Effects of the invasive *Lupinus polyphyllus* on species-rich mountain meadows and options for their restoration

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Contents

List of publications

This thesis is based on the following three research articles:

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2) Ludewig, K., Hansen, W., Klinger, Y.P., Eckstein, R.L. & Otte, A. (2020). Seed bank offers potential for active restoration of mountain meadows. Restoration Ecology 29(1):e13311. doi:10.1111/rec.13311
3) Hansen, W., Klinger, Y. P., Otte, A., Eckstein, R. L., Ludewig, K. (2022): Constraints in the restoration of mountain meadows invaded by the legume *Lupinus polyphyllus*. Restoration Ecology 30(8). doi:10.1111/rec.13682

For papers one and three, I had the main responsibilities of conceptualization, data sampling and analysis and writing. I conducted most of the experimental work. For the second paper I had a leading role in the data analysis and writing. All co-authors contributed constructive suggestions and helpful comments.

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4) Klinger Y.P., Harvolk-Schöning S., Eckstein, R.L., Hansen, W., Otte, A. & Ludewig, K. (2019). Applying landscape structure analysis to assess the spatio-temporal distribution of an invasive legume in the Rhön UNESCO Biosphere Reserve. Biological Invasions 21: 27352749. doi: 10.1007/s10530-019-02012-x*
5) Klinger, Y.P., Eckstein, R.L., Hansen, W., Kleinebecker, T., Otte, A. & Ludewig, K. (2021). Mowing machinery and migratory sheep are complementary dispersal vectors for grassland species. Applied Vegetation Science 24:e12579. doi:10.1111/avsc.12579
6) Ludewig, K. et al. (2022), Phenology of the invasive legume *Lupinus polyphyllus* along a latitudinal gradient in Europe. Neobiota 78:185-206. doi: 10.3897/neobiota.78.89673
7) Eckstein, L.R., Welk, E., Klinger, Y.P., Lennartson, T., Wissmann, J., Ludewig, K., Hansen W., Ramula S.: Biological Flora of Central Europe (2023) - *Lupinus polyphyllus* Lindl. *Perspectives in Plant Ecology, Evolution and Systematics*. doi:10.1016/j.ppees.2022.125715

Chapter 1

Effects of the invasive *Lupinus polyphyllus* on species-rich mountain meadows and options for their restoration: a synthesis

Introduction

Semi-natural grasslands

Grasslands are among the most species rich ecosystems worldwide at small spatial scales (Bardgett et al., 2021). Covering more than 25% of the earth's surface, they provide important ecosystem services, including water regulation, food, fodder and freshwater supply, erosion control, pollinator promotion and carbon sequestration. They offer scenic beauty and harbour many rare and endangered plant and animal species. More than 18% of Europe's vascular plant species and two thirds of European butterfly species are bound to grasslands (Habel, Dengler, et al., 2013). In contrast to 'natural' grasslands that are climatically determined and characterized by minimal human interference, 'semi-natural' grasslands have been shaped by centuries of human activities. 'Intensely managed' grasslands, in turn, have been substantially modified for agriculture (Bardgett et al., 2021). The centuries-long grazing or hay production on semi-natural grasslands took place on sites which otherwise would have been occupied by forests or woody species. This low-intensive land use in combination with little fertilizer input has significantly contributed to high species diversity and consequently, many of the typical grassland species evolved in response to human activity (Feurdean et al., 2017). Like other semi-natural ecosystems, semi-natural grasslands thus rely on the continuation of traditional land-use practices to prevent successional processes. Especially in Europe, this grassland type is of great importance for nature conservation. Besides their high importance for biodiversity, semi-natural grasslands are often valued for their aesthetics and are associated with a sense of identity, place and heritage (Bardgett et al., 2021).

Nowadays, grassland ecosystems are heavily degraded due to agricultural activity, such as land-use intensification but also abandonment, urbanization, or other anthropogenic factors (e. g. Andrade et al. 2015; Dudley et al. 2020). Another major issue affecting grassland ecosystems is the invasion of exotic plant species that can act as ecosystem engineers and decrease plant community diversity (Gibbons et al., 2017). Climate change is further aggravating grassland degradation, through more frequent and intense fires, droughts or changes in precipitation patterns (Bardgett et al., 2021). Degradation is defined as a state of land resulting from persistent decline or loss in biodiversity, ecosystem functions or ecosystem services (IPBES, 2018). The multiple drivers of degradation resulted in alarming losses of global grassland area and biodiversity during the last decades (IPBES, 2019). As a consequence, approximately 49% of the grassland area worldwide are classified as degraded to some extent (Bardgett et al., 2021) and 50-70% of the European grasslands have been replaced during the past 30 years (Török et al., 2021). In Germany, 18,000 ha of species-rich semi-natural meadows were lost, all in protected areas (NABU, 2014). The remaining areas are often remote and isolated patches. Degradation and loss of grasslands may have major impacts on a wide range of ecosystem services, water security, soil stabilization (Dudley et al., 2020), and especially in the case of semi-natural grasslands, on biodiversity (Ludewig et al., 2021). Moreover, the conversion of grasslands to arable cropland as well as disturbance due to overgrazing or invasive species can lead to substantial soil carbon losses (Bardgett et al., 2021). Abandonment and even de-intensification are often followed by the expansion of a few dominant species, including invasives (Pruchniewicz & Żołnierz, 2016). These can considerably change the diversity and composition of biotic communities (Ehrenfeld, 2010; Otte & Maul, 2005).

Grassland Restoration

Grassland degradation represents a major global challenge and poses an enormous threat to people who rely on grassland for food, fuel, fibre and medical products (Bardgett et al., 2021). It is crucial to address these problems in order to achieve the ambitious goals set by biodiversity agendas such as the Aichi Biodiversity Targets of the Convention on Biological Diversity (CBD), or the United Nations Sustainable Development Goals (SDGs).

The urgency of the situation is underlined by the recent launch of the "UN Decade on Restoration of Ecosystems 2021-2030", which was initiated to prevent, halt and reverse the degradation of ecosystems worldwide. Aichi target number 15 states that "by 2020 ecosystem resilience and the contribution of biodiversity to carbon stocks shall be enhanced, through conservation and restoration of at least 15 per cent of the degraded ecosystems, thereby contributing to climate change mitigation and adaptation and to combating desertification", but has not yet been reached. A post-2020 target, similar in content, has therefore been established (CBD, 2021).

According to the Society of Restoration (SER), ecosystems are considered restored when they contain:

- An assemblage of species characteristic of a reference system
- Indigenous species to the extent possible

- All necessary functional groups
- Reproducing populations
- Normal functioning for the developmental stage
- Integration and interaction with a larger landscape matrix
- No threats to the ecosystems' health and integrity from the landscape
- Resilience to stress and natural disturbance
- The potential to persist under contemporary environmental conditions (SER, 2004).

In order to restore degraded semi-natural grassland ecosystems and prevent the further accumulation of biodiversity decline, a range of restoration techniques exist. Active revegetation, for instance, may be used for reestablishing the native plant community (Kettenring & Adams, 2011). Revegetation might include sowing, green hay transfer or seed bank activation. Green hay transfer is a widely used method for reestablishing meadow vegetation (Török et al., 2021). In doing so, freshly cut plant material from nearby species-rich meadows that belong to the same vegetation type and harbor a large proportion of the target species is applied to degraded, species-poor sites. Thus, the ecologically adapted flora and fauna can be transferred to degraded meadows. In some cases, topsoil removal might be necessary in order to remove nutrient-rich soil layers, for example from agriculturally used areas (Hölzel & Otte, 2003). Soil seed banks might serve as a reservoir for conserving biodiversity as they are potential sources for native target species (Ludewig et al., 2021). Via soil disturbance, e. g. through harrowing, favorable germination conditions concerning water and light are created.

Restoration of invaded ecosystems is especially challenging since it requires significant reduction of the invasion as well as recovery of the native species community at the same time. Commonly used methods for invasive species control in grasslands include different cutting techniques, such as mowing, weed whipping or string trimming, hand pulling, grazing or more drastic actions such as burning and herbicide application (Brehm, 2004; Pysek et al., 2007; Thomas et al., 2019). Simply stated, invasive plant species present one of the most critical barriers for restoring native ecosystems (D'Antonio & Meyerson, 2002; Weidlich et al., 2020).

Invasive species

Invasive alien species (IAS) are considered a major threat to biodiversity on a global scale (Banks et al., 2018; Mack et al., 1999). Alien species are organisms that have been transferred to ecosystems outside their natural range. Through the globalization of trade and travel, the spread of alien species has been exacerbated across the globe and thus, organisms have been transferred between ecosystems that were previously isolated, e. g. by natural barriers (Keller et al., 2011). Plants, animals, disease agents and other organisms that are introduced outside of their natural range by people, either deliberately or not, may become destructive to the environment or to human livelihoods. In these cases, alien species are termed "invasive" (IUCN, 2018). In recent decades, the number of IAS has increased significantly (Seebens et al., 2018). Of more than 14,000 alien species worldwide in the year 2015, approximately 2,500 (18%) were considered invasive (Pagad et al., 2015). These species cover a large taxonomic range – viruses, bacteria, fungi, animals and plants – and affect nearly all regions worldwide (Keller et al., 2011; Mack et al., 1999). Awareness of the negative impacts caused by IAS, such as effects on ecosystems or human wellbeing, has also reached politics. Thus, framework conditions for impact resolution have been set on different political levels, e. g. the International Union for Conservation of Nature (IUCN), which includes thousands of both governmental and non-governmental organizations, has established an invasive species specialist group (ISSG) and maintains a global invasive species inventory and database in addition to the Red List of Threatened Species (http://www.iucngisd.org/gisd/). Similarly, the Convention on Biological Diversity (CBD), a secretariat of the United Nations, includes Aichi Target 9 in their Strategic Plan for Biodiversity, saying that "by 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment" (https://www.cbd.int/aichi-targets/target/9). As this was not achieved, a Post-2020 Global Biodiversity Framework under the CBD was set up, in which the original targets are to be further pursued until 2050 (CBD, 2020; Essl et al., 2020). These aims have also been implemented in international and national laws, e.g. EU Regulation 1143/2014 on the prevention and management of the introduction and spread of invasive alien species.

The consequences of invasive species vary widely and include, for animals, extinction of native species through predation, grazing, competition and habitat alteration (Mack et al., 1999). For plants, consequences include alterations of fire regimes, nutrient cycling, hydrology and energy budgets (Weidlich et al., 2020). The House Mouse *(Mus musculus)*, for example, consumes a great amount of seabird chicks and eggs on Gough Island, where it was introduced to in the 19th Century. This leads to a low burrow occupancy and low breeding success and consequently to significant declines of seabird populations (Caravaggi et al., 2019). The mite *Varroa destructor,* native to Asia and historically limited to the Asian honey bee, is now the most serious pest of Honeybee populations *(A. mellifera)* worldwide, and can destroy a colony in a few years if left unchecked (James & Li, 2012). Almost two thirds of the established plant species in Europe were introduced intentionally for ornamental, horticultural or agricultural purposes (Keller et al., 2011). Many of these plant species escaped from gardens and horticultural activities and were able to establish themselves, even in protected areas. In total, biodiversity was threatened by invasive plant species in 487 protected areas worldwide in 2007 (Pyšek et al., 2020). Therefore, invasive alien plant species are regarded as a major threat to biodiversity by managers in protected areas (Goodman, 2003). The invasive success of many of these species is attributed to their superior competitive ability, given their capacity to form a larger biomass and grow faster than native species (Gioria et al., 2018). Invasive plant species can also interfere with the establishment of native plant communities' structure and assembly. The invasive palisade grass (*Urochloa brizantha*), for example, alters the frequency and intensity of fire regimes in South American savannas (Thomas et al., 2019). Cheatgrass (*Bromus tectorum*) changes soil properties and leads to phosphate enrichments, but decreases almost all other plant nutrients and subsequently affects plant species diversity on invaded sites (Gibbons et al., 2017). Japanese knotweed (*Reynoutria japonica*) may change vegetation architecture and plant communities and thereby degrade habitat quality, e. g. for green frogs *(Rana clamitans)* by indirectly reducing arthropod abundance (Maerz et al., 2005). The American Bramble (*Rubus cuneifolius*) causes changes in nutrient cycling, increases in soil erosion, reductions in rangeland carrying capacity and viability, as well as effects on natural plant succession, fire patterns and hydrological processes (Rajah et al., 2019). In Europe, the perennial legume *Lupinus polyphyllus* Lindl. has quickly become invasive since its introduction in the 19th Century (Eckstein et al., 2023). It invades semi-natural grasslands and often outcompetes the native plant species through its high stature and fast growth. It decreases the diversity of small-statured plant species (Thiele et al., 2010), alters the vertical vegetation structure (Otte & Maul, 2005) and finally leads to more productive habitats (Hansen et al., 2020).

Objectives

The overall aim of this dissertation was to analyse the effects of *L. polyphyllus* on semi natural native plant communities and assess possibilities to manage *L. polyphyllus* stands and restore invaded meadows. Therefore, three research objectives were addressed. As a first step, an assessment was made of the affect *L. polyphyllus* has on the species and the functional composition of the vegetation in three main characterizing vegetation types in the study. The second study evaluated whether the seed bank was suitable for active restoration of the invaded mountain meadows. Finally, methods for reducing *L. polyphyllus* cover and restoring the three mentioned vegetation types were investigated and guidelines for a restoration concept were developed.

In particular the dissertation focuses on the following objectives:

i) Assess the impacts of *L. polyphyllus* on the native semi-natural aboveground vegetation in three different mountain meadow vegetation types (Chapter 2).

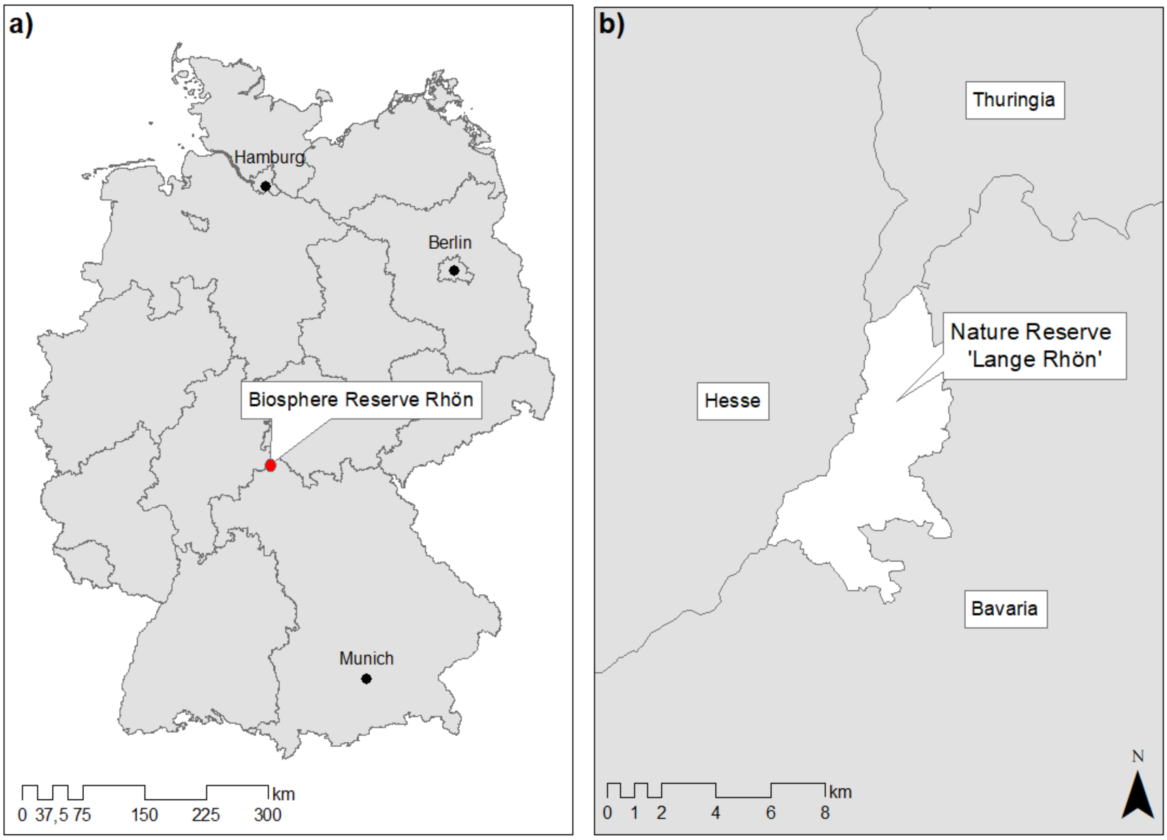
ii) Assess the impacts of *L. polyphyllus* on the seed banks in the three mountain meadow vegetation types and analyse the potential of the seed banks for active restoration (Chapter 3).

iii) Test combined methods of grassland restoration on invaded meadows and develop ideas for a concept for the restoration of the invaded mountain meadows in the Biosphere Reserve Rhön (Chapter 4).

Study area

The UNESCO Man and the Biosphere reserve Rhön (2.433,23 km²) was founded in 1991 and is located in Central Germany among the federal states of Bavaria, Thuringia, and Hesse. Biosphere reserves work as "learning places for a sustainable development under diverse ecological, social and economic contexts (Fig. 1.1a). They promote solutions reconciling the conservation of biodiversity with its sustainable use" (https://en.unesco.org/biosphere). Thus, the region is split into three areas: The core area, where nature protection has the highest priority and human activity is limited to research, monitoring and education. The buffer zone is used prudently, e. g for ecological agriculture and forestry, environmental education, landscape conservation or nature-friendly tourism. In the third zone, termed the transition zone, the usual legal requirements for agriculture, industry and trade/business apply. Here, on a voluntary basis, sustainable approaches for land-use and economy shall be tested here (https://en.unesco.org/biosphere/about). For the second zone, a care and development plan focuses on the conservation of the valuable and sensitive grassland ecosystems.

The study area is the nature reserve "Lange Rhön", the northern part of the High Rhön, a north-south running and scarcely populated plateau (600-900 masl). The "Lange Rhön" (2.657 ha) was previously designated as a nature conservation area in 1982 (Fig. 1.1b). It is situated in the Bavarian part of the biosphere reserve at the border with Hesse and covers an area of 32.7 km².



https://gdz.bkg.bund.de/index.php/default/digitale-geodaten/verwaltungsgebiete/nuts-gebiete-1-250-000-stand-31-12-nuts250-31-12.html

Figure 1.1: Location of a) the Biosphere Reserve Rhön in Central Germany and b) the study area, the Nature Reserve 'Lange Rhön'

Basaltic rocks of tertiary volcanic origin (Klausing, 1988) form the High Rhön. Thus, Basalt as well as the weathering products of basalt and loess clay form the parent rock. Normally, soils that develop on basaltic rocks are well supplied with cations; however, traditional land-use in combination with high precipitation have led to low nutrient availability and very low pH values in large parts of the Rhön region.

The Rhön can be found in the temperate climate zone and occupies a transition between Atlantic and Continental climates. The High Rhön is subjected to easterly cold air inflows. The highest peak in the area is Mt. Wasserkuppe with 950 m asl. It receives 1,176 mm of precipitation per year, with the precipitation peak in summer, and the mean annual temperature is 5.4° (DWD, 2020).

Originally a deciduous forested area, the Rhön Biosphere Reserve now comprises 8900 ha of low-intensity managed species-rich semi-natural grasslands, which have been created by repeated clearing periods between 1180 and 1725. Much of the clearing occurred due to the Thirty Years' War (1618-1648), when there was a great need for wood (Volz, 2003). The three characterizing vegetation types in the area are *Nardus* grasslands and mesic and wet mountain hay meadows. Because of their species richness, these areas are protected by the European Habitats Directive (Habitats Directive 92/43/EEC, habitat type 6230: species rich *Nardus* grassland and habitat type 6520: mountain hay meadows). Currently, only low-intensity landscape maintenance measures are conducted, e.g. using the grasslands for hay making and for pastoral sheep herding. Mountain meadows are mown once or twice per year (Klinger et al., 2019). When the Biosphere reserve was founded in 1991, the original mowing dates for hay making were postponed later into the year in order to protect, among others, the largest population of ground-nesting black grouse (*Tetrao tetrix)* in Central Europe. This change of the mowing regime gave the invasive species *L. polyphyllus* the opportunity to flower and spread into the surrounding meadows. Nowadays, *L. polyphyllus* forms large stands with covers of more than 90%. From 1998 to 2016, the area covered by *L. polyphyllus* has more than doubled (Klinger et al., 2019). Consequently, at present, the ongoing spread of *L. polyphyllus* poses a serious threat to the biodiversity of the mountain meadows.

Lupinus polyphyllus – Garden lupine

The perennial legume *Lupinus polyphyllus* Lindl. is currently one of the most common invasive plant species in Europe (Eckstein et al., 2023). Originating from the pacific North America, where it occurs mainly on mountain meadows, it was introduced as an ornamental plant in Europe in the early 19th century. Later, it was frequently used for ameliorating soil conditions in spruce forests, due to its nitrogen fixing abilities (Volz, 2003). Its use in forestry as well as in agriculture and road construction led to its introduction in different ecosystems and as a result it has become dominant in many places. Nowadays, it is invasive in a number of European countries e. g. Norway (Fremstadt, 2010), Lithuania (Vyšniauskiene et al., 2011), Finland (Valtonen et al., 2006), Germany (Kowarik & Rabitsch, 2010) and the Czech Republic (Hejda et al., 2009). Currently, *L. polyphyllus* is among the 100 worst plant invaders in Europe (Nentwig et al., 2018) and listed on the blacklist of invasive plant species in Germany (Nehring, 2010). With a maximum height of up to 1.5 m (Ludewig et al., 2022), it shades and overgrows smaller plant species that have high light requirements (Hejda, 2013; Otte & Maul, 2005). Nitrogen fixation in symbiosis with nodule bacteria may change the nitrogen budget of soils in the long-term and thus displace species that are adapted to nutrient-poor sites (Eckstein et al., 2023). Hence, the species has the ability to act as an ecosystem engineer and negatively impact native floras and change plant communities. These features are especially threatening for semi-natural grasslands that are characterized by a large number of small stature plant species with low nutrient requirements.

Chapter outline

This thesis is based on three manuscripts which have all been published in international peer-reviewed scientific journals and form Chapters 2, 3, and 4 of this thesis. The study in Chapter 2 aims at gaining basic knowledge of the impacts of *L. polyphyllus* on native plant communities and the study in Chapter 3 tests the potential of the soil seed banks in the study area for active restoration. The study in Chapter 4 investigates possibilities for restoring invaded meadows and the potential implications of restoration management.

This section gives a brief outline of the contents of the manuscripts and provides an overview of the applied methods. The findings and conclusions are presented in the subsequent sections.

Chapter 2 Invasive legume affects species and functional composition of mountain meadow plant communities

This manuscript presents an investigation of the effects of *L. polyphyllus* invasion on the species and functional diversity of the vegetation communities on three main grassland types in the Rhön Biosphere reserve. A comparison is made for the species diversity (richness, evenness, and effective species number), functional diversity (functional richness, evenness, divergence and dispersion) and similarity of 84 vegetation plots sampled in 2016 with different *L. polyphyllus* cover classes (Cover class 1: 0%, 2: 1 – 25%, 3: 26 – 75%, 4: >75% *L. polyphyllus* cover). Differences between cover classes were assessed by ANOVAs and post-hoc Tukey tests. Community weighted means (CWMs) of single plant traits and plots against the *L. polyphyllus* cover classes are also presented. In order to derive the main floristic gradients, a non-metric multidimensional scaling (NMDS) ordination was calculated and distances between the centroids of the cover classes were assessed and compared to one another. Finally, an investigation is made of the functional composition (proportions of legumes, grasses, herbs, and *L. polyphyllus*) of plots with different *L. polyphyllus* cover, resulting in a calculation and visualization of the relative share of each functional group within each vegetation type.

Chapter 3 Seed bank offers potential for active restoration of mountain meadows

This research analyses the effects of increasing cover of *L. polyphyllus* on the seed bank of mountain meadows, and the potential of the seed bank of these stands for active restoration of invaded mountain meadows in terms of species composition and species number. To this end, a seed bank analysis on 84 plots with increasing cover of *L. polyphyllus* in three vegetation types (*Nardus* grasslands, mesic mountain hay meadows, wet mountain hay meadows) was conducted. For each plot, nine soil cores of 2.8 cm diameter were pooled, which resulted in a 55.4 m^2 sample area for each plot. Prior to pooling, the litter layer was removed, and the soil cores separated into layers of 0-5 and 5-10 cm soil depth, in order to classify the longevity of the seeds from different species in the seed bank. Thus, 168 seed bank samples of 0.277 L soil volume were obtained. These were kept in the refrigerator until further processing. The number of viable seeds was determined using the emergence method in a greenhouse experiment. Typical native and rare (Red List) mountain meadow species occurring in the area were identified by appropriate literature and formed the target species pool. Seed banks of the species were classified as transient, short-term persistent, and long-term persistent depending on their presence in the aboveground vegetation and the horizontal distribution in the soil. Non-metric multidimensional scaling (NMDS) was used to analyse the composition of the seed bank and the aboveground vegetation. Mixed effects models tested the effects of *L. polyphyllus* and vegetation type (fixed factors) and meadow (random factor) on the overall seed density (sum of the seed density of all species per plot), and on the densities of typical mountain meadow species and Red List species. Kruskal-Wallis tests were performed to investigate the influence of *L. polyphyllus* cover on the variability of the relative abundance of the 51 most abundant species that occur in the meadows. In order to determine differences in similarity in species composition, the Soerensen index for similarity between seed bank and aboveground vegetation for the three vegetation types was calculated. Lastly, permutational multivariate analyses of variance (PerMANOVAs) for the seed bank and aboveground vegetation were performed separately for each vegetation type to analyse whether the species composition differed according to the *L. polyphyllus* cover classes.

Chapter 4 Constraints in the restoration of mountain meadows invaded by the legume *Lupinus polyphyllus*

This manuscript aims at identifying restoration methods for invaded mountain meadows. We investigated restoration techniques that support native plant communities and control IAS at the same time. For this purpose, a restoration experiment was implemented in 2017 on heavily invaded meadows (*L. polyphyllus* cover > 50%) belonging to the three main vegetation types. With two meadows for each vegetation type, this resulted in six restoration sites. In split-plot designs, this study tested the combination of two frequently used techniques in grassland restoration: Seed bank activation and green hay transfer. On each restoration site, two parallel strips were set up each containing six 5x5 m vegetation plots and each restoration method was applied on two of the plots. On two of the plots in each strip, the seed bank was activated with a rotary harrow to a depth of 10 cm. On two other plots, we removed the *L. polyphyllus* plants with weeding forks prior to seed bank activation. Finally, the remaining two plots were left with no treatment as a control. On one of the strips, green hay from species-rich uninvaded meadows was applied on the same day as the harvest. In a greenhouse experiment, the number of viable seeds in composite green hay samples of each vegetation type was assessed using the emergence method. Vegetation surveys were taken in 2017 prior to the experiment and in three years after the experiment (2018, 2019, 2021). The typical and rare mountain meadow and Red List species of study 2 also served as target species in this study. The focus lay on the development of the *L. polyphyllus* cover and the cover of target species. Generalized linear mixed effect models to evaluate the effects of the restoration measures on the *L. polyphyllus* and target species cover were calculated. Subsequently, the significance of the fixed variables was tested using Wald Chi square tests (Fox & Weisberg, 2018). Vegetation-type-specific treatment effects were obtained by calculating contrasts to the respective treatment group in 2017 within the vegetation types.

Main Results

Study 1 revealed that *L. polyphyllus* has measurable impacts on the aboveground current vegetation. In all three investigated vegetation types, it fosters the growth of high-growing, competitive species and as a result, increases the similarity of the different vegetation types (study 1). These results show that there is a need for action with regard to restoration of the endangered mountain meadows habitats.

However, study 2 demonstrated that *L. polyphyllus* has so far, no impacts on the belowground vegetation, i.e., the seed bank, as very few *L. polyphyllus* individuals emerged from seed bank samples in a greenhouse experiment. Meanwhile, typical species were still present and emerged in high numbers. Thus, the seed banks can be used in restoration via activation (study 2).

Consequently, restoration via seed bank activation in combination with green hay transfer and manual removal of *L. polyphyllus* individuals were tested in study 3. The intention was to increase the cover of typical and rare mountain meadow species (target species) and decrease the cover of *L. polyphyllus* at the same time. The results show that restoring invaded semi-natural mountain meadows is challenging since the target species could not be fostered in all three vegetation types. On the contrary, in *Nardus* grassland the cover of the target species was even reduced. The cover of *L.*

polyphyllus could be reduced in *Nardus* grassland and mesic mountain hay meadows, but it increased again during the course of the monitoring.

General Discussion

In general, invasive plant species are termed to threaten natural habitats and to be one of the main causes of global biodiversity loss (Thomas et al., 2019), e.g. by decreasing native plant species richness (D'Antonio & Meyerson, 2002). In accordance, study 1 showed that *L. polyphyllus* significantly reduces species richness of the invaded semi-natural grasslands. Nevertheless, in *Nardus* grasslands and wet mountain hay meadows, *L. polyphyllus* increased the overall species number in the first place. Similar effects of initially increased local plant species diversity due to invasion by exotic plant species were observed in studies conducted in Arizona (USA), Australia and Uruguay (Sax & Gaines, 2003). These supposedly positive effects lead to contradictory conclusions regarding invasive species. Some studies even speak of 'novel ecosystems' emerging due to the co-occurrence of long-term residents and non-native species and where invasive species contribute different benefits such as genetic diversity, novel hybrids and increased local biodiversity (e. g. Pearce 2016; Davis et al. 2011; Hobbs et al. 2006; Kowarik & Rabitsch 2010). However, in higher *L. polyphyllus* cover classes, species richness and effective species number tended to decrease. This may be due to small species being shaded out and outcompeted when *L. polyphyllus* becomes more dominant (Thiele et al., 2010). *L. polyphyllus* impacts the species and functional compositions of the mountain meadows of this study. Moreover, functional divergence was also influenced by the *L. polyphyllus* invasion. Single traits of the community that changed due to high *L. polyphyllus* cover were seed releasing height, LDMC (leaf dry matter content), and SLA (specific leaf area), which was only increased in *Nardus* grasslands. A low LDMC has been associated with fast growing species that show strong responses to an increased nutrient supply (Gross et al., 2007). Study 1 showed that heavily invaded sites harbour taller species with a lower LDMC, which highlights the increased competition capacity of co-occurring species. A higher SLA leads to a higher biomass production (Beest et al., 2015). While invasive plants may decrease plant species richness, they often simultaneously increase net primary productivity of invaded sites (Gibbons et al., 2017). Taken together, increasing seed releasing height and decreasing LDMC in all high *L. polyphyllus* cover vegetation sites provide evidence for higher productivity in *L. polyphyllus* dominated sites.

Hence, *L. polyphyllus* fosters species that have higher competitive abilities. Supporting these results, it was shown that mesic hay meadows were least affected by the *L. polyphyllus* invasion. Here, no significant effects on species diversity could be found. Frequent species on this vegetation type are often invasive in other regions of the world, such as *Anthoxanthum odoratum, Trisetum flavescens, Festuca rubra* and *Holcus lanatus* (Gross et al., 2010). It can be deduced that these species have highly competitive abilities, such as large growing-height, and can thus probably better cope with high *L. polyphyllus* cover. A main reason for this is that only a specific set of species can co- exist with the dominant invader (Hejda, 2013) and that these species need a certain suite of functional traits matching the conditions created by the dominant species. Moreover, invasive species may alter natural selection in the invaded system. Such effects have been found for native grasses in sites invaded by *Bromus tectorum* in the US (Leger & Goergen, 2017). Here, changes in phenotypic traits associated with greater survival or plant size led to a higher survival rate of the native grasses in the invaded sites. Such effects on some native species could also be true for *L. polyphyllus* and further research on this topic would be interesting.

The fact that species with highly competitive abilities can best co-occur with *L. polyphyllus* in mountain meadows, leads to a homogenization of the different vegetation types. This was shown by an increased similarity index between *Nardus* grasslands and mesic and wet mountain hay meadows with a high *L. polyphyllus* cover (Study 1). A homogenization of different invaded plant communities has been reported by other studies too (La Sorte et al., 2014; Yang et al., 2021). As a consequence of globalisation, common species are distributed, whereas highly specialized endemic species (only found in a limited area) may become extinct (Klingenstein, 2004). The resulting loss of floristic uniqueness due to alien species invasion is a global phenomenon (Yang et al., 2021) and matter of concern for several reasons. For example, invasive species can alter food webs in the native community. Although a high density of *L. polyphyllus*, for instance, attracts bumblebees on the one hand (Ramula & Sorvari, 2017), it can also lead to a lower abundance of *Lepidoptera* species (Valtonen et al., 2006) and arthropods in total on the other hand (Ramula & Sorvari, 2017). Moreover, species-rich plant communities show a higher resilience to drought (Chen et al., 2022), which is predicted to occur more often in the course of climate change (Stanik et al., 2020).

Generally, many invasive plant species are predicted to benefit from global warming (Catford et al., 2012; Hellmann et al., 2008). They mostly have large native geographic ranges (Leishman & Gallagher, 2015), broad environmental tolerance and tend to be phenotypically plastic (Leishman & Gallagher, 2015; Ludewig et al., 2022). Higher maximum growing heights, larger leaves and a higher SLA are traits that are favoured in grasslands under warmer climates (Catford et al., 2019; Sandel & Dangremond, 2012). These traits can partly be attributed not only to the species promoted by *L. polyphyllus,* but also to *L. polyphyllus* itself (Eckstein et al., 2023; Ramula & Kalske, 2020). Hence, *L. polyphyllus* might even expand faster in the near future and species that are fostered by it may profit from global warming. Additionally, whether an elevated atmospheric CO_2 level is especially advantageous to legumes depends on a variety of factors and is still under discussion, but for many of the investigated legumes, an increase in seed yield could be observed under long-term elevated CO_2. For example, in *Lupinus perennis,* a close relative of *L. polyphyllus*, increases in biomass and net photosynthetic rates could be found (Singer et al., 2020). Still, whether legumes in general become more abundant due to an elevated CO_2 level is highly dependent on resource availability (Leishman & Gallagher, 2015) and summer drought may just as well limit the symbiotic N_2 fixation (Serraj et al., 1999) and thus hamper growing rates. This was partially confirmed by Vetter et al. (2019) who found that 44 days of drought did not affect the survival of adult *L. polyphyllus* plants, but did negatively affect the performance (senescence, lower number of leaves and lower leaf size) of adult plants and seedlings. The production of seedlings was, however, not impaired (Vetter et al., 2019). Nevertheless, concrete effects of climate change on the performance *L. polyphyllus* are currently missing and would be interesting to investigate.

Overall, the invasion of *L. polyphyllus* leads to a loss of floristic uniqueness in protected and rare vegetation types. Sites that are actually low in nutrients increase in productivity and the functional composition changes. In all, *L. polyphyllus* has a strong influence on the native plant communities. In combination with the ongoing invasion of *L. polyphyllus* (Klinger et al., 2019) and the collective influences of climate change, it can be concluded that the selective pressure against native vegetation has increased and will continue to do so. Given this, management concepts to decrease the cover of *L. polyphyllus* are urgently needed.

Pysek et al. (2007) have shown that the complete removal of invasive species individuals is often a successful measure for their reduction and was well suited to reduce *L. polyphyllus* cover in *Nardus* grasslands and mesic hay meadows (Study 3). This is even more important as herbicide application, a common and often successful, but generally controversial method of reducing invasive plant species (Thomas et al., 2019), is not an option for large-scale application in nature conservation areas in Germany. Soil disturbance via seed bank activation reduced the *L. polyphyllus* cover significantly in mesic hay meadows, at least in the first four years following the experiment (Study 3). Nevertheless, the cover had increased again by the end of the monitoring period. Since *L. polyphyllus* can resprout from small (< 3 cm) pieces of its belowground stems (Saba, 2018), it is possible that the fragmentation of the plants caused by the soil disturbance might have been conducive to the resprouting process. In wet hay meadows, *L. polyphyllus* cover was not affected by any of the applied restoration techniques at all. This might be related to a higher water use efficiency of *L. polyphyllus* under drought conditions, when compared to some of the native species (Vetter et al., 2019). Generally, methods employed to control invasive plant species in restoration may not always result in the expected outcomes (Weidlich et al., 2020) and four years after the restoration had taken place, *L. polyphyllus* cover had again increased on most sites. Removal actions and soil disturbance in the course of seed bank activation probably left favorable conditions for re-invasion, i.e. open soil and light availability. Thus, repeated removal of *L. polyphyllus* individuals would most probably be necessary to achieve a lasting reduction on all vegetation sites. Repetition of invasion treatment is also recommended by other studies (Thomas et al., 2019). For instance, experiments to control the invasive *Prunus serotina* were only successful after five to six years of repeated treatments (Brehm, 2004). Also, manual removal methods are often used in smaller restoration projects, since labor costs are high at larger spatial scales (Weidlich et al., 2020). Hence, uprooting of *L. polyphyllus* should be concentrated on small stands and single plants. Large invaded areas on the other hand, should be managed with a suitable mowing regime that prevents *L. polyphyllus* from developing viable seeds, by including early and if necessary repeated mowing (see also Klinger et al. 2019).

Management actions in the study area were almost exclusively focused on the reduction of *L. polyphyllus* cover at the time of this study (Klinger, 2021). The reduction of *L polyphyllus* cover is undoubtedly an important aspect of biodiversity conservation. Still, seeing that *L. polyphyllus* has the ability to increase the similarity between different vegetation types (study 1), a comprehensive approach for restoration management is needed. Such an approach would require both invader control and restoration of the species composition of the three predominant grassland types at the same time. Therefore, study 3 focused not only on decreasing the cover of *L. polyphyllus* but also on increasing the cover of native target species taking into account the combined effects of seed bank activation, green hay transfer and the uprooting of *L. polyphyllus* plants. Uprooting *L. polyphyllus* plants was performed to decrease the *L. polyphyllus* cover, while green hay transfer and seed bank activation were primarily applied in order to increase the cover of target species.

Seed banks are often considered an important potential seed source for the restoration of plant communities and their activation is cheap and easy to apply (Bossuyt & Honnay, 2008). Via soil disturbance, which opens the soil and increases light availability, suitable germination and establishment conditions are created. Since the seed bank can also be a source of undesirable ruderal and competitive species (Bossuyt & Hermy, 2003) including invasive species, seed banks of invaded sites should be investigated carefully (Bossuyt & Hermy, 2003; Ludewig et al., 2021).

Fortunately, while its impact on the aboveground vegetation is large (study 1), *L polyphyllus* has relatively little impact on the soil seed bank (study 2). *L. polyphyllus* did not reduce the overall seed density, the seed density of typical mountain-meadow species, the proportion of typical species from all species, or the richness of the soil seed bank. Additionally, since very few of its seeds were found primarily in the upper 5 cm of the soil, the seed bank of *L. polyphyllus* was classified as transient. While persistent soil seed banks are commonly formed by flowering invasive species (D'Antonio & Meyerson, 2002; Gioria et al., 2021) and some literature references classified seeds of *L. polyphyllus* to be log-term viable in the soil (Volz, 2003), the results of study 2 did not support these findings. Other studies report greater impacts of certain invasive species on the aboveground vegetation than on the seed bank (Gioria et al., 2012; Thompson et al., 1995). Consistent with this, Gioria and Pyšek (2016) propose a delay between the time of the plant invasion and its effect on the seed bank. Accordingly, *L. polyphyllus* did not build a persistent seed bank in the study area, thus the existing seed banks can be used for restoration purposes.

Logically, the potential for restoration also depends on the composition of the soil seed bank, but it is often found that nearly half of the species present in the aboveground grassland vegetation do not contribute to the seed bank at all. Consequently, the similarity between the aboveground vegetation of grasslands and the seed bank is often not more than 50 – 60% (Bakker & Berendse, 1999). Grassland species generally have a low seed longevity and low densities in the soil (Bossuyt & Hermy, 2003). This may explain why a higher species number was found in the aboveground vegetation compared to the seed bank in this study (study 2).

In the long term, higher air temperatures resulting from climate change that result in significantly higher soil temperatures could accelerate the decline of seed viability. Changes in rainfall patterns may also determine the relative success of recruitment, with lower levels of success producing net losses to see bank longevity (Ooi, 2012). Thus, increasing precipitation with longer periods between events (Baer et al., 2019) and increasing temperature may raise the extinction risk of some species in the seed bank, further degrading natural restoration abilities and ultimately ecosystem resilience (Ma et al., 2020).

To overcome seed bank limitation, the additional transfer of seed bearing freshly cut green hay from nearby donor sites was chosen to further boost the target species communities (see also Kiehl et al. 2010; Donath et al. 2007). However, in none of the investigated vegetation types did the target species cover increase as a result of the applied measures. In fact, the cover of target species in *Nardus* grasslands and wet hay meadows was lowered. It is possible that the soil samples taken in study 2 were not a realistic representation of the actual species composition of the soil seed banks. Thus, the promising results of study 2 were contradicted by the results of the restoration experiment. This may be due to small-scale variation in the soil seed bank and the overall usually limited sample volume that can be drawn (Bossuyt & Hermy, 2003). Thus, the soil samples may only weakly reflect the current species composition of the soil seed banks.

In order to activate the soil seed bank and create favourable conditions for germination in the green hay, soil disturbance via harrowing was performed. While the number of microsites is often reported to be a bottleneck in restoration projects (Donath et al., 2007) and soil disturbance is stated to enable better species establishment (Schmiede et al., 2012), no positive effect of species establishment due to soil disturbance could be found in the course of study 3. Even if soil disturbance contributed to successful species establishment in short-term studies (Schmiede et al., 2012), in the long term, this positive effect may disappear (Harvolk-Schöning et al., 2020; Sommer et al., 2023). Moreover, large open gaps may be susceptible to year-specific effects such as drought, which may have lasting negative effects on the restoration success (Groves et al., 2020). Gaps are less shaded in the centre, which might also hamper germination, especially when summer drought occurs. In 2018, during the course of this study, there were atypically hot and dry weather conditions, which could have impeded the germination of target wet meadow species. Generally, drought is a main source for germination failure (Török et al., 2021) and high seedling mortality (Ludewig et al., 2018; Moles & Westoby, 2004).

Although green hay samples from the wet hay meadows had the highest number of target species seedlings, green hay transfer had no benefits in terms of target species cover at wet and mesic mountain hay meadow restoration sites (study 3). The application of green hay has been carried out successfully in numerous restoration studies in flood meadows (Donath et al., 2007; Kiehl et al., 2010). However, Sommer et al. (2023) found many target species in a floodplain restoration project established only poorly or not at all, in spite of their frequent occurrence at donor sites. Akin to this, Klimkowska et al. (2007) compared restoration techniques across a range of wet meadow restoration studies and found that the overall restoration success was rather limited. In *Nardus* grassland, the cumulative cover of target species was even lower in plots where green hay was applied. Here again, germination requirements might impair species establishment (see also Wagner et al. 2021). However, the restoration of *Nardus* grasslands is generally difficult and restoration efforts had no beneficial effects in an experiment in the Netherlands even after a period of 25 years (Bakker et al., 2009). Reasons for the partial failure may also lay in the method of green hay transfer itself: less frequent species on the donor site have a smaller probability of transfer to the recipient site. Also, if the grassland type harbours plants with a wide range of flower timing, not all species will be captured by the method with a single cut. Finally, species whose seeds are present in the transferred hay, but need a longer time to germinate, cannot use the microsites prepared in advance of the green hay transfer (Wagner et al., 2021). Given this information, it could be helpful for some species to create additional microsites in the first spring after the hay transfer and after seeds with primary seed dormancy have experienced cold stratification (Wagner et al., 2021).

Alternative common grassland restoration methods are seeding or plantings (Kiehl et al., 2010). Classic seed mixtures, contain 25 - 40 of the most common species, depending on the site conditions and do not often consider rare or endangered species (Dolnik et al., 2020), which are especially important for mountain meadow restoration. Plantings are suitable for species that are difficult to establish from seed and where only little initial seed is available, which circumvents the often very high natural failure rate during germination and development. Nevertheless, some of the target species of studies 2 and 3, such as *Arnica montana* (see also Stanik et al. 2020) or other endangered species such as *Pulsatilla vulgaris* or *Serratula tinctoria,* that occur in the Rhön region, are generally difficult to establish even via seeding or planting, but with results for plantings slightly better (Dolnik et al., 2020). For other species, such as *Alchemilla* spp. and *Succisa pratensis,* plantings are a suitable method for successful establishment. However, it must be considered that plantings are cost and labour intensive and that a cultivation of mother plants must be practical. Some species, such as *Aira caryophyllea* are just too small, other species might not be suitable for single propagation due to hemi-parasitism (*Euphrasia* spp. or *Pedicularis* spp.). For these species, green hay transfer turned out to be useful (Dolnik et al., 2020). Thus, to increase the establishment success of certain species, additional planting (*Arnica montana, Thymus pulegioides, Succisa pratensis)* or seeding *(Dianthus deltoides)* could be considered.

Semi-natural grasslands in mountain regions are considered highly vulnerable to the effects of climate change, such as increases in mean annual temperature and decreases in precipitation (Tasser et al., 2017). These effects lead to changes in species range limits, species composition and diversity and probably even local extinctions of species (Stanik et al., 2020). Some species have shown adaptations to global warming, e.g. *Brassica rapa* that responded with earlier flowering to drought experiences (Franks et al., 2007; Hamann et al., 2018). However, these adaptations developed at the price of losing "fitness", such as producing fewer seeds. Other species like the endangered *Arnica montana* perform worse when exposed to drought and higher temperatures (Stanik et al., 2020). In these cases, low mountains like those present in the study area, do not allow species to move upslope to avoid unfavorable environmental conditions (Pauli et al., 2012).

Generally, species-rich communities resist extreme weather events better than species-poor ones (Chen et al., 2022; Isbell et al., 2015). Hence, greater species and functional diversity should increase the likelihood of including species that are resilient to climate change (Baer et al., 2019; Wilsey, 2021). Against this background it becomes even more important to increase species diversity in invaded sites. However, restoration projects are often oriented on a pre-industrial era status of the vegetation (Wilsey, 2021). Since these conditions often can no longer be met, restoration projects should also consider the resilience of the vegetation community against climate warming. One option could be to proactively consider which species are expected to do well under future climate scenarios and adjusting management practices to accommodate restoration goals (Baer et al., 2019). Moreover, managers should consider fitness-related impacts of climate change on vulnerable species, identify high impact and refugial areas and stabilise climatically exposed populations (Stanik et al., 2020). Here, further research is necessary.

Seed banks provide a vital source of ecosystem resilience to global environmental change (Ma et al., 2020) and are an important supplementary component in restoration. Thus, expanding the knowledge of impacts of climate change on seed-bank dynamics is important for predictions of future species dynamics and extinction risks (Ooi, 2012). However, years of extreme weather will likely result in different outcomes concerning the species composition of restoration projects (Wilsey, 2021). Dominant species might differ between dry and average years (Manning & Baer, 2018) and hence every year might be unique for restoration (Stuble et al., 2017). Lastly, an important prerequisite for successful restoration via green hay application may be the low productivity of restoration sites (Sommer et al., 2023). The increase in atmospheric CO_2, which is likely to continue, has major impacts on primary productivity of grasslands (Wilsey, 2021; Polley et al., 2019), although the effect of increased CO_2 may be diminished by e. g. decreasing soil moisture (O'Mara, 2012). Additionally, study 1 has shown an increased productivity in *L. polyphyllus* invaded sites. Reversing invasion by exotic species can be one of the most challenging restoration goals. Especially if there has been sufficient time for feedback to develop between the invasive species and the soil, and for those feedbacks to inhibit growth and reproduction of native species (Baer et al., 2019). Against this background, restoration goals should be split, with the first step being a sustainable reduction of *L. polyphyllus* cover and productivity of the invaded sites. Afterwards, repeated seed bank activation and green hay application should be performed. Additional seedings and plantings of target species can be considered. Another crucial requirement in restoration projects is time: the establishment of species is quite variable and highly dependent on e.g. weather conditions. Persistence of the restoration and the ability to self-seed can only be assessed after long periods (Türk et al. 2021, Wilsey 2021).

In conclusion, it can be stated that the restoration of invaded mountain meadows is challenging and dependent on a complex interplay of biotic and abiotic factors. Suitable weather is especially important for successful species germination and establishment. Particularly, under climate warming conditions, increased stochasticity must be expected, meaning that the restoration success may depend on years with abnormal rainfall amounts or patterns. Therefore, to avoid shortcomings, it should be considered to repeat seed bank activation and green hay transfer, if necessary. For certain species, planting or seeding could be necessary for successful establishment. Unfortunately, within the scope of study 3, only a short period of time could be monitored. Nevertheless, long-term observations and monitoring of restoration projects are indispensable to evaluate the effects of the measures undertaken (Sommer et al., 2023). Climate change effects interact with management practices, as both factors influence not only ecosystem functioning but also the resilience of ecosystem services (Stanik et al., 2020). Thus, future management of semi-natural grasslands in the study area will have to master different challenges at the same time. The first is to maintain ecosystems and species under current climate change conditions. Second is to strengthen the resilience of the ecosystems against future climate change. Finally, semi-natural grasslands invaded by *L. polyphyllus* need to be restored.

Conclusions and practical implications

L. polyphyllus strongly affects the current aboveground vegetation by fostering high-growing and competitive species and homogenizing different vegetation types. Knowing the effects an invasive species has on a native plant species community provides important information to adjust restoration management (D'Antonio & Meyerson, 2002). In this case, it could be shown that the native plant communities should be promoted in order to counteract homogenization, especially in light of global loss of species and floristic uniqueness (Yang et al., 2021). Thus, it is necessary to implement an integrated management plan that increases native species cover and decreases the cover of the invasive *L. polyphyllus*. Studies investigating restoration measures that meet these requirements are rather scarce and thus knowledge about effective management is urgently needed (Habel et al., 2013). So far, seed banks in the Rhön mountains are not significantly affected by the *L. polyphyllus* invasion (Ludewig et al., 2021). Therefore, it was assumed that using this resource could provide a partial solution for restoration management via seed bank activation. It was furthermore suggested that detected depletions of some species in the soil seed bank could be countered via green hay transfer. Although they are often successfully applied in numerous restoration studies (e. g. Donath et al. 2007), study 3 showed that the combination of seed bank activation and green hay transfer cannot be readily used for restoration of invaded mountain meadows. Neither restoration measures favored target species communities in any of the investigated vegetation types. On the contrary, the cover of native target species was lowered. Constraints to success included harsh weather conditions and perhaps the method of green hay transfer itself. Weather conditions cannot be influenced, but seeding or planting could be helpful for some target species.

Manual removal of *L. polyphyllus* plants proved to be partly successful. Its cover could be initially lowered in *Nardus* grasslands and mesic mountain hay meadows, however a tendency for recovery could be observed in the last monitoring year (2021). However, manual removal of *L. polyphyllus* plants is work- and thus cost intensive (e. g. Weidlich et al. 2020) and must be undertaken with the utmost care, since even small pieces of the belowground stems (ca. 3 cm) can re-sprout (Saba, 2018). Hence, this method should be used to destroy single plants and small stands, which exert the strongest invasive pressure (Klinger et al., 2019). The cover of large and heavily invaded sites should be reduced by early and possibly repeated mowing to prevent *L. polyphyllus* individuals from producing ripe seeds (Klinger et al., 2021). Mowing is further indispensable in enhancing seed dispersal of native vegetation (Klinger et al., 2021). Management plans that include removal of harmful exotic organisms are often not linked to a post-removal revegetation plan, which often leads to reinvasion processes (D'Antonio & Meyerson, 2002). Therefore, once the sites are free of *L. polyphyllus* and less productive*,* promotion of the native communities with seed bank activation and green hay transfer should be applied. After that, native mountain meadow species have a better chance to establish again and reinvasion of *L. polyphyllus* is less likely.

Since some invasive species may benefit from climate change, their distribution and accompanying problems will increase (e. g. Catford et al. 2019). Thus, restoration of invaded ecosystems will become even more important in the future. Legal requirements and many concepts, methodologies and positive examples of restoration already exist. If we manage to implement these now and establish an efficient network of research results and practice, we might be able to stop the loss of species and ecosystems. This is necessary in order to achieve successful and lasting restorations, at least of the valuable semi-natural mountain meadows in the Rhön region.

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Chapter 2

Invasive legume affects species and functional composition of mountain meadow plant communities

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Abstract

Plant invasions are among the key drivers of global biodiversity and ecosystem change. They often cause reductions in native species richness and overall biodiversity. Nitrogen-fixing plants are problematic as they affect soil nutrient availability and outcompete species of nutrient-poor sites. Here we assessed the impacts of the legume *Lupinus polyphyllus* on species and functional diversity of mountain meadow communities in the UNESCO Biosphere Reserve Rhön. We compared species diversity (richness, evenness and effective species number), functional diversity (functional richness, evenness, divergence and dispersion) and similarity of plots in three characteristic vegetation types (*Nardus* grassland, mesic and wet mountain hay meadows) between different lupine cover classes. We calculated community weighted means (CWMs) of single plant traits and plotted them against lupine cover classes. The invasion of *L. polyphyllus* homogenizes vegetation composition since the similarity among plots of the different vegetation types increased with increasing lupine cover. It significantly affected species diversity in terms of richness and effective species number and the functional divergence of the vegetation. The trait set of species occurring together with lupine was shifted towards more competitive trait values. We demonstrate strongly negative impacts of *L. polyphyllus* on different mountain meadow vegetation types since *L. polyphyllus*, fosters the growth of competitive species and leads to overall more productive plant communities.

Introduction

Plant invasions are considered one of the major drivers of ecosystem modification and biodiversity change at the global scale (Davis et al. 2011; Duraiappah et al. 2005; Hejda and Pyšek 2009; Keller et al. 2011). The immediate and long-term consequences of invasions may often be detrimental for the invaded ecosystems, as plant invasions have led to substantial declines in biodiversity and ecosystems functioning. Invasive plant species may also affect native plant communities through altered habitat structure and related changes in light conditions (Otte and Maul 2005) or effects on water availability (Drenovsky et al. 2012) and nutrient supply (Ehrenfeld 2010). As a result, invasive species may alter the species composition of native communities (Hejda 2013; Otte and Maul 2005; Thiele et al. 2010). They are generally considered to reduce species richness or even erode whole gene pools by the extinction of endemics (Vilà et al. 2010). Consequently, dominance of invasive plants may lead to biotic homogenization of the resident communities, which manifests as an increase in genetic, taxonomic or functional similarity (Tordoni et al. 2019). However, Sax and Gaines (2003) pointed out that non-native species might also increase species richness at regional scales.

Species diversity, measured as species richness and species evenness, is related to productivity and population dynamics and, thus, ecosystems functions and services (Mace et al. 2012). Measuring changes in these indices therefore constitutes a first approach to assess impacts of invasive species on native communities. Functional traits reflect species interactions with the biotic and abiotic environment (Brym et al. 2018) and variation in traits is therefore strongly related to species composition (Tordoni et al. 2019). As invasive plant species may increase community similarity between invaded sites, they may also change the functional trait composition of invaded communities and thereby affect ecosystem functioning. A high functional diversity as "the range of the functional traits of the organisms in a given ecosystem" (Tilman 2001) may be beneficial for resistance to invasion (Mason et al. 2005). Functional diversity indices, calculated from plant traits, are thus tools that adequately reflect the different components of functional diversity and are, therefore, useful to detect impacts of plant invasions.

One of the most common alien invasive species in Europe is *Lupinus polyphyllus* Lindl. Introduced as an ornamental and for soil melioration, it has spread in many countries and has become dominant in various ecosystems. It has become invasive in several European countries like Norway, Lithuania, Latvia and Germany, but also on other continents e.g. in New Zealand (Fremstadt 2010). Further, *L. polyphyllus* is among the 15 most common plant invaders and listed on the blacklist of invasive species in Germany (Nehring et al. 2013). As a legume, it may increase the nitrogen availability of soils (Holdaway and Sparrow 2006), which will deteriorate conditions for species adapted to nutrient poor sites. It may furthermore outcompete smaller species for light due to its tall stature (Otte and Maul 2005) and thus reduce species richness (e.g. Ramula and Pihlaja 2012).

In the study area, the Rhön UNESCO Biosphere reserve, *L. polyphyllus* was introduced in spruce forests in order to meliorate soil conditions. A change of the mowing regime due to the foundation of the Biosphere reserve in 1991 was followed by a rapid spread of *L. polyphyllus*. Today, it dominates the landscape, building stands with covers up to 90% on many meadows. Especially affected by the invasion are *Nardus* grasslands (Habitats Directive 92/43/EEC, habitat type 6230: species-rich *Nardus* grasslands) and mesic and wet mountain hay meadows (habitat type 6520: mountain hay meadows; Klinger et al. 2019; Otte and Maul 2005; Volz 2003), which constitute the typical vegetation types of the Biosphere reserve.

Assessing the impacts of plant invasions on the community level, but also on species and functional diversity of resident communities, is crucial in order to coordinate restoration efforts and utilize resources as efficient as possible (Hejda 2013; Vilà et al. 2010). Particularly in sensitive areas, which host many rare and endangered plant species and are hence vulnerable to biodiversity loss, information on the impacts of invasive plant species are necessary. While previous studies have mainly focused on the impacts of *L. polyphyllus* on species diversity and community composition (Hejda 2013; Otte and Maul 2005), the simultaneous effects of *L. polyphyllus* on species and functional diversity are still poorly understood. To our knowledge, few studies addressed potential habitat-specific responses to the invasive *L. polyphyllus* (Thiele et al. 2010).

Therefore, we present a comprehensive study testing the impacts of *L. polyphyllus* on community composition, species diversity and functional diversity of grasslands. Since the impact of invasive plants may vary among invaded plant communitites, and different communities may be driven by different environmental constraints, we explored the effects of *L. polyphyllus* on the vegetation separately for *Nardus* grasslands, mesic and wet mountain hay meadows.

We ask the following research questions:

a. Does *L. polyphyllus* affect the species composition of mountain meadows?

We hypothesize that *L. polyphyllus* will increase the similarity across plant communities of the different meadow types.

b. Does *L. polyphyllus* affect the species diversity of the mountain meadows?

We hypothesize that *L. polyphyllus* increases species diversity (expressed by species richness, evenness, and effective species number) at low cover of *L. polyphyllus* by creating new niches, while all three variables will decrease at high cover.

c. Does *L. polyphyllus* affect multivariate functional diversity as well as community-weighted means of single traits of mountain meadows?

We hypothesize that with increasing lupine cover, sites become more productive, which will be reflected by the respective plant traits (SLA, seed releasing height, LDMC, seed number, flowering duration, life persistence). Functional diversity as well as the proportion of certain plant traits in the vegetation types will decrease with increasing cover of *L. polyphyllus.*

Materials and Methods

Study area

The study was carried out in the mountainous region of the UNESCO Rhön Biosphere Reserve in Central Germany, which was founded in 1991 and comprises an area of approx. 2400 km². Tertiary sands and clays shaped the geology by building an elevated shelf, which is covered by basaltic rocks (Klausing 1988). Soils over basaltic bedrocks are usually well supplied with cations. However, in the Rhön region, high precipitation and traditional land use resulted in low nutrient availability and low pH values (Puffe and Zerr 1988). The mean annual precipitation at the highest elevation in the area, Mt. Wasserkuppe (950 m a.s.l.), amounts to 1,135 mm (mean of 1981–2010; DWD 2019) and the mean annual temperature reaches no more than 5.5 °C (mean of 1981–2010; DWD 2019).

Traditional land-use such as regular mowing and pastoral sheep-herding in combination with low fertilizer input shaped the landscape and formed extended semi-natural grasslands with high conservation value (Otte and Maul 2005). The Rhön Biosphere Reserve comprises 8,900 ha of low-intensively used species-rich grasslands and thus plays an important role in nature conservation (Grebe 1995).

Vegetation data

Vegetation was sampled in *Nardus* grasslands, mesic mountain hay meadows and wet mountain hay meadows. These types are characterized by high abundance of the following plant species: mesic mountain meadows—*Geranium sylvaticum*, *Trisetum flavescens* and *Alchemilla monticola*; wet mountain meadows – *Persicaria bistorta, Trollius europaeus* and *Deschampsia cespitosa*; *Nardus* grasslands—*Nardus stricta*, *Potentilla erecta* and *Galium saxatile*. The vegetation types differ in productivity with *Nardus* grasslands usually yielding 3 t * ha^{-1}, while mountain hay meadows produce about 6 t * ha^{-1} annually (Dierschke et al. 2002). In total, we used 84 vegetation plots (5 m x 5 m) sampled during the growing season in 2014 and 2016. Within each vegetation type, we selected plots with four levels of *L. polyphyllus* cover (class 1: 0%, class 2: > 0–25%, class 3: > 25–75%, class 4: > 75–100%) with seven replicates, resulting in 84 sampled vegetation plots. Selection of the plots sampled in 2014 took place shortly before the sampling. Plots sampled in June 2016 were selected based on their lupine cover in September 2015, therefore, slight differences between the original estimation and the sampled lupine cover might occur. Plant species cover was estimated using the approach of Braun-Blanquet (1964) and converted it into percentage values for further analysis (r = 0.01%, + = 0.5%, 1 = 3.0%, 2 = 15%, 3 = 38%, 4 = 62.5%, 5 = 87.5%). Plant nomenclature follows (Jäger et al. 2017).

Data analysis

Community composition

To obtain the main floristic gradients, we performed a non-metric multidimensional scaling (NMDS) ordination. We chose NMDS as a robust, distance-based method that accurately displays the vegetation data. NMDS was calculated based on Bray-Curtis distances as dissimilarity measure with 20 random starts and three dimensions using the metaMDS function as implemented in the R vegan package 2.5-5 (Oksanen et al. 2019). We included *L. polyphyllus* in the ordination (for an NMDS without *L. polyphyllus*, see Fig. 7 in "Appendix"). The vegetation types and the lupine cover classes were used as grouping variables in the NMDS analysis. To avoid noise in the dataset we excluded species with less than three occurrences. We included weighted Ellenberg L (light), N (nutrients), R (reaction) and M (moisture) indicator values (including *L. polyphyllus*) as well as species diversity indices in the analysis in order to further evaluate the effects *L. polyphyllus* has on the vegetation composition.

To quantify the effects of *L. polyphyllus* on the community composition in the NMDS, we calculated the average distance of all plots in one cover class to the respective cover class centroid and compared these distances among the cover classes. Furthermore, we compared the distances of lupine cover class centroids with each other. In order to determine whether increasing cover of *L. polyphyllus* homogenizes the community composition, we estimated the similarity based on quantitative Sörensen dissimilarity (similarity = 1 – Sörensen dissimilarity) between all vegetation types within the cover classes. In order to account for environmental effects on similarities, we analyzed environmental alongside lupine effects on the similarity by means of a random intercepts linear mixed effect model without interaction term using the lme4 package (Bates et al. 2015). We calculated multivariate Gower distances between environmental variables (topographic wetness index and topographic position index derived from a digital elevation model and pH measured in 2015) of each plot using the FD package (Laliberté and Legendre 2010). *L. polyphyllus* cover classes and environmental distances served as fixed effects, whereas plot, i.e. vegetation type combinations, constituted the random effect. Statistical significance was obtained via bootstrapped *p* values based on 500 bootstrap samples from likelihood ratio tests between the full model with effects in question against reduced models without the effects in question.

Additionally, we calculated permutation-based analysis of variance (PerMANOVA, adonis function, Oksanen et al. 2019) in order to test for differences in species composition between lupine cover classes. Additionally, we used pH, topographic wetness index and position index as covariates to account for potential environmental effects. Subsequently, pairwise differences in the species composition of the different lupine cover classes in each of the three vegetation types were tested using the pairwise.adonis function (Martinez Arbizu 2020). We ran PerMANOVAs and following pairwise comparisons with 1000 permutations and Bray-Curtis distances as dissimilarity measure. We obtained adjusted *p* values using Bonferroni corrections for the pairwise comparisons.

Species diversity

To test for changes in species diversity among lupine cover classes, we calculated species richness, effective species number based on Shannon diversity (Jost 2006), and evenness (Hill 1973). After visually inspecting diagnostic plots (Zuur et al. 2010), differences in effective species number and evenness were tested through one-way ANOVAs separately for each vegetation type and Post-hoc Tukey tests. In the case of species richness, we computed generalized linear models (GLMs) for Poisson distributions for the single vegetation types and pairwise post-hoc Tukey tests.

Functional diversity and traits

We preselected 14 traits that we considered being responsive to changing lupine cover (life persistence, leaf dry matter content, seed number, flowering onset, end of flowering, reproduction type, leaf persistence, flowering duration, strategy type, life form, canopy height, releasing height, seed mass, specific leaf area). We obtained trait data from the open source trait databases LEDA and

BIOLFLOR (Kleyer et al. 2008; Klotz et al. 2002). Several studies have shown that using trait data from databases provides valuable estimates and meaningful results (Bernhardt-Römermann et al. 2011; Bernhardt-Römermann et al. 2008; Busch et al. 2018; Hattermann et al. 2019). In order to gain a reduced set of traits, we performed a double canonical correspondence analysis (double CCA) following the approach of Kleyer et al. (2012), using lupine cover as environmental gradient. By visual inspection of the resulting ordination plots, we identified six traits that best reflected functional responses to increasing lupine cover: (1) seed releasing height as a proxy for plant height, (2) LDMC (leaf dry matter content), (3) seed number, (4) SLA (specific leaf area), (5) flowering duration and (6) life persistence. Missing trait values were either obtained from literature or the mean value of the whole genus was taken. This was necessary in 23 (5 times canopy height, 10 times LDMC, 6 times seed releasing height, 2 times seed number) of 1020 cases. Four species (*Tephroseris helenitis, Ranunculus polyanthemos, Ranunculus nemorosus, Crepis mollis*) had to be omitted because of lacking data. In order to achieve normal distribution of the data, seed number was log-transformed.

We calculated the diversity indices functional richness, i.e. the amount of niche space filled by the species in the community, functional evenness, i.e. the degree to which the biomass of a community is distributed in niche space, functional divergence, i.e. the degree of niche differentiation (Mason et al. 2005) as well as functional dispersion, i.e. the spread of the species in the trait space (Laliberté and Legendre 2010). Calculations were done using the dbFD function of the R package FD 1.0–12 (Laliberté et al. 2014) and included the most important dimensions of functional diversity (Mason et al. 2005). In order to test for differences in functional diversity indices between the lupine cover classes for each vegetation type, we performed ANOVAs and post-hoc Tukey tests after visual inspection of diagnostic plots (Zuur et al. 2010).

In addition, we examined if single traits were influenced by lupine cover through calculating the community weighted mean (CWM) for each trait, i.e. the averaged trait values, weighted by the relative abundance of each species (Garnier et al. 2015). In order to detect the direction of change within the single traits we plotted the CWMs against the lupine cover classes and added weighted linear least squares regression curves. We calculated linear models to assess the strength and significance of the relation between CWMs and *L. polyphyllus* cover.

Since we were furthermore interested in the proportions of functional groups on plots with different lupine cover we estimated the cover fractions of legumes (*Fabaceae*), separated into *L. polyphyllus* and other legumes, grasses (*Poaceae, Juncaceae, Cyperaceae*), and herbs (remaining species including some dwarf shrubs). For each vegetation type, we summarized the cover of all species belonging to each functional group within each vegetation type and cover class and used this as 100% cover, so that the results are represented as relative cover values of each functional group within each vegetation type.

All statistical analyses were carried out in the R statistical environment version 4.0.0 (R core team 2020).

Results

Community composition

The NMDS ordination plot (Fig. 1a) depicted floristic variation among and within the three main vegetation types. Across vegetation types, the centroid of lupine cover class 1 was closer to cover class 2 than to class 3 and 4, while the latter were situated very close together (Table 1).

Table 1: Distances of NMDS *L. polyphyllus* cover class centroids to one another. With cover classes 1: 0%, 2: 1–25%, 3: 26–75%, 4: 76–100%

L. polyphyllus cover class	2	3	4
1	0.010	0.120	0.190
2		0.060	0.112
3			0.008

In relation to the respective class centroid, mean distance of all single plots within each lupine cover class was largest in class 1 (0.789) and smallest in class 4 (0.297). One-way ANOVA showed significant differences in distances to centroids ($F_{3,80} = 21.1; p < 0.001$) among cover classes. Pairwise significant differences (Tukey test) between classes 1 and 2 ($p < 0.01$), 3 and 1 ($p < 0.001$), as well as 4 and 1 ($p < 0.001$) and 4 and 2 ($p < 0.01$) showed that the vegetation composition within the plots becomes more similar with higher lupine cover. In addition, the three vegetation types showed strong overlaps for plots with higher lupine cover (Fig. 1a). In *Nardus* grasslands, high lupine cover plots were less scattered than those with low cover (Fig. 1b). Richness and lupine cover correlated strongest with axis one (Richness: $R^2 = 24\%$, lupine cover: $R^2 = 61\%$). Effective species number and evenness on the other hand showed the strongest correlation with axis two (Effective species number: $R^2 = 24\%$, Evenness: $R^2 = 26\%$). In mesic hay meadows, high lupine cover plots were situated very closely together whereas low lupine cover plots were rather scattered (Fig. 1c). Effective species number and evenness in mesic hay meadows had an R^2 of 19% and 36%, respectively, and correlated with axis one, as did lupine cover and the N value (R^2 = 68%, R^2 = 38%, respectively). Mesic hay meadows had higher Ellenberg R and L values in plots with lower lupine cover. They correlated strongest with axis two (R^2 = 50%, and 17% respectively). Species richness was also correlated with axis two (R^2 = 19%). Similar to *Nardus* grasslands and mesic hay meadows also wet hay meadows showed a clear pattern, with plots with high lupine cover being located close together (Fig. 1d). Significant variables in wet hay meadows were Ellenberg indicator values N, R, L and M as well as lupine cover, all of which correlate with axis one (N: R^2 = 23%, R: R^2 = 61%, L: R^2 = 38%, M: R^2 = 64%, lupine cover: R^2 = 31%).

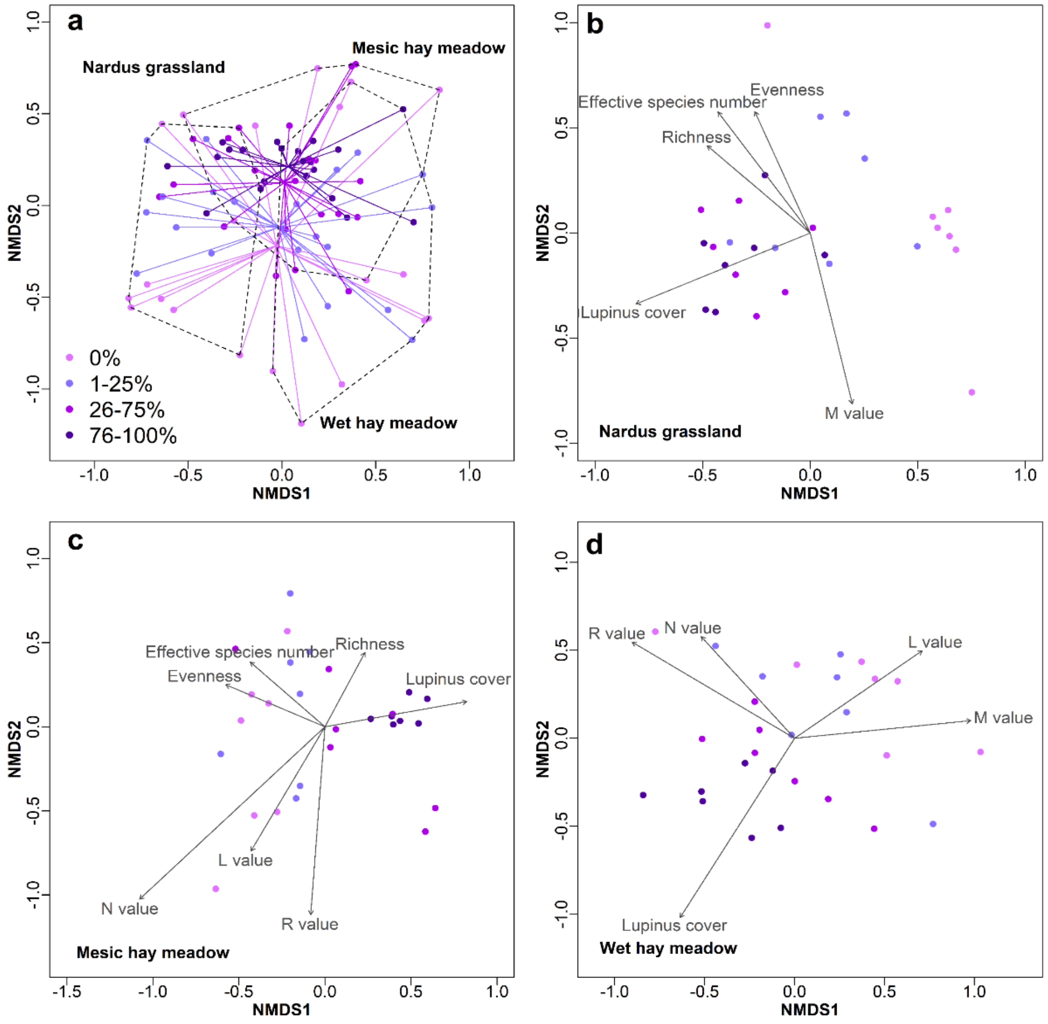

Figure 1: NMDS ordination diagram of the main floristic gradients. The color scheme represents the lupine cover classes, the respective centroids of the classes are marked by a spider web. Vegetation types are encircled with dashed lines in (**a**). The arrows point in the direction of the strongest change in Ellenberg indicator values (light, reaction, moisture, nitrogen) and species diversity indices (species richness, effective species number, species evenness) in (**b**–**d**). The length of the arrows represents the relationship between ordination and gradient with a significance level of $p \leq 0.05$

Mean similarity among vegetation types increased with increasing lupine cover, roughly doubling from plots without lupine to plots with > 70% cover (Fig. 2). Similarity was significantly affected by lupine cover (bootstrapped $p < 0.01$), while the environmental distance among the plots had no effect ($p = 0.93$).

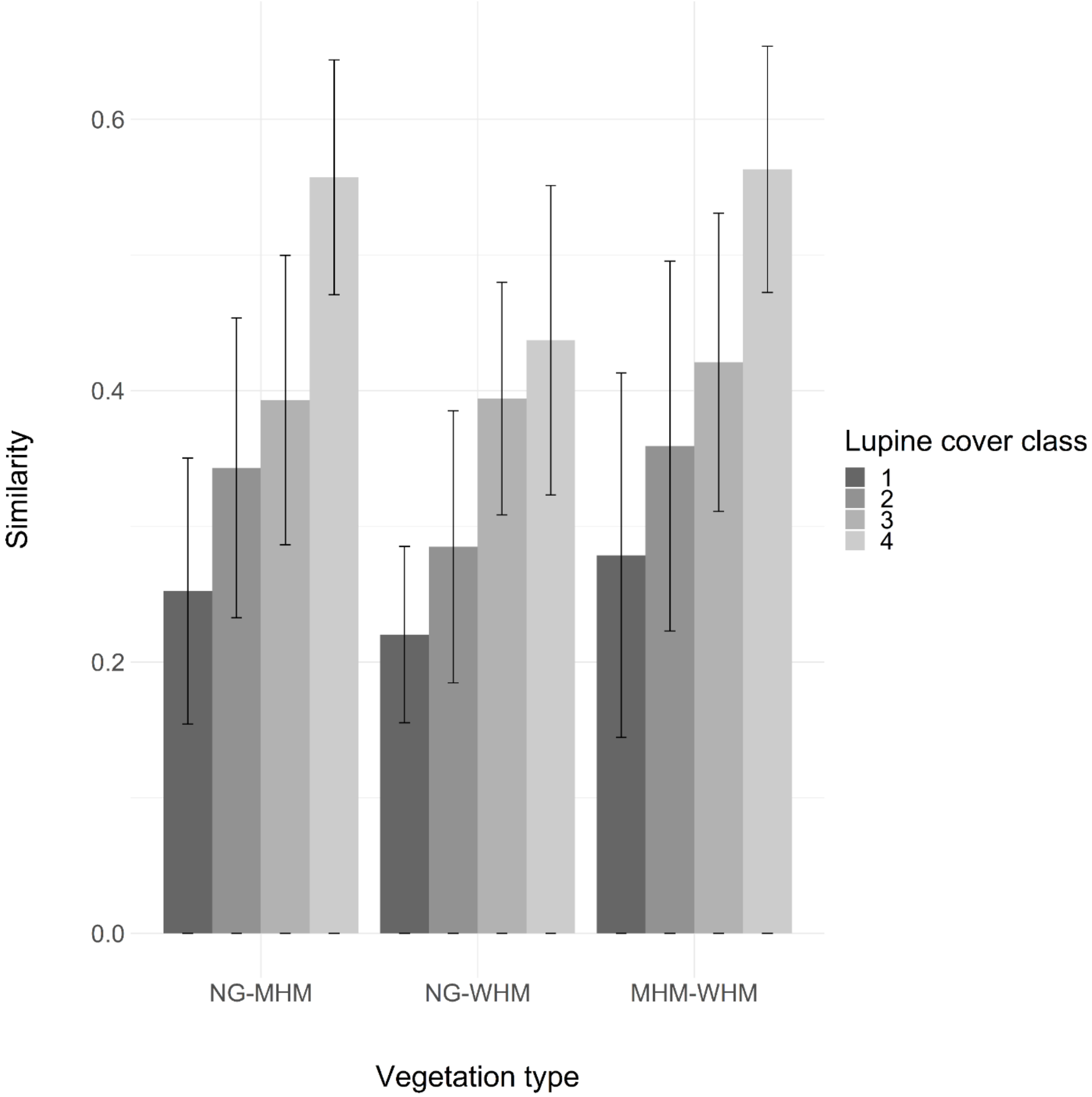

Figure 2: Barplot of mean Sörensen similarity compared between vegetation types and lupine cover classes (1: 0%, 2: 1–25 %, 3: 26–75 %, 4: 76–100 %), where NG, Nardus grassland; MHM, mesic hay meadow, WHM, wet hay meadow. Bars show means ± standard error

Species composition between lupine cover classes differed significantly within the three vegetation types and these differences solely depended on *L. polyphyllus* cover classes across all vegetation types (PerMANOVA: *Nardus* grassland: $F_{3,27} = 3.5$, $p < 0.001$; mesic hay meadow: $F_{3,27} = 3.0$, $p < 0.001$;

wet hay meadow: $F_{3,27} = 2.8$, $p < 0.001$), while environmental variables were not significant (*Nardus* grassland: cti: $F_{3,27} = 0.95$, $p = 0.44$, tpi: $F_{3,27} = 0.70$, $p = 0.75$; pH: $F_{3,27} = 1.15$, $p = 0.28$; mesic hay meadow: cti: $F_{3,27} = 1.19$, $p = 0.28$, tpi: $F_{3,27} = 1.27$, $p = 0.23$, pH: $F_{3,27} = 1{,}69$, $p = 0.09$; wet hay meadow: cti: $F_{3,27} = 0.72$, $p = 0.75$, tpi: $F_{3,27} = 1.10$, $p = 0.35$, pH: $F_{3,27} = 0.82$, $p = 0.61$). Pairwise comparisons of species composition differed significantly between cover classes 1 and 4 as well as 2 and 4 and 1 and 3 in *Nardus* grasslands, between cover classes 1 and 4 in as well as 2 and 4 in mesic hay meadows and between cover classes 1 and 4 as well as 1 and 3 and 2 and 4 in wet hay meadows (Table 2).

Table 2: Results of pairwise PerMANOVA comparisons between plant species compositions of four *L. polyphyllus* cover classes (1: 0%, 2: 1–25%, 3: 26–75%, 4: 76–100%)

Pairs	*Nardus* grassland				Mesic hay meadow				Wet hay meadow			
	df	F	R^2	p. adj.	*df*	F	R^2	p. adj.	*df*	F	R^2	p. adj.
1 versus 2	1	2.26	0.16	0.090	1	1.07	0.08	1.000	1	1.51	0.11	0.702
1 versus 3	1	5.9	0.33	0.006 **	1	1.96	0.14	0.300	1	2.62	0.18	0.036 *
1 versus 4	1	6.33	0.35	0.006 **	1	6.79	0.36	0.006 **	1	4.85	0.29	0.006 **
2 versus 3	1	2.29	0.16	0.114	1	1.59	0.12	0.756	1	1.91	0.14	0.162
2 versus 4	1	3.08	0.20	0.024 *	1	5.98	0.33	0.006 **	1	4.27	0.26	0.006 **
3 versus 4	1	1.03	0.08	1.000	1	1.74	0.13	0.708	1	1.97	0.14	0.108

Species diversity

The 84 vegetation plots contained 173 plant species. Species richness ranged from 19 (*Nardus* grassland) to 57 (wet hay meadow) species per plot. Species richness and effective species number were significantly affected by *L. polyphyllus* in *Nardus* grasslands (richness: $F_{3,24} = 9.8$, $p < 0.001$; effective species number: $F_{3,24} = 7.8$, $p < 0.001$, Fig. 3a) and wet hay meadows (richness: $F_{3,24} = 3.4$, $p < 0.05$; effective species number: $F_{3,24} = 4.3$, $p < 0.05$, Fig. 3b). In both vegetation types, richness and effective species number were significantly higher at intermediate lupine cover classes than in plots without lupine. In mesic hay meadows, lupine cover had no significant effects on richness and effective species number (richness: $F_{3,24} = 2.3$, $p = 0.11$; effective species number: $F_{3,24} = 1.02$, $p = 0.40$; Fig. 3a, b). Evenness showed no clear pattern and did not vary significantly among lupine cover classes (*Nardus* grassland: $F_{3,24} = 0.90$, $p = 0.46$; mesic hay meadow: $F_{3,24} = 1.1$, $p = 0.38$; wet hay meadow: $F_{3,24} = 1.19$, $p = 0.33$; Fig. 3c).

Functional diversity and traits

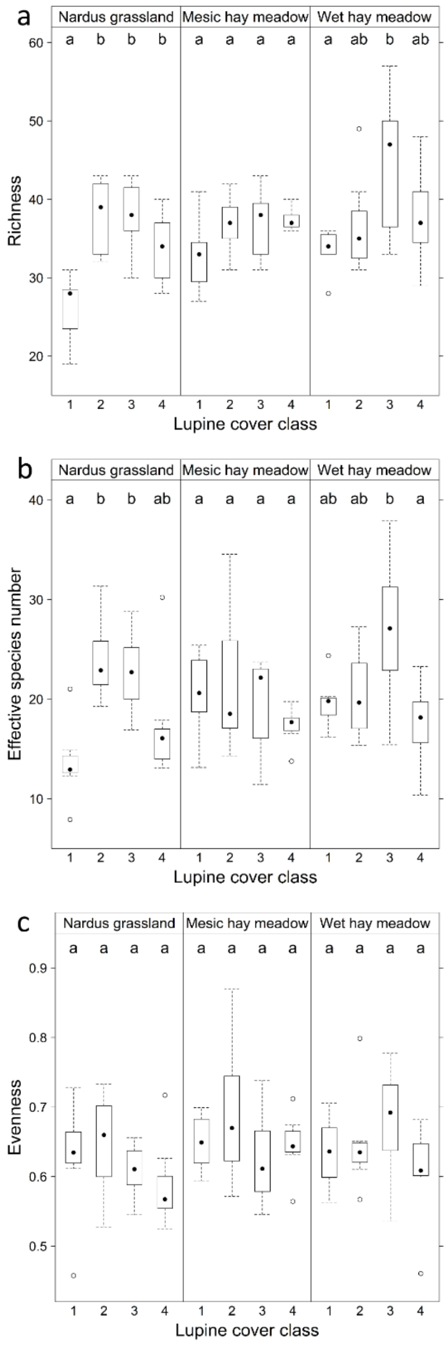

Figure 3: Box whisker plots of the species diversity indices, with dots representing outliers. Whiskers represent minimum and maximum of the data except for the outliers, the box upper and lower quartiles and the black dot the median. Equal letters indicate homogenous groups based on Tukey post hoc tests

All indices varied largely within vegetation types and cover classes. Functional richness and functional evenness were not significantly affected by lupine cover (*Nardus* grasslands: FRichness: $F_{3,24} = 0.27, p = 0.85$, FEveness: $F_{3,24} = 0.12$, $p = 0.95$; mesic hay meadows: FRichness: $F_{3,24} = 0.36, p = 0.78$; FEveness: $F_{3,24} = 2.98$, $p = 0.06$, wet hay meadows: FRichness: $F_{3,24} = 1.67$, $p = 0.2$, FEveness: $F_{3,24} = 1.91$, $p = 0.16$; Fig. 4a and b). Functional divergence was significantly higher in cover class four than in cover class one, two and three in mesic hay meadows ($F_{3,24} = 8.98$; $p < 0.001$, Fig. 4c) and differed significantly between cover classes two and four in wet hay meadows ($F_{3,24} = 3.42$, $p < 0.05$, Fig. 4d). It showed similar but non-significant patterns in *Nardus* grasslands ($F_{3,24} = 2.5$, $p = 0.08$). Functional dispersion differed significantly between cover classes two and four in mesic hay meadows ($F_{3,24} = 3.3, p < 0.05$) and was insignificant in *Nardus* grasslands and wet hay meadows (*Nardus* grasslands: $F_{3,24} = 0.41$,

$p = 0.75$; wet hay meadow: $F_{3,24} = 0.19$, $p = 0.91$).

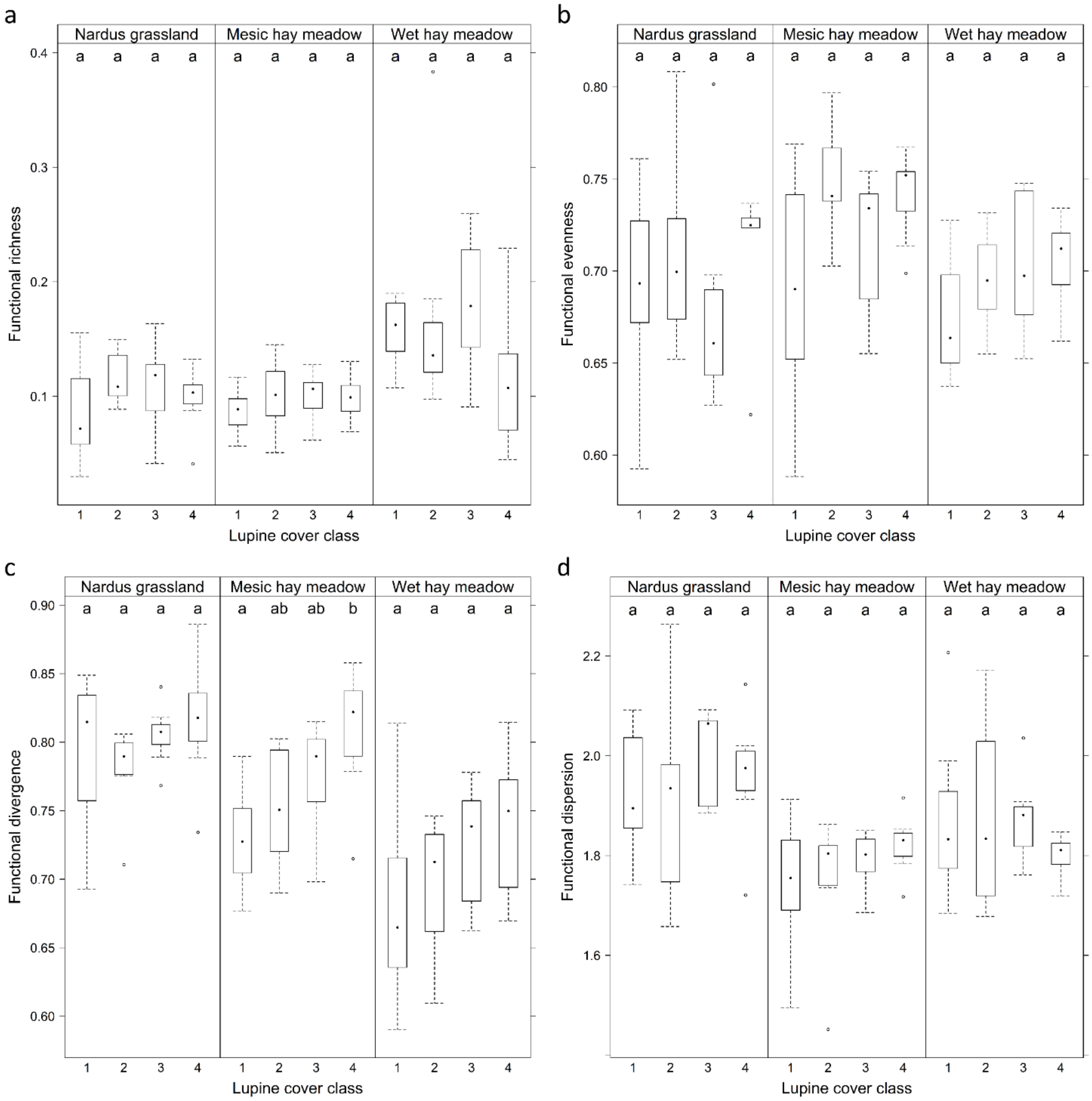

Figure 4: Box whisker plots of the functional diversity indices, with dots representing outliers. Whiskers represent minimum and maximum of the data except for the outliers, the box upper and lower quartiles and the black dot the median. Equal letters indicate homogenous groups

Community weighted means of single traits showed different responses among vegetation types (Fig. 5). In *Nardus* grassland communities, LDMC ($R^2 = 0.52$) decreased whereas releasing height ($R^2 = 0.784$) and SLA ($R^2 = 0.29$) increased significantly with increasing lupine cover. In mesic and wet hay meadows, releasing height was significantly affected, showing the same behavior as in *Nardus* grasslands. LDMC decreased with higher lupine cover in both vegetation types, but only significantly in mesic hay meadows.

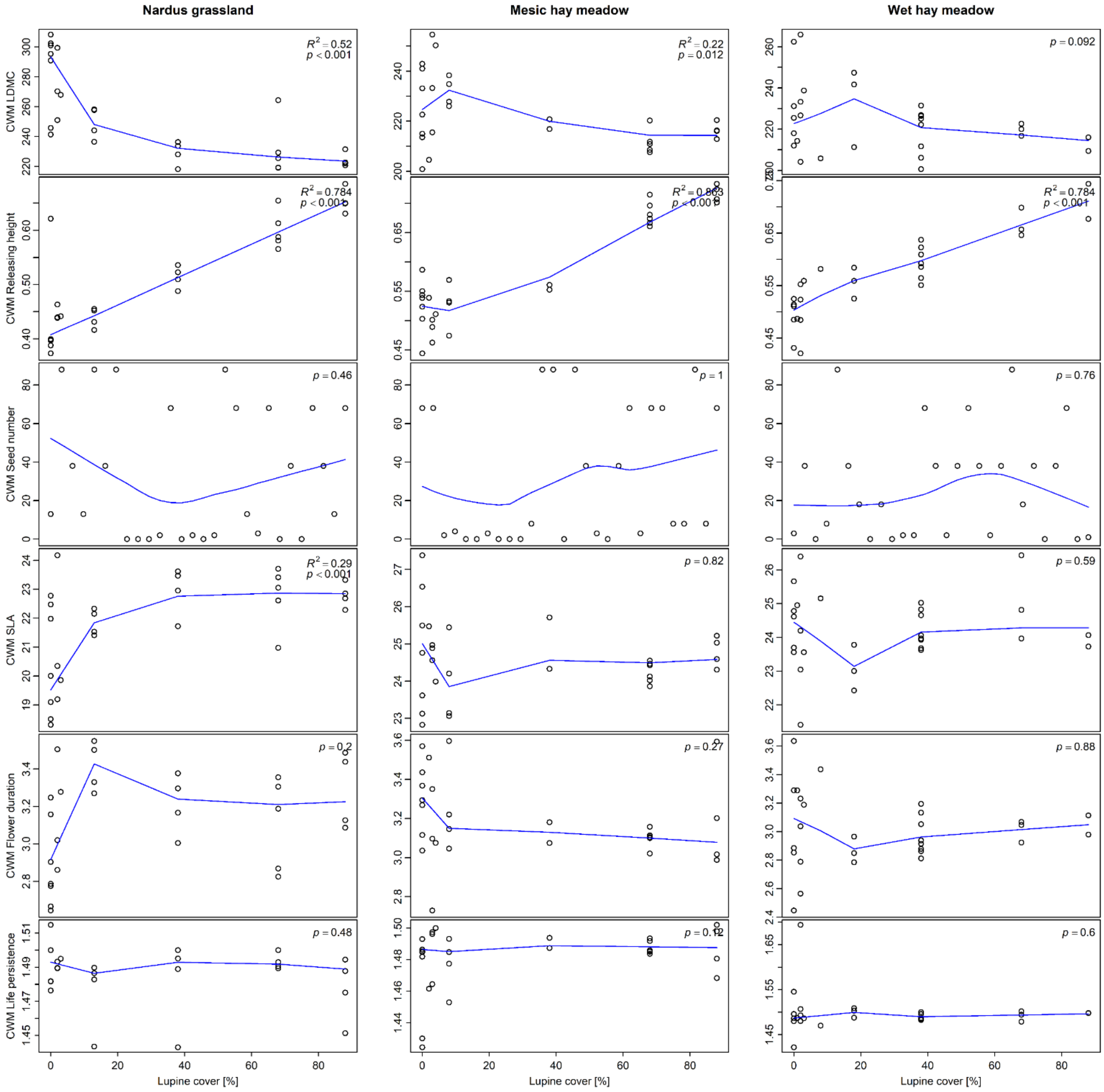

Figure 5: Scatterplot of community weighted means (CWM) of single plant functional traits in relation to *L. polyphyllus* cover. The blue line indicates the weighted linear least squares regression curve (lowess). R² is given when p values are significant

In all three vegetation types, the proportion of grasses was considerably smaller (0.24–0.26) in the highest *L. polyphyllus* cover class than in the lowest cover class (0.34–0.50), whereas the fractions of herbs declined to a minor extent. Other legumes were only affected and reduced in mesic and wet hay meadows (Fig. 6).

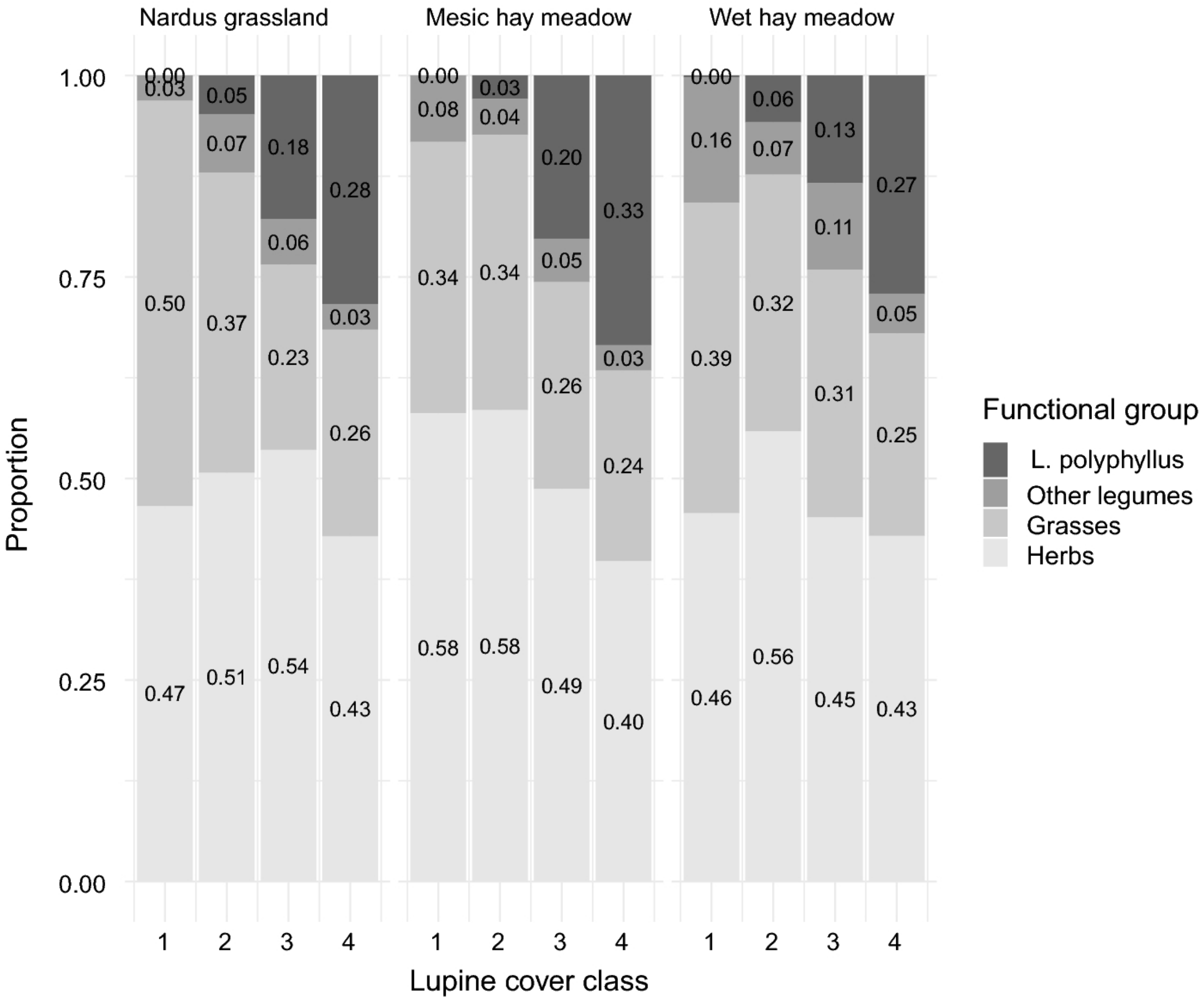

Figure 6: Proportion of the plant functional groups within vegetation types and *L. polyphyllus* cover classes

Discussion

Our results suggest that the invasive *L. polyphyllus* has significant effects on species composition of mountain meadow communities. When *L. polyphyllus* becomes dominant, many species of the native communities drop out (e.g. Thiele et al. 2010). The similarity among different vegetation types increased, which was reflected in the ordination and through direct comparison of centroid distances. This homogenization of native vegetation communities after plant invasion has been reported in several studies (La Sorte et al. 2014). This is most probably because only a specific set of other plants are able to co-exist with dominant non-native species (Hejda 2013), because they have a certain suite of functional traits (see below) matching with the conditions created by the dominant species (mass ratio hypothesis, Grime 1998). In contrast to Ramula and Pihlaja (2012), who could not show an effect of *L. polyphyllus* on community composition, using pooled relevés from meadows, forests, road verges and wastelands, we found a homogenization of the vegetation with increasing cover of *L. polyphyllus* among vegetation types. Moreover, our results showed that the species composition differed significantly within the vegetation types as lupine cover increased.

In *Nardus* grassland and wet hay meadow, the Ellenberg M value was lower in sites with a dense lupine cover. As the species itself has an Ellenberg moisture indicator value of 5 (Ellenberg et al. 1992) and hence an ecological optimum on mesic sites, *L. polyphyllus* apparently prefers sites with lower moisture in *Nardus* grassland and wet hay meadows. In mesic and wet hay meadows Ellenberg L value was higher where lupine cover was lower. Due to its tall growth, it fosters shade-adapted species that have a rather low light indicator value (Otte and Maul 2005; Thiele et al. 2010). Accordingly, species with high light value occur only in low lupine cover sites, where illumination is relatively high. Surprisingly, also the N value was higher in low lupine cover stands, while one would expect a higher N value in dense lupine stands, since *L. polyphyllus* increases plant available nitrogen (Hiltbrunner et al. 2014). The supposed discrepancy between expected and actual N value may result from the calculation of weighted Ellenberg values since *L. polyphyllus* has not been assigned an Ellenberg N value. Other species that co-occur with lupine and show high covers in mesic and wet mountain hay meadows have rather low or no N value, such as *Deschampsia cespitosa, Anthoxantum odoratum, Alopecurus pratensis, Cirsium palustre* or *Plantago lanceolata*. While our study was not designed to examine the soil conditions of lupine stands, these results may suggest that the change in vegetation composition is rather related to changing light and not soil conditions.

Many studies have shown that the character of the invaded community is decisive for the magnitude of the impact of invasive species (e.g. Hejda 2013; Mason et al. 2009). As pointed out by Thiele et al. (2010) especially *L. polypyllus* strongly interacts with the respective habitat type, which is in line with our findings. Species diversity was mainly affected in *Nardus* grasslands and wet hay meadows, where the introduction of *L. polyphyllus* increased the overall number of species. Similar results were found in studies conducted in Arizona (US), Australia and Uruguay, where exotic species increased local plant species diversity (Sax and Gaines, 2003 and see Davis, 2009). However, species richness and effective species numbers tended to decrease in the highest cover class, probably because small species will be shaded and outcompeted in the long term (Hejda 2013; Thiele et al. 2011). Mesic hay meadows were least affected by *L. polyphyllus*, as no significant impacts on species diversity indices were found. Species that co-occur here with lupine are often invasive in other regions of the world, such as *Anthoxantum odoratum, Trisetum flavescens, Festuca rubra* and *Holcus lanatus* (Gross et al. 2010; Hejda 2013). Thus, these species have high competitive abilities and can probably better cope with high lupine cover (Hejda 2013).

Multivariate functional diversity was not much affected by the lupine invasion. Functional divergence in mesic hay meadows and wet hay meadows showed increasing values in high lupine cover classes. This pattern of functional divergence may indicate a higher degree of niche differentiation in stands with high lupine cover (Funk et al. 2017) due to the increasing competition for light through lupine. Functional dispersion showed a similar pattern in mesic hay meadows which may indicate a change of the spread of species in trait space (Laliberté and Legendre 2010) that may be caused by *L. polyphyllus* changing the location of the center of this trait space due to its high abundance. Concerning the CWMs of single traits, all three vegetation types were significantly influenced by *L. polyphyllus*. With high cover of lupine, they harbored taller species with a lower LDMC. Large plant height implies high competition capacity (Bernhardt-Römermann et al. 2008), highlighting the increased competition capacity of co-occurring species in dense lupine stands. Species with low LDMC are associated with rather productive environments (Kleyer et al. 2008). In *Nardus* grasslands, lupine cover also significantly increased SLA, which may indicate higher growth rates, higher leaf N concentrations (Westoby et al. 2002), and a higher productivity (Duru et al. 2014). Moreover, species with a high SLA quickly invest nutrients in high-quality biomass and growth (Westoby et al. 2002). Taken together, increasing seed releasing height, decreasing LDMC in all high lupine cover vegetation types and increasing SLA in *Nardus* grasslands present evidence for higher productivity of lupine-dominated sites. In accordance with Tordoni et al. (2019), our study suggests that a homogenization of the vegetation communities as well as significant shifts of single functional traits among the vegetation types occurred due to the *L. polyphyllus* invasion; yet the multivariate functional space itself was not affected. We suggest that since *L. polyphyllus* originates from mountain meadows (Starfinger and Kowarik 2003) it might, generally, have a similar set of functional traits as the invaded community pool. Hence, *L. polyphyllus* does not change the dispersion of traits around the community mean in multivariate space, but it does promote a change of the mean value itself of certain traits, as it changes species composition and fosters the growth of competitive co-occurring species.

Our results showed that the proportion of functional plant groups changed substantially and consistently in all vegetation types with increasing *L. polyphyllus* cover. In unfertilized one-cut meadows the fraction of legumes is typically 10% (Voigtländer and Jacob 1987), while grasses and herbs have both a fraction of 45%. In the present study, the total proportion of legumes rose considerably with increasing *L. polyphyllus* cover, mainly to the disadvantage of grasses, which strongly declined. All three vegetation types hosted more than 30% of legumes and are thereby outside the range of unfertilized farmed grassland (Voigtländer and Jacob 1987). The changes of functional groups induced by *L. polyphyllus* again mirrors its shaping and homogenizing of the vegetation types.

Conclusions

Increasing cover of *L. polyphyllus* changed community composition and led to a homogenization of all three vegetation types. However, plots with low cover of lupine showed higher local species diversity. The primary effect of the *L. polyphyllus* invasion on functional diversity was a significant shift of certain species' traits to a more competitive suite of traits. Compared to mountain grasslands without lupine, the proportion of grasses dropped significantly in plots dominated by *L. polyphyllus*, whereas legumes increased. Since the study comprises vegetation types of high conservation value, hosting many rare and endangered species, measures to control the invasion and reduce the *L. polyphyllus* number are urgently needed.

Acknowledgments

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Appendix

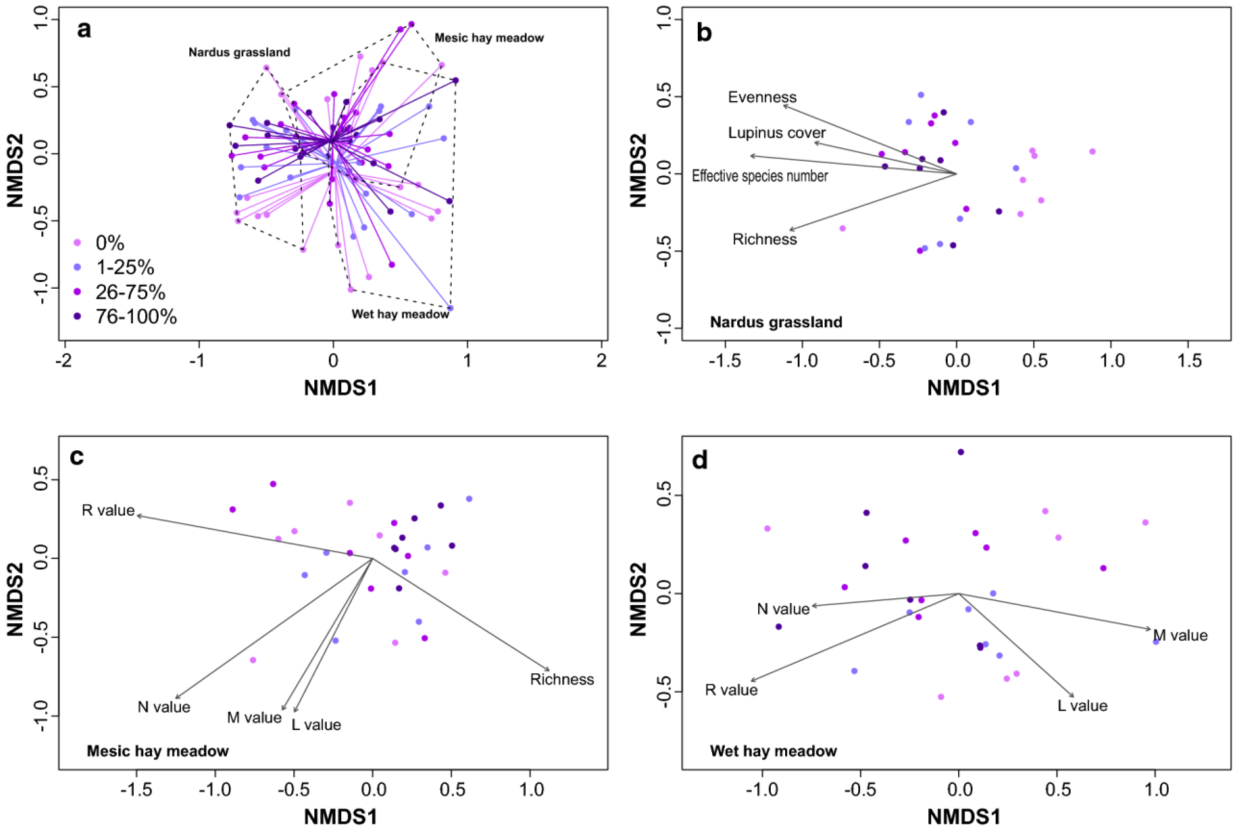

Figure 7: NMDS ordination diagram of the main floristic gradients and excluding L. polyphyllus. The color scheme represents the lupine cover classes, the respective centroids of the classes are marked by a spider web. Vegetation types are encircled with dashed lines in (**a**). The arrows point in the direction of the strongest change in Ellenberg indicator values (light, reaction, moisture, nitrogen) and species diversity indices (species richness, effective species number, species evenness) in (**b**–**d**). The length of the arrows represents the relationship between ordination and gradient with a significance level of $p \leq 0.05$

Chapter 3

Seed bank offers potential for active restoration of mountain meadows

Kristin Ludewig, Wiebke Hansen, Yves P. Klinger, R. Lutz Eckstein, Annette Otte

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Abstract

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.

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).

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 × 100; seed bank: seed density/m^2 of each species/sum of total seed density/m^2 × 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.

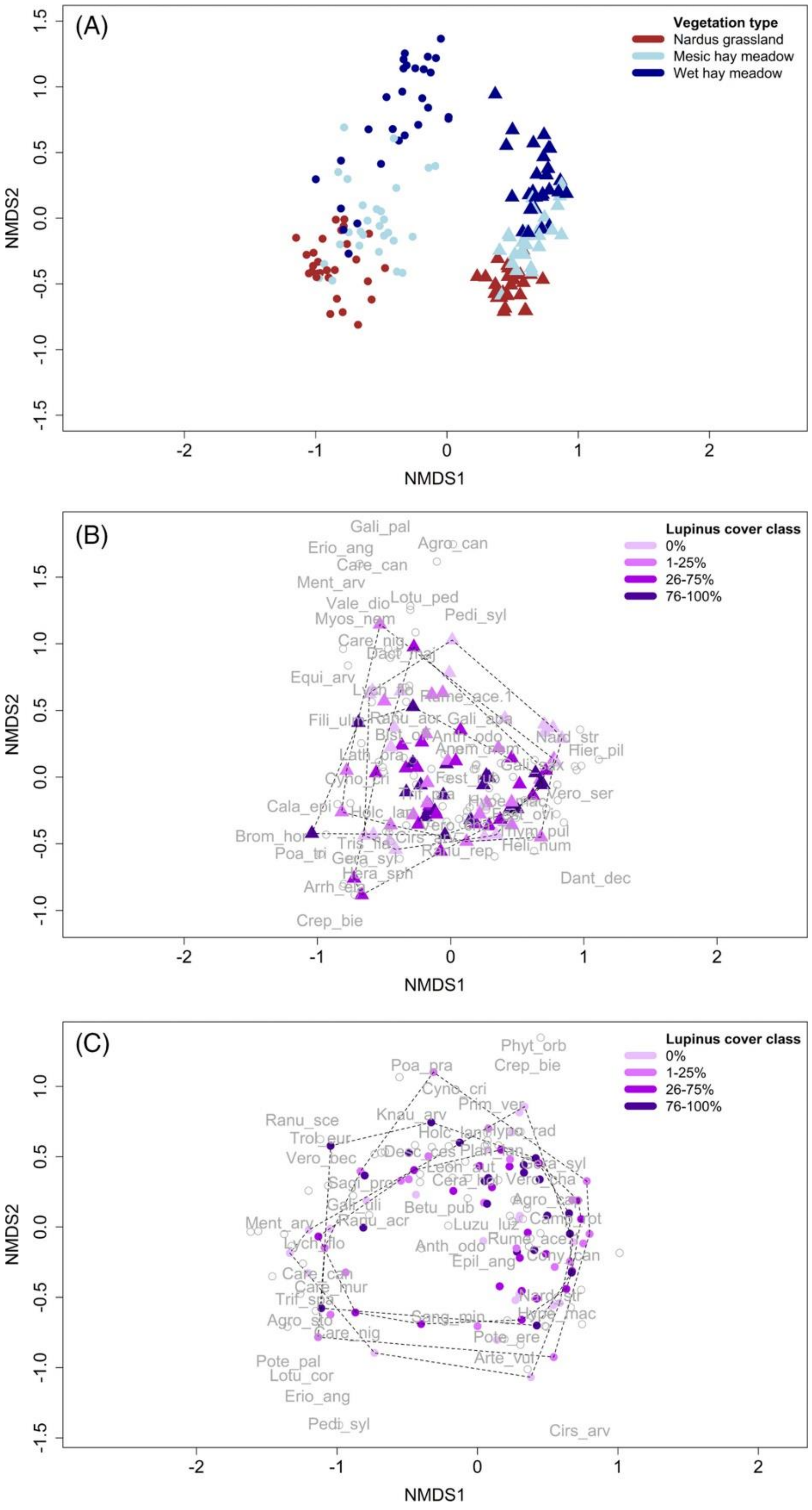

Figure 1: NMDS ordination with the composition of (A) the aboveground vegetation (triangles) and the seed bank samples (circles, stress-value 0.11) grouped according to vegetation type; (B) aboveground vegetation (stress-value 0.12); and (C) seed bank samples (stress-value 0.11), both grouped according 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.

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 ± 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 ± 11,918) than in mesic meadows (11,136 ± 1,279) and *Nardus* grasslands (11,388 ± 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 ± 306) than in the plots with <25% *L. polyphyllus* cover (1,839 ± 768), with >75% (1,143 ± 459) or without *L. polyphyllus* cover (3,832 ± 1,518), but these differences were not significant in the post hoc test.

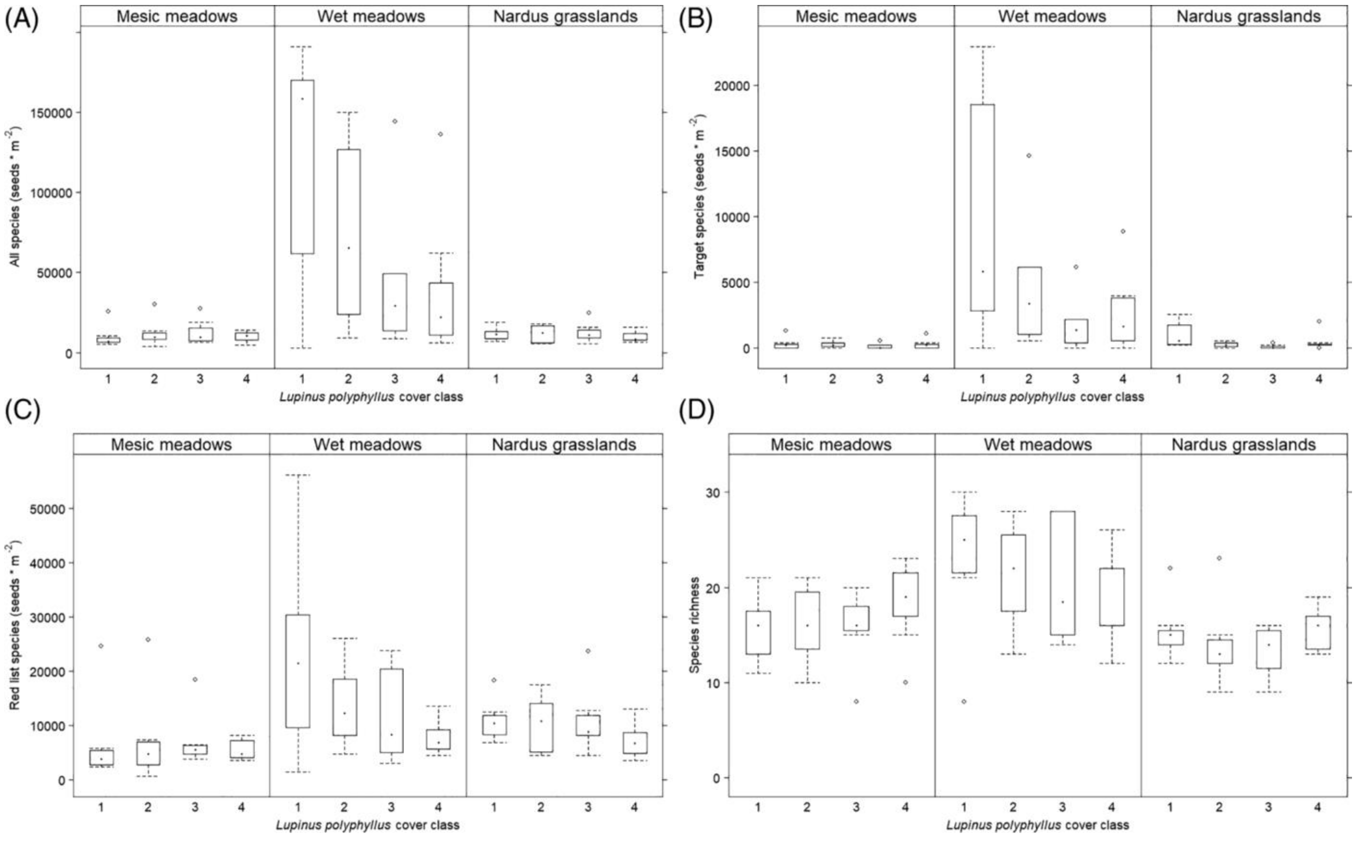

Figure 2: Boxplots of the response variables: (A) seed densities of all species; (B) seed densities of target species; (C) seed densities of Red List species; and (D) species richness of the plots according to vegetation type and Lupinus polyphyllus cover class (1 = 0%, 2 = 1–25%, 3 = 26–75%, and 4 = 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.

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

Table S1: List of all plant species in the seed bank (SB) and the aboveground vegetation (AV) of the 84 study plots. The species are in alphabetic order within three groups (group 1: in SB and AV, group 2: only in SB, group 3: only in AV). Further, the acronym, the longevity of the seed bank (if calculated) and the binding to mountain meadow vegetation types (Oberdorfer 1977, Peppler-Lisbach and Petersen 2001) of the species is given, and the information whether they are threatened according to the Red List (Metzing et al. 2018).

Species	**Group**	**Acronym**	**SB-type**	**typical**	**RL**
Achillea millefolium ag.	1	Achi_mil	(transient)	1	
Agrostis canina	1	Agro_can	short-term persistent		
Agrostis capillaris	1	Agro_cap	short-term persistent		
Agrostis stolonifera	1	Agro_sto	short-term persistent		
Ajuga reptans	1	Ajug_rep	short-term persistent	1	
Alchemilla vulgaris ag.	1	Alch_vul	long-term persistent	1	
Anthoxanthum odoratum	1	Anth_odo	short-term persistent	1	
Bellis perennis	1	Bell_per	(transient)	1	
Bistorta officinalis	1	Bist_off	(transient)		V
Calluna vulgaris	1	Call_vul	long-term persistent	1	
Campanula rotundifolia	1	Camp_rot	short-term persistent	1	
Cardamine pratensis	1	Card_pra	short-term persistent		
Carex canescens	1	Care_can	short-term persistent		
Carex muricata	1	Care_mur	short-term persistent		
Carex nigra ag.	1	Care_nig	short-term persistent		
Carex pallescens	1	Care_pal	short-term persistent	1	
Carex panicea	1	Care_pan	short-term persistent		V
Carex pilulifera	1	Care_pil	short-term persistent	1	
Cerastium holosteoides	1	Cera_hol	short-term persistent	1	
Cirsium arvense	1	Cirs_arv	(transient)		
Cirsium palustre	1	Cirs_pal	short-term persistent		
Crepis biennis	1	Crep_bie	(long-term persistent)		
Crepis mollis	1	Crep_mol	transient	1	3
Cynosurus cristatus	1	Cyno_cri	transient	1	
Danthonia decumbens	1	Dant_dec	short-term persistent		V
Deschampsia cespitosa	1	Desc_ces	short-term persistent	1	
Eriophorum angustifolium	1	Erio_ang	short-term persistent		V
Festuca pratensis	1	Fest_pra	(long-term persistent)	1	
Festuca rubra ag.	1	Fest_rub	transient	1	
Galium aparine	1	Gali_apa	short-term persistent		
Galium palustre	1	Gali_pal	short-term persistent		
Galium saxatile	1	Gali_sax	short-term persistent		
Galium uliginosum	1	Gali_uli	long-term persistent		
Geranium sylvaticum	1	Gera_syl	(transient)	1	
Helianthemum nummularium	1	Heli_num	(transient)		V
Helictotrichon pratense	1	Heli_pra	(transient)		V
Helictotrichon pubescens	1	Heli_pub	(transient)		
Hieracium pilosella	1	Hier_pil	(long-term persistent)	1	
Holcus lanatus	1	Holc_lan	short-term persistent	1	
Hypericum maculatum	1	Hype_mac	short-term persistent		
Hypochaeris radicata	1	Hypo_rad	(long-term persistent)		
Juncus articulatus	1	Junc_art	long-term persistent		
Juncus effusus	1	Junc_eff	short-term persistent		
Knautia arvensis	1	Knau_arv	(transient)		
Lathyrus linifolius	1	Lath_lin	(long-term persistent)		V
Lathyrus pratensis	1	Lath_pra	(long-term persistent)		
Leontodon autumnalis	1	Leon_aut	short-term persistent	1	
Leucanthemum vulgare ag.	1	Leuc_vul	short-term persistent		
Lolium perenne	1	Loli_per	transient		
Lotus corniculatus	1	Lotu_cor	transient	1	
Lupinus polyphyllus	1	Lupi_pol	short-term persistent		
Luzula luzuloides	1	Luzu_luz	short-term persistent		
Lychnis flos-cuculi	1	Lych_flo	short-term persistent		
Medicago lupulina	1	Medi_lup	(transient)		
Mentha arvensis	1	Ment_arv	short-term persistent		
Myosotis nemorosa	1	Myos_nem	short-term persistent		
Nardus stricta	1	Nard_str	short-term persistent	1	V

Pedicularis sylvatica	1	Pedi_syl	transient		3
Phyteuma orbiculare	1	Phyt_orb	(long-term persistent)		3
Phyteuma spicatum	1	Phyt_spi	short-term persistent	1	
Pimpinella saxifraga	1	Pimp_sax	(transient)		
Plantago lanceolata	1	Plan_lan	short-term persistent	1	
Poa chaixii	1	Poac_hai	short-term persistent	1	
Poa palustris	1	Poap_alu	(long-term persistent)		
Poa pratensis	1	Poap_rat	short-term persistent		
Poa trivialis	1	Poat_riv	short-term persistent		
Potentilla erecta	1	Pote_ere	short-term persistent	1	
Potentilla palustris	1	Pote_pal	short-term persistent		
Primula veris	1	Prim_ver	(transient)		V
Ranunculus acris	1	Ranu_acr	short-term persistent		
Ranunculus sceleratus	1	Ranu_sce	(long-term persistent)		
Rumex acetosa	1	Rume_ace	short-term persistent	1	
Rumex acetosella	1	Rume_ac2	long-term persistent	1	
Sanguisorba minor	1	Sang_min	short-term persistent		
Sanguisorba officinalis	1	Sang_off	short-term persistent		V
Saxifraga granulata	1	Saxi_gra	long-term persistent		V
Taraxacum Sec. Ruderalia	1	Tara_off	short-term persistent	1	
Thymus pulegioides	1	Thym_pul	(transient)	1	
Trifolium dubium	1	Trif_dub	short-term persistent	1	
Trifolium pratense	1	Trif_pra	short-term persistent	1	
Trifolium repens	1	Trif_rep	long-term persistent	1	
Trifolium spadiceum	1	Trif_spa	short-term persistent		2
Trollius europaeus	1	Trol_eur	(transient)		3
Vaccinium myrtillus	1	Vacc_myr	short-term persistent	1	
Valeriana dioica	1	Vale_dio	transient		
Veronica arvensis	1	Vero_arv	transient		
Veronica chamaedrys	1	Vero_cha	short-term persistent	1	
Veronica officinalis	1	Vero_off	short-term persistent	1	
Veronica serpyllifolia	1	Vero_ser	long-term persistent		
Viola palustris	1	Viol_pal	long-term persistent		
Viola riviniana	1	Viol_riv	short-term persistent		
Arabidopsis thaliana	2	Arab_tha	long-term persistent		
Artemisia vulgaris	2	Arte_vul	(long-term persistent)		
Betula pubescens	2	Betu_pub	long-term persistent		
Capsella bursa-pastoris	2	Caps_bur	(long-term persistent)		
Carex disticha	2	Care_dis	long-term persistent		
Chenopodium glaucum	2	Chen_gla	long-term persistent		
Conyza canadensis	2	Cony_can	(long-term persistent)		
Epilobium angustifolium	2	Epil_ang	long-term persistent		
Epilobium ciliatum	2	Epil_cil	long-term persistent		
Epilobium hirsutum	2	Epil_hir	(long-term persistent)		
Glyceria declinata	2	Glyc_dec	long-term persistent		
Juncus filiformis	2	Junc_fil	(long-term persistent)		V
Persicaria hydropiper	2	Pers_hyd	(long-term persistent)		
Persicaria maculosa	2	Pers_mac	long-term persistent		
Plantago media	2	Plan_med	(long-term persistent)	1	
Poa annua	2	Poaa_nnu	long-term persistent		
Prunella vulgaris	2	Prun_vul	long-term persistent	1	
Rumex obtusifolius	2	Rume_obt	(long-term persistent)		
Sagina procumbens	2	Sagi_pro	long-term persistent		
Scleranthus annuus	2	Scle_ann	(long-term persistent)		
Sonchus arvensis	2	Sonc_arv	(long-term persistent)		
Spergularia rubra	2	Sper_rub	(long-term persistent)		
Stellaria alsine	2	Stel_als	long-term persistent		
Stellaria aquatica	2	Stel_aqu	long-term persistent		
Stellaria media	2	Stel_med	long-term persistent		
Urtica dioica	2	Urti_dio	long-term persistent		
Veronica beccabunga	2	Vero_bec	long-term persistent		
Viola arvensis	2	Viol_arv	long-term persistent		
Aegopodium podagraria	3	Aego_pod			
Alopecurus pratensis	3	Alop_pra		1	
Anemone nemorosa	3	Anem_nem		1	
Angelica sylvestris	3	Ange_syl			
Anthriscus sylvestris	3	Anth_syl		1	
Arnica montana	3	Arni_mon		1	3
Arrhenatherum elatius	3	Arrh_ela			
Betonica officinalis	3	Beto_off			V
Briza media	3	Briz_med		1	
Bromus hordeaceus	3	Brom_hor			
Calamagrostis epigejos	3	Cala_epi			
Caltha palustris	3	Calt_pal			V
Carum carvi	3	Caru_car		1	
Centaurea jacea	3	Cent_jac			

Centaurea montana	3	Cent_mon		
Cirsium vulgare	3	Cirs_vul		
Colchicum autumnale	3	Colc_aut		
Crepis paludosa	3	Crep_pal		
Dactylis glomerata	3	Dact_glo	1	
Dactylorhiza majalis	3	Dact_maj		3
Deschampsia flexuosa	3	Desc_fle	1	
Epilobium tetragonum	3	Epil_tet		
Equisetum arvense	3	Equi_arv		
Equisetum fluviatile	3	Equi_flu		
Equisetum palustre	3	Equi_pal		
Equisetum sylvaticum	3	Equi_syl		
Festuca ovina	3	Fest_ovi		V
Filipendula ulmaria	3	Fili_ulm		
Galium album	3	Gali_alb		
Galium boreale	3	Gali_bor		V
Galium verum	3	Gali_ver		
Genista tinctoria	3	Geni_tin		V
Geranium palustre	3	Gera_pal		
Geum rivale	3	Geum_riv		
Heracleum sphondylium	3	Hera_sph	1	
Hieracium lachenalii	3	Hier_lac	1	
Juncus conglomeratus	3	Junc_con		
Koeleria pyramidata	3	Koel_pyr		V
Leontodon hispidus	3	Leon_his	1	
Lilium martagon	3	Lili_mar		
Lotus pedunculatus	3	Lotu_ped		
Luzula campestris ag.	3	Luzu_cam	1	V
Lythrum salicaria	3	Lyth_sal		
Pastinaca sativa	3	Past_sat		
Platanthera chlorantha	3	Plat_chl		3
Polygala serpyllifolia	3	Poly_ser	1	3
Polygala vulgaris	3	Poly_vul	1	V
Ranunculus nemorosus	3	Ranu_nem	1	V
Ranunculus repens	3	Ranu_rep		
Rhinanthus minor	3	Rhin_min	1	
Scabiosa columbaria	3	Scab_col		
Stellaria graminea	3	Stel_gra	1	
Succisa pratensis	3	Succ_pra	1	V
Tephroseris helenitis	3	Teph_hel		2
Thesium pyrenaicum	3	Thes_pyr		3
Tragopogon pratensis	3	Trag_pra		
Trifolium medium	3	Trif_med		
Trisetum flavescens	3	Tris_fla	1	
Vaccinium vitis-idaea	3	Vacc_vit	1	
Valeriana officinalis	3	Vale_off		
Vicia angustifolia	3	Vici_ang		
Vicia cracca	3	Vici_cra		
Vicia hirsuta	3	Vici_hir		
Vicia sativa	3	Vici_sat		
Vicia sepium	3	Vici_sep	1	
Viola tricolor	3	Viol_tri		

Table S2: List of all plant species in the seed bank (SB) and the aboveground vegetation (AV) of the 84 study plots. The species are in alphabetic order within three groups (group 1: in SB and AV, group 2: only in SB, group 3: only in AV). Seed density (seeds m^{-2}) of the species and mean cover in the AV (%) is given for the vegetation types mesic mountain meadows (GF), wet mountain meadows (GN) and Nardus-grasslands (BG) and different cover classes of *L. polyphyllus* (1 = 0%, 2 = 1-25%, 3 = 26-75% and 4 = 76-100% cover at date of plot selection). Full names of species are given in Table S1.

Veg. type	**GF**				**GF**				**GN**				**GN**				**BG**				**BG**			
Compartment	**SB**	**SB**	**SB**	**SB**	**AV**	**AV**	**AV**	**AV**	**SB**	**SB**	**SB**	**SB**	**AV**	**AV**	**AV**	**AV**	**SB**	**SB**	**SB**	**SB**	**AV**	**AV**	**AV**	**AV**
Lupinus cover class	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Group 1:																								
Achi_mil	0	0	0	0	7.0	2.6	2.7	2.6	26	0	0	0	0.0	0.6	1.6	3.6	0	0	0	0	0.7	2.7	8.7	4.3
Agro_can	0	0	0	26	0.0	0.0	0.0	0.0	593	567	490	155	0.6	0.0	0.0	0.0	0	26	0	0	0.0	0.0	0.0	0.0
Agro_cap	1444	3454	1340	2114	0.4	1.1	0.6	0.3	438	876	928	1057	0.4	0.0	0.0	0.9	3532	2320	3403	2604	1.3	0.7	0.0	0.7
Agro_sto	0	0	0	0	0.0	0.0	0.0	0.0	129	567	26	0	1.4	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Ajug_rep	26	284	52	412	0.9	3.0	2.4	3.0	516	155	26	52	2.6	1.9	2.3	2.1	0	26	0	77	0.7	0.9	0.7	0.9
Alch_vul	335	438	464	670	5.9	8.4	10.0	4.1	103	103	129	335	1.0	1.3	3.1	2.9	26	103	26	387	0.0	3.7	3.1	3.3
Anth_odo	129	103	155	103	7.0	3.0	8.0	3.7	387	180	438	180	15.1	6.7	5.9	4.7	180	103	0	103	12.4	10.9	7.0	6.6
Bell_per	0	0	0	0	0.4	0.7	0.0	0.0	0	26	0	0	0.1	0.6	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Bist_off	0	0	0	0	14.7	20.4	7.9	8.7	0	26	0	0	18.7	45.9	15.9	16.9	0	0	0	0	15.0	1.3	3.1	3.3
Call_vul	26	0	0	0	0.0	0.0	0.0	0.0	103	52	954	0	0.3	0.0	0.0	0.0	180	1108	387	52	0.6	1.6	0.0	0.0
Camp_rot	258	1108	1108	748	0.9	0.7	0.4	0.0	0	26	155	103	0.0	0.3	0.4	0.0	464	3454	2732	1521	1.9	3.7	5.4	4.4
Card_pra	129	26	52	155	0.7	1.6	1.7	1.9	335	77	26	180	1.6	2.3	1.4	1.9	0	0	26	335	0.3	0.3	0.4	0.3
Care_can	103	52	0	26	0.0	0.0	0.0	0.0	3325	2140	541	541	1.6	1.4	0.0	0.4	206	0	77	0	0.0	0.0	0.0	0.0
Care_mur	0	52	26	52	0.0	0.0	0.0	0.0	5053	1031	438	206	0.6	0.4	0.4	0.0	129	155	77	0	0.0	0.4	0.3	0.0
Care_nig	0	0	0	0	0.0	0.0	0.0	0.0	1495	2088	490	387	2.7	4.3	1.3	0.9	309	26	52	129	0.0	0.0	0.0	0.0
Care_pal	0	0	0	0	0.0	0.0	0.0	0.0	0	129	26	232	0.0	1.4	0.3	0.3	0	0	0	0	0.0	0.0	0.0	0.0
Care_pan	0	0	0	0	0.0	0.0	0.0	0.0	516	464	258	180	7.1	2.9	1.9	0.9	0	0	0	0	0.0	0.0	0.0	0.0
Care_pil	180	52	52	103	0.0	0.0	0.0	0.0	52	0	52	52	0.0	0.3	0.0	0.0	799	180	309	284	0.0	0.3	0.0	0.3
Cera_hol	129	309	155	103	1.1	2.0	1.7	1.7	103	180	438	103	0.9	1.4	2.1	1.4	0	77	206	129	0.0	1.4	1.7	1.3
Cirs_arv	0	0	0	0	0.4	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0	26	0	0	0.0	0.0	0.0	0.0
Cirs_pal	0	26	26	0	0.0	0.0	0.7	0.6	26	26	77	129	1.4	2.3	3.3	1.3	0	0	26	0	0.0	0.3	0.3	0.0
Crep_bie	26	0	0	0	1.9	0.4	1.4	0.0	26	0	0	0	0.4	0.3	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Crep_mol	26	26	0	0	3.7	2.4	2.6	1.6	26	0	0	0	1.0	1.7	1.9	1.6	103	0	0	0	2.7	2.1	2.6	1.6
Cyno_cri	103	52	0	0	8.4	7.0	4.1	2.9	77	77	0	0	15.3	1.9	2.0	0.7	0	0	0	0	0.0	0.0	0.0	0.0
Dant_dec	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	26	103	0	0	0.0	0.3	0.0	0.0
Desc_ces	284	644	258	619	0.0	1.0	1.0	0.7	1083	980	1005	1186	0.6	2.1	3.3	4.1	0	77	26	77	0.0	2.7	4.1	4.4
Erio_ang	0	0	0	0	0.0	0.0	0.0	0.0	541	180	26	0	0.0	1.4	0.0	0.3	0	0	0	0	0.0	0.0	0.0	0.0
Fest_pra	26	0	0	0	0.4	0.0	0.0	0.0	0	0	0	0	1.9	0.4	0.4	0.3	0	0	0	0	0.0	0.0	0.0	0.0
Fest_rub	0	0	0	0	16.6	20.9	10.0	7.6	0	0	0	0	10.1	15.7	19.4	10.9	77	26	0	0	13.7	20.1	15.9	18.3
Gali_apa	0	0	0	0	0.0	0.0	0.0	0.0	258	464	258	619	0.4	0.0	0.0	0.1	0	0	0	0	0.0	0.0	0.0	0.4
Gali_pal	0	0	0	0	0.0	0.0	0.0	0.0	26	0	26	0	0.4	0.4	0.0	0.0	644	77	232	77	0.0	0.0	0.0	0.0
Gali_sax	0	0	0	0	0.4	1.1	0.0	4.1	0	26	0	0	1.9	1.6	2.3	2.0	490	0	258	52	5.0	4.4	18.7	8.9
Gali_uli	0	0	0	0	0.0	0.0	0.0	0.0	52	52	77	0	0.0	0.0	0.4	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Gera_syl	0	0	0	0	13.1	12.6	23.3	2.1	0	0	0	26	5.7	5.1	3.7	14.6	0	0	0	26	0.4	2.3	2.9	0.6
Heli_num	0	0	26	0	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0.0	1.1	1.9	1.9
Heli_pra	0	0	0	0	2.9	0.7	0.3	1.4	26	0	0	0	0.0	0.7	1.3	1.1	0	0	0	0	0.7	6.0	5.4	2.3
Heli_pub	0	0	0	0	0.9	2.1	2.6	2.4	0	0	26	0	0.7	1.0	1.7	1.6	0	0	0	0	0.0	0.9	0.0	0.4

Hier_pil	0	0	0	0	0.4	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	26	26	0	0	2.9	1.7	0.4	0.0
Holc_lan	335	335	541	825	14.7	10.3	11.1	10.9	1212	361	361	696	4.7	7.3	9.4	19.4	0	0	0	26	1.1	1.3	4.9	2.6
Hype_mac	3171	284	2836	412	4.3	2.4	4.6	4.4	26	103	3274	155	0.0	0.4	1.9	1.3	1263	1186	2165	644	3.3	7.1	10.7	5.0
Hypo_rad	0	0	26	0	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.6	1.1	0.7
Junc_art	0	0	0	0	0.0	0.0	0.0	0.0	387	438	52	77	0.4	0.0	0.0	0.0	0	26	0	0	0.0	0.0	0.0	0.0
Junc_eff	232	464	26	619	0.0	0.0	0.0	0.0	85558	52278	38693	26371	4.1	4.6	0.7	0.4	155	438	155	490	0.0	0.0	0.0	0.0
Knau_arv	0	26	0	0	2.1	3.6	2.0	2.7	0	0	0	0	0.0	1.6	1.7	1.6	0	0	0	0	2.4	2.9	4.0	4.4
Lath_lin	0	0	0	26	0.3	0.7	1.0	2.1	0	0	0	0	0.0	0.7	0.9	1.0	26	0	0	0	2.3	3.1	4.3	3.6
Lath_pra	0	0	0	0	3.7	0.7	1.6	1.1	26	0	0	0	4.1	4.1	4.7	2.1	0	0	0	0	0.0	0.4	0.0	0.4
Leon_aut	0	26	26	0	1.1	0.0	0.1	0.3	0	0	26	26	0.1	0.0	0.1	0.0	0	26	0	0	0.0	0.0	0.3	0.1
Leuc_vul	232	103	129	284	2.6	3.1	2.4	2.0	206	26	0	52	0.0	0.9	1.3	1.1	0	180	180	335	0.7	2.6	2.7	4.0
Loli_per	0	0	77	0	0.4	0.4	0.0	0.0	0	0	26	0	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Lotu_cor	0	0	0	0	0.0	0.3	0.0	0.0	26	0	26	26	0.0	0.0	0.3	0.0	0	0	0	0	0.0	1.0	0.4	0.9
Lupi_pol	0	0	52	26	0.0	5.7	46.7	73.7	0	0	0	0	0.4	12.6	30.1	65.1	26	0	26	0	0.0	10.4	47.3	71.6
Luzu_luz	155	180	258	180	0.4	0.4	0.4	1.7	335	387	387	284	0.3	0.9	1.3	0.9	180	309	284	284	2.3	1.9	0.3	0.3
Lych_flo	103	52	52	77	0.6	0.1	0.4	0.4	4898	1701	464	1160	2.1	2.9	2.4	0.9	0	0	0	26	0.0	0.0	0.0	0.0
Medi_lup	0	26	0	0	0.0	0.3	0.0	0.0	0	0	0	0	0.0	0.4	0.4	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Ment_arv	0	0	0	0	0.0	0.0	0.0	0.0	52	103	26	103	0.0	0.4	0.1	0.3	0	0	0	0	0.0	0.0	0.0	0.0
Myos_nem	0	0	0	0	0.1	0.0	0.0	0.0	1108	387	52	103	2.4	1.9	1.0	1.0	0	0	0	0	0.0	0.0	0.0	0.0
Nard_str	0	0	0	52	0.4	0.4	0.0	1.3	26	0	0	52	2.7	7.7	3.0	0.4	722	232	0	155	43.0	17.3	12.6	11.0
Pedi_syl	0	0	0	0	0.0	0.0	0.0	0.0	77	0	0	0	0.6	0.0	0.4	0.0	0	0	0	0	0.9	0.0	0.0	0.0
Phyt_orb	26	0	0	0	0.9	0.9	0.4	0.0	0	0	0	0	0.0	0.0	0.0	0.3	0	0	0	0	0.0	0.3	0.6	0.6
Phyt_spi	103	0	52	26	3.4	1.7	1.9	2.1	0	0	0	26	0.4	0.6	1.1	0.3	129	26	103	26	2.4	2.3	2.4	2.7
Pimp_sax	0	0	0	0	0.3	0.1	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.4	0	26	0	0	0.0	0.4	0.1	0.0
Plan_lan	361	954	180	155	6.3	4.7	4.0	4.0	26	77	77	52	2.4	2.0	1.1	1.0	26	77	52	155	2.3	8.7	7.7	6.3
Poac_hai	26	52	26	52	2.9	2.1	4.9	12.3	0	0	26	0	1.0	1.1	4.9	1.6	0	0	0	0	2.9	2.6	2.7	4.7
Poap_alu	0	0	26	0	0.0	0.0	0.0	0.0	0	26	0	0	0.0	0.0	0.0	0.3	0	0	0	0	0.0	0.0	0.0	0.0
Poap_rat	0	155	52	26	0.9	6.3	1.1	0.3	129	52	26	284	1.1	1.6	1.4	2.0	0	0	52	0	0.4	0.0	0.0	0.3
Poat_riv	180	26	335	26	0.0	0.3	1.6	0.0	180	309	26	541	0.3	0.4	0.7	1.7	0	0	0	26	0.0	0.0	0.0	0.0
Pote_ere	103	52	0	77	1.0	1.1	1.3	2.0	541	464	258	26	1.3	3.0	1.6	1.7	799	361	361	206	4.3	6.7	5.9	6.3
Pote_pal	0	0	0	0	0.0	0.0	0.0	0.0	52	26	26	0	0.9	0.3	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Prim_ver	26	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.1	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Ranu_acr	129	129	232	103	4.6	3.0	3.9	3.1	1263	464	361	670	16.0	10.3	10.0	5.0	103	0	0	77	1.3	2.0	2.6	2.6
Ranu_sce	0	0	0	0	0.4	0.0	0.1	0.0	0	26	0	26	0.4	0.4	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Rume_ace	52	77	129	180	7.1	3.1	4.7	4.6	0	0	52	52	3.7	1.7	2.3	2.7	0	0	0	26	1.6	5.0	5.6	3.0
Rume_ac2	26	52	77	52	0.0	0.0	0.0	0.0	0	26	26	77	0.0	0.0	0.1	0.0	26	26	155	155	0.0	0.0	0.0	0.0
Sang_min	0	0	0	0	0.0	0.4	0.0	0.0	26	26	0	0	0.0	0.0	0.0	0.0	0	0	26	0	0.0	0.0	0.0	0.0
Sang_off	26	0	0	0	8.4	9.3	4.0	6.7	26	26	0	0	2.9	7.6	8.7	5.0	0	0	0	0	15.3	11.9	8.7	9.4
Saxi_gra	0	77	77	103	0.4	0.7	1.0	1.6	103	26	77	258	0.4	0.7	1.1	1.1	0	0	0	232	0.0	0.0	0.0	0.3
Tara_off	0	26	77	52	1.9	2.4	2.3	1.1	0	0	0	26	1.9	0.9	0.7	0.6	0	0	26	77	0.0	1.3	0.7	0.0
Thym_pul	0	0	0	0	0.0	0.4	0.4	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0	52	0	0	0.0	1.0	5.7	2.6
Trif_dub	0	52	0	0	0.0	0.4	0.0	0.0	0	26	0	0	0.0	0.4	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Trif_pra	180	52	155	0	7.3	4.9	7.6	1.6	129	103	26	52	6.0	3.4	3.6	1.6	0	0	0	0	0.9	5.7	5.4	1.7
Trif_rep	541	26	232	206	3.1	0.9	0.7	1.7	129	155	77	77	4.7	1.9	1.4	1.3	0	26	26	103	0.0	4.6	2.6	1.4
Trif_spa	0	26	0	0	0.0	0.0	0.0	0.0	825	464	335	516	7.1	2.1	2.0	1.3	0	0	0	26	0.0	0.0	0.0	0.0
Trol_eur	0	0	0	0	1.1	0.1	1.0	0.9	0	26	0	0	1.6	5.0	4.0	2.1	0	0	0	0	0.0	0.0	0.0	0.0
Vacc_myr	26	0	155	0	0.0	0.4	0.0	0.0	0	0	0	0	0.0	0.3	0.0	0.3	103	52	26	52	3.3	3.1	2.7	4.9
Vale_dio	0	0	0	0	0.0	0.0	0.0	0.0	77	0	26	0	3.9	1.3	1.7	1.1	0	0	0	0	0.0	0.0	0.0	0.0
Vero_arv	0	26	1495	0	0.0	0.4	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	26	26	0.0	0.0	0.0	0.0

Vero_cha	129	206	232	309	6.9	8.6	6.1	8.3	26	52	52	77	1.6	0.6	2.1	7.4	26	284	77	77	3.3	4.6	4.3	3.7
Vero_off	0	0	0	0	0.0	0.0	0.0	0.1	0	0	0	26	0.0	0.0	0.0	0.0	412	77	232	52	1.9	0.3	0.6	0.4
Vero_ser	0	361	284	26	0.0	0.0	0.0	0.0	26	206	0	0	0.0	0.0	0.0	0.0	26	0	0	0	0.0	0.9	3.1	2.3
Viol_pal	0	0	0	0	0.0	0.0	0.0	0.0	155	155	26	26	0.4	0.4	0.3	0.0	0	0	0	0	0.0	0.0	0.0	0.0
Viol_riv	0	0	26	26	0.1	0.5	0.6	0.0	0	0	26	26	0.0	0.0	0.4	0.0	0	26	52	0	0.3	0.9	0.4	0.4
Group 2:																								
Arab_tha	0	335	26	0					0	26	0	722					0	0	0	0				
Arte_vul	0	0	0	0					0	0	0	0					0	52	0	0				
Betu_pub	52	0	26	26					52	129	103	103					26	26	52	0				
Caps_bur	0	0	26	0					0	0	0	0					0	0	0	0				
Care_dis	0	0	0	0					0	0	284	0					0	0	0	0				
Chen_gla	26	0	26	26					0	0	0	0					0	0	0	0				
Cony_can	0	26	0	0					0	0	0	0					26	0	0	0				
Epil_ang	129	77	52	103					26	412	129	103					335	26	77	206				
Epil_cil	0	77	52	129					103	335	206	77					26	26	52	180				
Epil_hir	0	0	0	0					26	0	0	0					0	0	0	0				
Glyc_dec	0	0	0	0					26	77	0	0					0	0	0	0				
Junc_fil	0	0	0	0					52	0	0	0					0	0	0	0				
Pers_hyd	0	0	26	0					0	0	0	0					0	0	0	0				
Pers_mac	0	0	0	0					0	0	0	26					26	0	0	26				
Plan_med	26	0	0	0					0	0	0	0					0	0	0	0				
Poaa_nnu	0	26	0	26					103	26	0	0					0	0	0	0				
Prun_vul	0	0	0	0					77	0	0	0					0	0	0	0				
Rume_obt	0	0	26	0					0	0	0	0					0	0	0	0				
Sagi_pro	52	464	0	0					3119	619	129	0					0	0	232	206				
Scle_ann	26	0	0	0					0	0	0	0					0	0	0	0				
Sonc_arv	0	0	0	26					0	0	0	0					0	0	0	0				
Sper_rub	0	0	0	26					0	0	0	0					0	0	0	0				
Stel_als	77	438	103	77					0	26	309	129					0	52	52	52				
Stel_aqu	0	0	0	0					0	26	0	0					0	0	26	26				
Stel_med	0	0	77	0					0	0	0	0					0	0	0	0				
Urti_dio	129	129	696	129					180	0	103	129					0	52	0	26				
Vero_bec	0	0	0	0					0	77	0	26					0	0	0	0				
Viol_arv	0	52	0	284					0	0	0	0					0	26	52	52				
Group 3:																								
Aego_pod					0.0	0.0	0.1	0.0					0.0	0.0	0.0	0.0					0.0	0.0	0.0	0.0
Alop_pra					2.1	2.4	5.9	0.4					2.1	1.1	2.0	1.3					0.0	0.0	0.0	0.0
Anem_nem					1.4	1.3	2.3	1.7					0.9	3.9	1.3	1.9					2.9	1.4	3.1	1.6
Ange_syl					0.0	0.0	0.0	0.0					0.6	0.4	2.3	1.0					0.0	0.0	0.4	0.0
Anth_syl					2.9	0.4	0.4	0.0					0.3	0.0	0.0	0.0					0.0	0.0	0.0	0.0
Arni_mon					0.0	0.0	0.0	0.0					0.0	0.0	0.0	0.0					0.0	0.0	0.9	0.0
Arrh_ela					2.4	0.4	1.6	0.0					1.9	0.4	0.4	0.3					0.0	0.0	0.4	0.0
Beto_off					0.4	0.0	0.0	0.0					0.0	0.0	0.0	0.0					0.0	0.4	0.1	0.4
Briz_med					0.0	0.0	0.0	0.0					0.0	0.0	0.0	0.7					0.0	0.9	0.4	0.4
Brom_hor					0.0	1.1	0.0	0.0					0.0	0.0	0.1	0.4					0.0	0.0	0.0	0.0
Cala_epi					0.0	0.0	0.0	0.0					0.0	0.0	0.3	0.6					0.0	0.0	0.0	0.0
Calt_pal					0.0	0.0	0.0	0.0					2.7	0.6	0.6	5.4					0.0	0.0	0.0	0.0
Caru_car					0.4	0.0	0.0	0.0					0.0	0.0	0.0	0.0					0.0	0.0	0.0	0.0
Cent_jac					0.4	0.4	0.1	0.0					0.0	0.4	0.0	0.0					0.0	0.0	0.0	0.0

Cent_mon	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.1	0.3	0.0
Cirs_vul	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.0	0.0
Colc_aut	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crep_pal	0.4	0.6	0.3	0.3	0.0	0.0	0.0	0.4	0.0	0.3	0.0	0.0
Dact_glo	2.7	0.9	0.7	0.0	0.4	0.3	0.4	0.1	0.0	0.3	0.0	0.3
Dact_maj	0.0	0.0	0.0	0.0	0.7	0.1	0.7	0.4	0.0	0.0	0.0	0.0
Desc_fle	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.6	3.3	2.9	5.1
Epil_tet	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Equi_arv	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
Equi_flu	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Equi_pal	0.4	0.0	0.0	0.0	0.3	0.0	0.1	0.3	0.0	0.0	0.0	0.0
Equi_syl	0.0	0.0	0.0	0.0	0.4	0.0	1.1	0.0	0.0	0.0	0.0	0.0
Fest_ovi	0.4	1.4	0.6	1.4	0.3	0.4	2.0	1.1	3.6	8.9	1.7	5.0
Fili_ulm	0.3	0.0	0.6	0.0	1.0	1.9	0.4	1.6	0.0	0.0	0.0	0.0
Gali_alb	0.4	0.7	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
Gali_bor	0.6	1.1	1.6	1.9	1.3	1.0	3.0	1.3	0.4	0.3	1.4	2.3
Gali_ver	0.4	0.9	0.9	1.4	0.0	0.0	0.7	1.1	0.3	0.0	0.3	0.3
Geni_tin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0
Gera_pal	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Geum_riv	0.4	0.1	0.3	0.6	0.7	0.0	1.4	2.0	0.0	0.0	0.0	0.0
Hera_sph	1.3	0.0	0.7	0.3	0.4	0.3	0.0	0.0	0.0	0.0	0.4	0.0
Hier_lac	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Junc_con	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
Koel_pyr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Leon_his	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.9	0.0
Lili_mar	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lotu_ped	0.0	0.0	0.0	0.0	8.0	0.6	10.3	1.9	0.0	0.0	0.0	0.0
Luzu_cam	1.3	2.3	2.0	2.6	1.7	2.0	2.7	1.1	3.0	2.1	2.4	1.6
Lyth_sal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Past_sat	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Plat_chl	0.9	0.0	0.3	0.0	0.3	0.4	0.4	0.4	0.7	0.6	0.6	0.3
Poly_ser	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Poly_vul	0.4	0.1	0.6	0.0	0.0	0.3	0.7	0.0	0.9	5.3	4.1	2.7
Ranu_nem	0.3	0.0	0.4	0.1	0.4	0.9	0.3	0.6	0.4	1.3	0.9	1.4
Ranu_rep	0.9	0.3	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.4	0.4	0.0
Rhin_min	5.1	2.6	2.4	2.3	3.1	1.4	2.1	0.9	2.3	3.4	3.6	3.9
Scab_col	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
Stel_gra	2.1	1.1	1.1	1.0	0.0	0.0	1.4	0.7	0.0	0.4	1.4	1.4
Succ_pra	0.1	0.0	0.1	0.0	1.1	0.9	1.0	0.3	0.0	0.0	0.0	0.0
Teph_hel	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
Thes_pyr	0.3	0.3	0.9	1.3	0.0	0.0	0.4	0.0	0.0	2.9	1.3	2.1
Trag_pra	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trif_med	0.0	0.7	0.4	0.0	0.0	0.0	0.0	2.3	2.4	0.4	2.3	0.0
Tris_fla	6.7	2.6	2.7	2.3	0.7	0.4	1.7	1.0	0.0	0.1	0.0	0.0
Vacc_vit	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.1
Vale_off	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Vici_ang	0.0	0.0	0.4	0.4	0.7	0.0	0.7	0.3	0.0	0.0	0.0	0.0
Vici_cra	1.9	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vici_hir	0.4	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Vici_sat	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vici_sep	1.1	0.0	0.7	0.0	0.4	0.4	0.0	0.1	0.0	0.0	0.1	0.0
Viol_tri	0.3	1.3	0.6	1.9	0.0	0.0	0.0	0.3	0.4	0.4	0.4	0.4

Chapter 4

Constraints in the restoration of mountain meadows invaded by the legume *Lupinus polyphyllus*

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Abstract

Semi-natural grasslands invaded by the legume *Lupinus polyphyllus* need the introduction of target species in order to promote highly endangered native target plant communities. However, which techniques are best suited to achieve both invader control and the introduction of target species at the same time? Few studies have investigated restoration techniques that support native plant communities in mountain meadows and control the invader simultaneously. We employed the restoration techniques seed bank activation and green hay transfer in combination with manual removal of the invasive *L. polyphyllus* on three types of grassland (*Nardus* grassland, mesic and wet mountain hay meadows) in the low mountain ranges of the Rhön UNESCO Biosphere Reserve in central Germany. Vegetation reacted differently to specific measures depending on the type of grassland. *L. polyphyllus* cover could be effectively reduced by *L. polyphyllus* removal in *Nardus* grassland and mesic hay meadow sites, but not in wet meadows. In *Nardus* grassland, the cover of target species was lowered by the application of green hay. The target species cover of wet mountain hay meadows declined in response to seed bank activation. When restoring mountain meadows, restoration practitioners should thus first consider the specific vegetation types. As our study indicates that target mountain meadow species may react negatively to restoration measures in the short term, the long-term effects of the application of green hay should be studied.

Implications for Practice

- Green hay application might not be a suitable tool for restoring *Nardus* grassland since small species might not be able to grow through the plant material layer; in that case it should be removed before the following growing season.

- *Lupinus polyphyllus* cover on restoration sites can be reduced by manual removal of all parts of the plants, but a lasting reduction requires at least repeated applications.
- Reinvasion of *Lupinus polyphyllus* into restoration sites must be prevented with an appropriate management, e.g. early and/or repeated mowing.
- Active restoration through seed bank activation failed to promote mountain meadow target species and reduced the cover of wet mountain hay meadow target species.

Introduction

Semi-natural grassland ecosystems are among the most species-rich habitats in Central Europe (Habel et al. 2013). Semi-natural mountain meadows, in particular, provide habitats for many rare and endangered species. They are of high conservation value for both plant and animal species (Müller et al. 2014) but threatened by land use change and abandonment. Especially, land abandonment and deficient management might foster the spread of invasive species (Matus et al. 2003).

Since invasive species often act as ecosystem engineers, they may change environmental conditions and gain competitive advantages leading to the local disappearance of native plant species (Vilà et al. 2011; Lee et al. 2017). Consequently, they are regarded as a threat to biodiversity (Gallardo & Aldridge 2013). In many regions, invasive species massively complicate biodiversity and nature conservation efforts (Vilà et al. 2010) and invasive species control is hence a priority in the execution of many restoration projects (D'Antonio & Meyerson 2002).

Among the most common invasive species in Europe is *Lupinus polyphyllus* Lindl. (Nentwig et al. 2017). Being a tall-growing legume, *L. polyphyllus* has the potential to change the nutrient flows of ecosystems and to act as an ecosystem engineer. Thus, it meets the IUCN criteria for invasive species and is listed on the blacklist of invasive species in Europe (Nehring et al. 2013). Originating in Pacific North America, *L. polyphyllus* was brought to Europe in the nineteenth century to be grown as green manure and for ornamental purposes. However, it escaped from gardens and fields and is nowadays a common sight in road ditches, riverbanks, and meadows of European low mountain ranges (Valtonen et al. 2006). In the UNESCO Rhön Biosphere Reserve, the invasion of semi-natural species-rich mountain meadows by *L. polyphyllus* has been observed during the last three decades (Volz 2003; Otte & Maul 2005; Klinger et al. 2019). Located in central Germany, the Rhön Mountains are among the last refuges for Central European semi-natural mountain meadow ecosystems and their associated plant species. The ongoing spread of *L. polyphyllus* poses a serious threat to these mountain meadow species (Volz 2003; Otte & Maul 2005). *L. polyphyllus* was introduced to ameliorate soils of Rhön spruce afforestations in the 1940s. In recent years, it has spread and invaded the surrounding mountain meadows, probably due to changes in the traditional mowing regime (Volz 2003; Klinger et al. 2019). The UNESCO Biosphere Reserve Rhön was founded in 1991 in order to protect, among others, the largest Central European population of Black Grouse (*Tetrao tetrix*; Müller et al. 2007). To this end, annual mowing, traditionally carried out in July, was delayed to August or September. While early mowing had inhibited the spread and establishment of *L. polyphyllus* in the meadows, the shift to a later mowing date gave it opportunity to reproduce and disperse ripe seeds before mowing. Over time, this altered management for conservation resulted in a strong increase of *L. polyphyllus* cover. Nowadays many meadows in the nature conservation area are heavily invaded by *L. polyphyllus*. As it forms stands with up to 90% cover, it shades and outcompetes small-stature light-demanding species (Thiele et al. 2010) and thereby alters the vertical vegetation structure (Otte & Maul 2005). Moreover, tall-growing competitive species that benefit from *L. polyphyllus* invasion homogenize the diversity within and among the different vegetation types (Hansen et al. 2020). To address this issue, since 2015 the mowing dates have been gradually brought forward again (T. Kirchner, personal communication, 2017).

Generally, in order to control invasive plant species in grassland ecosystems, different management techniques have proven successful, e.g. grazing, mowing, cutting of stems or reproductive parts, or even chemical control (Tyler et al. 2006; Nielsen et al. 2007; Ramula 2020; and see also Kettenring & Adams 2011). Thereafter, target communities may be promoted by sowing, turf or green hay transfer or soil seed bank activation (Kiehl et al. 2010; Klaus et al. 2018; Wagner et al. 2021). Many plant species build up persistent soil seed banks to overcome unfavorable times (Thompson et al. 1997). Hence, depending on the number of target species lost from the vegetation, their respective densities, and, to a lesser extent, overall seed density, species richness, and evenness in the soil seed banks, mechanical sward disturbance for soil seed bank activation may be a simple and inexpensive restoration tool. However, grassland communities are considered rather stable and therefore grassland species do not typically produce long-term persistent or many seeds (Bossuyt & Honnay 2008; Klaus et al. 2018). Accordingly, the soil seed banks of grassland sites targeted for restoration have to be investigated, to determine their suitability for restoration (Bossuyt & Honnay 2008). The restoration potential of the soil seed banks of the three most characteristic vegetation types in the Rhön Biosphere Reserve has been studied by Ludewig et al. (2021). Since the seed banks were specific to the vegetation types in question and contained the respective target species, they were deemed suitable for restoration. Moreover, the authors could not find any effects of the invasive *L. polyphyllus* on seed density and species richness of the seed bank. However, they recommended to actively introduce species not present in the soil seed bank, for instance by the application of green hay, that is, seed containing plant material transferred directly after cutting (Ludewig et al. 2021).

Restoration of degraded ecosystems is costly and hence projects must be planned carefully (D'Antonio & Meyerson 2002; Nielsen et al. 2007). To ensure that resources are used with maximum efficiency, the effects of restoration measures have to be tested and evaluated. In the case of invaded ecosystems, it is crucial to test restoration techniques that simultaneously control invasive species and promote the recovery of native species. Moreover, studies focusing on concrete management actions are urgently needed (Habel et al. 2013; Catalano et al. 2019). While many studies applied the abovementioned restoration measures on floodplain meadows (e.g. Donath et al. 2007; Klimkowska et al. 2007; Harnisch et al. 2014), only few studies used them to restore mountain meadows (but see Scotton 2019; Sullivan et al. 2020). To our knowledge, there have been no studies simultaneously exploring the effects of these restoration measures and of invasive plant removal across a range of mountain meadow communities. The present study focuses on restoring three different types of mountain meadows invaded by the legume *Lupinus polyphyllus* Lindl. in the UNESCO Rhön Biosphere Reserve. We applied a combination of different grassland restoration methods to restore plant species composition of three characteristic vegetation types in the Rhön region (namely *Nardus* grassland, mesic and wet mountain hay meadows). We used seed bank activation combined with application of green hay from uninvaded donor sites to overcome possible seed bank depletion. We investigated the vegetation development before (2017) and in 3 years after the treatments (2018, 2019, and 2021). We could not sample in 2020 due to the Covid pandemic.

We address the following research questions:

1. Is seed bank activation an effective method for restoring different invaded mountain meadow vegetation types, that is, for reducing *L. polyphyllus* cover and/or promoting typical mountain meadow species?
2. Is the additional application of green hay necessary for restoring the different mountain meadow vegetation types, that is, to reduce *L. polyphyllus* cover and/or promote typical mountain meadow species?
3. Is manual removal of *L. polyphyllus* needed in order to reduce *L. polyphyllus* cover and hence to promote native species effectively?

Methods

Study Area

The study area is located in the UNESCO Rhön Biosphere Reserve in Central Germany, which was founded in 1991 and comprises an area of 2,400 km^2. The Rhön region is characterized by extended grassland areas used as hay meadows or for pastoral sheep herding. Low fertilizer input over centuries and the low-intensity land use resulted in species-rich semi-natural grasslands. The most frequent vegetation types that developed here are *Nardus* grasslands, mesic and wet mountain hay meadows (Otte & Maul 2005). As these vegetation types form habitats for many rare and endangered species they are of high conservation value and thus protected by the European Habitats Directive (92/43/EEC, habitat type 6,520: mountain hay meadows and 6,230: species rich *Nardus* grasslands). The dominant bedrock types in the Rhön region are Tertiary basalt rock, Triassic sandstones, and shell limestone (Klausing 1988). Although soils that developed on basaltic parent material are usually well supplied with cations, high precipitation and a traditional land use have resulted in low nutrient availability and low pH values in the area (Puffe & Zerr 1988). The precipitation on the highest peak in the region (Mt. Wasserkuppe with 950 m above sea level) amounts to 1,135 mm (mean of 1981–2010; DWD 2019) and the mean annual temperature is 5.5°C (mean of 1981–2010; DWD 2019).

The investigated vegetation types (i.e. *Nardus* grassland, mesic mountain hay meadow and wet mountain hay meadow) are located in the nature conservation area "Lange Rhön" that has been designated as such in 1982. All grasslands within the nature reserve are mown between 15 June and 1 August, with mowing dates varying between individual grasslands. Mowing may not be carried out before 15 June due to the protection of ground-nesting birds. Aftermath grazing by migratory sheep flocks is carried out after 15 August (Klinger et al. 2021). In the High Rhön, mountain hay meadows deliver annual yields between 2.5 and 4 tons dry matter/ha (Volz 2003). *Nardus* grasslands and mountain hay meadows developed on formerly forested sites after cutting and forest pasturing. *Nardus* grasslands can be found on shallow soils with low nutrient supply, whereas mesic and wet hay meadows occur on more base-rich soils with a good water and nutrient supply. The vegetation types are characterized by the regular occurrence of the following plant species: *Nardus* grasslands—*Nardus stricta*, *Potentilla erecta*, and *Galium saxatile*; mesic mountain meadows—*Geranium sylvaticum*, *Trisetum flavescens*, and *Alchemilla monticola*; wet mountain meadows—*Bistorta officinalis*, *Trollius europaeus*, and *Deschampsia cespitosa* (Peppler-Lisbach & Petersen 2001). The seed density of the soil seed bank (of all species and the target species) varies between the vegetation types with wet mountain hay meadows having higher seed densities than mesic mountain hay meadows and *Nardus* grasslands having the lowest density (Ludewig et al. 2021). This might influence restoration success via seed bank activation. Differences between these grassland types were also mentioned by Hansen et al. (2020), who showed that they responded differently to *L. polyphyllus* invasion.

Experimental Design

We set up experimental blocks in each of the investigated vegetation types. For each vegetation type, we selected two heavily invaded meadows and thus, restoration treatments were applied in six experimental blocks. In each restoration block we implemented two parallel strips separated by a guard row of 10 m. Each of the strips contained six 5 × 5–m permanent subplots (arranged in line) with a buffer of 5 m between the subplots, respectively. In each strip, two subplots each were assigned at random to each of three disturbance treatments, including: (1) soil seed bank activation—the soil seed bank of the upper 10 cm was activated with a cultivator, increasing the amount of bare soil and thus creating gaps in the vegetation; (2) seed bank activation plus *L. polyphyllus* removal—we manually removed all *L. polyphyllus* plants prior to the seed bank activation. *L. polyphyllus* plants were removed with weeding forks carrying two prongs of 27 cm, enabling us to uproot the whole plants and (3) control plots without any treatment. Finally, one of each pair of strips was covered with green hay from donor sites (Fig. 1). Thus, four repetitions for every treatment were set up. For each vegetation type, the two restoration sites were located no more than 5 km from their corresponding donor site (Fig. 2). The donor sites were *L. polyphyllus*-free, species-rich, and belonged to the three investigated vegetation types, thus each restoration block received green hay from the corresponding vegetation type. The green hay of 5,000 m^2 was freshly cut and transferred to the restoration sites on the same day. The chosen amount enabled spreading of green hay from one donor site onto two recipient sites. The green hay was loosely spread, and the resulting hay layer was between 10 and 20 cm thick, with some variability due to the texture of the material, as well as target vegetation type. The restoration measures (seed bank activation and green hay application) for each vegetation type were carried out at the same day between July and the end of August (*Nardus* grassland: 8 July 2017; mesic hay meadows: 8 August 2017; wet hay meadows: 30 August 2017). This was the first cut of these sites in that year.

Vegetation surveys on the 5 × 5–m subplots were carried out in 2017 prior to the restoration experiment and repeated at the end of May in 2018, 2019, and 2021, resulting in 72 vegetation relevés per year. On each donor site the vegetation was surveyed on three 5 × 5–m plots in 2017 in those areas where the green hay was cut, resulting in nine donor site plots. Plant composition and cover were estimated using the modified Braun-Blanquet scale (Braun-Blanquet 1964). Taxonomy was based on Wisskirchen and Haeupler (1998).

Green Hay Sampling

In order to analyze the seed content of the transferred green hay, for each vegetation type, a composite sample of 160 L of plant material was taken after transfer. Germinable seed content of these samples was determined using the emergence method (Ter Heerdt et al. 1996). The green hay samples were threshed, and the fine material was spread in layers of approximately 10 mm on a sterile potting compost (Fruhstorfer Pikiererde LD80 Archut, Hawita Group)–sand mixture (2:1 relation) in styrofoam trays (18 × 28 cm). For the samples of each vegetation type, we prepared 30 trays in

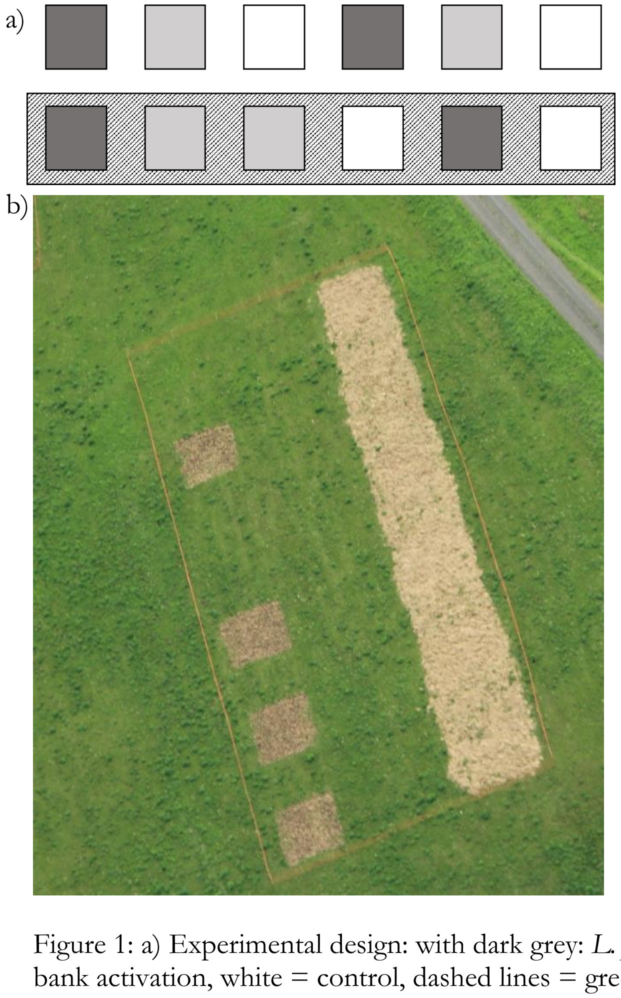

Figure 1: a) Experimental design: with dark grey: *L. polyphyllus* removal, light grey: seed bank activation, white = control, dashed lines = green hay application b) the restoration experiment conducted on Nardus grassland; each treatment square is 5 x 5 m

this way. To control for windborne seeds, additionally six trays only with sterile potting compost were randomly placed among the others; species that germinated in these trays were excluded from further analysis. In total, 96 styrofoam trays were exposed to optimized controlled greenhouse conditions for temperature (T_{day}: 18–24°C, T_{night}: 12–18°C), light (>10,000 lx from 06:00 to 22:00 hours) and humidity (<70%) and watered every third day. Emergence took place from November to the end of December 2017, subsequently the green hay samples were cold-wet stratified under outdoor conditions. After stratification, the experiment continued until no more seedlings emerged in August 2018. Emerged seedlings were identified using the key of Muller (1978). Seedlings that could not be identified were planted into pots and grown until identification was possible with Jäger et al. (2017).

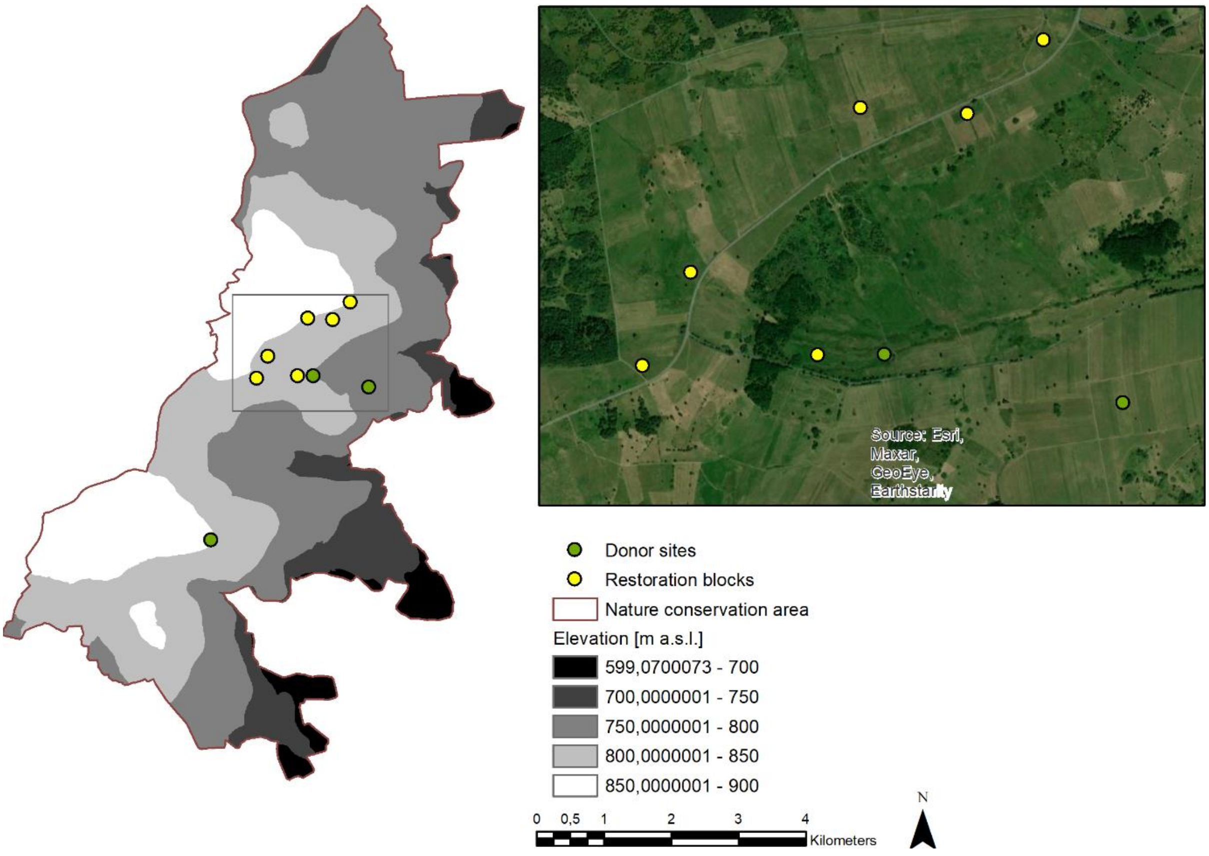

Figure 2: Location of the restoration blocks and donor sites in the nature conservation area and a zoom-in insert map

Statistical Analyses

Phytosociological character species of the investigated vegetation types serve as target species in this study and were identified using appropriate literature (Oberdorfer 1977; Peppler-Lisbach & Petersen 2001; see Tables S2–S4). The vegetation cover data were transformed into percentage values (*r* = 0.01%, + = 0.5%, 1 = 3.0%, 2 = 15%, 3 = 38%, 4 = 62.5%, 5 = 87.5%) for statistical analyses.

In order to investigate the effects of the different treatments on *L. polyphyllus* and target mountain meadow species, we ran four mixed effect models. *L. polyphyllus* cover and cumulative wet mountain hay meadow target species cover were log+1-transformed and the models calculated with the lmer function of the lme4 package (version 1.1.27.1; Bates et al. 2015). The cumulative cover of *Nardus* grassland species and the cumulative cover of mesic mountain hay meadow target species were analyzed with beta distribution (Damgaard & Irvine 2019), logit-link generalized linear mixed effect models using the glmmTMB function of the glmmTMB package (version 1.1.2; Brooks et al. 2017). The model structure was the same in all four models: we entered *L. polyphyllus* cover and the cover of the three groups of target species as response variable, vegetation type (three levels: *Nardus* grassland, mesic hay meadow, and wet hay meadow), disturbance treatments (three levels: i.e. *L. polyphyllus* removal, seed bank activation, and control), the transfer of green hay (two levels: yes and no), and year of the vegetation surveys (four levels: 2017, 2018, 2019, and 2021) with interactions served as explanatory variables. Hay strip ID (12 levels) nested in Block ID (six restoration sites) formed the random structure of the models. By this we accounted for the similarity in environmental conditions due to the spatial proximity between the strips within one restoration site. The significance of the fixed variables was tested with Wald chi-square test using the analysis of variance function of the car package (Fox & Weisberg 2019). Vegetation-type-specific treatment effects were obtained by calculating contrasts to the respective treatment group in 2017 within the vegetation types using the emmeans package (version 1.6.3; Lenth et al. 2021).

We computed nonmetric multidimensional scaling (NMDS) ordinations for each type separately with 20 random starts and three dimensions based on a Bray–Curtis dissimilarity matrix and including *L. polyphyllus* to identify main floristic gradients and changes within the vegetation types over time using the vegan package (version 2.5.6; Oksanen et al. 2019). We had one NMDS ordination calculated with the donor sites included to see if the restoration plots would shift in this direction over time, that is, donor and restoration sites become more similar regarding their vegetation composition (Fig. S1a) and one NMDS without the donor sites to enhance visibility of the single restoration relevés (Fig. S1b).

Species composition of restoration sites was compared to species composition of donor sites in order to assess performance of the different restoration measures. To this end, we calculated the mean Bray–Curtis similarity index (1 − Bray–Curtis dissimilarity) between the vegetation relevés of the restoration areas and the relevés surveyed on the donor sites (see Fig. S2).

All data analyses were carried out in the R statistical environment (version 4.0.3; R Core Team 2020).

Results

In total, we found 120, 137, 131, and 147 plant species in 2017, 2018, 2019, and 2021 in the aboveground vegetation, respectively. Numbers of species found in the three vegetation types and years are listed in Table S1. The total numbers of species increased slightly, whereas the numbers of target species in all three vegetation types stayed roughly the same during the investigation period.

A total of 25,769 seedlings belonging to 57 species germinated from the green hay samples. In total 29 species, 8 of which were target species, germinated from *Nardus* grassland samples; 37 species including 8 target species germinated from the mesic; and 42 species including 11 target species germinated from the wet hay meadows green hay samples. The highest number of individuals of target species germinated from the wet hay meadow samples, the lowest in the *Nardus* grassland samples (Table S2).

Species that were not present on the restoration sites in 2017 and only appeared in subsequent years are *Phyteuma orbiculare* in mesic hay meadows, *Agrostis canina*, *Galium palustre*, *Pedicularis sylvatica*, *Poa palustris*, and *Stellaria alsine* in wet hay meadows (Table S3). In turn, *Juncus effusus* was very abundant on wet hay meadows in 2017 but vanished completely in 2019 and 2021 (Table S4) just like *Cynosurus cristatus* in mesic hay meadows (Table S3).

Effects of the Restoration Measures on the Target Species Cover

The cover of *Nardus* grassland target species was significantly affected by the vegetation type (which could be expected), green hay application, and the interaction between year and green hay application. Year, green hay application, the interaction between year and green hay application, as well as the interaction between disturbance treatment and green hay application had a significant effect on the cover of characteristic mesic mountain hay meadow species. The cover of wet mountain hay meadow target species was significantly affected by the vegetation type, disturbance treatment, and the interaction between year and disturbance treatment (Table S5).

Generally, the data showed large variation, but some patterns could be revealed by comparisons to the plots surveyed in 2017 that serve as control plots. On *Nardus* grassland, the cover of target species was significantly lowered by the application of green hay material on control plots and on *L. polyphyllus* removal plots during all monitoring years, on seed bank activation plots target species cover was lower in 2018 and 2019 compared to the control plots in 2017. On plots where green hay was not applied, target species cover was lower on control and *L. polyphyllus* removal plots in 2021 (Fig. 3A). On mesic hay meadows the target species cover was significantly increased with the application of green hay on control and seed bank activation plots in 2019 compared to 2017. This effect disappeared again in 2021. On *L. polyphyllus* removal plots target species cover was significantly lowered in 2021 (Fig. 3B). Seed bank activation with and without green hay application as well as *L. polyphyllus* removal without green hay application resulted in a significantly lower target species cover compared to the respective treatment in 2017 on wet hay meadows (Fig. 3C).

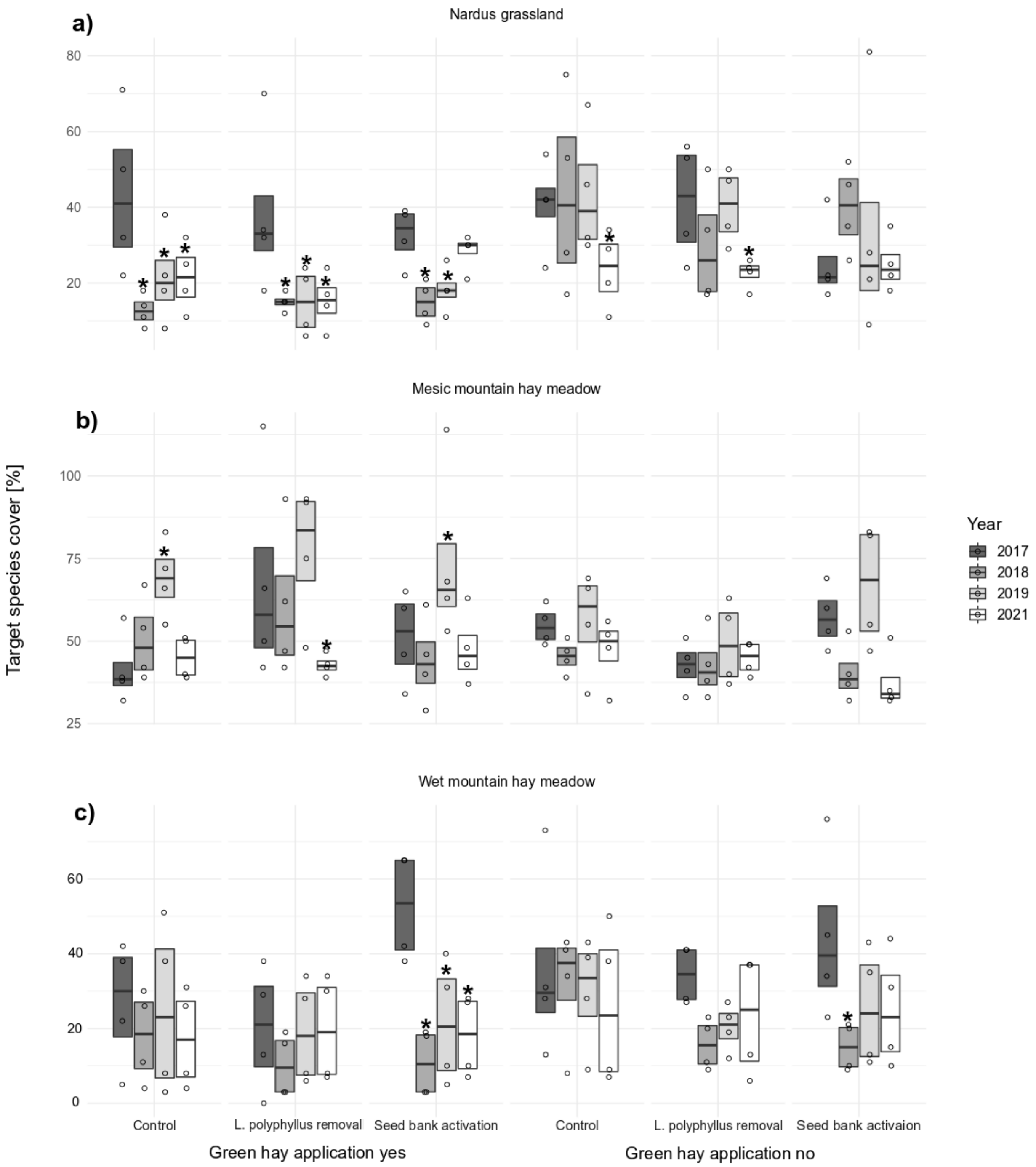

Figure 3: Box and scatter plots of the target species cover of a) Nardus grassland, b) mesic hay meadows, c) wet hay meadows during the study period. The boxes represent the upper and lower quartiles and the line the median. Asterisks indicate significant differences of pairwise comparisons within the vegetation type to the respective plots of 2017 ($p < 0.05$, contrasts to the respective treatment of 2017)

Effects of the Restoration Measures on the *L. polyphyllus* Cover

Year, disturbance treatment, and the interaction between vegetation type and year significantly affected *L. polyphyllus* cover (Table S5). *L. polyphyllus* cover was significantly lower in plots where the plant had been removed and green hay was applied in all monitoring years on *Nardus* grasslands and mesic hay meadows. Furthermore, *L. polyphyllus* cover was also lower on control plots and plots where the seed bank was activated, and green hay was applied in 2019 on mesic hay meadows. On sites where green hay was not applied *L. polyphyllus* cover was lower on plots where the plants had been removed on *Nardus* grasslands in 2018 and 2019 and on mesic hay meadows in all monitoring years. Apart from that mesic hay meadow control and seed bank activation plots had significantly lower *L. polyphyllus* cover in 2018 and 2019 (Fig. 4A & 4B). Removal of *L. polyphyllus* had no significant effects on its cover in subsequent years on wet hay meadows (Fig. 4C).

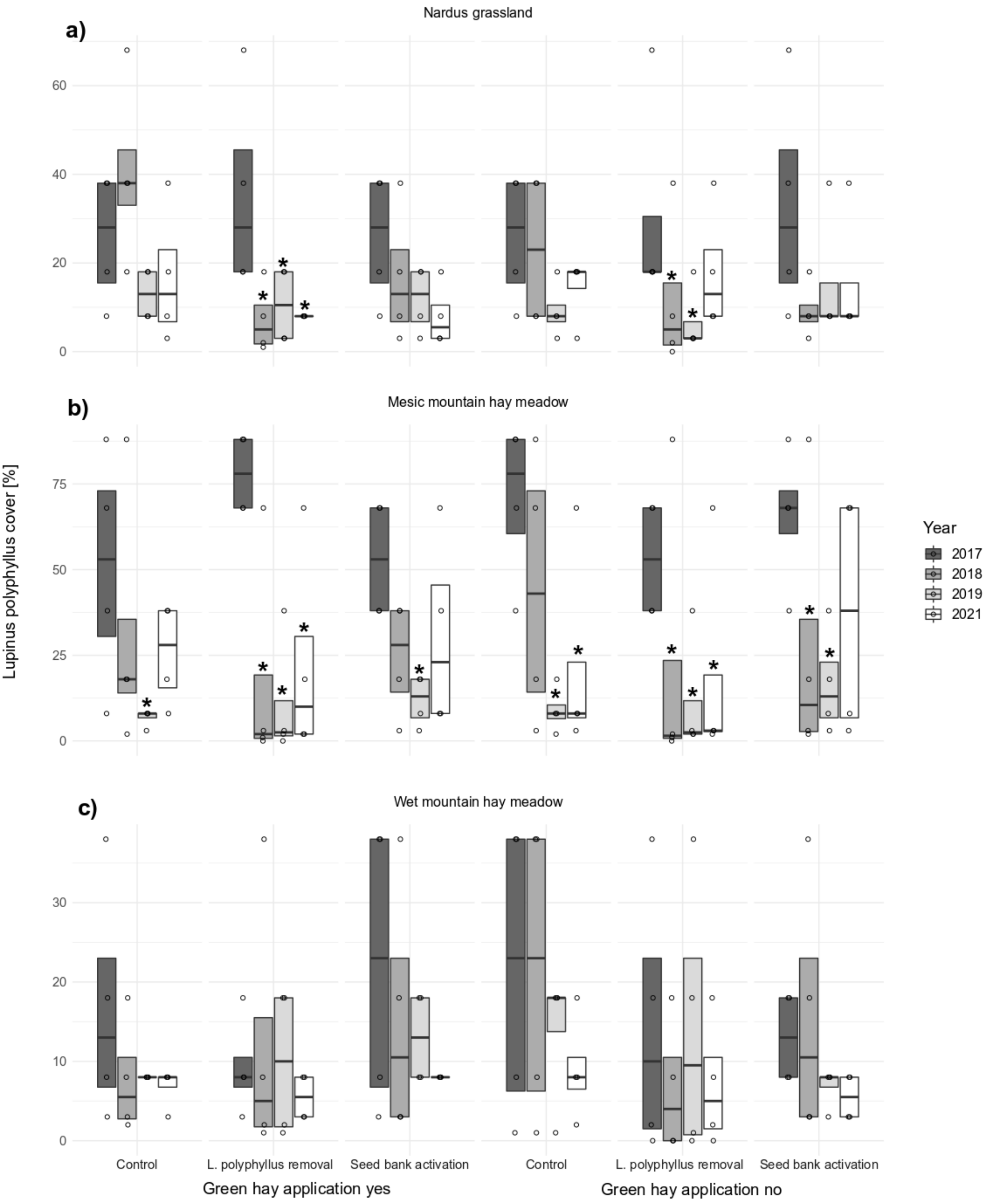

Figure 4: Box and scatter plots of the Lupinus polyphyllus cover of a) Nardus grassland, b) mesic mountain hay meadows, c) wet mountain hay meadows during the study period. The boxes represent the upper and lower quartiles and the line the median. Asterisks indicate significant differences to the respective plots of 2017 ($p < 0.05$, pairwise contrasts to the respective treatment of 2017)

Effects of the Restoration Measures on the Species Composition

The restoration relevés were situated very close together in the NMDS ordination plot, as opposed to the donor site relevés of *Nardus* grassland and mesic hay meadows that were rather scattered and furthermore far apart from the restoration sites. In all vegetation types a strong influence of time (i.e. the year of sampling) can be seen in the restoration relevés. Clear patterns of the restoration treatments are, however, absent. The vegetation relevés of the restoration sites shifted during the study period, but they did not move in the direction of the donor site relevés (Fig. S1a). The influence of year of sampling can also clearly be seen in Figure S1b where the samples of the year 2019 are outside of the range of the samples of the three remaining years.

The Bray–Curtis similarity between vegetation of donor and that of the restoration treatment sites was generally very low across all three vegetation types. There was no influence of the restoration treatments that would be indicated by an increase in similarity between restoration and donor sites during the study period (Fig. S2).

Discussion

Effects of the Restoration Measures on the Target Species Cover

The applied restoration measures did not increase target species cover in the three vegetation types. While some species that had been absent from the vegetation of the restoration plots prior to the experiment did establish during the study period, we could not determine if this was due to the restoration measures. Other species in turn disappeared completely. Accordingly, seed bank activation lowered the cumulative cover of target species in wet hay meadows. This contradicts results that had shown the suitability of seed banks, although slightly depleted in species, for restoration (Ludewig et al. 2021). However, the transferability of the results of seed bank analyses may be limited due to known limitations of soil seed bank sampling methods to adequately affect actual species composition due to small-scale variation in the soil seed bank and usually limited sample volume (Bossuyt & Honnay 2008). Thus, the soil samples may only weakly reflect the actual species composition of the soil seed bank. Moreover, germination conditions in the field differ from those in the greenhouse and despite the fact that they are optimized for germination, they might not be suitable for all species (Wagner et al. 2021). Large open gaps as we created in the framework of our study may be susceptible to year-specific effects such as drought, which may have lasting effects on restoration success (Groves et al. 2020). Moreover, gaps are less shaded in the center which could inhibit germination, especially during summer drought. Thus, the atypically hot and dry weather conditions in 2018 (DWD 2018) might have especially impeded target wet meadow species from germination and establishment. This would be in line with other studies that identified drought as a main source of germination failure (Lawesson 2000; Hölzel & Otte 2004) and high seedling mortality (Silvertown & Dickie 1981; Ryser 1993). In all, the results presented here suggest that seed bank activation via large gaps does not guarantee success in promoting the establishment of target species in mountain meadows.

Ludewig et al. (2021) suggested that species missing from the soil bank could be actively introduced. Species diversity in grasslands can often be enhanced by additional introduction of seeds (Müller et al. 2014) and green hay transfer is a very efficient method with regard to the high number of seeds that can be introduced (Scotton & Ševčíková 2017). Many of the target species still occurred on the restoration sites prior to the experiment, although at low cover. Thus, the aim of the green hay transfer was to boost the native plant communities, increase propagule pressure of native species, and increase competitive effects on *L. polyphyllus* plants. Contrary to this aim, in *Nardus* grasslands the cumulative cover of target species was lower in plots where green hay was applied. Since many of the target *Nardus* grasslands species are of low stature (Otte & Maul 2005; Peppler-Lisbach & Könitz 2017) and slow growing, they might struggle growing through the green hay layer (Schmiede et al. 2013). In fact, restoration of *Nardus* grassland is generally difficult (Finck et al. 2017) and restoration efforts had no beneficial effects in experiments in the Netherlands even after a period of 25 years (Bakker et al. 2009).

The application of green hay has been carried out successfully in numerous restoration studies in flood meadows (e.g. Donath et al. 2007; Kiehl et al. 2010; Schmiede et al. 2013). In contrast to these findings, and although in our study, the hay samples from the wet hay meadows had the highest numbers of target species seedlings, green hay application did not produce any benefits in terms of target species cover at wet and mesic meadow restoration sites. Klimkowska et al. (2007) compared restoration techniques across a range of wet meadow restoration studies and found that the overall restoration success was rather limited. However, they also stated that many of these were short-term studies, as was the case in our study. Other studies have shown that a number of species establish in the second or third year after green hay transfer due to initial dormancy of their seeds at the time of green hay transfer and an accompanying delayed germination (e.g. Wagner et al. 2021). Although we could not observe these effects 4 years after green hay transfer, larger effects of the restoration measures may be visible in the future. Similarity of restored vegetation to donor site vegetation remained low for all grassland types. These findings are similar to those of Sullivan et al. (2020) who could not find an increased similarity between most of their investigated donor and restoration meadows even after a period of 11 years. However, a strong effect of year is visible in the cover data of target species, which is most likely a strong influence of the weather conditions during the study period, which included years characterized by atypical weather.

Effects of the Restoration Measures on the *L. polyphyllus* Cover

Manual removal of *L. polyphyllus* was well suited to reduce *L. polyphyllus* cover in *Nardus* grasslands and mesic hay meadows. Soil disturbance via seed bank activation reduced *L. polyphyllus* cover significantly in mesic hay meadows. Other studies have shown that manual removal of invasive species can be a successful measure for their reduction (e.g. Pysek et al. 2007). However, as *L. polyphyllus* cover was not affected by any of the applied restoration techniques on the investigated wet hay meadows, these measures partly failed in the course of our restoration project. But given the low number of replications in the experiment, any observed reduction might simply have been too small to be statistically detectable. Also, favorable site conditions may have led to rapid recovery of *L. polyphyllus* plants, as in dry vegetation periods the soil conditions might tend to be more in the mesic range. Finally, initial *L. polyphyllus* cover at restoration sites at the start of the experiment differed between target vegetation types, and tended to be lower for wet hay meadows, probably since its competitive ability and vitality might be reduced under wet conditions (Klinger et al. 2019). However, repeated *L. polyphyllus* removal may be necessary to generally guarantee lasting reduction, as in our study, in 2021, *L. polyphyllus* cover had again increased at several sites. This could be problematic as manual removal of *L. polyphyllus* is time consuming and thus, costly. Furthermore, it should be done very thoroughly, as the species is able to sprout from even small rhizome parts of 2 cm (unpublished data), and single plants left unattended have the potential to spread rapidly (Klinger et al. 2019). Moreover, as *L. polyphyllus* has been an established species in the area for many years, reinvasion processes following control measures might occur in the long term. Given reduced levels of competition in depleted plant communities (Banks et al. 2018), the high number of *L. polyphyllus* plants on nearby grasslands, road verges, or other linear structures (e.g. clearance cairns and creek sides) may lead to high propagule pressure of the invader. Furthermore, as the grasslands used in our experiment were heavily invaded (with over 100 *L. polyphyllus* shoots removed on some 5 × 5–m experimental subplots), some plants may have survived experimental removal of shoots. Accordingly, the management regime has to be adapted to avoid that *L. polyphyllus* outcompetes newly established target species. If possible, this should include an early mowing of *L. polyphyllus* plants during full bloom, to prevent production of viable seeds. On sites where this is not possible due to the protection of ground-nesting birds, other measures could be used, e.g. removal with weeding forks or mowing with hand scythe for small *L. polyphyllus* stands or single plants. Larger stands that need to be mown with mowing machinery can also be mown after full bloom of *L. polyphyllus* as long as the seeds are green and soft. What should be avoided in any case is the dispersal of ripe *L. polyphyllus* seeds (characterized by brown to black color and hardseededness; Klinger et al. 2020) by mowing machinery or zoochory. Although another study found that mowing once annually reduced vital rates and biomass of *L. polyphyllus* (Ramula 2020), this could not be confirmed for the present study area (Klinger et al. 2019). Consequently, at heavily invaded sites, mowing more than once per year may be required. However, since preventing initial establishment is often more cost-effective than postestablishment control (Banks et al. 2018), small stands of *L. polyphyllus* or single plants should have the highest priority for control measures. Our results also revealed that *L. polyphyllus* cover was also significantly lower on control plots in the monitoring years. *L. polyphyllus* prefers sites with rather humid soil conditions, as reflected by its Ellenberg indicator value for moisture of five (Ellenberg et al. 1992) and thus the dry weather conditions might have resulted in lower *L. polyphyllus* cover.

Overall, our results provide mixed evidence for the effectiveness of the applied restoration measures. Interestingly, none of the applied measures seemed to be suitable to restore the investigated vegetation types, at least not over the limited duration of this study. On the contrary, the measures have rather led to the opposite effect, as green hay application on *Nardus* grassland and seed bank activation on wet hay meadows led to a decline in target species cover. Another surprising result is that *L. polyphyllus* management was not sufficient to foster the recovery of target species 3 years after the restoration efforts in all the investigated vegetation types. However, our study indicates several caveats that can hamper the success of restoration measures carried out in invaded meadows. These are, e.g. dry weather conditions or the potential of reinvasion in heavily invaded areas. Furthermore, our results underline the fact that different grassland types react differently to restoration techniques, which is why restoration measures should be carried out adaptively.

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Appendix

Table S3: Total number of species and the number of target species in the vegetation relevés of the investigated vegetation types during the investigation period

	Nardus grassland				Mesic hay meadow				Wet hay meadow			
Year	2017	2018	2019	2021	2017	2018	2019	2021	2017	2018	2019	2021
Total nr of species	87	95	93	98	59	66	79	78	80	95	91	99
Nr of target species	9	9	7	10	18	17	16	17	16	17	17	16

Table S2: Target species of *Nardus* grasslands and the average cover values (%) of the vegetation surveys in 2017, 2018, 2019 and 2021 on the donor sites (DS; 3 5 x 5 m plots on each vegetation type), control plots (C), soil disturbance plots (SD), *L. polyphyllus* removal plots (LR) with or without green hay application (green hay yes, green hay no) as well as seeds per m² of the seed bank trial by Ludewig et al (2021; SB) and seedling numbers that emerged from the hay samples of 160 l of green hay for each vegetation type (HS). Red list species are marked with an R.

Nardus grassland																											
	2017								2018						2019						2021						
	DS	Green hay yes			Green hay no			SB	HS	Green hay yes			Green hay no			Green hay yes			Green hay no			Green hay yes			Green hay no		
Target species		C	SD	LR	C	SD	LR			C	SD	LR	C	SD	LR	C	SD	LR	C	SD	LR	C	SD	LR	C	SD	LR
Arnica montana R	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Calluna vulgaris	2	-	-	-	-	-	-	432	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-
Carex pallescens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-
Carex pilulifera	-	0.5	1.0	0.5	-	0.5	1.8	393	-	-	-	-	0.3	-	-	-	-	-	-	-		-	0.5	-	1	-	-
Danthonia decumbens	-	-	-	-	-	-	-	32	5		-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	0.3
Festuca rubra	3	10.5	10.5	13	18	10.5	15.5	26	226	6.8	4.5	5.8	20.5	28	18	15.5	9.3	10.5	24.3	26.8	25.5	10.5	18	5.8	8.5	13	8
Helianthemum nummularium	3	9.5	0.8	2.0	-	0.8	1.5	-	3	1	0.8	1.3	-	0.8	1.5	-	-		-	-		0.8	1.3	1.3	-	1	1.8
Hieracium pilosella	0.65	1.0	-	1.3	0.8	-	1.3	13	6	0.3	0.8	1	1	1	1.8	0.8	1.5	-	2.3	1.3	3	-	1.3	1	0.8	-	2
Nardus stricta R	8.67	13.0	10.5	13.5	13.5	7.0	13.3	277	98	0.8	1.3	0.3	12.8	1.5	0.3	0.8	1.3	-	10.5	0.8	2	3	1	0.8	5	2.5	1.5
Potentilla erecta	3.34	3.3	3.5	3.5	3.3	3.0	3.5	432	13	2	3.3	3	3.3	3	2.8	1.8	3	3	3	1.8	3.3	2.3	3	3	3.3	3	3
Thymus pulegioides	1	3.0	3.0	3.0	3.0	2.3	3.3	13	3	1.3	2.5	2.5	3.5	2.8	-	0.8	1.5	1.5	2.3	3	2.8	4.3	1.8	2.8	3.5	3.8	3.8
Vaccinium myrtillus	2.67	2.0	1.8	1.0	2.0	-	1.5	58	-	0.8	0.5	0.5	2	-	0.8	1	0.8	-	0.5	0.8	0.8	0.8	0.5	0.8	1.5	0.5	0.8
Veronica officinalis	-	1.0	1.5	0.8	-	1.5	-	193	1	-	1.5	0.8	-	2.8	1.8	-	0.3	-	0.5	0.5	1	-	1	-	-	1.25	1.5

Table S3: Target species of mesic hay meadows and the average cover values (%) of the vegetation surveys in 2017, 2018, 2019 and 2021 on the donor sites (DS; 3 5 x 5 m plots on each vegetation type), control plots (C), soil disturbance plots (SD), *L. polyphyllus* removal plots (LR) with or without green hay application (green hay yes, green hay no) as well as seeds per m² of the seed bank trial by Ludewig et al (2021; SB) and seedling numbers that emerged from the hay samples of 160 l of green hay for each vegetation type (HS). Red list species are marked with an R.

Mesic hay meadows																											
	2017									2018						2019						2021					
	DS	Green hay yes			Green hay no			SB	HS	Green hay yes			Green hay no			Green hay yes			Green hay no			Green hay yes			Green hay no		
Target species		C	SD	LR	C	SD	LR			C	SD	LP	C	SD	LP	C	SD	LP	C	SD	LP	C	SD	LP	C	SD	LP
Agrostis capillaris	3	-	2.0	-	-	-	-	2088	1476	-	-	-	-	-	-	2.5	2	3.3	1.3	1.3	2	-	1.5	1.3	3.5	0.8	1.5
Alchemilla monticola	-	2.0	2.3	4.23	4.3	3.5	4.3	135	477	3	3.5	2.8	5.5	4.3	6.8	3.3	9.3	10.5	3	3	3	4.3	3.3	5.8	3.3	3.3	4.3
Anthoxanthum odoratum	4	2.3	3.0	3	3.0	2.3	3.0	19	122	2	1.3	0.8	1.5	2.3	3	3.8	4.3	2.3	3	4.3	4.5	4.3	3.3	3	2.5	3.3	2.5
Bistorta officinalis	4.67	7.3	8.0	13	13.0	16.8	8.0	-	-	18	10.5	20.5	5.5	5.5	6.8	28	20.5	20.5	15.5	20.5	10.5	7	7	6.8	9.5	7	5.8
Campanula rotundifolia	3	3.3	3.3	2.3	2.5	3.0	2.3	806	-	2.8	2.8	1.5	1.5	3	3	2.3	2.3	2.3	2	3	2.3	2.8	3.5	3	2.3	3.3	3.3
Carex muricata	-	-	-	-	-	-	-	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crepis mollis R	-	3.3	3.0	3	3.0	3.3	3.0	13	-	3	3	2.3	3	23	2.3	3	3	2	2.5	3	2	3	2.3	2.8	2.8	2.5	2.5
Cynosurus cristatus	-	0.5	-	-	-	-	-	39	-	0.25	-	-	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Galium saxatile	-	1.3	0.8	0.8	1.8	1.5	2.0	-	3	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-
Geranium sylvaticum	1	3.5	4.5	21.8	6.8	3.3	0.5	-	-	3.5	3	6.8	7.8	3.3	1.5	6	6	14.3	7.3	1.5	2.3	3.5	2.8	3.3	3.5	1	1.5
Helictotrichon pratense R	-	-	-	0.8	0.8	1.5	0.8	-	6	1.5	-	1.5	0.8	0.8	-	-	-	-	-	-	-	-	1.8	0.8	0.5	-	1.5
Helictotrichon pubescens	-	2.0	2.3	2.3	2.3	1.5	1.5	-	-	1.5	2	0.8	2.3	2.3	3.3	2.3	2.5	1.5	1.8	1.8	1.8	1.3	1.5	2.5	1.5	2.3	1.5
Hypericum maculatum	14.7	0.8	6.5	1.8	1.3	2.3	1.5	1676	802	1.5	2.8	0.8	2.3	1.5	2.3	2.8	1.5	0.8	2	1.3	3	1.5	3	0.8	18	0.8	2.8
Lathyrus linifolius	-	-	0.8	0.8	2.0	1.0	0.8	6	-	1	-	0.8	0.5	0.3	-	0.5	0.5	0.8	1.8	1.3	3	1	0.5	1.3	1.3	-	1.3
Luzula luzuloides	-	-	-	-	0.8	-	0.8	193	-	-	-	-	0.5	0.5	0.8	-	0.3	-	-	-	-	-	-	-	0.5	-	-
Phyteuma orbiculare R	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	0.5	0.3	-	-	0.5	0.5	-	0.5	0.8	-	0.8

Phyteuma spicatum	2.67	1.5	3.0	3	3.0	1.5	2.8	45	-	3	3	3	3	2.3	2.8	2	2	3	3	2.5	2.5	2.5	2	1.8	2.8	2.5	3
Poa chaixii	0	6.8	3.0	2.3	3.0	3.0	3.0	39	112	3	3	3	4.3	2.8	3	4.3	6.8	5.5	6.8	13	3	10.5	9.5	6.8	3.5	5.5	6
Sanguisorba officinalis R	8.67	6.0	8.0	9.3	6.8	11.8	6.8	6	201	5.8	8.5	15.5	5.5	7.3	5.5	6	10.5	7	5.5	6.8	7.3	2.8	4.5	3.8	5.8	4.5	5
Saxifraga granulata R	-	1.3	1.0	0.3	0.8	1.3	1.8	64	-	1.3	0.8	1.3	0.8	0.8	1.5	1.5	2.8	2.8	0.8	3.5	4.3	1.5	3	1.5	3	3.5	3.3

Table S4: Target species of wet hay meadows and the average cover values (%) of the vegetation surveys in 2017, 2018, 2019 and 2021 on the donor sites (DS; 3 5 x 5 m plots on each vegetation type), control plots (C), soil disturbance plots (SD), *L. polyphyllus* removal plots (LR) with or without green hay application (green hay yes, green hay no) as well as seeds per m² of the seed bank trial by Ludewig et al (2021; SB) and seedling numbers that emerged from the hay samples of 160 l of green hay for each vegetation type (HS). Red list species are marked with an R.

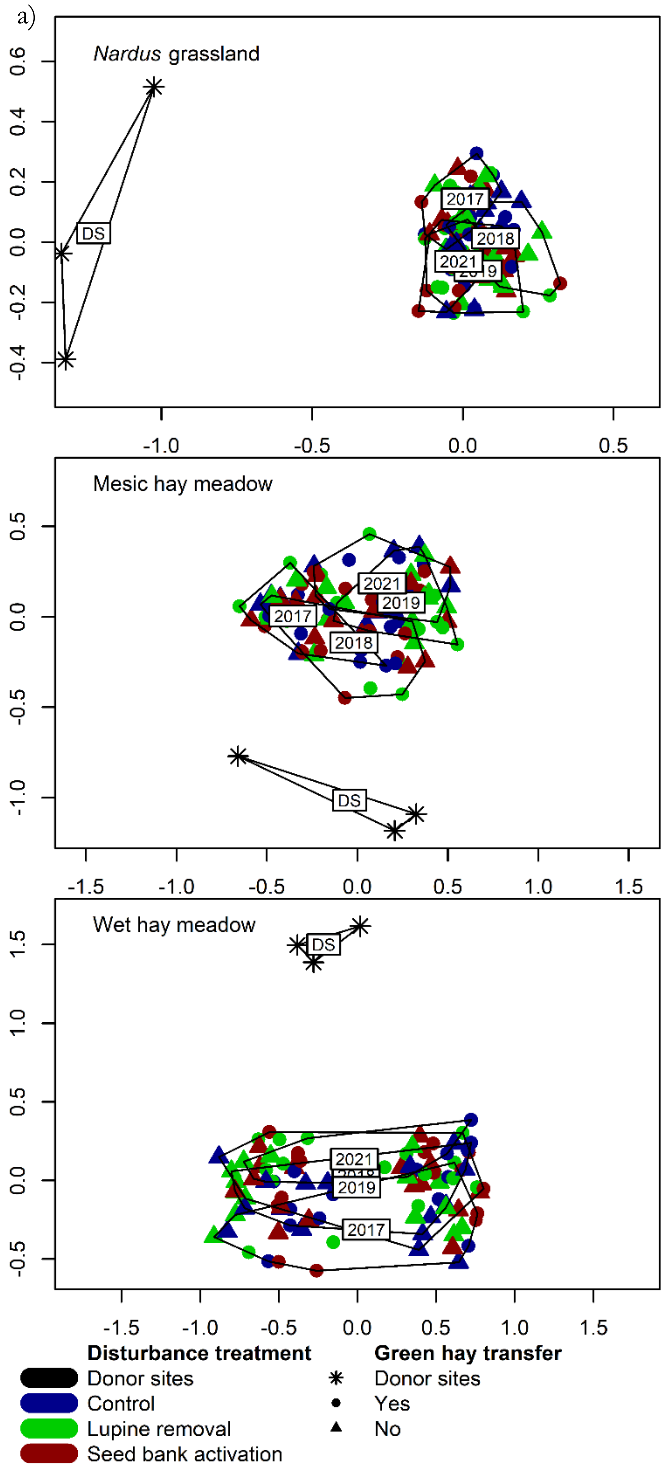
Wet hay meadows																											
	2017									2018						2019						2021					
Target species	DS	Green hay yes			Green hay no			SB	HS	Green hay yes			Green hay no			Green hay yes			Green hay no			Green hay yes			Green hay no		
		C	SD	LR	C	SD	LR			C	SD	LR	C	SD	LR	C	SD	LR	C	SD	LR	C	SD	LR	C	SD	LR
Agrostis canina	-	-	-	-	-	-	-	451	293	-	-	-	-	-	-	0.5	-	-	-	-	-	-	1.5	2	1.8	1.8	1.5
Carex canescens R	-	-	0.8	-	1.0	-	0.5	1637	-	0.8	1.3	-	1.3	-	0.3	-	-	-	1	-	-	-	-	-	0.8	-	0.5
Carex disticha	0.33	2.0	2.0	1.8	1.8	1.8	0.8	71	-	1.5	1.5	1.3	2.8	1.5	1.3	1.5	0.3	1.3	0.5	1.3	0.8	1.5	1.5	1.5	1.5	1.3	1.5
Carex nigra ag.	1	1.5	1.5	0.5	1.5	1.5	1.5	1115	-	0.8	1.3	0.5	1.5	1	1.5	0.8	1.5	-	1.3	1	1.3	1.5	1.5	0.8	1.5	1	1.5
Carex panicea R	2.67	1.5	1.5	1.5	1.8	1.5	1.5	354	-	0.8	-	-	1	0.5	1	0.5	-	-	1.5	0.5	1	0.8	1	0.5	1.5	1.5	1.8
Cirsium palustre	1.3	1.3	1.5	1	1.8	2.0	1.0	64	6	1.3	0.3	0.3	1.3	18	0.8	1.3	2	1.8	1.8	1.8	1.5	1	1.5	2	1.8	2.8	2.8
Comarum palustre R	-	0.8	1.3	0.5	0.3	0.8	0.8	26	-	-	0.5	0.3	0.8	0.8	0.8	0.8	-	-	0.3	1.3	1.3	0.3	-	0.8	0.8	1.3	1
Deschampsia cespitosa	8	12.5	33	4.8	21	25.5	20.5	1063	446	4.3	3	3	13	4.3	5.5	5.5	4.8	3	13.3	6.8	7	33	4.3	3.5	5.8	6	5.5
Eriophorum angustifolium	-	-	-	-	0.5	-	0.5	187	-	-		-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Galium palustre	-	-	-	-	-	-	-	13	12	-	-	-	-	-	-	0.5	-	-	0.8	-	-	-	-	-	-	-	-
Galium uliginosum	2	2.3	1.5	2.3	2.8	3.0	2.3	45	-	1.8	0.8	0.3	2.3	1.3	2	1.5	1	1.3	2.3	1.5	1.3	2	2	2.8	2.3	2.3	2
Glyceria declinata	-	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Juncus articulatus	-	-	-	-	-	-	-	238	84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Juncus effusus	3.67	1.5	5.3	1.8	0.8	2.3	1.5	50725	3.860	1	0.8	0.8	-	1.3	1.3	-	-	-	-	-	-	-	-	-	-	-	-
Juncus filiformis	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mentha arvensis		0.5	1.5	0.5	-	1.25	-	71	7	1	1.5	1.3	1.3	0.3	0.3	2.8	2.8	1.8	1.5	0.5	-	1.5	1.5	1.5	0.5	1.5	1.5
Myosotis nemorosa	1	-	-	0.8	-	-	-	412	-	-	-	-	-	-	-	1.5	2.8	0.8	0.8	1.5	1.8	-	-	-	-	-	-
Pedicularis sylvatica	-	-	-	-	-	-	-	19	-	-	-	-	-	-	-	-	0.5	0.5	-	0.5	-	-	-	-	-	0.3	-
Poa palustris	-	-	-	-	-	-	-	6	-	-	0.3	-	0.8	0.5	1.3	-	-	-	-	-	-	0.8	0.8	0.8	-	-	0.5
Silene flos-cuculi R	-	1.5	1.8	1.5	2	1.3	1.5	2056	61	0.5	0.3	0.3	2	0.5	0.8	0.8	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2	1.5	2	1.8
Stellaria alsine	-	-	-	-	-	-	-	116	39	-	0.3	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trifolium spadiceum R	2	1.5	-	2.5	0.8	1.5	1.5	535	323	0.8	0.3	1.3	1.5	1.3	1.3	2.8	2.8	5	1.3	4	3	1.8	1.8	1.5	2.8	1.8	1.8
Trollius europaeus	3	1.3	1.3	1.8	1.3	1.5	0.5	6	-	3.8	0.3	0.5	2.8	1.5	0.3	3.3	1	1.8	1.8	3	0.8	2.3	0.8	1.3	2.5	2.3	0.8
Valeriana dioica R	0.67	0.8	1.8	0.8	1.0	2.5	0.8	26	-	1.5	0.8	1.5	0.8	1	0.5	1.3	0.8	1.3	1.3	1.3	-	0.8	-	0.5	1.8	1	0.5
Veronica beccabunga	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Viola palustris	-	-	-	-	-	-	-	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table S5: Results from the linear mixed effects models and generalized linear mixed models used to examine the effects of restoration measures, i.e. green hay application and soil disturbance treatments on the cover of *Lupinus polyphyllus* and the cover of target species of the three investigated vegetation types over three years.

Response variable	Explanatory variable	X^2	*df*	*p*
Lupinus cover	Vegetation type	1.02	2	0.584
	Year	70.17	3	< 0.001
	Disturbance treatment	20.35	2	< 0.001
	Green hay application	0.13	1	0.722
	Vegetation type:Year	26.91	6	< 0.001
	Veg_type:dist_treat	2.95	4	0.566
	Year:dist_treat	14	6	0.05
	Veg_type:green_hay	0.08	2	0.961
	Year:green_hay	0.16	3	0.983
	Dist_treat:green_hay	0.47	3	0.789
	Veg_type:Year:dist_treat	7.83	12	0.798
	Veg_type:Year:green_hay	3.83	6	0.699
	Veg_type:dist_treat:green_hay	2.88	4	0.578
	Year:dist_treat:green_hay	1.44	6	0.963
	Veg_type:Year:dist_treat:green_hay	3.60	12	0.99
Character *Nardus* grassland species cover	Vegetation type	13.45	2	< 0.01
	Year	20.05	3	< 0.001
	Disturbance treatment	0.48	2	0.789
	Green hay application	52.33	1	< 0.001
	Vegetation type:Year	66.21	6	< 0.001
	Veg_type:dist_treat	7.44	4	0.114
	Year:dist_treat	4.7	6	0.583
	Veg_type:green_hay	2.06	2	0.357
	Year:green_hay	33.8	3	< 0.001
	Dist_treat:green_hay	1.04	3	0.595
	Veg_type:Year:dist_treat	12.44	12	0.411
	Veg_type:Year:green_hay	8.99	6	0.174
	Veg_type:dist_treat:green_hay	6.60	4	0.158
	Year:dist_treat:green_hay	2.76	6	0.838
	Veg_type:Year:dist_treat:green_hay	9.50	12	0.659
Character mesic hay meadow species cover	Vegetation type	1.64	2	0.440
	Year	38.93	3	< 0.001
	Disturbance treatment	1.75	2	0.417
	Green hay application	19.04	1	< 0.001
	Vegetation type:Year	37.58	6	< 0.001
	Veg_type:dist_treat	7.1	4	0.133
	Year:dist_treat	11.42	6	0.076
	Veg_type:green_hay	3.1	2	0.217
	Year:green_hay	8.59	3	< 0.05
	Dist_treat:green_hay	10.23	3	< 0.01
	Veg_type:Year:dist_treat	13.10	12	0.362
	Veg_type:Year:green_hay	11.94	6	0.063

	Veg_type:dist_treat:green_hay	6.91	4	0.141
	Year:dist_treat:green_hay	12.16	6	0.059
	Veg_type:Year:dist_treat:green_hay	11.6	12	0.478
Character wet hay meadow species cover	Vegetation type	38.24	2	< 0.001
	Year	11.38	3	< 0.01
	Disturbance treatment	2.11	2	0.355
	Green hay application	1.0.1	1	0.373
	Vegetation type:Year	16.87	6	< 0.01
	Veg_type:dist_treat	4.74	4	0.303
	Year:dist_treat	4.83	6	0.589
	Veg_type:green_hay	1.98	2	0.458
	Year:green_hay	2.73	3	0.445
	Dist_treat:green_hay	3.05	3	0.226
	Veg_type:Year:dist_treat	21.35	12	< 0.05
	Veg_type:Year:green_hay	2.54	6	0.864
	Veg_type:dist_treat:green_hay	4.44	4	0.34
	Year:dist_treat:green_hay	4.46	6	0.614
	Veg_type:Year:dist_treat:green_hay	6.36	12	0.907


a)
Nardus grassland
DS
2017
2018
2021
2019
0.6
0.4
0.2
0.0
-0.2
-0.4
-1.0
-0.5
0.0
0.5
Mesic hay meadow
2021
2019
2017
2018
DS
0.5
0.0
-0.5
-1.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Wet hay meadow
DS
2021
2018
2019
2017
1.5
1.0
0.5
0.0
-0.5
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Disturbance treatment
Donor sites
Control
Lupine removal
Seed bank activation
Green hay transfer
Donor sites
Yes
No

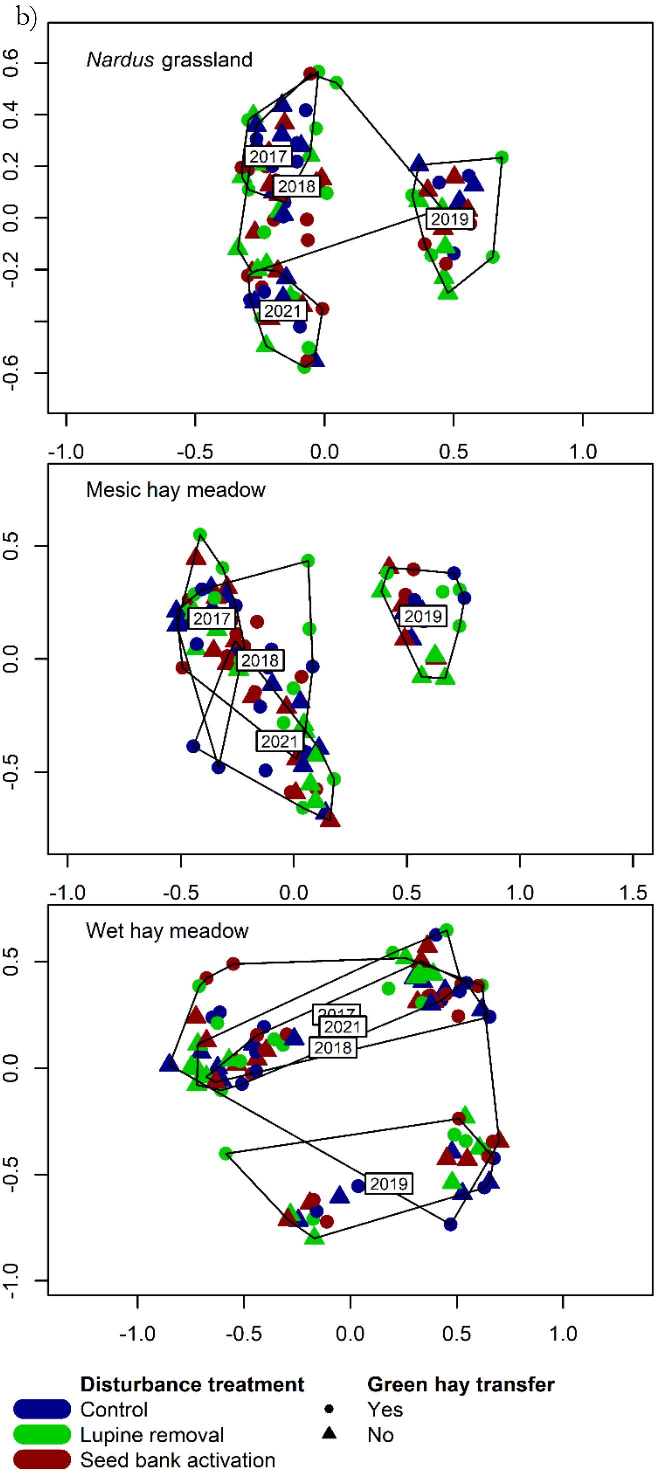


Figure S5: NMDS ordination graphic of the main floristic gradients in the three vegetation types. The color scheme represents the disturbance treatment, triangles indicate green hay application yes, circles indicate green hay no, hulls represent the sampling year, a) with donor sites included, b) without donor sites to enhance visibility of the restoration site relevés.

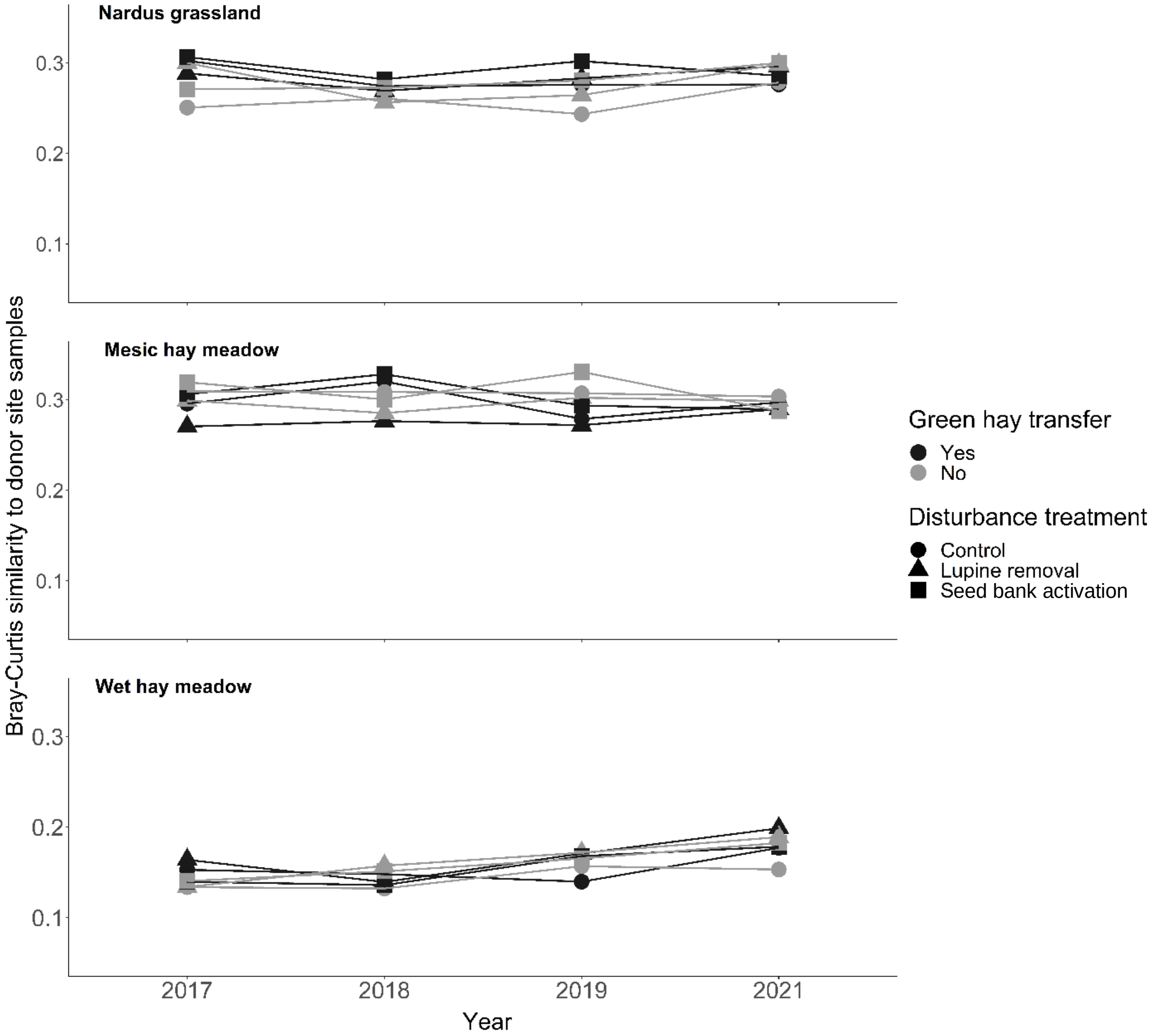


Figure S6: Bray-Curtis similarity between vegetation relevés of the restoration sites and the donor site relevés of the three vegetation types.

Abstract

Semi-natural grasslands are one of the last remnants of historical cultural landscapes in Central Europe. Due to century-long traditional management like mowing and grazing with low fertilizer input, they belong to the most diverse and species-rich ecosystems. However, because of anthropogenic changes such as intensification of abandonment, they are nowadays heavily endangered. For example, abandonment or de-intensification may lead to the expansion of invasive species that may change the productivity and species composition. After it had been originally sown for soil improvements in spruce forest, the invasive *Lupinus polyphyllus* (Lindl.), for instance, was able to invade the surrounding mountain meadows of the Rhön Biosphere Reserve as a consequence of a change in the mowing regime. Here, it outcompetes small-statured plant species and thus alters the composition of the native plant communities. This thesis investigates the impacts of *L. polyphyllus* on the functional and species diversity of mountain meadow plant communities in the Rhön Biosphere Reserve. It further assesses the usefulness of the soil seed banks for restoration and tested different restoration methods in field experiments. Data analysis was performed using univariate and multivariate statistical approaches.

In general, the results showed that *L. polyphyllus* impacts the native plant communities since it increases the similarity between different invaded vegetation types (*Nardus* grassland, mesic hay meadows, wet hay meadows) and fosters competitive species. However, while the impact on the current vegetation was high, *L. polyphyllus* has, so far, a small impact on the soil seed bank. Therefore, activation of the soil seed banks can be used for restoration. However, the results demonstrated that the seed banks are depleted in species compared to the current vegetation. That is why green hay transfer was used to additionally boost the native plant communities. Moreover, *L. polyphyllus* individuals were removed with weeding forks in order to reduce their cover. The *L. polyphyllus* cover could be reduced in the short term in *Nardus* grasslands by manual removal but not in mesic and wet hay meadows. It was not possible to increase the cover of native target species in any of the vegetation types. This may be due to the dry and unfavorable weather conditions during the experiment. The method of green hay transfer itself may also be a reason, since not all species might be harvested at the appropriate time and not all species are able to use the relatively small time window for germination before the created microsites are overgrown.

For further restoration plannings it should be considered to first reduce the *L. polyphyllus* cover by an adequate mowing management. Manual removal should be performed on small stands and single plants since it is work and cost -intensive. After the cover has been reduced, seed bank activation and green hay transfer can be used to restore the native plant communities. To assist species with delayed germination soil disturbance can be repeated in the first spring after the experiment has been conducted and after cold-stratification has taken place. For plant communities with a wide range of phenology a second mowing date could be useful. For some species (e.g. *Arnica montana*) green hay transfer does not work well. Here, additional plantings or seedings should be taken into consideration. Nevertheless, changing climatic conditions pose additional challenges to restoration plannings and management. Here, further research is necessary. In the end, long-term monitoring is crucial in order to make sure that the restoration of the mountain meadows is lasting.

Zusammenfassung

Naturnahes Grünland ist eines der letzten Relikte der historischen Kulturlandschaft in Mitteleuropa. Durch jahrhundertelange traditionelle Bewirtschaftung wie Mahd und Beweidung mit geringem Düngereinsatz gehört naturnahes Grünland hier zu den vielfältigsten und artenreichsten Ökosystemen. Aufgrund anthropogener Veränderungen wie Intensivierung oder Nutzungsaufgabe landwirtschaftlicher Flächen ist es heute jedoch stark bedroht. So können beispielsweise Landnutzungsänderungen zur Ausbreitung von invasiven Arten führen, die die Produktivität und Artenzusammensetzung der invadierten Standorte verändern. Ursprünglich zur Verbesserung der Böden in Fichtenforsten ausgesät, konnte sich beispielsweise die invasive Stauden-Lupine (*Lupinus polypyhllus* Lindl.) nach einer Änderung des Mahdregimes in den Naturnahen Bergwiesen des Biosphärenreservats Rhön ausbreiten. Hier verdrängt sie kleinwüchsige Pflanzenarten und verändert so die Zusammensetzung der Artengemeinschaft.

Die vorliegende Arbeit befasst sich daher mit den Auswirkungen von *L. polyphyllus* auf die Pflanzengesellschaften der Bergwiesen im Biosphärenreservat Rhön. Darüber hinaus wurden die Eignung der Bodensamenbanken für die Restituierung bewertet und verschiedene Restituierungsmethoden in Feldversuchen getestet. Die Daten wurden mit einer Reihe von univariaten und multivariaten statistischen Ansätzen analysiert.

Insgesamt zeigten die Ergebnisse, dass *L. polyphyllus* einen starken Einfluss auf die heimischen Pflanzengemeinschaften hat. Sie erhöht die Ähnlichkeit zwischen verschiedenen untersuchten invadierten Vegetationstypen (Borstgrasrasen, mesische Bergwiesen und nasse Bergwiesen). Arten, die hier gemeinsam mit *L. polyphyllus* vorkommen, zeigen eine große Konkurrenzstärke. Während die Auswirkungen auf die derzeitige Vegetation hoch waren, hat *L. polyphyllus* bisher keine Auswirkungen auf die Bodensamenbank. Daher kann die Bodensamenbank durch Aktivierung zur Wiederherstellung der Vegetationstypen genutzt werden. Die Ergebnisse zeigten weiter, dass die Bodensamenbanken im Vergleich zur oberirdischen Vegetation artenarm sind. Deshalb wurde eine Mahdgutübertragung von artenreichen Spenderwiesen angewendet, um die einheimischen Pflanzengemeinschaften zusätzlich zu stärken. Außerdem wurden die *L. polyphyllus*-Pflanzen mit Ampferstechern manuell entfernt, um ihren Bewuchs zu reduzieren. Durch die manuelle Entfernung konnte die *L. polyphyllus*-Dichte kurzfristig in den Borstgrasrasen reduziert werden, nicht aber in den mesischen und feuchten Heuwiesen. Bei keinem der Vegetationstypen war es möglich, den Anteil der einheimischen Ziel-Arten zu erhöhen. Dies könnte auf die trockene Witterung während des Restituierungsexperiments zurückzuführen sein. Auch die Methode der Mahdgutübertragung selbst könnte ein Grund sein, da vermutlich nicht alle Arten zum richtigen Zeitpunkt geerntet wurden und

nicht alle Arten das relativ kleine Zeitfenster für die Keimung nutzen konnten, bevor die durch Bodenstörung geschaffenen Mikrohabitate zugewachsen waren.

Bei weiteren Restituierungsprojekten sollte in Betracht gezogen werden, zunächst die *L. polyphyllus*-Dichte durch ein angemessenes Mahdregime zu reduzieren. Die manuelle Entfernung sollte bei kleinen Beständen und Einzelpflanzen durchgeführt werden, da sie arbeits- und damit kostenintensiv ist. Nach der Reduzierung des Bewuchses können Samenbankaktivierung und Mahdgutübertragung zur Wiederherstellung der einheimischen Pflanzengemeinschaften eingesetzt werden. Zur Förderung von Arten mit verzögerter Keimung kann die Bodenstörung im ersten Frühjahr nach Durchführung des Versuchs und nach erfolgter Stratifizierung wiederholt werden. Bei Pflanzengemeinschaften mit einer großen weiten Phänologie könnte ein zweiter Mahdtermin sinnvoll sein. Für einige Arten (z. B. *Arnica montana*) ist die Mahdgutübertragung nicht geeignet. Hier sollten zusätzliche Anpflanzungen oder Aussaaten in Betracht gezogen werden. Zusätzlich stellen die sich ändernden klimatischen Bedingungen zusätzliche Herausforderungen für die Restituierungsplanung und das Management dar. Hier ist weitere Forschung notwendig. Letztendlich ist eine langfristige Überwachung von entscheidender Bedeutung, um sicherzustellen, dass die Restituierung der Bergwiesen dauerhaft ist.

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Declaration

I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of an anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University Giessen "Satzung der Justus-Liebig-Universität Giessen zur Sicherung guter wissenschaftlicher Praxis" in carrying out the investigations described in this dissertation.

Kiel, May 23, 2023

Wiebke Hansen