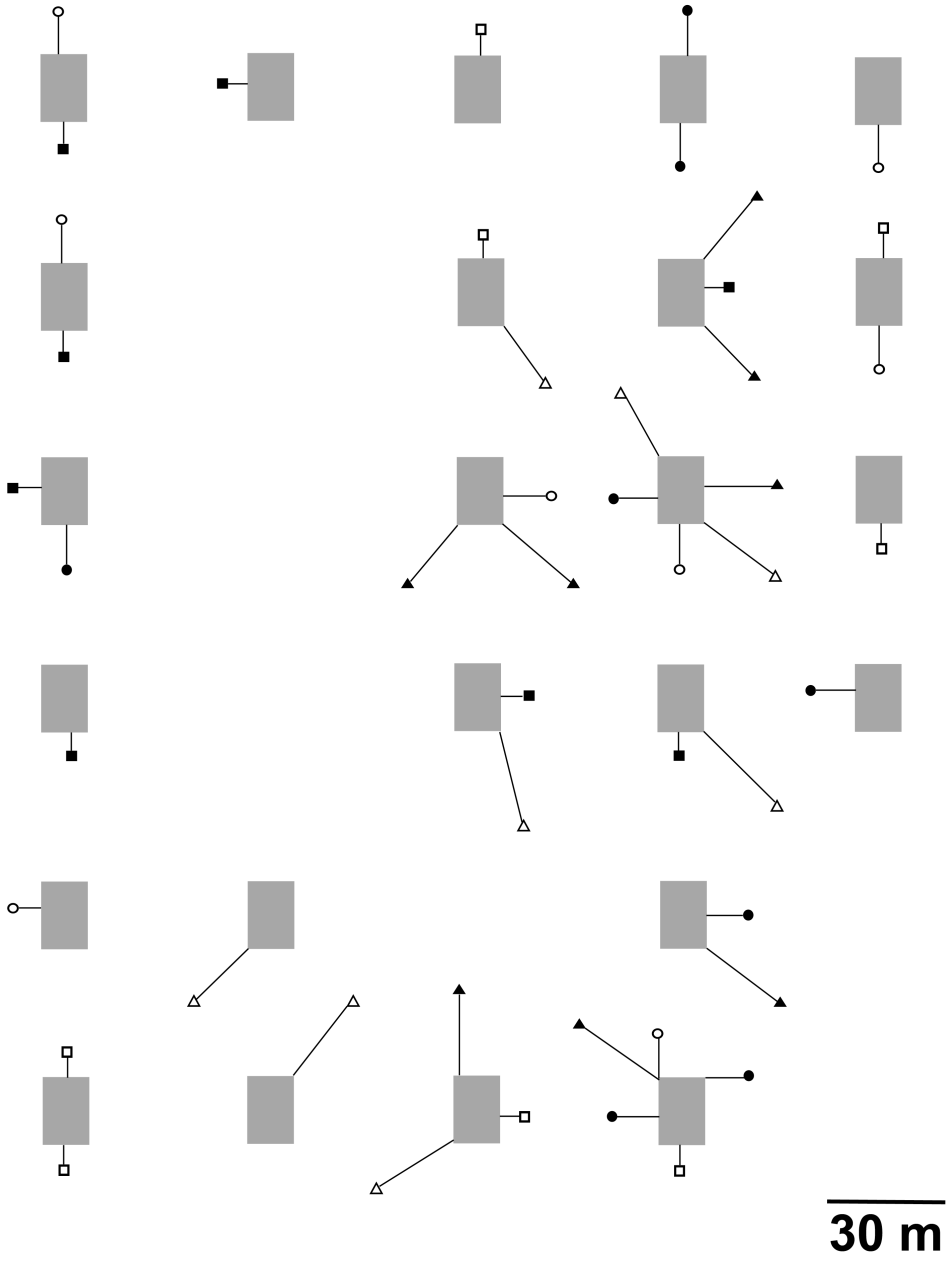
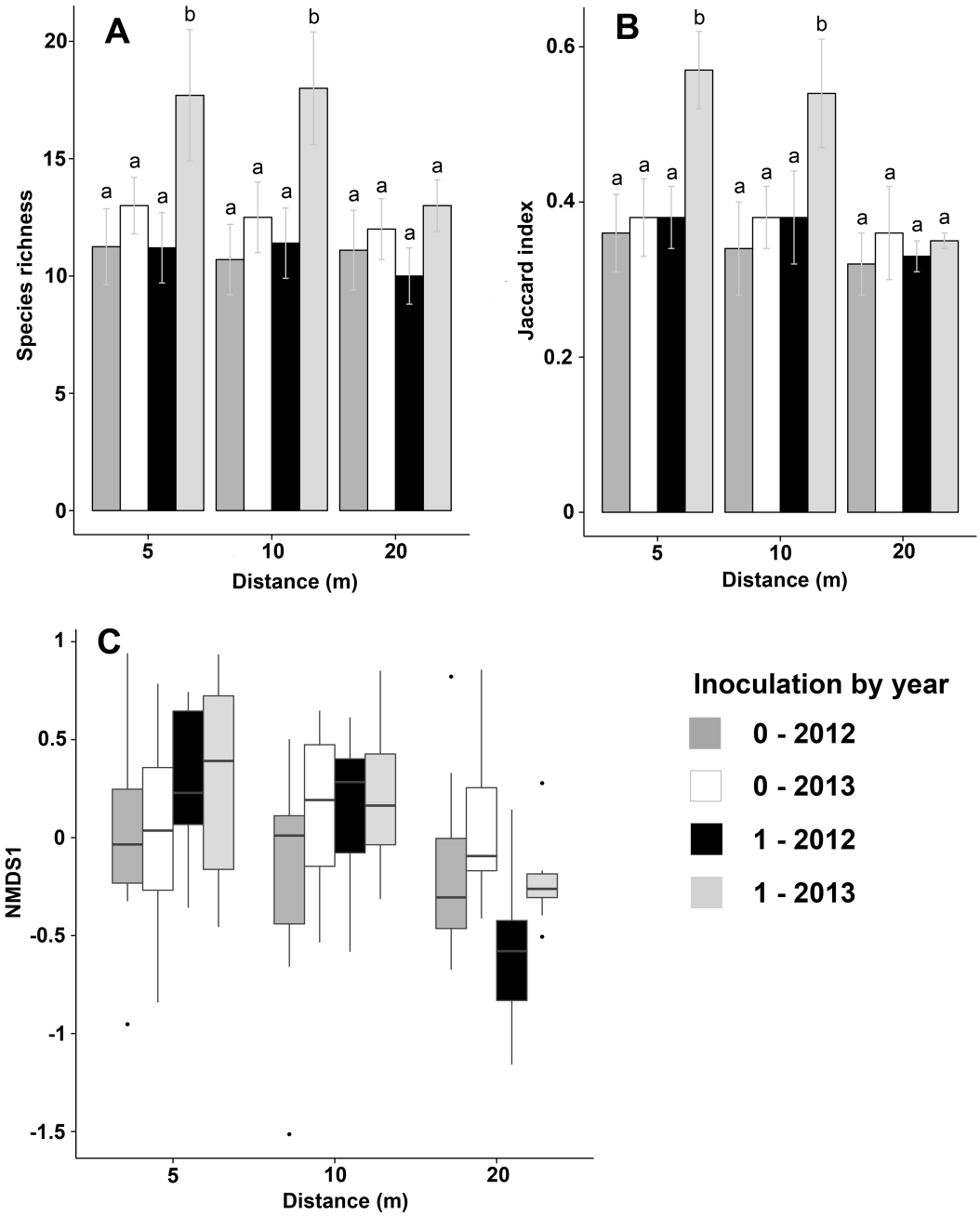


Figure 1. Spatial arrangement of the study plots relative to the intact grassland remnants (grey rectangles). Squares represent study plots at 5 m, circles at 10 m, and triangles at 20 m from the closest intact remnant grassland. Inoculated plots are represented by solid symbols and non-inoculated plots by open symbols.



747
748 **Figure 2.** Inoculum addition generates strong effects on plant species richness
749 (A), similarity with intact remnant grassland communities (B), and community
750 composition (C) for all plant species, but not in the plots furthest (20 m) from the
751 intact grassland remnants. Inoculated plots: 1; control plots: 0. Error bars
752 represent ± 1 SE. Different lower case letters denote significant differences
753 between years within treatments.
754
755
756

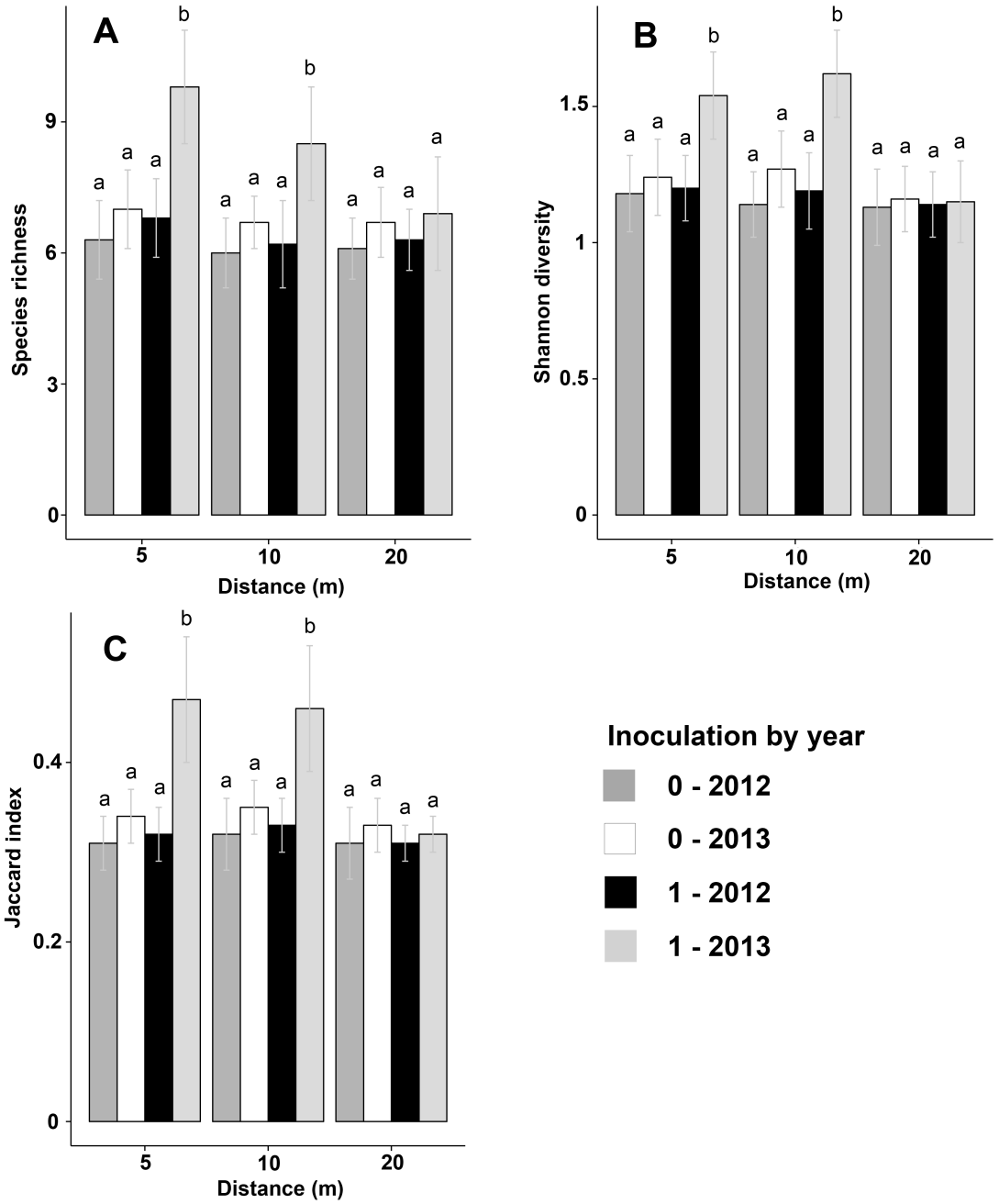


Figure 3. Inoculum addition generates strong positive effects on plant species richness (A), Shannon diversity (B) and similarity with intact remnant grasslands (C) for AMF-dependent plant species, but not in the plots furthest (20 m) from the intact grassland remnants. Inoculated plots: 1; control plots: 0. Error bars present ± 1 SE. Different lower case letters denote significant differences between years within treatments.

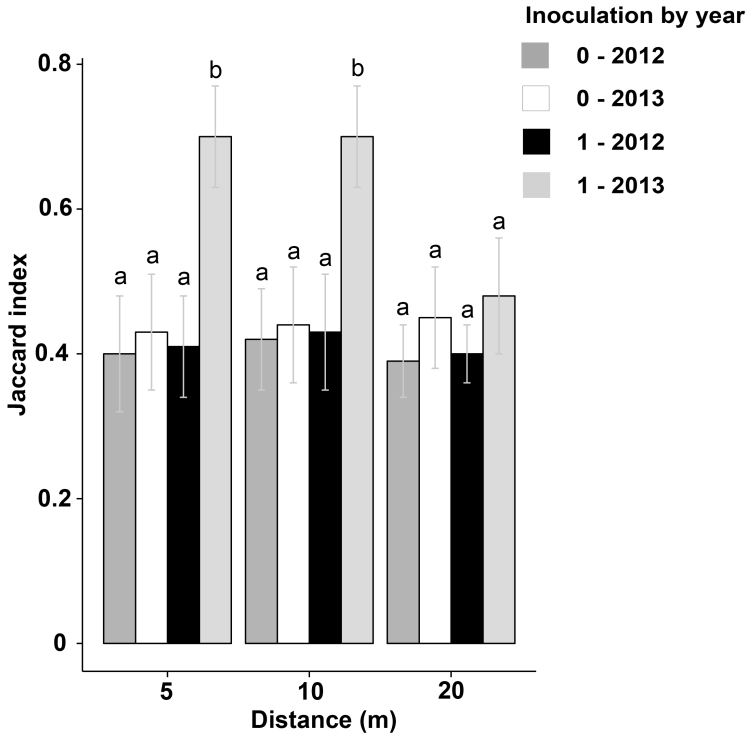


Figure 4. Strong effect of inoculum addition on the plant community similarity between plots and intact remnant grassland patches for specialist plant species of nutrient-poor grasslands, but not in the plots furthest (20 m) from the intact remnant grasslands. Inoculated plots: 1; control plots: 0. Error bars represent ± 1 SE. Different lower case letters denote significant differences between years within treatments.

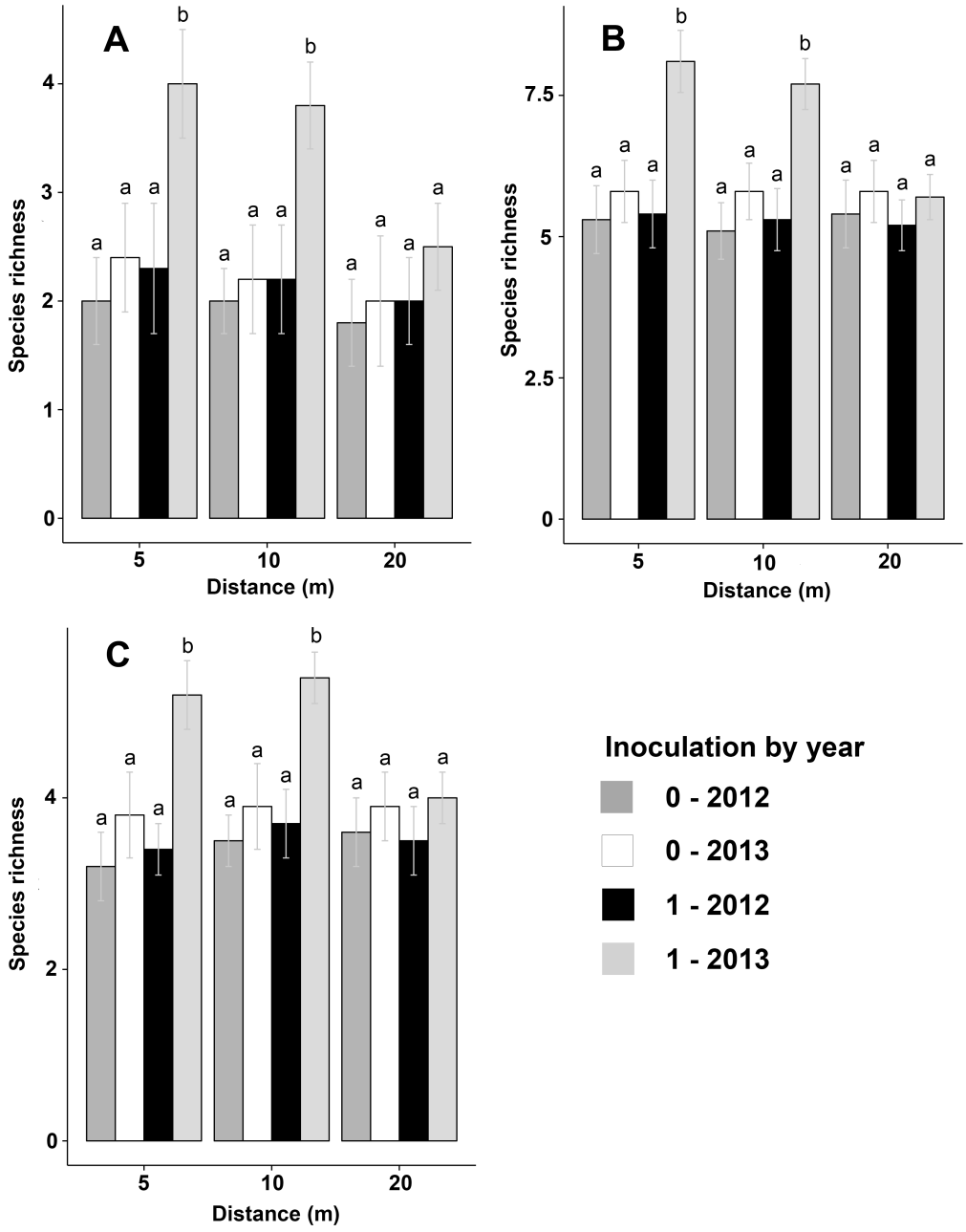


Figure 5. Strong effects of inoculum addition on the richness of plant species dispersed by ants (A), wind (B) and dung (C), at every distance class, except at 20 m from the intact remnant grassland patches. Inoculated plots: 1, and control plots: 0; Error bars represent ± 1 SE. Different lower case letters denote significant differences between years within treatments.

784 **Supplementary material**

785

786 **Table S1.** Plant species frequencies in intact remnant grassland patches and in the plots. AMF-dependent plant species,
787 specialist plant species of the nutrient-poor grassland and well-dispersed plant species are indicated, and main the seed
788 dispersal mode is provided.

Species	Intact remnant grasslands	5 m		10 m		20 m		AMF-dependent	Specialist	Dispersal type	Well-dispersed
		2012	2013	2012	2013	2012	2013				
Achillea millefolium	2	0	1	0	0	0	0	X		Wind	
Agrostis canina	2	0	0	1	0	1	0	X		Wind	
Agrostis capillaris	14	8	14	10	12	0	0	X	X	Wind	
Aira caryophyllea	3	0	1	0	0	0	0	X		Wind	
Anagallis arvensis	7	2	6	2	4	1	2	X		Wind	
Aphanes australis	7	3	4	2	6	0	3	X			
Arenaria serpyllifolia	9	7	7	4	7	3	4			Wind	
Artemisia vulgaris	2	0	1	0	0	0	0	X		Wind	
Betula pendula	11	8	10	7	10	6	8			Wind	
Buddleja davidii	4	1	3	0	0	0	1			Wind	
Calluna vulgaris	15	8	11	7	11	11	13			Wind	X
Campanula rotundifolia	1	1	0	0	0	0	0	X	X	Wind	
Cardamine hirsuta	2	1	0	1	0	0	0	X		Dung	
Carduus crispus	2	0	0	1	0	0	0			Ant	
Carex pilulifera	15	8	11	8	13	12	13		X	Ant	X
Centaurium erythraea	7	2	6	1	1	1	3	X		Wind	
Centunculus minimus	2	2	0	0	0	0	0				
Cerastium fontanum	2	2	0	1	0	0	0	X		Dung	
Cerastium glomeratum	1	0	0	1	0	1	0			Dung	

Cirsium arvense	1	0	0	1	0	0	0	X		Wind	
Conyza canadensis	2	2	0	1	0	1	0	X		Wind	
Crepis capillaris	2	2	0	0	0	0	0	X		Wind	
Cirsium spp.	3	0	1	0	3	0	0	X			
Cytisus scoparius	11	6	7	5	10	2	6	X	X	Ant	
Epilobium spp.	1	0	1	0	0	0	0	X			
Equisetum arvense	3	2	2	2	2	1	1	X			
Erodium cicutarium	2	1	1	0	1	0	0	X		Wind	
Fragaria vesca	5	4	4	1	3	0	2	X		Dung	
Gnaphalium uliginosum	2	2	0	1	0	0	0	X		Wind	
Holcus lanatus	15	1	12	3	8	0	11	X		Wind	X
Holcus mollis	15	3	8	2	8	3	5	X		Wind	
Hypericum humifusum	3	3	0	3	2	1	2			Wind	
Hypericum perforatum	13	9	12	10	13	7	9	X		Ant	X
Hypochaeris radicata	4	1	4	1	4	0	1	X	X		
Juncus bufonius	5	0	5	2	4	3	4	X		Dung	
Juncus effusus	14	12	13	11	14	13	14	X		Dung	X
Juncus tenuis	10	6	10	4	7	3	6			Dung	
Linaria vulgaris	3	0	2	0	2	0	3	X		Wind	
Lotus corniculatus	1	1	1	0	0	0	0	X	X	Dung	
Luzula multiflora	15	13	11	14	15	8	13		X	Dung	X
Medicago lupulina	6	0	6	0	6	0	1	X		Dung	
Molinia caerulea	13	5	11	5	9	7	13	X		Wind	X
Myosotis spp.	1	0	1	0	0	0	0	X			
Ornithopus spp.	2	0	2	0	2	0	0		X		
Pinus sylvestris	15	15	16	14	16	11	16			Wind	X
Plantago major	5	4	3	1	1	1	1	X		Wind	

Poa annua	9	0	5	2	8	1	6	X		Wind	
Polygala serpyllifolia	7	0	0	0	0	0	0				
Potentilla erecta	2	0	1	0	0	0	0	X	X		
Potentilla reptans	2	1	0	0	0	0	0	X		Ant	
Prunella vulgaris	5	3	4	0	3	0	1	X		Ant	
Pteridium aquilinum	4	0	0	1	3	1	2	X			
Quercus robur	2	0	0	0	1	0	0			Dung	
Ranunculus repens	5	4	3	4	3	0	0	X		Wind	
Rubus fruticosus agg.	13	11	11	10	12	11	12	X		Dung	X
Rumex acetosella	6	0	2	0	5	0	3	X	X	Wind	
Sagina procumbens	11	5	9	5	8	2	3			Dung	
Salix spp.	10	8	8	2	5	2	3				
Scrophularia nodosa	4	3	1	1	2	0	1	X		Wind	
Solidago virgaurea	4	0	3	0	1	0	1	X		Ant	
Spergula arvensis	1	0	1	0	1	0	0	X		Dung	
Spergularia rubra	1	0	0	1	0	0	0			Dung	
Stellaria spp.	8	0	7	0	5	0	2	X			
Taraxacum officinale agg.	5	0	1	0	4	0	0	X			
Teucrium scorodonia	10	6	5	6	8	5	5	X		Dung	
Thymus pulegioides	10	0	0	0	0	0	0				
Trifolium dubium	5	1	1	1	1	0	0	X		Wind	
Vaccinium myrtillus	2	1	0	0	0	0	0			Dung	
Vaccinium spp.	2	0	1	0	1	0	0				
Verbascum thapsus	1	0	0	1	2	0	1	X		Wind	
Veronica officinalis	8	5	6	5	4	1	3	X	X	Ant	
Veronica serpyllifolia	9	3	4	5	7	0	1	X		Ant	
Vicia hirsuta	2	0	1	0	1	0	0	X		Dung	

Asteraceae sp 1	1	0	1	0	0	0	0				
Asteraceae sp 2	1	0	0	0	1	0	0				
Asteraceae sp 3	1	1	0	0	0	0	0				
Brassicaceae sp.	1	0	0	0	0	0	1				
Caryophyllaceae spp.	1	0	1	0	0	0	0				
Lamiaceae spp.	1	0	0	0	1	0	0				
Poaceae spp.	1	0	0	0	0	0	3				