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RESEARCH ARTICLE

Seed bank offers potential for active restoration of mountain meadows

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The nitrogen-fixing legume *Lupinus polyphyllus* invaded semi-natural mountainous grasslands across Europe during the last decades. This invasion resulted in degraded habitats through changes in the structure and function of the mountain meadow vegetation. In our study, we analyzed (1) the effects of increasing cover of *L. polyphyllus* on the seed bank of mountain meadows, and (2) the potential of the seed bank of these stands for active restoration of mountain meadows in terms of species composition and number. We conducted a seed bank analysis on 84 plots with increasing cover of *L. polyphyllus* in three mountain-meadow types of the Rhön Biosphere Reserve, Germany. Seedlings from 119 species germinated from the seed bank samples, including 17 Red List species but only a few seedlings of *L. polyphyllus*. The species composition of the seed bank matched distinct patterns of the three meadow types, but differed from the species composition of the current aboveground vegetation in a nonmetric multidimensional scaling ordination. While the influence of *L. polyphyllus* on the current vegetation was visible, no effects on the seed bank were apparent. *L. polyphyllus* had no influence on total seed density, seed density of typical mountain-meadow species, or species numbers in the seed bank. Only the seeds of the Red List species were significantly related to the cover of *L. polyphyllus*. We conclude that the seed bank offers potential for active restoration of species-rich mountain meadows, but species absent from the seed bank have to be added by other measures.

Key words: active restoration, invasive *Lupinus polyphyllus*, mountain-meadow restoration, nitrogen-fixing legume, seed-bank activation, semi-natural ecosystems

Implications for Practice

- *Lupinus polyphyllus* had no large effects on the seed bank, in terms of species composition, total seed density, seed density of typical mountain-meadow species, or species numbers.
- Active restoration could include activating the seed bank through soil disturbance to re-establish a part of the specific mountain-meadow plant communities after reducing *L. polyphyllus*.
- Typical mountain-meadow species absent from the soil seed bank have to be actively introduced via other measures such as seed sowing or the transfer of seed-containing plant materials

Introduction

European semi-natural habitats such as agriculturally unimproved grasslands contribute greatly to the species diversity of landscapes (Billeter et al. 2008; Liira et al. 2008). Mountain meadows are typical species-rich semi-natural grasslands with many rare and endangered plant species, for instance *Arnica montana* (Asteraceae), *Crepis mollis* (Asteraceae), or *Trollius europaeus* (Ranunculaceae). A strong decline in the species richness and diversity of mountain meadows has been observed in the last decades, similar as in other European semi-natural grasslands (Gillet et al. 2016). Generally, the main causes for loss of species-rich grasslands are land-use changes such as intensification (e.g. Wesche et al. 2012), neglect, and abandonment (e.g. Jensen & Schrautzer 1999). Consequently, mountain grasslands are of high conservation value, and certain types are protected by the Habitats Directive (92/43/EEC, habitat type 6,520: mountain hay meadows; and 6,230: species-rich *Nardus* grasslands).

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Neglect and abandonment of the used grasslands can lead to the expansion of few dominant species, and further, to the expansion of invasive species (Pruchniewicz & Żołnierz 2016), which often outcompete the site-specific flora (Ramula & Pihlaja 2012). Plant invaders can change the diversity and composition of biotic communities, thus altering ecosystem structure and functions (e.g. Ehrenfeld 2010; Gooden & French 2014). Therefore, biological invasions are recognized as one component of current global change (e.g. Pyšek & Richardson 2010). To prevent further species loss, the restoration of these (formerly) species-rich ecosystems is a major concern for nature conservation (e.g. Bossuyt & Hermy 2003). In this context, soil seed banks have received increased attention in restoration ecology (e.g. Bakker et al. 1996; Bakker & Berendse 1999; Bossuyt & Hermy 2003; Bossuyt & Honnay 2008; Metsoja et al. 2014; Godefroid et al. 2018; Kiss et al. 2018).

Since seeds can disperse through time as a "memory" of the former vegetation (Bakker et al. 1996), soil seed banks may serve as a reservoir for conserving biodiversity (Vandvik et al. 2016). Thus, soil seed banks are potentially a source for target species during active restoration of species-rich plant communities. However, the absence of seeds of target species often hampers the success of restoration projects. Whether the seed bank can be used as a seed source in restoration projects is still under debate. In some studies, the seed bank contains a large proportion of target species for the potential restoration of the studied semi-natural grasslands (Kalamees et al. 2012; Metsoja et al. 2014). In other studies, restoration could not rely on the seed bank alone, as not all species from the actual vegetation were also present in the seed bank (Bossuyt & Hermy 2003; Bossuyt & Honnay 2008; Toth & Hüse 2014). Therefore, this relationship has to be analyzed for every planned restoration project and knowledge about the species composition of soil seed banks, the ratio of target species to non-target species, and the longevity of seeds is thus important to predict restoration success (Strykstra et al. 1998). Surprisingly, there is no general understanding of how plant invasion may influence the seed bank of different habitats in general (but see Gioria et al. 2014; Gioria & Pyšek 2016) or of mountain meadows in particular (Pruchniewicz et al. 2016). Additionally, it is also unknown whether the activation of the seed bank for restoration could promote germination of the invasive species, in case that the invasive species can build up a seed bank.

In our study, we investigated the effects of *Lupinus polyphyllus* (Fabaceae) on the soil seed bank of mountain meadows in the Rhön Biosphere Reserve in Central Germany. *L. polyphyllus* is a perennial legume from western North America introduced to Europe as an ornamental plant in the 19th century. The species fulfills the criteria to be classified as an invasive species, defined by the International Conservation Union as a species establishing in natural and semi-natural ecosystems or habitats, threatening native biodiversity, and therefore being an agent of change (McNeely et al. 2001). Further, *L. polyphyllus* is included in a list of the 149 worst alien species (not only plants) with the highest environmental and socioeconomic impact in Europe (Nentwig et al. 2018).

In the Rhön Biosphere Reserve, in the 1940s, seeds of *L. polyphyllus* were sown into spruce (*Picea abies*) plantations for soil improvement through N-fixation (Volz 2003). From these plantations, *L. polyphyllus* invaded the adjacent meadows and pastures. In recent years, *L. polyphyllus* cover strongly increased, probably due to changes in land-use regimes (Volz 2003). For example, the area invaded by *L. polyphyllus* doubled from 1998 to 2016 in a 407-ha part of the study region (Klinger et al. 2019). This invasion threatens the diversity particularly of low-growing plant species (Thiele et al. 2010) and alters the structure and functions of the affected mountain meadows (Otte & Maul 2005), resulting in more productive (Hansen et al. 2020) and, from a nature conservation point of view, degraded habitats. Current projects aim at managing *L. polyphyllus* and restoring the species composition of the mountain meadows. It is unclear whether the seed bank of meadows invaded by *L. polyphyllus* is similar in composition to that of uninvaded meadows. If so, the seed bank would potentially represent the local species pool for restoration without a requirement of active species introduction.

We aim at analyzing the potential of the seed bank—concerning species composition and species richness—to re-establish species absent in the actual vegetation. We conducted a seed bank analysis on 84 mountain-meadow plots of three vegetation types (mesic and wet mountain hay meadows, and *Nardus* grasslands) with four levels of *L. polyphyllus* cover (including controls without *L. polyphyllus*) and compared the species composition of the soil seed bank to that of the current aboveground vegetation. We addressed the following research questions: Does the invasion by *L. polyphyllus* affect the species composition of the seed bank and the aboveground vegetation of mesic and wet mountain meadows and *Nardus* grasslands?; How does the invasion by *L. polyphyllus* affect the similarity between aboveground vegetation and the soil seed bank of the mountain meadows?; Does the invasion by *L. polyphyllus* reduce species richness and seed density of the soil seed bank? Does the invasion by *L. polyphyllus* affect the seed densities of all species, typical mountain-meadow species, and rare species in a different way?; How persistent is the seed bank of typical mountain meadow species?.

Methods

Study Area and Study Sites

This study was conducted in the mountainous region of the UNESCO Rhön Biosphere Reserve in Central Germany. This Biosphere Reserve comprises an area of approximately 2,400 km^2 (for more information: http://biosphaerenreservat-rhoen.de). In the so-called High Rhön, a plateau between 600 m and 950 m above sea level (from 50°26′N to 50°32′N and from 09°54′E to 10°05′E), the landscape is characterized by grasslands mainly used as meadows and pastures (Otte & Maul 2005). These semi-natural grasslands of high conservation value are the result of regular mowing, pastoral sheep-herding, and very low fertilizer input for centuries. With still 8,900 ha of low-intensively managed species-rich grasslands, the Rhön

Biosphere Reserve plays an important role in nature conservation and protection of these ecosystem types, also providing habitats for ground-nesting birds such as the Black Grouse *Tetrao tetrix* (Planungsbüro Grebe 1995). The management of large parts of the grasslands was optimized for the conservation of *T. tetrix*, that is meadows were mown later in summer since the early 1990s. This was beneficial for seed ripening of *Lupinus polyphyllus* (Klinger et al. 2020) and may be a reason for the invasion success of this species.

The landscape of this mountainous region forms an elevated shelf which is superimposed by basaltic rocks of tertiary volcanic origin building the parent material in the Rhön (Klausing 1988). Soils that developed on basaltic bedrocks are generally well supplied with cations. However, high precipitation and traditional land use resulted in low nutrient availability and very low pH in a large part of the Rhön Region (Puffe & Zerr 1988). Mt. Wasserkuppe (at 950 m the highest mountain in the region) receives 1,176 mm of precipitation per year (mean of 1980–2010; DWD 2016) and has a relatively low mean annual temperature of 5.4°C (mean of 1980–2010; DWD 2016).

In the High Rhön, we selected 84 study plots (size: 5 × 5 m) in three vegetation types (mesic and wet mountain hay meadows and *Nardus* grasslands) on 61 different meadows, extending 11 km from north to south, and 13 km from east to west. The chosen vegetation types are the three most typical mountain-meadow types in this region. These types are characterized by regular occurrence of the following plant species: mesic mountain meadows: *Geranium sylvaticum* (Geraniaceae), *Trisetum flavescens* (Poaceae), and *Alchemilla monticola* (Rosaceae); wet mountain meadows: *Bistorta officinalis* (Polygonaceae), *Trollius europaeus* (Ranunculaceae), and *Deschampsia cespitosa* (Poaceae); *Nardus* grasslands: *Nardus stricta* (Poaceae), *Potentilla erecta* (Rosaceae), and *Galium saxatile* (Rubiaceae). Within the three vegetation types, we selected plots with four levels of *L. polyphyllus*-cover (0%, 1–25%, 26–75%, and 76–100%) and used seven replicates. As the cover of *L. polyphyllus* increased with time, denser stands of this species probably have been the result of more time having passed since initial colonization (see Klinger et al. 2019).

Seed Bank and Vegetation Sampling

In September 2015, we collected soil samples from the 84 study plots. We pooled nine soil cores of 2.8 cm diameter, resulting in a sample area of 55.4 cm^2 for each plot. Before pooling, we removed the litter layer and separated the soil cores into layers of 0–5 cm and 5–10 cm soil depth. This resulted in 168 seed bank samples of 0.277 L soil volume (0.554 L soil volume from each of the 84 plots), which were kept in the refrigerator until further processing. The numbers of germinable seeds in the soil samples were determined using the emergence method (Roberts 1981). Roots and rhizomes were carefully removed from the soil samples, which were then spread in a layer of 1–2 mm on a 3–4 cm layer of sterile potting compost (Fruhstorfer Erde LD80 Archut)–sand mixture (2:1 relation) in two styrofoam trays of 18 × 28-cm size. The trays were exposed to controlled temperature (T_{day}: 18–24°C, T_{night}: 12–18°C), light (>10,000 lux from 06:00 hours to 22:00 hours), and humidity (<70%) conditions in a greenhouse and were watered every third day. We added six trays containing only sterile garden soil to control for wind-borne seeds. We excluded species germinating in these control trays from the analyses. We kept the seed bank samples in the greenhouse from October to December 2015 for germination. Then the soil samples were cold-wet stratified under outdoor conditions under a dense gauze to prevent wind-borne seed input. After stratification, we kept the samples in the greenhouse again from March to July 2016 until no more seedlings germinated. The emerging seedlings were counted and identified using the key of Muller (1978). Seedlings that could not be identified were transplanted into pots and grown until identification was possible with the appropriate literature (Conert 2000; Klapp & Opitz von Boberfeld 2013; Jäger 2017). For each species, we used the number of seeds in the soil samples to calculate seed density of the uppermost 10 cm of soil as seeds/m^2.

In June 2015 and 2016, we carried out vegetation relevés on the study plots. We estimated plant species abundances and cover following the scale of Braun-Blanquet (1964). Plant nomenclature follows Jäger (2017).

Data Analysis

To determine the relative share of typical mountain-meadow species in the seed bank, we assigned the species to socio-ecological species groups (Ellenberg et al. 1992). We identified typical species of mesic and wet mountain hay meadows and *Nardus* grasslands based on the literature (Oberdorfer 1977; Peppler-Lisbach & Petersen 2001). We recorded which of the occurring species are listed in the Red List of vascular plants for Germany (Metzing et al. 2018). The typical mountain-meadow species and the Red List species form the target species pool of this study.

In order to assess the longevity of the seeds from different species in the seed bank, we used the key by Thompson et al. (1997). The species occurring in the seed bank were classified as transient, short-term persistent, and long-term persistent by comparing the presence of the species in the aboveground vegetation and the horizontal distribution of seeds as quantified in our study for the 0–5 cm and 5–10 cm soil layers (Thompson et al. 1997). Seed banks were classified as transient when a species was present only in the upper seed bank horizon; as short-term persistent when they were also present in the lower horizon but more abundant in the upper than the lower horizon; and as long-term persistent when there were at least as many seeds in the lower horizon as in the upper horizon. Species present in the vegetation and absent from the seed bank were not considered in the classification of seed bank longevity, as recommended by Jensen (2004). Further, species with less than three occurrences in the seed bank samples were excluded from this analysis.

We used nonmetric multidimensional scaling (NMDS) to analyze the species composition of the seed bank and aboveground vegetation. We excluded species with less than three occurrences in the dataset from the NMDS to reduce the disproportional influence of rare species. Additionally, we excluded *Juncus effusus* (Juncaceae) and *L. polyphyllus*, the former since its seeds dominate the seed bank and the latter in order to visualize its effect on the species composition of the invaded meadows. We transferred the Braun-Blanquet values of the vegetation relevés into percentages (r = 1%, + = 2%, 1 = 3%, 2 = 13%, 3 = 38%, 4 = 68%, 5 = 88%). To be able to compare species composition of the vegetation (cover) and the seed bank (seed density/m^2), we standardized all data relative to sample total (i.e. relative abundance; vegetation: cover of each species/cover sum of all species $\times$ 100; seed bank: seed density/m^2 of each species/sum of total seed density/m^2 $\times$ 100) for each plot. Finally, we used 153 species, and "Bray–Curtis" as distance measure in the NMDS. As the stress values were acceptable with four dimensions, we used four dimensions in the NMDS ordinations.

We used mixed effect models to test if vegetation type and *L. polyphyllus*-cover (fixed factors) and meadow (random factor) had an effect on the overall seed density (sum of the seed density of all species per plot), on the densities of typical mountain meadows species and Red List species, and on species richness. To fulfill the requirement of homoscedasticity, the overall seed density, the density of the target species, and the density of the Red List species were $\log10(x + 0.01)$-transformed. For the 51 most abundant species of the seed bank study (with at least 20 individuals in all seed bank samples together), we analyzed whether the relative abundance of species varied between *L. polyphyllus*-cover classes with nonparametric Kruskal–Wallis tests. In order to analyze differences in similarity in species composition, we calculated the Sørensen index for similarity between seed bank and aboveground vegetation for the three vegetation types. Finally, we conducted permutational multivariate analyses of variance (PerMANOVAs) for the seed bank and aboveground vegetation separately for each vegetation type to analyze whether the species composition differed according to the *L. polyphyllus*-cover classes. The PerMANOVAs were run without the species *L. polyphyllus* and with 1,000 permutations, and with "Bray–Curtis" as distance measure. We conducted the univariate statistical tests with the lmerTest and the *multcomp* package, and the NMDS and PerMANOVAs with the *vegan* package using R 3.4.2 (R Core Team 2016).

Results

Altogether 14,341 seedlings belonging to 119 species germinated from the seed bank samples (Tables S1 & S2). Seventeen of these species are listed as endangered in the Red List of vascular plants of Germany (Metzing et al. 2018): *Trifolium spadiceum* (Fabaceae) with Red List status 2; *Crepis mollis* (Asteraceae), *Pedicularis sylvatica* (Orobanchaceae), *Phyteuma orbiculare* (Campanulaceae), and *Trollius europaeus* (Ranunculaceae) as vulnerable, status 3; and 13 species as near threatened, status 5 (Table S1). The dominant species in the seed bank samples were *Juncus effusus* (Juncaceae, contributing 56% of the seedling total), followed by *Agrostis capillaris* (Poaceae, 6%), *Hypericum maculatum* (Hypericaceae, 4%), *Campanula rotundifolia* (Campanulaceae, 3%), and *Lychnis flos-cuculi* (Caryophyllaceae, 2%).

We found 91 species in the aboveground vegetation and the seed bank whereas 66 species exclusively occurred in the aboveground vegetation (Table S2). Only 28 species germinated (mostly with few individuals) from the seed bank samples, but were not present in the aboveground vegetation, e.g. *Sagina procumbens* (Caryophyllaceae), *Epilobium angustifolium* (Onagraceae), and *Urtica dioica* (Urticaceae).

The species composition of the soil seed bank was different from that of the aboveground vegetation, resulting in a clear separation of both groups in the NMDS ordination (Fig. 1A). In the aboveground vegetation, plots with high percentage of *Lupinus polyphyllus* were more congregated in the ordination graph compared to the other cover classes, indicating relatively high similarity of species composition of plots with >75% *L. polyphyllus* cover (Fig. 1B). Additionally, the species composition of the aboveground vegetation did differ significantly between *L. polyphyllus*-cover classes within each vegetation type (PerMANOVA without *L. polyphyllus*: mountain mesic meadows: F = 2.45; $p < 0.01$, mountain wet meadows: F = 3.20; $p < 0.01$, *Nardus* grasslands: F = 2.28; $p < 0.05$). In the seed bank samples, no such effects of *L. polyphyllus* on the species composition were visible (Fig. 1C). Accordingly, the species composition of the seed bank did not differ significantly between *L. polyphyllus*-cover classes within each vegetation type (PerMANOVA without *L. polyphyllus*: mountain mesic meadows: F = 0.78; p = 0.77, mountain wet meadows: F = 2.28; p = 0.06, *Nardus* grasslands: F = 1.50; p = 0.11). The Sørensen index of similarity between seed bank and aboveground vegetation for the three vegetation types was 0.65 in mountain mesic meadows, 0.62 in wet meadows, and 0.66 in *Nardus* grasslands.

Soil seed bank density of the mountain meadows ranged from 3,068 seeds/m^2 to 191,094 seeds/m^2, while the mean seed density of all sites was 30,803 $\pm$ 4,987 seeds/m^2. It varied significantly between meadow types (F = 25.0; $p < 0.001$; Fig. 2A), with higher seed density in wet mountain meadows (69,885 $\pm$ 11,918) than in mesic meadows (11,136 $\pm$ 1,279) and *Nardus* grasslands (11,388 $\pm$ 927). The cover of *L. polyphyllus* had no significant effect on seed densities (F = 1.1; p = 0.34). The same pattern was found for the seed density of the target species, which was affected only by the vegetation type (F = 6.7, $p < 0.01$; Fig. 2B), but not by the cover of *L. polyphyllus* (F = 1.0, p = 0.4). However, *L. polyphyllus* cover was significantly related to the seed density of Red List species (F = 3.6, $p < 0.05$; Fig. 2C). In the plots with 25–75% cover of *L. polyphyllus*, fewer seeds of Red List species were detected (653 $\pm$ 306) than in the plots with <25% *L. polyphyllus* cover (1,839 $\pm$ 768), with >75% (1,143 $\pm$ 459) or without *L. polyphyllus* cover (3,832 $\pm$ 1,518), but these differences were not significant in the post hoc test.

Species richness of the seed bank samples was significantly higher in the wet mountain meadows (21.1 $\pm$ 1.1) than in the mesic meadows (16.5 $\pm$ 0.75) and *Nardus* grasslands

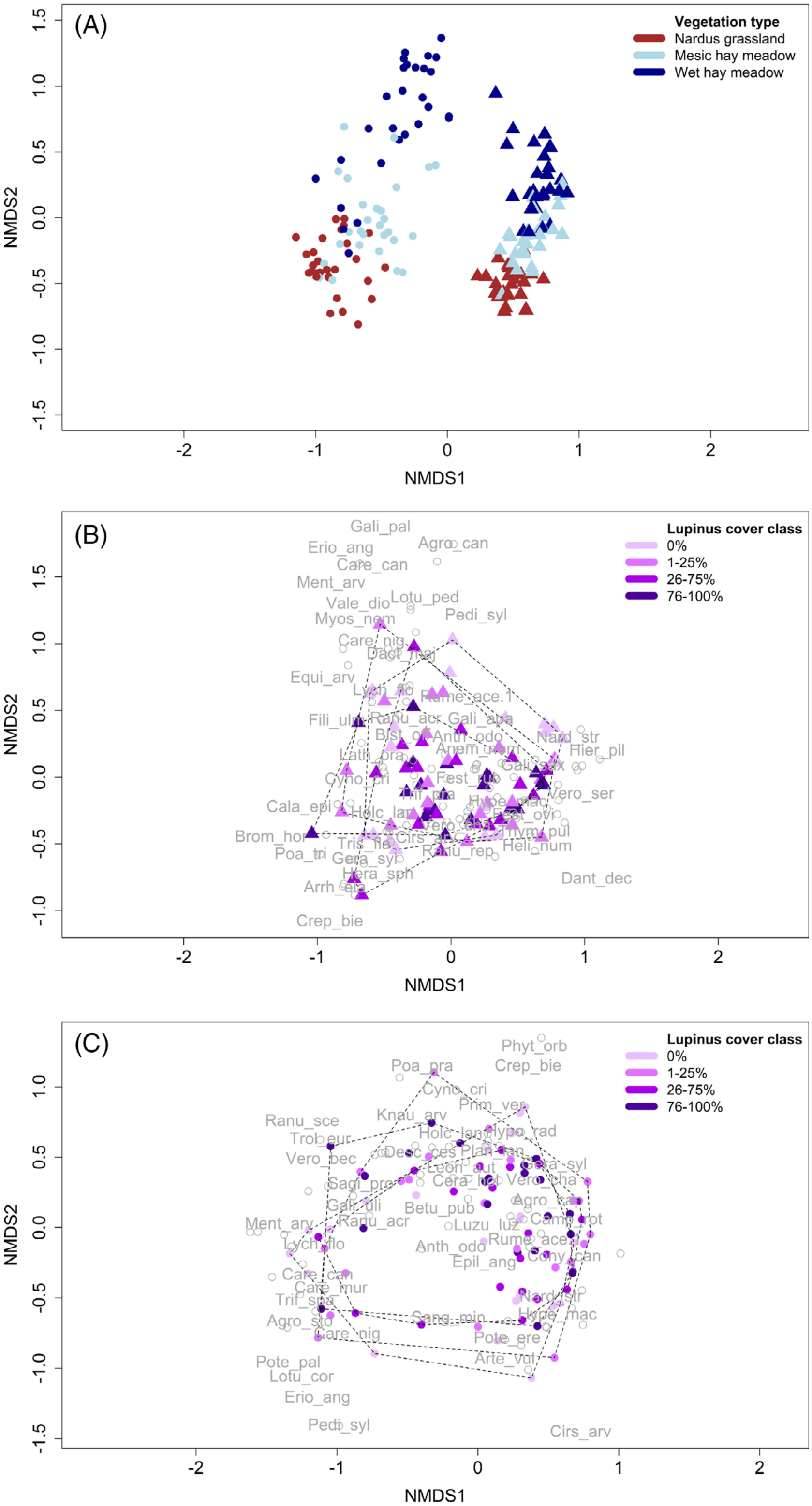

Figure 1. NMDS ordination with the composition of (A) the aboveground vegetation (triangles) and the seed bank samples (circles, stress-value 0.11) grouped according to vegetation type; (B) aboveground vegetation (stress-value 0.12); and (C) seed bank samples (stress-value 0.11), both grouped according to *Lupinus polyphyllus* cover class. In (B) the smallest hull is laid around the cover class with highest cover of *L. polyphyllus* and in (C) no differentiation according to *L. polyphyllus* cover class is visible. The most abundant species are labeled, for full species names see Table S1.

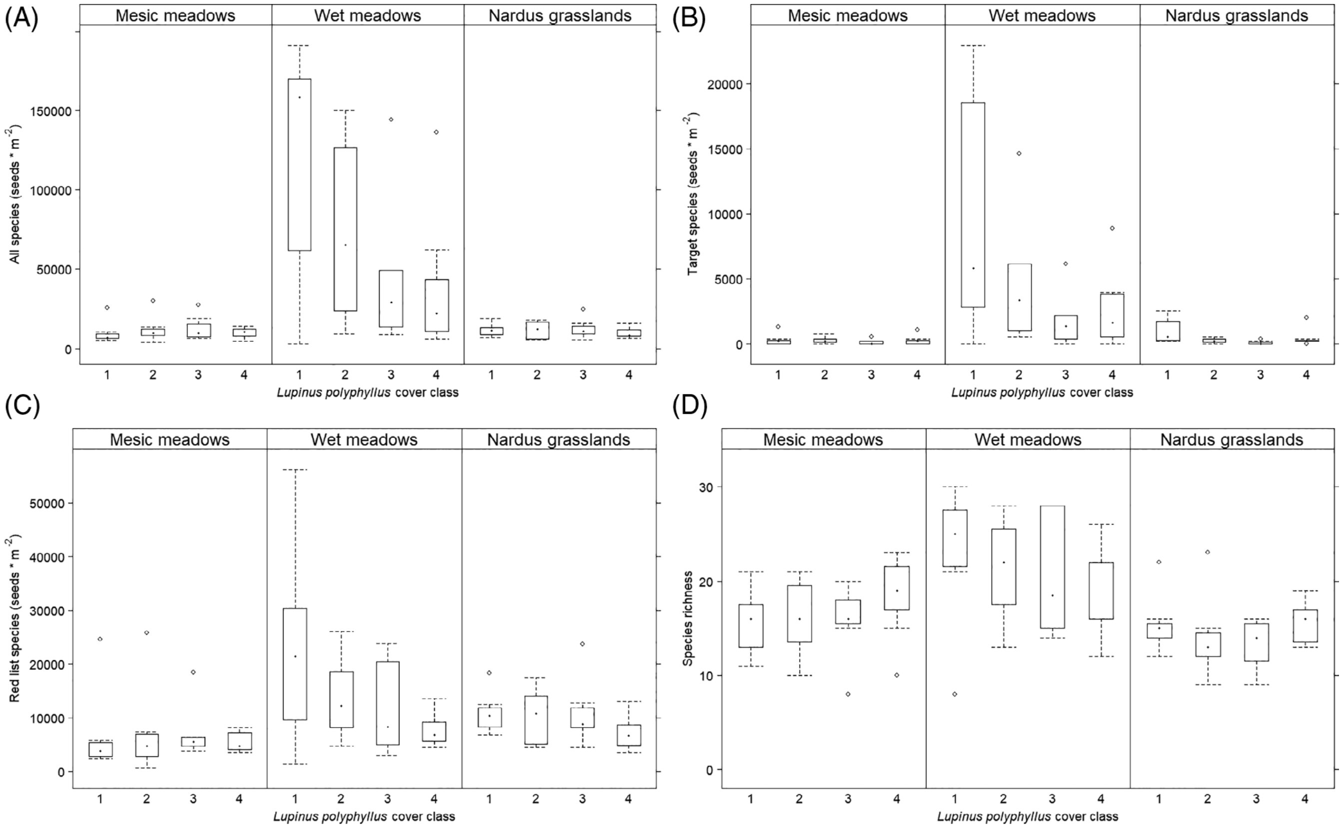

Figure 2. Boxplots of the response variables: (A) seed densities of all species; (B) seed densities of target species; (C) seed densities of Red List species; and (D) species richness of the plots according to vegetation type and *Lupinus polyphyllus* cover class (1 = 0%, 2 = 1–25%, 3 = 26–75%, and 4 = 76–100% cover of *L. polyphyllus* at date of plot selection). The black dot is the median, the box represents upper and lower quartiles, and the whiskers are the minimum and maximum of the data except for the outliers, which are also shown.

(14.6 ± 0.6) ($F = 14.8$; $p < 0.001$), but the cover of *L. polyphyllus* had no effect on species richness of the seed bank ($F = 0.5$; $p = 0.66$).

Of the 51 most abundant species of the seed bank (with at least 20 individuals in the seed bank samples) only the seed density of *Nardus stricta* was significantly influenced by the *L. polyphyllus* cover (Kruskal–Wallis test, $H = 8.75$; $p < 0.05$). While *Nardus stricta* had most seeds in the plots with highest *L. polyphyllus* cover (approximately 1,000 seeds/m), least seeds were found in plots with 25–75% cover of *L. polyphyllus* (approximately 700 seeds/m), but in multiple group comparisons this difference was not significant.

Of the species with more than two seedlings in the seed bank study, eight species had a transient seed bank, 51 species (including *L. polyphyllus*) a short-term persistent seed bank, and 26 species a long-term persistent seed bank (Table S1).

Discussion

Lupinus polyphyllus did not yet affect the seed bank composition of the mountain meadows. While a homogenizing effect of *L. polyphyllus* on the aboveground vegetation was visible, we found no effect of *L. polyphyllus* on the seed bank composition. This matches other studies, which reported greater impacts of certain invasive species on the actual vegetation than on the seed bank (Thompson et al. 1995; Gioria et al. 2012). Accordingly, Gioria and Pyšek (2016) propose a delay between the time of plant invasion and its effect on the seed bank and they conclude that the processes underlying this delay need further investigation.

The invasion by *L. polyphyllus* did not affect the similarity between aboveground vegetation and the soil seed bank of the mountain meadows. Generally, the Sørensen similarity between the species composition of the seed bank and the aboveground vegetation (approximately 0.65) was in a usual range for grasslands (Hopfensperger 2007). Nevertheless, the seed bank samples and the aboveground vegetation were clearly separated in the ordination. This was probably due to the different numbers of species in these two compartments, with 69 (36.7%) species solely found in the aboveground vegetation. In contrast, in the seed bank and aboveground vegetation of alpine grasslands in the Caucasus, the same number of species was detected (Onipčenko 2004) and more species were detected in the seed bank compared to the aboveground vegetation in a North American mixed-grass prairie (Robertson & Hickman 2012). Nevertheless, species composition of the soil seed bank and the current aboveground vegetation varied strongly among vegetation types in our study, indicating that the species in the seed banks are characteristic for their vegetation type. Therefore, and because of the small number of *L. polyphyllus* seeds in the seed bank we rate the seed bank as suitable for restoring the different typical plant communities of mountain meadows after reducing *L. polyphyllus* aboveground by suitable management. Suitable management could be earlier and maybe repeated mowing (Volz 2003).

L. polyphyllus had only weak effects on different characteristics of the mountain-meadow seed bank. The invasion by *L. polyphyllus* did not reduce the overall seed density, the seed density of the typical mountain-meadow species, or the proportion of typical species from all species, nor the species richness of the soil seed bank. The rather small effect of expansive species (including *L. polyphyllus*) on different characteristics of the seed bank is in line with a study in mountain meadows of the Central Sudetes (Pruchniewicz et al. 2016). A significant effect was found for the seed density of Red List species, but the relationship with *L. polyphyllus* cover was not monotonous, as we found fewer seeds of Red List species in plots with low or medium cover of *L. polyphyllus* and more seeds in plots without or with large cover of *L. polyphyllus*. The seeds of rare species are often dispersed in a patchy manner (Fenner & Thompson 2005; Burmeier et al. 2011). Stochastic processes may be more important for these species than the occurrence of an invasive species. Overall, the seed densities of typical mountain-meadow species were similar on sites with and without *L. polyphyllus*.

In our study, most species had short-term persistent seed banks. Grasslands as relatively stable plant communities are mainly inhabited by species that do not produce long-lived seeds (Fenner & Thompson 2005; Hopfensperger 2007). The stability of grassland communities is therefore a main reason for the differences in species composition between the aboveground vegetation and the seed bank (Hopfensperger 2007), which was also obvious in our study. Consequently, a similarity index of approximately 0.65 indicated that many species were underrepresented in the seed bank compared to the aboveground vegetation. Hence, it can be argued that activation of the soil seed bank only will not be sufficient to restore the full plant communities (Bakker & Berendse 1999). As proposed in several other studies, re-establishment of target species could be additionally promoted via transfer of seed-containing plant material (Donath et al. 2007; Kiehl et al. 2010; Klaus et al. 2018). Active seed addition seems to be most important in relatively undisturbed habitats characterized by species with transient seed bank (Kiss et al. 2018).

A successful restoration of the species-rich mountain meadows could be hampered, when seeds of the invasive species germinate and establish after the restoration measure. Therefore, the seed longevity of the invasive species under concern is a crucial factor. As *L. polyphyllus* is a perennial plant, regular re-establishment from soil seed bank and seed persistence may not be essential for its survival. This would explain why *L. polyphyllus* only occurred in four out of 12 seed bank variants and seed densities were also low. In contrast to the study of Sapra et al. (2003), in which a seed viability of approximately 50 years was projected for *L. polyphyllus* from the artificial conditions of seed storage in a gene bank, we classified the seeds of this species as short-term persistent, due to their occurrence in the upper soil layer. However, it has to be kept in mind that the seeds of a colonizing species will first accumulate in the top layer of soil during initial colonization. This means that the seed longevity of *L. polyphyllus* as being short term could be underestimated. Long-term seed burial experiments are needed to test the longevity of *L. polyphyllus* seeds. If seed viability would decrease significantly within the first 5 years after burial, as suggested by the classification as short-term persistent, preventing seed shedding could rapidly reduce new germination and establishment events of *L. polyphyllus* in our study region. Also, the right timing of management actions plays an important role in preventing the incorporation of the seeds into the seed bank, as seeds of *L. polyphyllus* from meadows being cut early tend to germinate in autumn, seeds from meadows being cut late express higher levels of dormancy, and could potentially accumulate in the soil seed bank (Klinger et al. 2020). To this end, early and, if necessary, repeated mowing of the mountain meadows would be crucial.

Overall, activation of the soil seed bank could facilitate a subset of the typical mountain-meadow species in active restoration of mountain meadows invaded by *L. polyphyllus*. Beyond seed bank activation, targeted introduction particularly of the seeds of species not represented in the seed bank would be beneficial for restoring species-rich meadow communities in mountainous areas in Central Europe.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. List of all plant species in the seed bank: the acronym, the longevity of the seed bank, and the binding to mountain meadow vegetation types of the species is given.

Table S2: List of all plant species in the seed bank: seed density of the species and mean cover in the AV is given.

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