

**Distribution, dispersal, and management of the invasive
legume *Lupinus polyphyllus* in the UNESCO Biosphere
Reserve Rhön**

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“Research means that you don’t know, but are willing to find out.”

Charles F. Kettering

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List of Publications

This thesis is based on the following three papers:

1. 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: 2735–2749. doi: 10.1007/s10530-019-02012-x*
2. Klinger, Y.P., Eckstein, R.L., Horlemann, D., Otte, A. & Ludewig, K. (2020). Germination of the invasive legume *Lupinus polyphyllus* depends on cutting date and seed morphology. *NeoBiota* 60: 79-95. doi: 10.3897/neobiota.60.56117
3. 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

Author Contributions:

For all three of the papers listed above, I had the main responsibilities of conceptualization, field work, data analysis, and paper writing. In the first publication, Kristin Ludewig and Annette Otte contributed valuable ideas to the concept of the study, and Lutz Eckstein and Sarah Harvolk-Schöning contributed to the statistical analysis. In the second study, K. Ludewig and A. Otte conceived the research idea, K. Ludewig, David Horlemann, and R.L. Eckstein contributed to data preparation and statistical analysis. In the third study, I conceived the research idea, K. Ludewig contributed to the field work, K. Ludewig, R.L. Eckstein, Wiebke Hansen, and Till Kleinebecker contributed to statistical analysis. All co-authors of the respective studies contributed by providing constructive suggestions and helpful comments to the manuscripts.

Furthermore, I contributed to the field work, data analysis, and manuscript writing of the following studies, which explore the ecology of the invasive garden lupine (*L. polyphyllus*) and its management:

4. 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:e13311. doi:10.1111/rec.13311
5. Hansen, W., Klinger, Y.P., Otte, A., Eckstein, R.L. & Ludewig, K. (submitted). Applying combined restoration methods on invaded mountain meadows.

6. Ludewig, K., Klinger, Y.P., Donath, T.W., Eckstein, L.R., et. al. (submitted). Flowering phenology of the invasive legume *Lupinus polyphyllus* along a latitudinal gradient in Europe.

Chapter 1

Distribution, dispersal, and management of *Lupinus polyphyllus*: a synthesis

Introduction

Semi-natural grasslands

Semi-natural grasslands are among the most species-rich ecosystems in the world at small spatial scales (Wilson et al. 2012). Globally, grassland ecosystems provide various ecosystem services, as they support the production of domestic animals, provide genetic resources, and store about 30% of the carbon stocks of all terrestrial ecosystems (European Commission 2008). In the European Union, semi-natural grasslands cover approximately one-third of the total agricultural area and are thus considered a key element of ‘high nature value farmland’ (Paracchini et al. 2008). Unimproved, low-productive grasslands are important remnants of the historical cultural landscapes of Central Europe and provide habitat for vast numbers of vascular plants, insects, and bird species (Dengler et al. 2014). Among semi-natural grasslands, mountain grasslands are particularly species-rich and therefore protected by the Habitats Directive (e.g. 92/43/EEC, habitat types 6520: mountain hay meadows and 6230: species-rich *Nardus* grasslands). They significantly contribute to the species diversity of modern intensively used landscapes (Billeter et al. 2008).

Semi-natural grasslands are threatened by different aspects of global change, such as land-abandonment, agricultural intensification (Bakker & Berendse 1999), climate change, and invasive species (Pruchniewicz 2017). During the last century, the area of semi-natural grasslands has decreased drastically (Pärtel et al. 2005; Dengler et al. 2014), and the current distribution of well-preserved semi-natural grasslands in Central Europe is often restricted to remote, mountainous landscapes or protected areas. This makes many of the remaining grasslands vulnerable to the negative effects of isolation, which may lead to further species extinctions (Pärtel et al. 2005). As a consequence of this isolation, both the number of well-preserved grasslands and the associated species diversity are continuously declining (Dahlström et al. 2008; Gillet et al. 2016). Despite considerable efforts to conserve semi-natural grasslands, they remain among ecosystems that show the most deteriorating trends in Europe (European Environment Agency 2020).

One reason for the ongoing negative trends may be that the conservation of semi-natural grasslands relies on the continuation of traditional land-use practices that originally led to the formation of these ecosystems over the course of centuries (Poschlod et al. 2005). Such practices comprise, for example, low-intensity mowing and haying and pasturing with very low nutrient

inputs. In the European Union, more than 60 habitat types depend on or benefit from the continuation of traditional land-use (Halada et al. 2011). Thus, the cessation of traditional practices due to abandonment or agricultural intensification has severe negative effects on semi-natural grasslands. In addition to their dependence on management on-site, the conservation and restoration of grasslands is hampered by factors that cannot be influenced at local scales but depend on the larger contexts. These are, for example, eutrophication by airborne nitrogen or dispersal limitation (Bakker & Berendse 1999), which may have delayed negative effects ('extinction debt') on grassland ecosystems (Kuussaari et al. 2009). Furthermore, against the background of modern high-yield agriculture, the management of semi-natural grasslands strongly relies on subsidies (Van Huylenbroeck & Durand 2003), and thus, many grasslands of high conservational value are located in protected areas. However, even in protected areas, semi-natural grasslands show deteriorating trends due to changes in environmental conditions or grassland management, which may then lead to changes in the species composition of these ecosystems. These changes are often accompanied by the spread of invasive species, which further increases the pressure on the remaining semi-natural grasslands (Pruchniewicz 2017).

Invasive species

Invasive alien species are presumed to be among the most significant drivers of the global biodiversity loss during the last century (Butchart et al. 2010). According to the IUCN, invasive alien species are defined as species that are introduced outside of their natural distribution range and negatively affect ecosystems, human health, or human economy in the invaded ranges (IUCN, 2021). The transport and introduction of species to new places is strongly tied to human activity and ongoing globalization. The growing global trade networks contribute significantly to the increase in invasive species (Seebens et al. 2017), and the number of introduced species is directly linked to anthropogenic factors such as population density and gross domestic product (Pyšek et al. 2020). Consequently, both Europe and North America host the highest numbers of established alien species and are at the same time the most common sources of invasive species in other regions of the world (van Kleunen et al. 2015).

Although the pathways of introduction differ between taxa, intentional introduction, escape from cultivation (for plants) and intentional release (for mammals) are the most significant introduction pathways of invasive species in Europe (Pergl et al. 2017). To address the threat imposed by invasive species, the Convention on Biological Diversity, signed by 150 governments in 1992, considered invasive species in Aichi Target 9, where it is stated that the identification and management of priority invaders and their introduction pathways should be achieved by the year 2020 (CBD, 2014). Consequently, measures to monitor and manage invasive species have been

established on the national and international levels, e.g. in the list of Invasive Species of Union Concern of the EU (European Union, 2019). However, recent research has shown that Aichi Target 9 was not achieved (Essl et al. 2020; Tittensor et al. 2014), and across all continents and taxonomic groups, both the number of introduced species and the number of invasive species are still rapidly increasing (Seebens et al. 2017).

Invasive species can negatively impact ecosystems, e.g., by reducing native species richness and disturbing ecosystem functioning (Gallardo et al. 2016), but also by negatively affecting human well-being and inflicting economic damage (Simberloff et al. 2013). However, non-native species introduced to new geographical ranges are not *per se* problematic, but their impact strongly depends on the ecological context (Bartz & Kowarik 2019; Sapsford et al. 2020). Factors influencing the outcome of an introduction are, for example, the traits of the non-native species in place, the circumstances of the introduction, and the colonized ecosystems. On the one hand, species from all taxonomic groups can become invasive, and the most problematic invasive species in Europe comprise examples from plants (e.g., *Acacia dealbata*), mammals (e.g., *Rattus norvegicus*), and arthropods (e.g., *Varroa destructor*; Nentwig et al. 2018). On the other hand, for many invasive species that are perceived as problematic, proof of severe ecological impact is scarce (Nentwig et al. 2018), e.g., for the widespread annual *Impatiens glandulifera* (Hejda et al. 2009). Additionally, the effects of invasive species are often hard to disentangle from other aspects of global change (Pyšek et al. 2020). Thus, the impact of single non-native species can vary greatly between different ecosystems or geographical regions (Blackburn et al. 2014). Some of the most problematic invasive species are ecosystem engineers, which are capable of altering ecosystems to gain a competitive advantage (Cuddington & Hastings 2004). For example, water-demanding invasive trees, such as *Eucalyptus* spp. and *Acacia* spp., are capable of lowering the groundwater table and considerably altering the hydrology of invaded sites in South Africa (Mack et al. 2000), and N₂-fixing invaders can increase nitrogen levels and accelerate nitrogen cycling in invaded ecosystems (Hiltbrunner et al. 2014). If the invaded ecosystem has been significantly altered, measures to restore the pre-invasion state may need to go far beyond the eradication of the invasive species and can be time and cost intensive.

A widespread and problematic non-native species in Europe is the perennial legume *Lupinus polyphyllus* Lindl., which is considered to be among Europe's 100 worst invasive species (Nentwig et al. 2018). It originates from Pacific North America and was introduced to Europe as an ornamental plant (Hegi 1924). *L. polyphyllus* is considered an ecosystem engineer (Cuddington & Hastings 2004) as it is capable of considerably altering invaded ecosystems, e.g., by its capability to affect nitrogen cycling (Hiltbrunner et al. 2014) or by altering the vertical vegetation structure of invaded sites (Otte & Maul 2005; Thiele et al. 2010). In many places, it negatively affects native

habitats, as it is able of overgrowing and shading the underlying vegetation, which may lead to a decline in the number of smaller species (Thiele et al. 2010; Hiltbrunner et al. 2014), and fosters the growth of tall-growing, nitrogen-demanding vegetation (Otte & Maul 2005; Hansen et al. 2020). This is why semi-natural grasslands, which are characterized by a high number of light-demanding, small-growing species (Dierschke 1997), are particularly threatened by *L. polyphyllus*.

Objectives

The described background underlines the importance of linking knowledge on the ecology of invasive species to their management in semi-natural grasslands. This thesis aims to improve the understanding of the ecology of the widespread invasive perennial, *L. polyphyllus*, its distributional patterns, and the mechanisms leading to these patterns against the background of more efficient species management. To this end, I present three studies dealing with a) the spatio-temporal distribution of *L. polyphyllus* and its link to landscape structure, b) the germination ecology of *L. polyphyllus* depending on management, and c) possible dispersal processes associated with the management of semi-natural grasslands.

Specifically, the objectives of this dissertation were to:

1. quantify the spatial distribution and the temporal changes in the distribution of *L. polyphyllus* in the nature reserve 'Lange Rhön'
2. assess the effect of different management regimes/mowing dates on the germination capability of *L. polyphyllus*
3. assess the species transported by different dispersal vectors in semi-natural grasslands considering the dispersal of both *L. polyphyllus* and typical grassland species

Study area

The studies were conducted in the Rhön Mountains that belong to the Central European low mountain ranges (Fig. 1.1a). The study area is part of the UNESCO Man and Biosphere Reserve Rhön, which comprises an area of approximately 2400 km² in the German federal states Bavaria, Hesse, and Thuringia. The Rhön Mountains are located within the temperate climate zone in the transitional area between the oceanic and continental climate zones. The climate of the study area is characterized by a low mean annual temperature of 5.4 °C (mean of 1980–2010 of Mt. Wasserkuppe, 950 m a.s.l.; DWD 2020), a high annual precipitation of 1176 mm (mean of 1980–2010; DWD 2020), and a short growing season. Triassic basalt rocks form the bedrock in the study area. Soils that develop on basaltic bedrocks are well supplied with base cations, but high precipitation as well as the traditional land-use have led to low nutrient availability and very low pH values in most of the Rhön region (Puffe and Zerr 1988, Volz 2003).

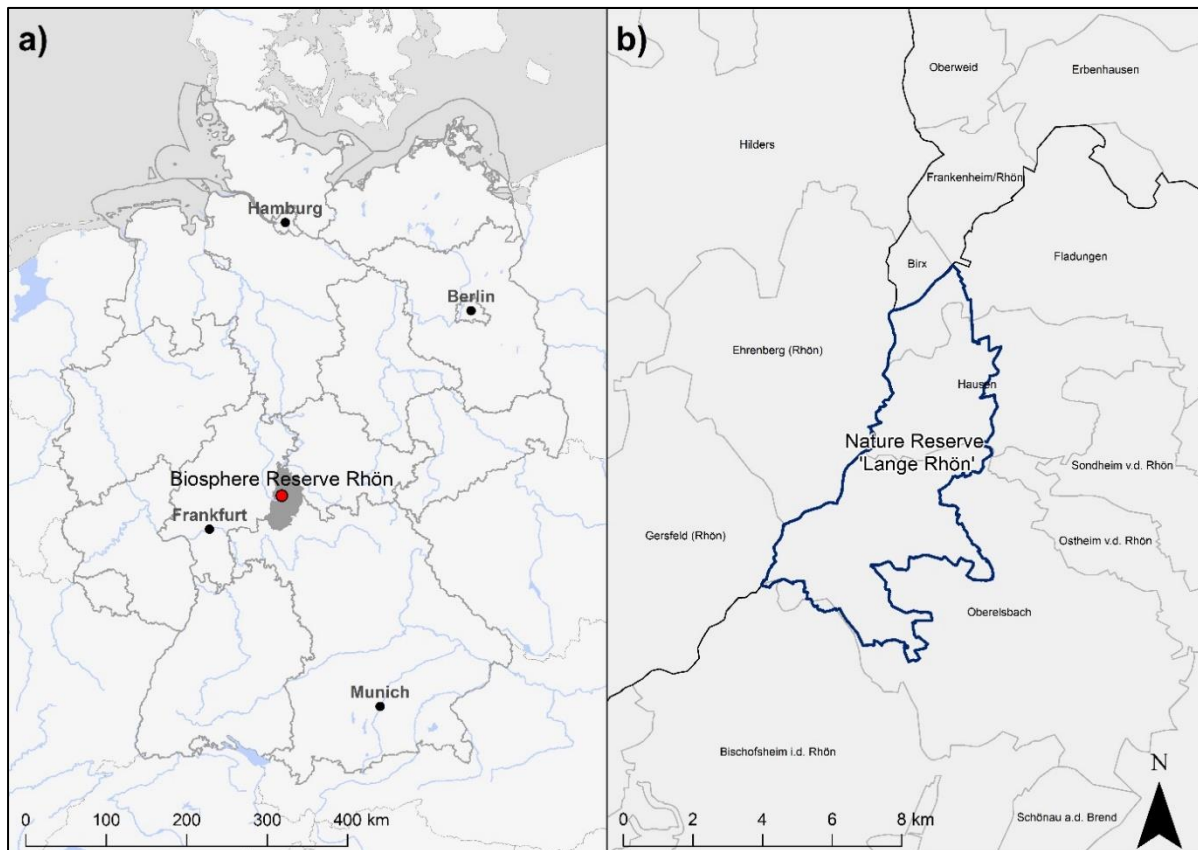


Figure 1.1 Location of a) the Biosphere Reserve in Central Germany and b) the Study Area, the Nature Reserve ‘Lange Rhön’

One of the most prominent characteristics of the Biosphere Reserve is 8900 ha (~5% of its total area) of semi-natural grasslands (e.g., Habitats Directive 92/43/ EEC, habitat type 6520: mountain hay meadows and 6230: species-rich *Nardus* grasslands) that are the result of a century-

long land-use history, which included traditional mowing and sheep-herding with low fertilizer inputs (Otte & Maul 2005). In the study area, the major share of semi-natural grasslands can be classified as mountain hay meadows or species-rich *Nardus* grasslands. Floristically, mountain hay meadows belong to the Geranio-Trisetetum association (Dierschke 1997), which is characterized by species such as *Trisetum flavescens*, *Geranium sylvaticum*, or *Phyteuma spicatum*. Species such as *Trollius europaeus*, *Caltha palustris*, and *Angelica sylvestris* can be found on sites with wet conditions (Dierschke 1997). *Nardus* grasslands in the study area largely belong to the Polygalo-Nardetum association (Petersen 2001) that is characterized by species such as *Nardus stricta*, *Polygala vulgaris*, and *Arnica montana*. Furthermore, many grasslands comprise transitions between these floristic associations, and the aspect of the vegetation can change on very small scales. Due to the widespread occurrence of these grasslands within the Biosphere Reserve Rhön, it is of supra-regional importance for the conservation of these habitat types in Central Europe (Grebe 1995). The main distribution of *L. polyphyllus* is located in the High Rhön plateau (from 50°26'N to 50°32'N and from 09°54'E to 10°05'E), between 600 and 950 m a.s.l., where the three studies were conducted (Fig. 1.1b).

***Lupinus polyphyllus* - Garden lupine**

The perennial legume *Lupinus polyphyllus* Lindl. originates from Pacific North America. Depending on site conditions, *L. polyphyllus* can reach a height of 0.9 to 1.5 m (Fremstad 2010). Inflorescences are formed in June and July, each consisting of 50 to 80 single flowers (Hegi 1924). *L. polyphyllus* develops seed pods with four to twelve seeds, which burst at seed maturity and spread the seeds ballistically over several meters (Volz 2003). Per plant, several hundred (Volz 2003; Ramula 2014) to 2500 seeds can be produced (Aniszewski 2001), and seed production is highly variable between individuals (Volz 2003).

Originally, *L. polyphyllus* was introduced to Europe as an ornamental plant in 1826 (Fremstad 2010). In Germany, it has been recorded as naturalized since 1890 (Hegi 1924). In addition to its use as an ornamental plant, it was commonly sown as nurse plants and used for soil melioration in spruce woods (Rehfuess et al. 1991), for greening along road verges (Volz 2003), and deliberately spread as wildlife fodder throughout Europe (Fremstad 2010, Hegi 1924). The species is considered naturalized in different regions all over the world, including Central and Northern Europe (Fremstad 2010), New Zealand (Holdaway & Sparrow 2006), and Chile (Meier et al. 2013). However, the Genus *Lupinus* consists of many of varieties and hybrids (Sawicka-Sienkiewicz et al. 2008), and considerable genetic variation between populations of *L. polyphyllus* has been found in Finland (Li, Vasemägi, and Ramula 2016). Consequently, there may be high uncertainties concerning the correct identification of *L. polyphyllus* populations throughout

Europe. Invaded habitats comprise road verges (Valtonen et al. 2006), riparian terraces (Meier et al. 2013), and mountain grasslands (Volz 2003; Otte & Maul 2005). In Germany, *L. polyphyllus* is one of the 15 most common non-native species (Nehring et al. 2013) and considered to be among Europe's 100 worst invasive species (Nentwig et al. 2018).

As *L. polyphyllus* overgrows and shades the underlying vegetation, its spread may lead to a considerable decline in species richness (Thiele et al. 2010; Hiltbrunner et al. 2014) and abundance of low-growing species (Valtonen et al. 2006). Furthermore, it fosters the growth of tall, nitrogen-demanding vegetation (Otte & Maul 2005; Hansen et al. 2020). The negative effects of *L. polyphyllus* on the native vegetation are correlated to stand size (Ramula & Pihlaja 2012) and lupine cover (Hansen et al. 2020). In particular, semi-natural grasslands are threatened by *L. polyphyllus*, as these ecosystems harbor a high number of light-demanding, small-growing species (Dierschke 1997). Overall, *L. polyphyllus* may induce a decline in species numbers, which may lead to the homogenization of the vegetation composition of different invaded grassland types (Hansen et al. 2020). Furthermore, due to its high water content and the presence of alkaloids in *L. polyphyllus*, invaded grasslands provide hay of low fodder quality (Hensgen & Wachendorf 2016). This makes the biomass harvested from lupine-invaded meadows less suitable as animal fodder and may lead to the disposal of the biomass, thus resulting in reduced interest in the conservation of invaded grasslands by farmers.

In the Rhön region, *L. polyphyllus* was introduced extensively in the 1930s to meliorate the nutrient supply in young spruce forests and to stabilize verges of a newly built road (Volz 2003). From this time on, it was commonly found along road verges, along forest edges, and on clearance cairns throughout the study area (Volz 2003). However, the species was not considered problematic until some decades later. In the 1990s, around the time when the Rhön UNESCO Biosphere Reserve was established, the traditional mowing date in early July for many sites was postponed to August and September, mainly in order to conserve the populations of protected ground-nesting birds such as black grouse (*Tetrao tetrix*), corn crake (*Crex crex*) or whinchat (*Saxicola rubetra*). Furthermore, due to the abovementioned changes in the traditional grassland use, semi-natural grasslands have decreased in economic importance since the 1950s. This led to the cessation of accompanying grassland management in addition to regular mowing, such as the control of problematic plants like *L. polyphyllus* (Volz 2003). Moreover, fallows became more frequent, as a considerable amount of grasslands in the study area cannot be mown by machinery due to stoniness or wet soil conditions (Kirchner, pers. communication). Additionally, the earlier start of the vegetation period (DWD 2021) may have favored the spread of the *L. polyphyllus*, as the traditional mowing date in the area was set under different climatic conditions. Overall, the

combination of these changes allowed *L. polyphyllus* to spread extensively in the grasslands of the study area. Due to the extent of the invasion and the negative effects of *L. polyphyllus* on invaded ecosystems, the invasion is considered a major threat for the biodiversity in the Biosphere Reserve (Otte & Maul 2005).

Chapter outline

This dissertation is based on three manuscripts, which were submitted to international peer-reviewed scientific journals. All three manuscripts presented in Chapters 2, 3, and 4 are already published. Study 1 analyzes the changes of the spatial distribution of *L. polyphyllus* during an 18-year period and its distributional patterns on a landscape scale. Study 2 investigates the effects of different cutting dates and easily assessable seed traits on the germination capability of lupine seeds, aiming at broadening time windows for grassland management. Study 3 explores the field dispersal processes of two of the most important management-driven dispersal vectors for species occurring in semi-natural grasslands: mowing machinery and sheep endozoochory. Further, I contributed essential knowledge on the interplay of different dispersal vectors and the soil seed bank in semi-natural grasslands. In this synthesis, I complement the findings of study 3 by assessing the dispersal potential of *L. polyphyllus* by these two vectors. Based on all three studies, I draw conclusions concerning the integration of invasive species control and the sustainable management of semi-natural grasslands.

In the following paragraphs, the contents and methods applied in each study are briefly introduced prior to the synthesis of the main results and conclusions of this dissertation.

Chapter 2

Applying landscape structure analysis to assess the spatio-temporal distribution of an invasive legume in the Rhön UNESCO Biosphere Reserve

This manuscript analyses the changes in spatial distribution of *L. polyphyllus* in a part of the Rhön Biosphere Reserve over an 18-year period (1998-2016). To this end, I mapped the lupine distribution in 2016 by means of aerial mapping combined with field mapping. I then quantified the development since 1998, based on a map by Otte and Maul (2005), using a change detection analysis. Each *L. polyphyllus* stand was assigned to one of three classes based on lupine cover: low cover stands (5% cover), medium cover stands (<5–50%), and high cover stands (>50% lupine cover). I then performed landscape structure analysis for the mapped lupine stands and for the invaded landscape in 1998 and 2016. I investigated the differences between the grasslands in the study area as well as the development of the lupine stands using principal components analyses (PCAs). Based on these results, I used generalized linear models (GLM) to assess if the

proportion of habitat patch covered by lupine was linked to grassland type or landscape metrics, such as the Euclidian distance to the next road, or size or shape of the respective grassland patch. Furthermore, to compare the proportion of habitat patch invaded between grasslands within each year, I calculated post-hoc pairwise Tukey tests.

Chapter 3

Germination of the invasive legume *Lupinus polyphyllus* depends on cutting date and seed morphology

This chapter analyzes the germination capability of *L. polyphyllus* seeds cut on different dates. Therefore, I collected seeds of *L. polyphyllus* from five locations weekly during a six-week period. This covered a phenological phase from when seeds were already formed, but not fully developed, until seeds are shed by the plants. For each cutting date, I determined seed traits (color/size/hardseededness) that may be associated with successful germination. These traits can be assessed by managers for an improved timing of lupine cutting. This may broaden time windows for management and can increase the efficiency of control measures. I combined two germination experiments: a common garden experiment and a climate chamber experiment. As response variables, I used germination percentage (%), mean germination time (days), and synchrony of germination (unitless). The effects of the experimental variables cutting date, seed size, seed color, and proportion of hard seeds on the response variables were analyzed using linear mixed-effect models (LMM) and generalized linear mixed-effect models (GLMM) for binomial distributions, for which sampling location was included as a random factor. To choose the best seed traits or trait combinations to explain germination success of *L. polyphyllus*, I compared these models via AIC and pairwise model ANOVA.

Chapter 4

Mowing machinery and migratory sheep herds are complementary dispersal vectors for grassland species

In this study, I assessed the role of two characteristic vectors of human-mediated dispersal in semi-natural grasslands: mowing machinery and migratory sheep herds. Both vectors are pivotal for the long-term conservation of semi-natural grasslands, as they transport seeds between grassland patches to overcome seed limitation. In grasslands invaded by *L. polyphyllus*, the benefits of seed dispersal may be diminished due to the dispersal of lupine seeds to uninvaded sites. However, the number of seeds that are transported in the field under the established grassland management is unclear. To this end, I sampled plant material from mowers after twelve mowing events at the beginning of July, a typical mowing date in non-intensively used grasslands in central Europe. For the sheep samples, I collected sheep dung from three sheep herds weekly

during a thirteen-week period. I assessed the species found in each vector in the greenhouse using the emergence method (Roberts 1986) over the course of 11 months. I compared the species compositions of the dispersal vectors to the aboveground and seed bank vegetation species pools of the study area by calculating the relative species abundances of each sample. I performed a trait-based analysis to explore the differences between the traits of dispersed species and non-dispersed species in both species pools. In these analyses, I focused on database traits relevant for dispersal ability by either vector (i.e. leaf dry matter content, maximum releasing height, seed longevity, seed volume, flowering duration, and Ellenberg indicator value for nutrients) and plant functional groups (i.e. proportion of herbs and grasses). Compositional differences were assessed using non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities, a PerMANOVA, and an indicator species analysis. The trait differences between dispersal vectors and species pools were assessed using log-response ratios of the unweighted mean trait values of each sample (mower, sheep dung) compared to the mean trait value of the non-dispersed species of each species pool (aboveground, seed bank) and tested using fixed-effects metaregressions.

Main Results and Conclusions

Protected areas like nature reserves are designated to safeguard native habitats, native species, and remnants of historic cultural landscapes from the negative effects of global change (Hannah et al. 2007). To present, many protected areas achieve this goal, for example by providing barriers against biological invasions (Gallardo et al. 2017). However, as the number of invasive species in protected areas is predicted to increase (Gallardo et al. 2017), monitoring new and established invasive species is pivotal for developing successful strategies that consider both invasive species control and the respective conservation targets (Foxcroft et al. 2017). One of the most fundamental resources for monitoring and assessing the risk of invasive species at a landscape scale are maps that display invasive species distributions. Although detailed maps of the spatio-temporal distribution of invasive species are urgently needed, they are seldom available, mainly due to high costs and organizational demands (Malmstrom et al. 2017). The results of the study on the distribution of *L. polyphyllus* (Chapter 2) reveal that, during the 18-year investigation period, *L. polyphyllus* had spread extensively, as the area covered by lupine stands had doubled and lupine cover had increased in most sites. While in 1998 lupine stands were characterized by small sizes, high edge densities, and compact form, lupine stands in 2016 were fewer, but much larger and less compact. These changes in landscape metrics indicate the transition from a mid- to a late-stage invasion (Clark et al. 2018), with formerly small satellite stands rapidly increasing in size and completely covering grassland patches. In the study area, both the high amount of semi-natural grasslands and the open landscape lacking dispersal barriers facilitated the spread of *L. polyphyllus*. Rapid growth in stand sizes is typical for many invasive species and has been observed for *L. polyphyllus* in other regions, e.g., in Chile (Meier et al. 2013). As the negative effects of *L. polyphyllus* increase with both higher lupine cover (Hansen et al. 2020) and larger stand sizes (Ramula & Pihlaja 2012), it is highly likely that the effects on the native vegetation have increased since 1998. Furthermore, the results indicate that all grasslands in the study area provide suitable habitat for the invader. However, the success of *L. polyphyllus* also depends on management decisions made by local farmers (e.g., mowing dates, additional invader control), and the establishment of the plant in a meadow can be prevented given adequate management (Volz 2003). Therefore, during the investigation period, management was ineffective in mitigating the spread of *L. polyphyllus* (Chapter 2). The change detection analysis revealed that the invasion had progressed and many parts of the study area were heavily invaded in 2016. Upon reviewing maps created by several Bachelor's and Master's students (Fey 2020, Horlemann 2017, Weber 2019), it became evident that the trends observed in chapter 2 were consistent throughout the study area (Fig. 1.2). To this end, the lupine distribution in the study area is characterized by large and dense

stands that are typically found on large grassland patches that are often located adjacent to roads. Based on these findings, the lupine invasion in the Rhön mountains can be considered one of the largest invasions of *L. polyphyllus* in semi-natural grasslands in Central Europe. Given the scale of the invasion, a rapid eradication of *L. polyphyllus* is highly unlikely. However, due to the detrimental effects of *L. polyphyllus* on grassland vegetation and its ability to alter invaded ecosystems, control and preventative measures should still be carried out, even when the chances of eradication are low (following Richardson et al. 2000). Due to the large area already invaded by lupine, it is likely that propagule pressure is high in many places and new sites will be invaded in the future.

In invasive species management, control measures during early invasion stages ('nascent foci', Moody and Mack 1988) are generally more effective and much less costly than control during later stages (Veitch & Clout 2002), for example due to high propagule pressure. Given the high potential of *L. polyphyllus* for self-dispersal of up to 6 meters via ballochory (Volz 2003) and its ability to spread vegetatively via rhizomes, small stands within a meadow can increase in size rapidly. Thus, management schemes in the Biosphere Reserve should primarily focus on monitoring uninvaded sites and target newly emerging stands. There are various methods available for fine-scale monitoring: mapping via aerial photographs provides detailed information, and in Germany, this data is available from the federal states every two to four years. However, capture dates vary between years and may be unsuitable for some sites in some years. Monitoring of *L. polyphyllus* via UAV-borne remote sensing seems to be a promising approach (Wijesingha et al. 2020), but at the current state is not possible on a landscape scale, mainly due to limited operating ranges and regulatory hurdles. Site managers could be involved in monitoring efforts, but this further complicates grassland management and may result in low data quality. When single lupine plants or small stands are identified, they can be removed mechanically by uprooting using weeding forks or by manual scything over several years (Kirchner, unpublished data). Chemical control has also proven successful (Walczak, unpublished data), but should be avoided on larger scales in the context of nature reserves.

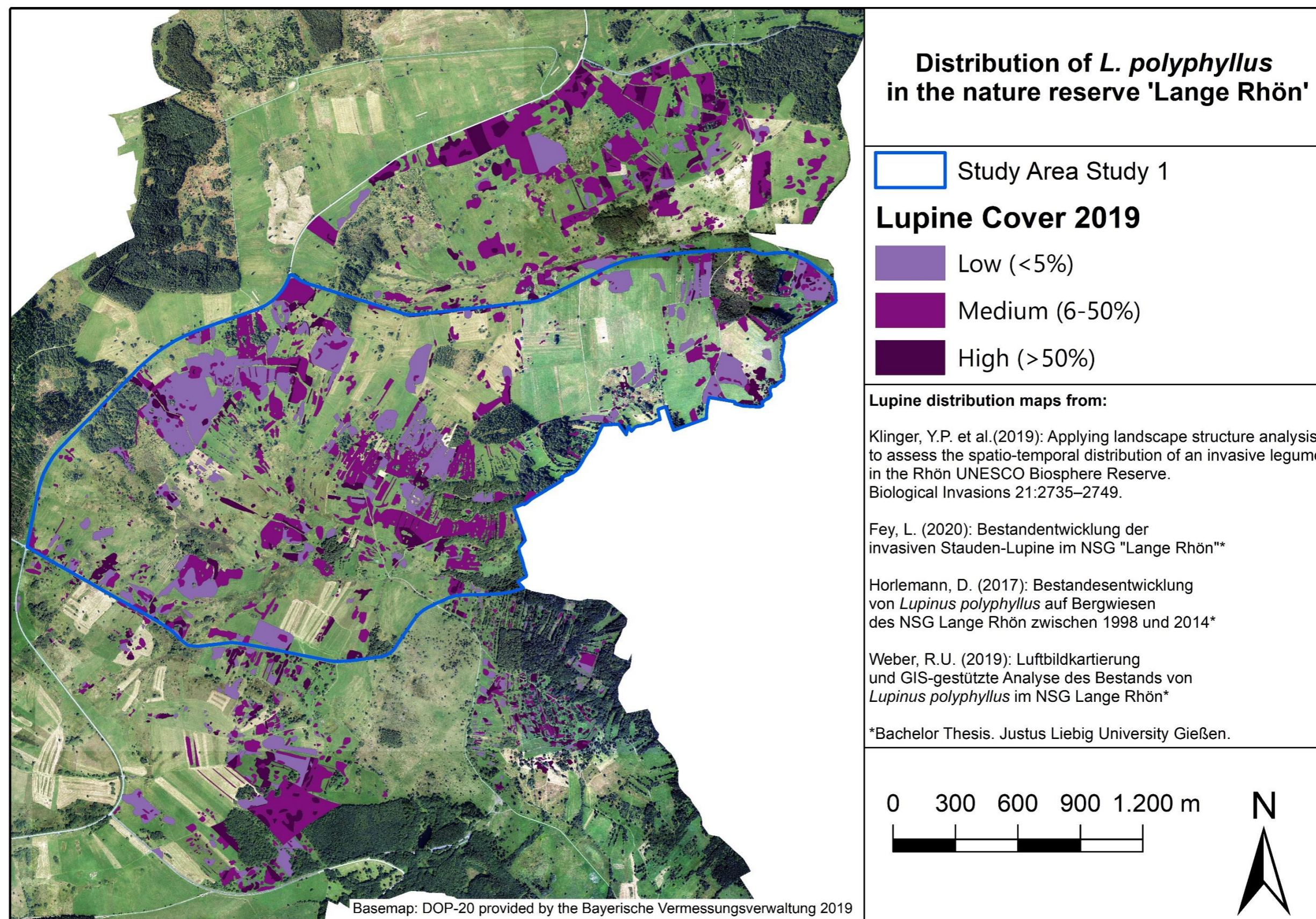


Figure 1.2 Map of the *L. polyphyllus* distribution 2019 in the Study Area.

Knowledge about the long-distance dispersal of invasive species is necessary to decrease the number of propagules that are transported to new sites. While there are many potential dispersal vectors available for *L. polyphyllus*, e.g., game, birds, hikers, or motor vehicles, grassland management has the highest potential to transport large amounts of seeds directly between suitable habitat patches (Poschlod & Bonn 1998). However, if and how well a species can be dispersed depends on its phenological status, i.e. the amount of viable seeds produced. Although invasive species managers aim to undertake control measures prior to seed production (i.e. during the flowering stage), this may not be possible when facing large-scale invasions or when other conservation goals must be considered. Study 2 (Chapter 3) explored the germination capability of *L. polyphyllus* seeds cut during different stages of development. One aim of the study was to broaden time windows for lupine control. To this end, seed traits that can be assessed by managers in the field were determined and checked for their relationship to germination capability (such as seed color, Fig. 1.3). Study 2 revealed that even unripe seeds of *L. polyphyllus* are capable of successful germination. However, germination capability increases significantly during the first three weeks after seed formation. Furthermore, the germination capability of lupine seeds was related to seed color and the amount of hard seeds, increasing with darker seed color and higher percentage of hard seeds. This indicates that plants carrying green and soft seeds may be cut when control during flowering is not possible. The production of black and hard seeds should be avoided, as the risk of dispersal of viable *L. polyphyllus* seeds is strongly increased in these seeds. Furthermore, as hard seeds express dormancy, they are more likely to germinate during favorable spring conditions (Masuda & Washitani 1992) or to be carried over to the soil seed bank (Russi et al. 1992a), which should be avoided.



Figure 1.3 Seed Colors Distinguished in the Germination Study (Chapter 3).

While the germination study (Chapter 3) revealed the potential of *L. polyphyllus* seeds of different development stages to be dispersed, it was widely unknown to what degree the grassland management leads to the dispersal of the species. The role of two vectors of human-mediated dispersal, mowing machinery and migratory sheep endozoochory, was investigated in

Chapter 4. Regular mowing and grazing by migratory sheep are pivotal management actions for the conservation of semi-natural grasslands (Kapfer 2010). The frequency and intensity of the disturbance induced by these actions shapes the composition of aboveground vegetation and soil seed bank (Klaus et al. 2018). In addition to influencing site conditions, both mowing machinery and migratory sheep herds usually move between different grassland patches. Thus, they provide the opportunity for seeds of typical species to be transported (Janzen 1984; Strykstra et al. 1997). In modern landscapes, the vast majority of dispersal processes have been lost compared to historical landscapes (Poschlod & Bonn 1998) and in particular remnants of semi-natural grasslands are affected by seed limitation (Bakker & Berendse 1999). In addition to these problems, plant invasions impose the challenge on grassland management to mitigate the spread of invaders without discontinuing the dispersal of typical species. As the land-use in the study area “Lange Rhön” consists of a combination of non-intensive mowing and migratory sheep herding, it makes for an interesting model landscape to study dispersal processes in semi-natural grasslands. In the case of *L. polyphyllus*, the benefits of seed dispersal by management may be diminished by the dispersal of lupine seeds to new sites. Thus, managers are looking for information on how to combine grassland management and invasive species control. The study on seed dispersal (Chapter 4) has shown the interplay of different dispersal vectors in semi-natural grasslands: While many plant species were transported by mowing machinery and by sheep endozoochory, there were significant differences between the species compositions of both groups. Furthermore, different functional traits were favorable for the transport by each vector. Thus, both dispersal vectors complement each other and the loss of one vector will lead to the loss of dispersal for typical species. Additionally, the study revealed that neither dispersal vector fulfills the role of the soil seed bank (‘dispersal in time’, see Plue and Cousins 2018), as there were significant differences between the species transported by the vectors and the species found in the soil samples.

Only two of more than 22,000 propagules determined in the seed dispersal study (Chapter 4) were *L. polyphyllus* seedlings. Similar results were found when studying the soil seed bank in the study area, where, despite high abundance of *L. polyphyllus* in the aboveground vegetation, only five lupine individuals were found among 14,400 seedlings (Ludewig et al. 2020). These low numbers may be explained by different factors: Generally, both abundance in the field and high seed production increase the probability that viable seeds are dispersed by any dispersal vector (Strykstra et al. 1996), or enter the soil seed bank (Bakker et al. 1996). In many plant species, high seed production is strongly associated with smaller seed sizes (Moles & Westoby 2006). Consequently, the effects of seed size and seed production on dispersal ability can hardly be

disentangled in most studies (Bruun & Poschlod 2006). *L. polyphyllus* is characterized by relatively large seeds (on average 3.9 mm for fully ripened seeds, Chapter 3) and a medium seed production (up to 2000 seeds per plant). This may lead to a low number of transported *L. polyphyllus* seeds (Tab. 1.1). However, the potentially low dispersal ability may be compensated by the high seed production per area in dense *L. polyphyllus* stands. Furthermore, a tradeoff between seed size and seed production is not found in *L. polyphyllus* (Aniszewski 2001; Söber & Ramula 2013), which means that large seeds are produced even by individuals with higher seed production. Large seed sizes are positively correlated with seedling survival (Moles & Westoby 2006), which may lead to high establishment rates. Given this factor, a low number of dispersed lupine seeds may be sufficient to colonize new sites.

Table 1.1 Potential of *L. polyphyllus* to be dispersed by different dispersal modes.

Dispersal mode	Potential for dispersal of <i>L. polyphyllus</i>	Relevant traits	Remarks	Literature
Ballochory	+	Seed size Seed production	Dispersal distances of up to 6 m. Seeds are shed over several weeks if lupine plants are left untouched.	Volz (2003), own unpublished data
Hydrochory	-	Buoyancy	Low buoyancy of lupine seeds	Otte et al. (2020)
Endozoochory	+/-	Seed size Seed production hardseededness	High seed survival in a feeding experiment, but only low numbers were found in the field	Otte et al. (2002), Chapter 3
Epizoochory	0	Releasing height Hooked appendages	Lack of data.	-
Hemerochory	+	Releasing height Seed production	High potential if ripe seeds are abundant in the field	Own unpublished data

As different mechanisms underlie the transport of propagules by different vectors (Poschlod & Bonn 1998), specific factors support the dispersal by mowers and sheep. For dispersal by mowers, it is evident that only species that carry viable seeds by the time of mowing are transported (Strykstra et al. 1997), while species that have not produced seeds suffer from biomass loss and do not benefit from dispersal. Concerning functional traits, high releasing heights are positively correlated with dispersal by mowers (Strykstra et al. 1997), which may also favor the tall-growing *L. polyphyllus*. Given the high amount of lupine in most of the sampled grasslands, the early phenological state of lupine plants during the sampling on July 4, 2017

provides evidence for this finding: By the time of mowing, most plants were still in full bloom or carried green seeds, which are characterized by low germination percentages (as shown in Chapter 3). Due to this, mowing machinery was sampled again on several mowing dates in the years 2018 and 2019 (unpublished results) and the adherent *L. polyphyllus* seeds were counted. I found between 30 and 130 lupine seeds per mowing event in mid July 2018 and 2019. Furthermore, later cutting dates led to an increased number of lupine seeds being dispersed. Additionally, other machinery that is employed in the study area may be relevant for the spread of *L. polyphyllus* seeds in the landscape, e.g., rakes and baling presses. For dispersal by sheep endozoochory, different factors may be relevant compared to mowers. As migratory sheep herding is carried out in the study area throughout the vegetation period, the dispersal of species may be less restricted by the timing of seed production. However, the results of the dispersal study (Chapter 4) showed that there may be stronger filtering concerning some functional traits. In particular, endozoochory seems to be highly restrictive concerning seed size, favoring species with very small seeds. Furthermore, according to the ‘foliage is the fruit’ theory (Janzen 1984), traits that influence palatability, such as nitrogen content, are deciding in which species are eaten and, thus, dispersed via the feces. Additionally, low releasing heights have been shown to increase seed uptake by ungulates (Albert, Auffret, et al. 2015) and traits such as hardseededness (Russi et al. 1992b) or the formation of mucilaginous seed surfaces (Lepková et al. 2018) can increase survival rates of seeds in the animal gut. The germination study (Chapter 3) has shown that lupine seeds quickly become hardseeded, which can increase the survival in the animal gut and in the soil seed bank. Additionally, endozoochory is complemented by epizoochory, the dispersal via hoofs or fur, which has been reported to be more selective concerning functional traits (Couvreux et al. 2005; Albert, Auffret, et al. 2015), but also more efficient for dispersal concerning the survival of seeds (Manzano & Malo 2006) compared to endozoochory. Thus, epizoochory increases the amount of species that can be dispersed and favors different traits compared to endozoochory, e.g., species with higher releasing heights or seeds with hooked appendages (Albert, Mårell, et al. 2015). Concerning *L. polyphyllus*, its high seed production, nitrogen content and hardseededness increase the probability of dispersal via sheep endozoochory (or by wild animals such as boars, deer, or birds). In contrast, its large seeds and high releasing height (up to 150cm) can be considered detrimental for dispersal via endozoochory, but not for epizoochory (Tab. 1.1). However, herd management (e.g., whether lupines carrying ripe seeds are grazed or not) is highly relevant and the low amounts of lupine seeds found in the sheep dung samples may give evidence of suitable grazing regimes in the study area. Finally, if a dispersed lupine seed will be able to germinate and establish successfully additionally depends on the ripeness of the seed (Chapter 3).

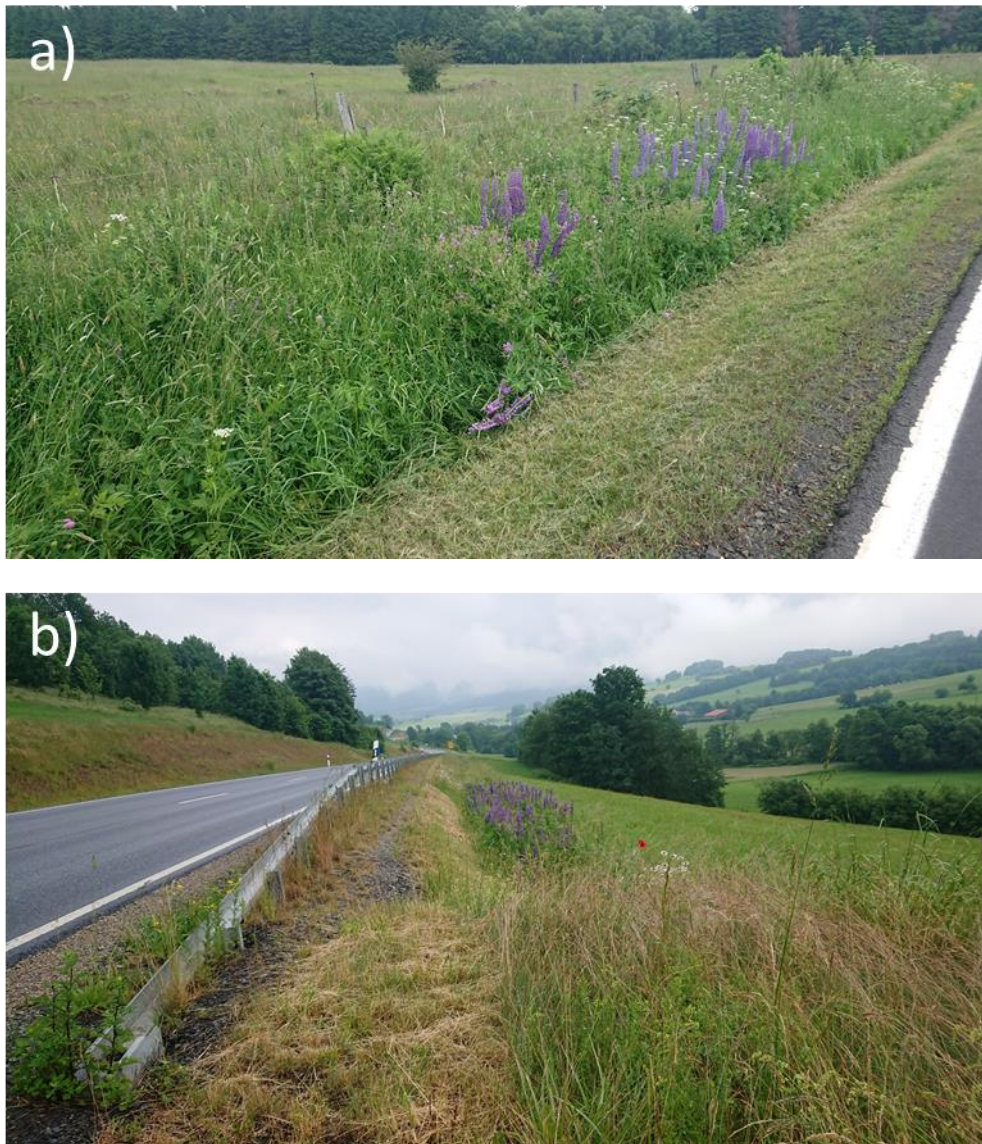


Figure 1.4 *L. polyphyllus* can frequently be found on the back slopes of roadsides a) inside and b) outside of the study area.

In the study area, the distance of a grassland patch to the road network was the most deciding factor explaining the lupine invasion during the investigation period. Roadsides are both habitats and corridors for *L. polyphyllus* in the study area, and thus influence factors such as propagule pressure on adjacent grassland patches (Lockwood et al. 2009). Roadside ecosystems are strongly altered by human activity, as they are characterized by high disturbance frequencies and strongly modified soils and may be imposed to strong fluctuations in habitat conditions such as water availability and salinity (Mederake et al. 1989). Due to this strong anthropogenic effect, roadsides are one of the most important habitats for non-native plant species (Lemke et al. 2019). Examples of invasive species persisting along roads comprise widespread species such as common ragweed (*Ambrosia artemisiifolia*; Lemke et al. 2019) and narrow-leaved ragwort (*Senecio inaequidens*; Ernst 1998) in Europe or smooth bedstraw (*Galium mollugo*) in North America

(Meunier & Lavoie 2012). In a landscape context, roadsides can be considered as both habitats and corridors for non-native species (Thiele et al. 2008), as they may host populations over long timespans. Additionally, roadside populations strongly facilitate human-mediated dispersal, for example via motor vehicles (Lemke et al. 2019). Furthermore, as roadsides are the primary pathways of invasive species introductions into protected areas (Spellerberg 1998), it is obvious that the management of nature reserves should comprise these ecosystems. In the case of *L. polyphyllus*, roads play a pivotal part for its spread (Chapter 2), as the species was deliberately used for greening along newly built roads when the local 'Hochrhönstraße' was built (Volz 2003). Usually, the invader grows in the back slope zone of road verges (Karim & Mallik 2008), where grassland vegetation is commonly found (Fig. 1.4). Recent mappings in the study area have shown that up to 27% of roadsides were invaded by *L. polyphyllus*, and roadside management in the Hessian part of the study area has been adapted to mitigate its further spread (Jolitz 2013). The success of the implemented measures has to be closely monitored during the following years. However, roads are also utilized by several potential dispersal vectors such as tractors and migratory sheep (Chapter 4) and may thus be important pathways even when roadside populations are managed.

Finally, management should be adapted on areas that are already heavily invaded. Given the scale of the invasion (Chapter 2), considerable work and time have to be involved. We have shown that *L. polyphyllus* has not invaded the soil seed bank in the study area yet (Ludewig et al. 2020), which strongly facilitates eradication. Study 2 underlines that time windows for adequate management are short, as seed ripening is finished 2-3 weeks after seed formation. Additionally, even unripe seeds of *L. polyphyllus* showed relatively high germination percentages under experimental conditions (~8%). Inadequate timing of management actions, i.e. when high amounts of ripe seeds have already been produced, may lead to the unwanted dispersal of the species. Thus, management has to consider the phenological stage of lupine stands when planning control measures. The results of the germination experiments (Chapter 3) have shown that the color of lupine seeds is a good indicator both of viability as well as of the timing of germination. Based on these results, it can be concluded that managing lupine populations carrying brown or black seeds should be avoided (or followed thorough cleaning of the equipment after management), whereas managing *Lupinus* stands carrying green seeds may be more feasible. However, as the study area is of high conservational value and hosts a vast amount of endangered vascular plants, insects, and ground-nesting bird species (Grebe 1995), other conservation targets have to be considered when planning invasive species control. This complicates the management of the invader and calls for a more flexible conservation framework

that allows yearly adjustments in conservation management. In addition to lupine control, typical species of the respective vegetation types that have disappeared or been greatly reduced in abundance may have to be reintroduced by restoration measures such as green hay transfer or sowing. However, negative effects of *L. polyphyllus* on the native vegetation may be hard to quantify in some cases, also due to the typical time lag in grasslands reacting to negative effects, such as isolation (Pärtel et al. 2005).

Implications for management

In invasive species management, measures to prevent invasive species from reaching new sites should be prioritized over invasive species control (Veitch & Clout 2002). Based on the findings of my dissertation discussed above, these landscape-scale management recommendations can be derived (ordered by priority):

- 1.) Monitoring of uninvaded sites and control of satellite populations
- 2.) Identifying dispersal vectors and mitigating long-distance dispersal
- 3.) Roadside management, including the back slope of roadsides
- 4.) Management adapted to the phenological status of *L. polyphyllus* on heavily invaded sites

In accordance with the overarching targets of the Convention on Biological Diversity, the monitoring of uninvaded sites and the mitigation of long-distance dispersal should be prioritized by nature conservation in the study area. Furthermore, in the context of protected areas, holistic management schemes should consider the landscape context more carefully. In particular, habitats such as roads and ditches are pathways of introduction of non-native species from other areas as well as a source of propagules of invasive species within a landscape (Pauchard & Alaback 2004) and should thus be included in management. The control of *L. polyphyllus* at a landscape scale will require considerable time and effort. The most promising approach for large-scale control (e.g., on roadsides and heavily invaded grasslands) comprises mowing adapted to the phenological status of *L. polyphyllus*. In most years, early mowing will be necessary, i.e. when the species is still flowering (Volz 2003). A single mowing event per year has also been suggested by other authors, e.g., by Ramula (2020). However, due to the favorable climatic conditions in the study area, *L. polyphyllus* is capable of resprouting and producing seeds after a second flowering period in late summer/early autumn, which was also observed in Norwegian lupine populations (Brobäck 2015). Thus, for an effective control, a single, early mowing event has to be complemented either by a second mowing, by aftermath grazing, or by additional manual lupine control. Management in the study area has shown first success in reducing the cover of *L. polyphyllus* in some sites, but evidence of success on larger scales is still missing.

This thesis has shown that invasive species, such as *L. polyphyllus*, impose an additional and underestimated threat on Central European semi-natural grasslands. With the ongoing globalization, invasive species are projected to become more problematic throughout the world in the future, also in protected areas. Science on invasive species in agriculturally used grasslands is still relatively scarce, maybe due to the low degree of invasion compared to other ecosystems (Axmanová et al. 2021) or due to the factor that negative effects of invasive species on grassland vegetation may take decades to manifest. Thus, more research on invasive species in grasslands is necessary to address upcoming challenges in the future.

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Chapter 2

Applying landscape structure analysis to assess the spatio-temporal distribution of an invasive legume in the Rhön UNESCO Biosphere Reserve

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Abstract

Landscape composition and structure may strongly affect the spread of invasive species in landscapes. Landscape analysis provides a powerful toolset for assessing invasive species invasions over time and for planning control measures. We applied a combination of aerial mapping and landscape analysis to assess the invasion of the legume, *Lupinus polyphyllus*, in the Rhön UNESCO Biosphere Reserve. The Biosphere Reserve contains different types of large and well-connected grasslands threatened by lupine invasion. We assessed the changes in lupine distribution between 1998 and 2016 in a strictly protected part of the Biosphere Reserve by means of landscape structure analysis. The area invaded by *L. polyphyllus* doubled from 1998 to 2016. While the number of lupine stands decreased by 25%, stand size on average increased by 300%; stands also became less compact during that period. Furthermore, the degree of invasion of different grassland types changed. In 1998, all investigated grassland types were invaded to equal extents, whereas in 2016, large and well-connected mesic grasslands located close to roads were more heavily invaded than small and remote wet grasslands. Our results show that landscape composition plays an important role for the spread of lupine. Specifically, invasive stand characteristics, such as stand size, form, and connectivity, are crucial for driving the invasion of lupine. Therefore, in addition to landscape composition, invasive stand characteristics should be included in the planning of conservation measures. Overall, aerial mapping combined with landscape analysis provides a cost-effective and practical tool for landscape managers to prioritize invasive control measures.

Keywords

Landscape structure, *Lupinus polyphyllus*, Mountain grassland, Plant invasions, Protected area

Introduction

Many factors influence the invasion success of exotic species. Plant species that are intentionally introduced by humans have a better overall chance to become established in new regions, mainly due to factors such as higher propagule pressure (Lockwood et al. 2009) and repeated introduction events over a longer timespan (Stohlgren et al. 2008). Therefore, these species have a high disposition to establish in areas outside their natural range and are more likely to become invasive than unintentionally introduced species (Lockwood et al. 2009). Nitrogen-fixing plants that were originally spread for soil melioration are particularly problematic, as they may drastically alter the nutrient conditions of the invaded habitats (Hiltbrunner et al. 2014). They are considered successful invaders, especially in habitats with low nutrient levels (Lee et al. 2017). Furthermore, they may substantially change the nitrogen turnover on different spatial scales, depending on the plant ecology of the invasive and the extent of the invasion. Possible consequences of an invasion by nitrogen-fixing plants include accelerated successional processes and shifts in species compositions, resulting in an overall loss of biodiversity (Thiele et al. 2010).

The analysis of invasion patterns and processes on varying spatio-temporal scales is important to understand the impact invaders have on species diversity at the landscape level. Especially in protected areas like nature reserves, the understanding of landscape-scale patterns underlying an invasion is a crucial prerequisite to develop successful long-term strategies for the regulation of invaders and to limit further spreading. Therefore, it is obvious that landscape-scale monitoring of invasions is important for landscape managers, e.g. farmers (Malmstrom et al. 2017) and conservationists (Foxcroft et al. 2017); however, this has rarely been done, mainly due to high costs and high organizational demands (Malmstrom et al. 2017).

Landscape metrics are an important toolset to analyze landscape structure and quantify land-use changes (Lang and Blaschke 2007). Therefore, they may contribute valuable information about the establishment of invasives in landscapes, i.e., spatial distribution, temporal spread, and connectivity of stands (Rodewald and Arcese 2016). Since measures to manage or control invasives vary greatly depending on stand size, shape, and connectivity, detailed maps of invaded ranges are needed (Malmstrom et al. 2017). Aerial mapping presents a cost-effective method to create detailed maps that provide crucial information for landscape managers. The combination of aerial mapping and the application of landscape metrics offers the potential to assess plant invasions over time. Currently, when assessing an invasive species invasion, the link between remote sensing/aerial mapping and (1) assessing the degree of invasion, (2) identifying sensitive habitats, and (3) identifying invasion pathways, is often missing.

The legume *Lupinus polyphyllus* Lindl. originates from Pacific North America. In addition to its widespread occurrence as ornamental plant outside its native range (Fremstadt 2010), it is commonly used for soil stabilization and to improve the nitrogen budget of nutrient-poor sites (Rehfuess et al. 1991). *L. polyphyllus* can be found worldwide, e.g. in Northern (Fremstadt 2010) and Central Europe (Hejda 2013), New Zealand (Holdaway and Sparrow 2006), and Chile (Meier et al. 2013). Due to its wide range of applications, the species has colonized many different habitats, e.g. linear habitats such as forest edges, road verges (Valtonen et al. 2006), and riparian terraces (Holdaway and Sparrow 2006), but also non-intensively used mountain grasslands (Otte and Maul 2005). In addition to changing soil nitrogen availability (Hiltbrunner et al. 2014), *L. polyphyllus* substantially affects the vertical structure of invaded grasslands (Otte and Maul 2005). It overgrows and shades the underlying vegetation and causes a considerable decline in the richness of small species (Thiele et al. 2010; Hiltbrunner et al. 2014), while promoting the spread of tall-growing, nitrogen-demanding vegetation (Otte and Maul 2005).

In the Rhön UNESCO Biosphere Reserve, the invasion of *L. polyphyllus* in non-intensively used grasslands (namely Habitats Directive 92/43/EEC, habitat types 6520: mountain hay meadows, and 6230: species-rich *Nardus* grasslands) was observed during the last two decades (Otte and Maul 2005), despite management efforts to control the species. Grasslands provide suitable habitats for *L. polyphyllus*, but it is not clear whether invasion success and intensity vary among grassland types, and if so, if these differences are due to habitat characteristics or landscape composition.

In our study, we first assessed how the spatial distribution of *L. polyphyllus* has changed since the first structured assessment of the invasion status in 1998. Then, we analyzed whether the distribution of *L. polyphyllus* over the different grassland types in the study area changed with time. Lastly, we analyzed the relationship between the occurrence of *L. polyphyllus* grasslands and linear landscape elements that may serve as potential source habitats, namely, road verges and clearance cairns.

To this end, we applied a combination of aerial mapping and landscape structure analysis both on the lupine stand and habitat patch level to quantify and analyze changes in spatial distribution of *L. polyphyllus* in a 407 ha area of the Rhön UNESCO Biosphere Reserve. We explicitly addressed the following questions:

1. How did the number, size, and cover of *L. polyphyllus* stands change during the last two decades? How did the spatial distribution and connectedness of *L. polyphyllus* stands change?

2. Which grassland types does *L. polyphyllus* invade most heavily? Did the level of invasion change among different grassland types during the last 18 years?
3. Do the size and shape of grasslands, and their proximity to linear habitats lead to a more frequent occurrence of *L. polyphyllus*?

Based on our analyses, we have developed management recommendations for conservation managers to reduce the impact of *L. polyphyllus* on local biodiversity and to limit its further spread.

Materials and methods

Study area

The study was conducted in the Rhön UNESCO Biosphere Reserve, which comprises an area of approximately 2400 km² in Central Germany. The study area, the “Leitgraben” (407 ha), is located in the High Rhön plateau (from 50°26'N to 50°32'N and from 09°54'E to 10°05'E), between 600 and 950 m a.s.l. It is one of the distribution centers of *L. polyphyllus* in the Biosphere Reserve and has been the subject of previous studies (Volz 2003; Otte and Maul 2005).

One of the prominent characteristics of the landscape is 8900 ha (~ 5% of the total area of the Biosphere Reserve) of spacious and well-connected mountain grasslands (e.g. Habitats Directive 92/43/EEC, habitat type 6520: mountain hay meadows and 6230: species-rich *Nardus* grasslands) with a long and continuous land-use history which included traditional mowing and sheep-herding with low nitrogen inputs (Otte and Maul 2005). Therefore, this region is of high importance for the conservation of these habitat types in Central Europe (Grebe 1995). Another pronounced feature is the high frequency of linear clearance cairns between grassland patches. These cairns consist of stones that were removed from the grassland by farmers to improve manageability of the sites.

The climate of the study area is characterized by a low mean annual temperature of 5.4 °C (mean of 1980–2010 of Mt. Wasserkuppe, 950 m a.s.l.; DWD 2016), high annual precipitation of 1176 mm (mean of 1980–2010; DWD 2016), and a short growing season. Basalt rocks form the bedrock in the study area. Soils that develop on basaltic bedrocks are well supplied with base cations. High precipitation as well as the traditional land-use lead to low nutrient availability and very low pH values in most of the Rhön region (Puffe and Zerr 1988).

L. polyphyllus was first introduced to Germany in the 1890s. Due to its high invasive potential, it is among the 15 most common non-native species today (Nehring et al. 2013). It was introduced to the Rhön region in the 1930s to meliorate the nutrient supply in spruce forests and to stabilize verges of newly built roads (Volz 2003). In 1991, the UNESCO established the Rhön

Biosphere Reserve to protect the species-rich, semi-natural mountain meadows and the ground-breeding black grouse (*Tetrao tetrix*) (UNESCO 2007). In an attempt to conserve the largest Central European population of black grouse outside the Alps, the annual mowing scheme of the mountain meadows was postponed. Thereafter, meadows that had traditionally been mown in early July were cut much later, i.e., in August and September. While the former early mowing dates inhibited the spread and establishment of *L. polyphyllus* in the meadows, the later mowing allowed most lupine plants to produce and spread ripe seeds before mowing. Overall, this land-use change due to nature conservation likely resulted in the extensive spread of *L. polyphyllus* and its establishment in many protected meadows. Today, large parts of the region are heavily invaded (Otte and Maul 2005), and this invasion is considered a major threat for the biodiversity of the mountain meadows in the Biosphere Reserve.

Mapping of *L. polyphyllus* stands

To quantify the spatial patterns of *L. polyphyllus* in the study area, we compared the distribution of *L. polyphyllus* between 1998 and 2016. During the flowering phase in July 1998, aerial photographs (Kodak SO 359) of the area were taken at a 1:2500 scale (Otte and Maul 2005). Based on these aerial photographs, mapping of *L. polyphyllus* stands was conducted by Otte and Maul (2005) using a stereoscope, followed by field validation. Each patch of *L. polyphyllus* ($n_{1998} = 993$) was assigned to one of three classes based on lupine ground cover: low cover stands with $\leq 5\%$, medium cover stands with $> 5\text{--}50\%$, and high cover stands with $> 50\%$. The same classification was used in the 2016 mapping of *L. polyphyllus* stands ($n_{2016} = 709$). To assess the present state of the *L. polyphyllus* distribution, we conducted aerial mapping followed by field validation. The aerial mapping was based on digital Orthophotos with 20 cm ground resolution (DOP-20) taken on September 28th, 2014, provided by the Bavarian Agency for Digitization, High-Speed Internet and Surveying. As the Orthophotos were taken after the main *L. polyphyllus* flowering period and some areas had not regrown after mowing by that time, we used a supplementary dataset of 41 non-orthorectified aerial photographs taken on June 18th, 2014 (during the main flowering period of *L. polyphyllus* and before mowing), provided by T. Kirchner from the Wildlandstiftung Bavaria. To verify the results of the aerial mapping and to update the dataset to represent the status of 2016, a field validation of the mapped lupine stands was carried out in June and September of 2016.

Spatial distribution and landscape analysis

To quantify the development of the spatial distribution of *L. polyphyllus* stands between 1998 and 2016, we calculated landscape metrics for all stands in each of the three lupine cover classes (low,

medium, and high lupine cover) between 1998 and 2016. Landscape metrics are widely used to quantify landscape patterns (Gustafson 1998). We applied the landscape metrics to describe lupine stand structure (size, number of stands) and stand form (edge/perimeter, shape index; Forman and Godron 1986; Lang and Blaschke 2007). To obtain information about the connectedness of *L. polyphyllus* stands to each other, we calculated the nearest neighbor distance (edge-to-edge Euclidean distance) and the proximity index (the size-to-distance ratio of the neighboring patches, see Gustafson and Parker 1994) using a buffer of 10 m, as lupine seeds are dispersed within this radius around the mother plant. Based on the obtained information at the lupine stand scale, we summarized these metrics at both the cover class and landscape levels (meaning over all cover classes). To quantify changes of the spatial distribution of stands between 1998 and 2016, we carried out a change detection analysis. Change detection is a widely applied tool that can be used to assess land-use land-cover changes over time (Wegmann et al. 2016). To this end, we transformed the two *L. polyphyllus* distribution maps from vector format to a raster format (using a cell size of 0.25 m²). For both years, each of the three lupine cover classes, as well as the class “no lupine cover,” were assigned unique numeric values. Afterwards, the 2016 raster was subtracted from the 1998 raster, resulting in a change-raster with 16 unique values that represent the 16 possible class changes between the two years. Calculations were completed using the V-LATE extension (Lang and Tiede 2003) for ArcGIS® 10.4 by Esri (www.esri.com).

Based on digital habitat-type maps of 1993 and 2006 (Luftbild, Umwelt, Planung GmbH 2008), the four most significant grassland types in our study area in terms of total area were *mesic mountain hay meadows* (38% of total area), *mesic Nardus grassland* (21%), *wet mountain hay meadows* (14%), and *wet Nardus grassland* (9%). During the field mapping in 2016, we updated the habitat type map to account for changes since 2006. We focused on these four grassland types in our analysis. For each grassland patch, we calculated landscape metrics (area, perimeter, shape index) and summarized these metrics at the grassland class and landscape levels. Furthermore, to quantify and standardize the overall area occupied by *L. polyphyllus*, we estimated the proportion of area covered by *L. polyphyllus* for each grassland patch. To this end, we merged the lupine distribution map of 1998 with the habitat-type map of 1993, and the distribution map of 2016 with the most recent habitat type map of 2006.

We mapped clearance cairns based on Orthophotos from 2014 (DOP-20) (Bavarian Agency for Digitization, High-Speed Internet and Surveying 2014) and extracted streets and agricultural roads from the automated real estate map. We assessed the occurrence of *L. polyphyllus* in clearance cairns and along roads in the study area. For each grassland patch, we calculated the shortest Euclidean distance to the nearest road and clearance cairn. Using this information, we

analyzed if *L. polyphyllus* occurrence in grasslands depended on the Euclidean distance of grassland patches to linear habitats.

Statistical analysis

We analyzed the differences in landscape metrics of lupine stands (namely, lupine stand size, stand edge/perimeter, stand shape, nearest neighbor distance, and stand proximity) between two years (1998 and 2016) and three cover classes (low, medium, and high cover). We carried out a principal component analysis (PCA). Each lupine stand was treated as a sample, and the data was standardized and centered. Stand size, stand edge, nearest neighbor distance, and stand proximity were log₁₀-transformed.

We carried out another PCA to analyze differences in landscape metrics (patch size, edge, shape, shortest Euclidean distance to roads and clearance cairns) between the four predominant grassland types (mesic mountain hay meadow, mesic *Nardus* grassland, wet mountain hay meadow, wet *Nardus* grassland) and two years (1998 and 2016). Each grassland patch was treated as a sample, variables were standardized and centered. Patch size, patch edge, shortest Euclidean distances to roads and to clearance cairns were log₁₀ transformed.

We used Generalized Linear Models (GLMs) for quasibinomial distributions to test if proportion of habitat patch covered by *L. polyphyllus* depended on grassland type and/or habitat patch landscape metrics (patch size, edge, and shape, as well as the shortest Euclidean distance to roads and clearance cairns) for each year. We then fitted models with these landscape metrics as factors, both in combinations and as single-factor models for each year. To choose the best grassland properties or property combination to explain the proportion of grassland patch covered by *L. polyphyllus*, we compared these models via Akaike Information Criterion (AIC) and pairwise model ANOVA. To compare the proportion of habitat patch invaded between grasslands within each year, we calculated post hoc pairwise Tukey tests. The final models included grassland type and shortest Euclidean distance to roads and clearance cairns as relevant factors. We visually checked for normality of residuals and homogeneity of variances using diagnostic plots (Zuur et al. 2010). All statistical analyses were carried out in R 3.4.4 (R Core Team 2016). Post-hoc analyses were completed using the “multcomp” package (Hothorn et al. 2008), and graphs were created with “ggplot2” (Wickham 2016).

Results

Lupine stand development

The area invaded by lupine approximately doubled from 1998 to 2016 (Fig. 2.1a, b). The largest shares of the invaded area in 2016 consisted of lupine stands of medium (44% of total area covered by lupine stands) and low cover (also 44%), while high cover stands made up a relatively small portion (12%). While the area covered by low and high cover stands approximately doubled, the area of medium cover stands increased disproportionately from 11.6 to 42.9 ha. Both in 1998 and 2016, lupines could be found in all parts of the study area, while only a few large meadows (> 5 ha) were not invaded in 2016. The variability of stand sizes was generally very high (ranging over several orders of magnitude in both years; 1998: 1 m² to 6.9 ha and 2016: 3 m² to 7.9 ha, Fig. 2.2).

The first and second PCA axes explain 52.1 and 26.2% of the variance, respectively (Fig. 2.3). The first axis mainly represents lupine stand size and stand edge, and the second axis represents the proximity index and the nearest neighbor distance. Average stand size differed between lupine cover classes and years, and increased (more than doubled, on average) for all classes between the two years (Fig. 2.2). Furthermore, the size of low cover stands increased more between the two years than the size of medium or high cover stands, so that over the course of the 18 years, low cover stands became larger than medium cover stands, which became larger than high cover stands (Fig. 2.3).

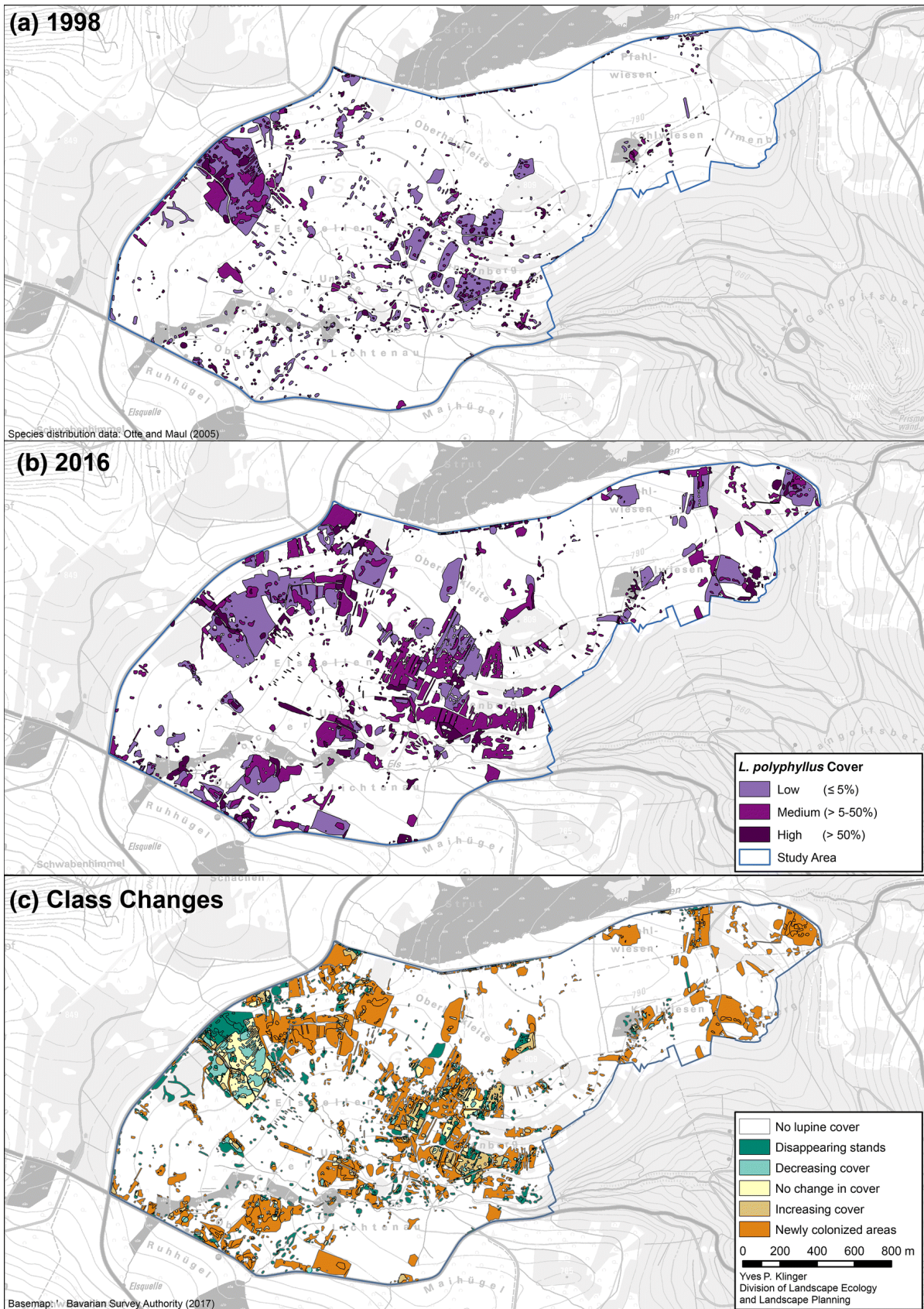


Figure 2.1 Map showing the distribution of *L. polyphyllus* between the years **a** 1998 and **b** 2016, and **c** the class changes 1998 versus 2016 in the study area. Lupine distribution increased strongly during the investigated period, with most 2016 stands being in relative proximity of 1998 stands. Lupine distribution data 1998 by Otte and Maul (2005)

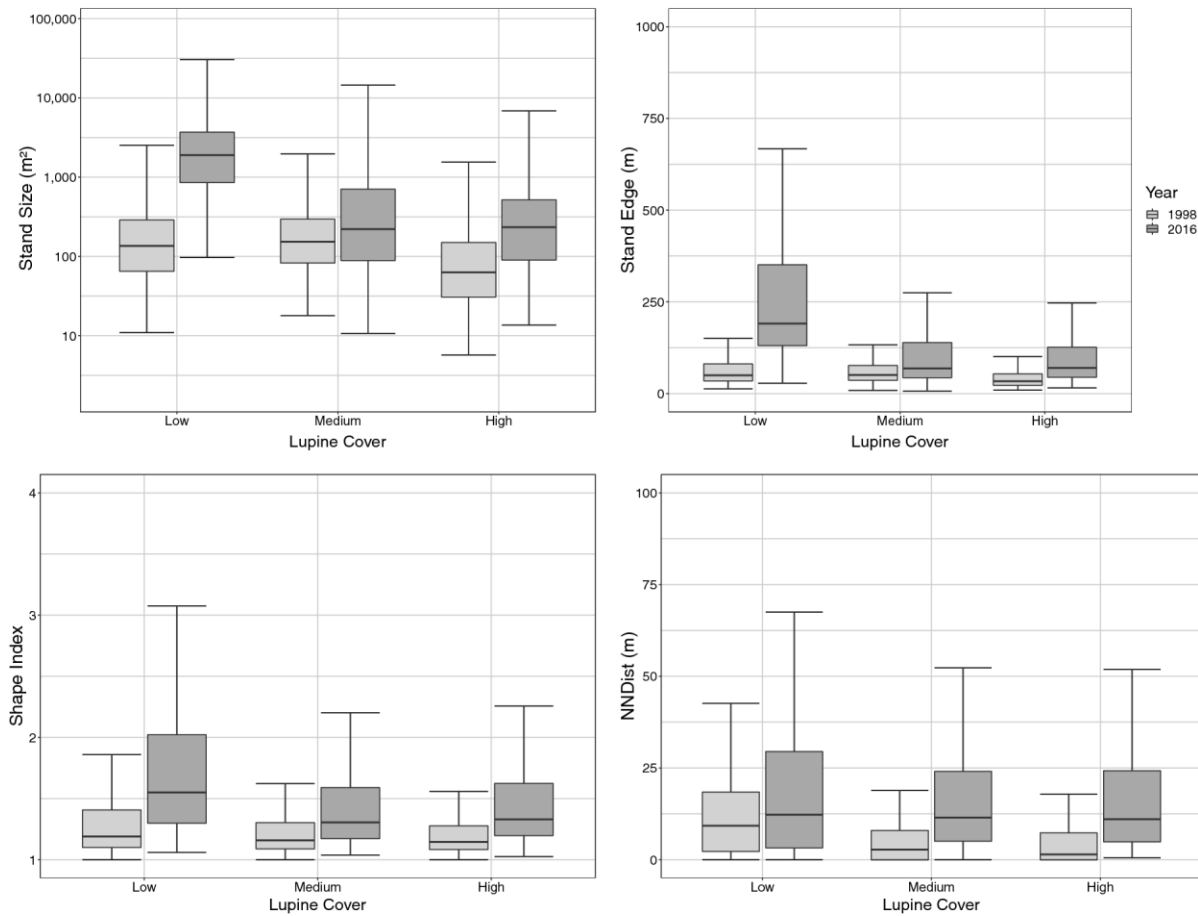


Figure 2.2 Box-Whisker Plot showing the increases of *L. polyphyllus* stand size, stand edge/perimeter, shape index, and nearest neighbor distance from 1998 (light gray) to 2016 (gray). The different classes display the lupine cover: low cover stands with $\leq 5\%$ lupine cover, medium cover stands with $> 5\text{--}50\%$ lupine cover, and high cover stands with $> 50\%$ lupine cover. The plot shows the Median, the box represents the Inter Quartile Range (IQR), and Whiskers show the 1.5-fold IQR

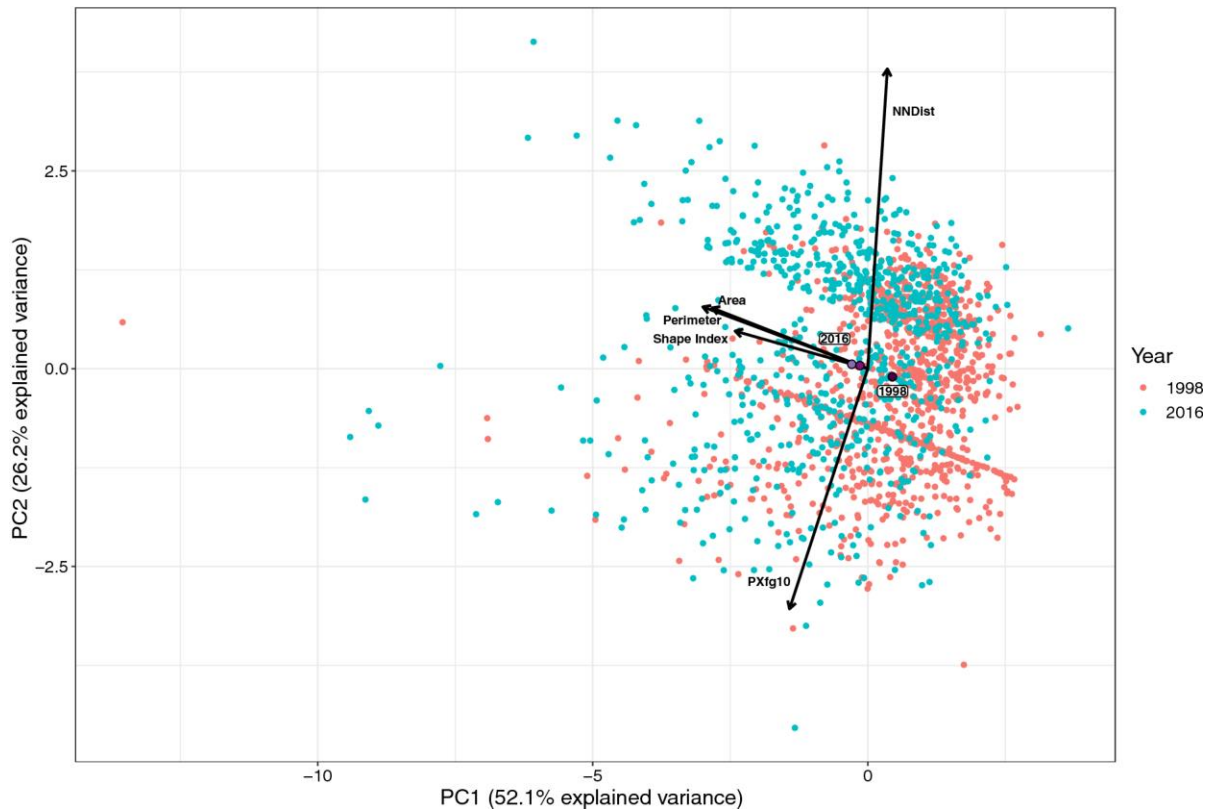


Figure 2.3 Principal component analysis (PCA) showing the changes in lupine stand landscape metrics between 1998 ($n = 993$) and 2016 ($n = 709$). Small points represent lupine stands, labels show centroids (means) of years, large circles show centroids of the three cover classes (light purple = low lupine cover, purple = medium lupine cover, dark purple = high lupine cover) over both years. The first axis (52.1% explained variance) represents changes in the stand area and stand edge/perimeter, and shape index. The second axis (26.2% explained variance) represents changes in nearest neighbor distance and proximity index

The total number of lupine stands decreased by approximately 25%. While in 1998 there were many small *L. polyphyllus* stands that were spatially separated from each other, by 2016, many of them have merged and form large stands covering entire meadows. This finding was supported by an increase in the mean patch proximity of *L. polyphyllus* stands from 1998 to 2016. Additionally, mean stand edge increased for all classes between years, mainly due to the increase in stand sizes during this period (Fig. 2.2). In contrast, total edge decreased between 1998 and 2016 for low cover stands and increased for medium and high cover stands. The edge density of lupine stands in our study area decreased from approximately 1800 to 1200 m ha^{-1} . This finding was accompanied by an increase in the shape indices of the *L. polyphyllus* stands between grassland classes and years (Fig. 2.2), meaning *L. polyphyllus* stands were on average less compact in 2016 than in 1998 (with landscape-level shape index increasing from 1.27 to 1.5). While the shape index of low and high cover stands increased, the shape index of medium cover stands only increased marginally.

Most of the invaded sites in 2016 were newly colonized during the investigated period, i.e., there was no occurrence of *L. polyphyllus* at these sites in 1998 (on 72.1 ha, Fig. 2.1c). About 80% of these newly colonized areas (57.5 ha) could be found close to lupine stands (within a 10 m edge-to-edge distance) that already existed in 1998, while 20% (14.6 ha) were located further away. Altogether, changes in lupine cover were detected on 115 ha of the study area (Fig. 2.4). The major share of these changes indicated increasing cover of *L. polyphyllus* (on 80.9 ha). This can be seen particularly in two sections in the western and central part of the study area that were already invaded in 1998, as these grew notably and increased in lupine cover. A smaller share of the changes consisted of areas with decreasing lupine cover (overall on 24.6 ha, ca. 20% of the invaded area) and no change in lupine cover (on 9.5 ha, thus only a small percentage of lupine stands did not change at all).

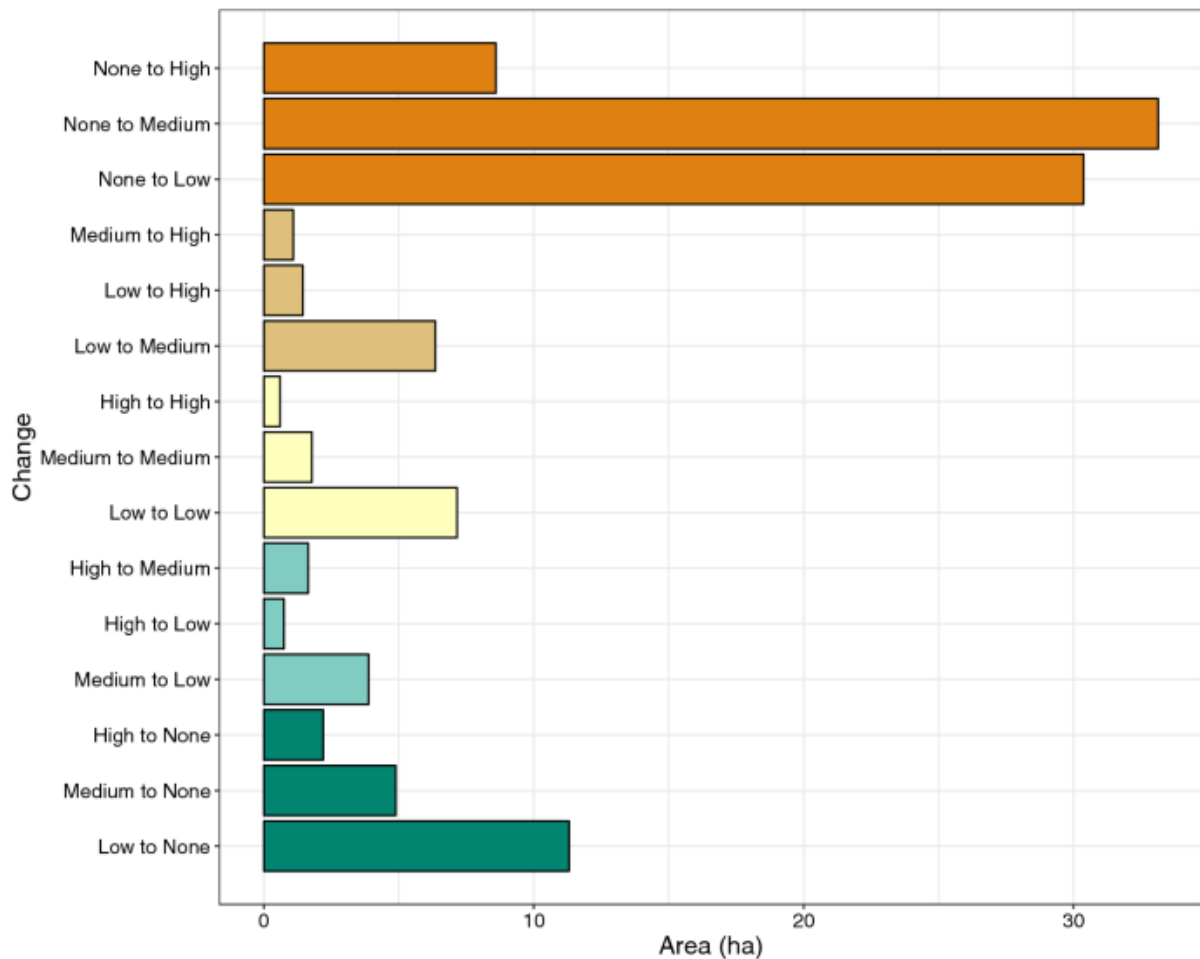


Figure 2.4 Total area of the 15 classes resulting from the change detection of the *L. polyphyllus* distribution 1998 versus 2016. The predominant class change was the colonization of new areas

Grassland types and *L. polyphyllus*

The first and second PCA axes explain 51.2 and 19.3% of the variance (Fig. 2.5). The first axis mainly represents patch size and patch edge, and the second axis represents distance to linear structures and shape index. Overall, the ordination shows that mesic grasslands were larger, more compact, and less remote than wet grasslands in our study area. This was shown by differences in patch sizes as well as decreases in the shape indices between mesic and wet mountain hay meadows. In contrast, mesic and wet *Nardus* grasslands were similar in shape. Furthermore, mesic grasslands were located closer to linear structures than wet grasslands. The mean Euclidean distance to the nearest road or clearance cairn differed between grassland types, with mesic mountain hay meadows being the closest to roads (often being located adjacent to them), followed by mesic *Nardus* grasslands. Both mesic hay meadows and *Nardus* grasslands were located similarly close to clearance cairns. In contrast, wet mountain hay meadows and *Nardus* grasslands were on average more remote and thus located further away from roads.

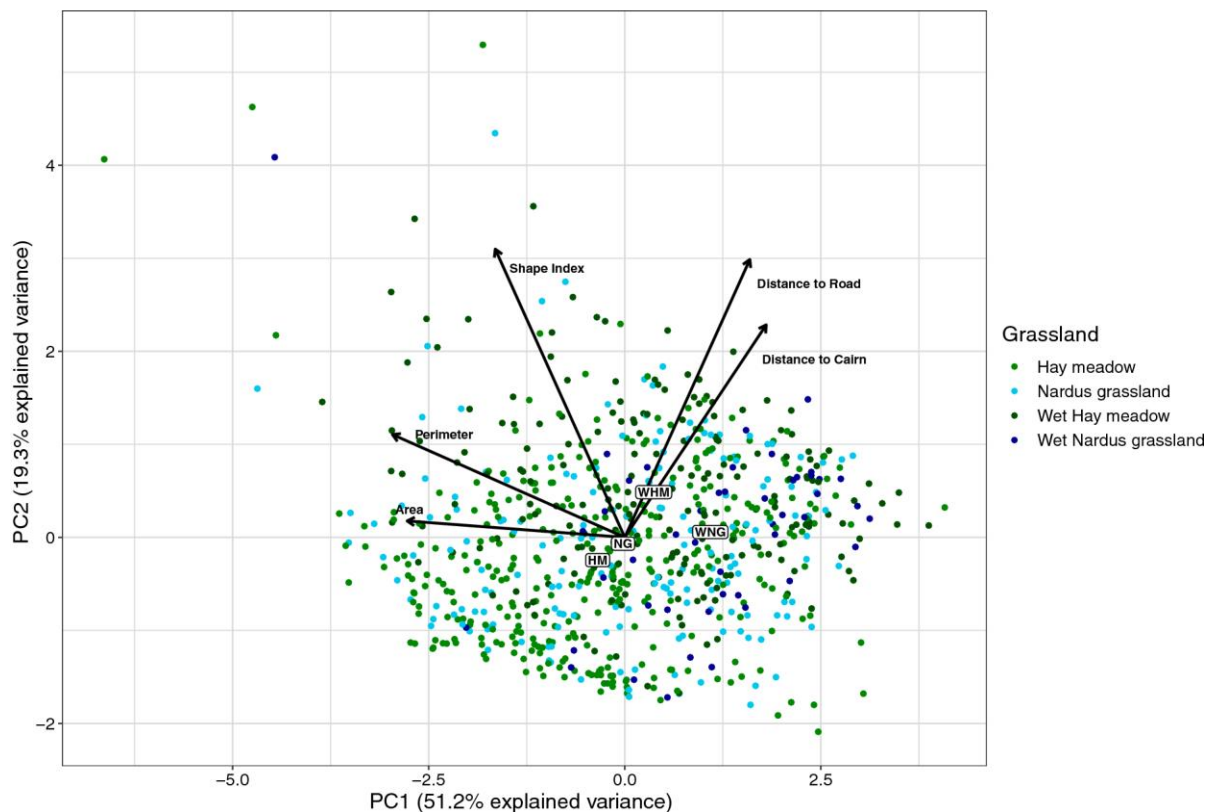


Figure 2.5 Principal component analysis (PCA) showing the changes grassland patch landscape metrics the four predominant grasslands (HM *mesic mountain hay meadows*, NG *mesic Nardus grassland*, WHM *wet mountain hay meadows*, and WNG *wet Nardus grassland*). Points represent grassland patches, labels show centroids (means) of different grasslands. The first axis (51.2% explained variance) represents changes in the patch size and patch edge/perimeter. The second axis (19.3% explained variance) represents changes in the shortest Euclidean distances to roads and clearance cairns as well as the shape index

The occurrence of *L. polyphyllus* in grasslands shifted substantially between 1998 and 2016. While there were no significant differences in the amount of habitat covered by lupine stands between the four grassland types in 1998, in 2016 there were very significant differences between grassland types ($p < 0.001$, Tab. 2.A1). In 1998, the percentage of habitat covered by *L. polyphyllus* was 14.6% on mesic mountain hay meadows, 13.3% on mesic *Nardus* grassland, 12.8% on wet mountain hay meadows, and 11.4% on wet *Nardus* grassland (Fig. 2.6). In 2016, the proportion of habitat covered by *L. polyphyllus* approximately doubled on mesic mountain hay meadows (31.2%) and mesic *Nardus* grassland (28.3%), while there was only a small increase in area covered on wet grasslands (13.9% on mountain hay meadows and 15% *Nardus* grassland) compared to 1998 (Fig. 2.6).

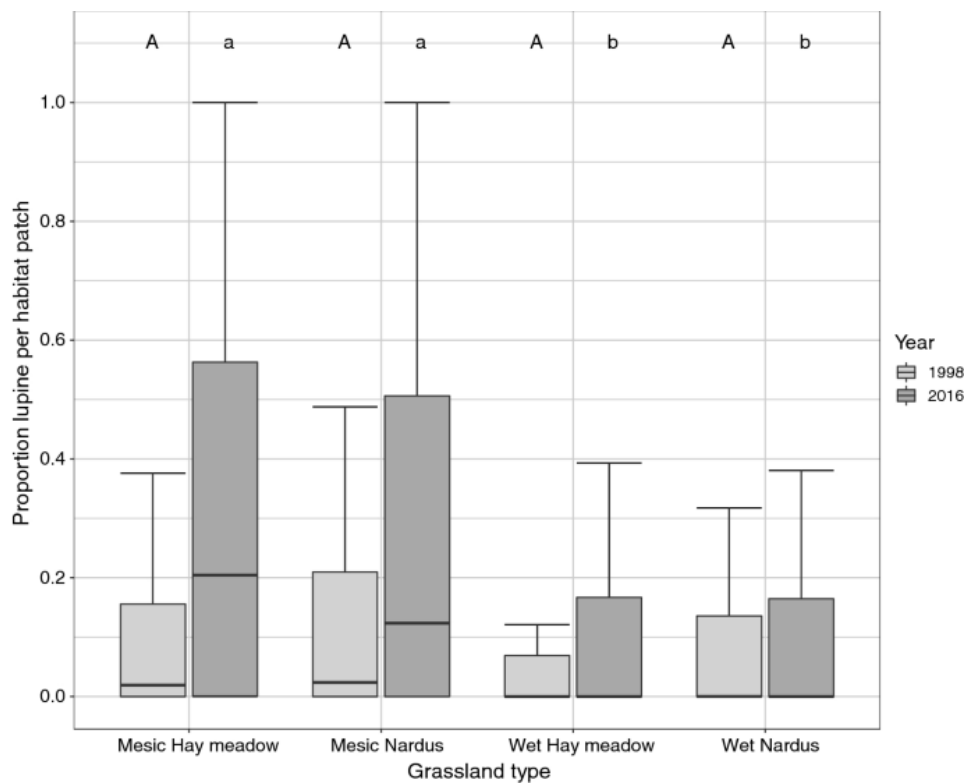


Figure 2.6 Box-Whisker Plot showing the differences in the proportion of habitat area invaded by *L. polyphyllus* of the four predominant grassland types 1998 (light gray) and 2016 (gray) (*mesic mountain hay meadows*, *mesic Nardus* grassland, *wet mountain hay meadows* and *wet Nardus* grassland). The plot shows the Median, the box represents the Inter Quartile Range (IQR), whiskers show the 1.5-fold IQR. Differences were tested within years using a glm and post hoc Tukey tests. While the proportion of invaded habitat area was the same for all grasslands in 1998 (capital letters), in 2016 mesic grasslands were more heavily invaded than wet grasslands (lowercase letters)

L. polyphyllus stands covered a higher proportion of habitat patches in grasslands close to linear structures. According to the model, grassland patches with a distance of 50 m to roads have approximately three times the lupine cover of patches that are located 200 m away from roads. There was a highly significant positive relation between the Euclidean Distance to the closest

road ($p < 0.001$, Tab. 2.A1) and the proportion of *L. polyphyllus* in grasslands in 2016. Nevertheless, *L. polyphyllus* stands did not occur more frequently or with higher cover on clearance cairns or along road verges compared to grasslands.

Discussion

1. How did the number, size, and cover of *L. polyphyllus* stands change during the last two decades? How did the spatial distribution and connectedness of *L. polyphyllus* stands change?

In the Rhön Biosphere Reserve, the area invaded by *L. polyphyllus* doubled during the last 18 years. This development is clearly reflected in the change of invasive stand landscape metrics. Stand size, shape index, and invasive stand proximity all increased during the transition from small infestations to larger, less compact stands, while stand number and nearest neighbor distance decreased over the course of the ongoing invasion (Figs. 2.2, 2.3). This coalescence, the fusion of small stands with larger ones (Clark et al. 2018), may indicate the change from a mid- to a late-stage invasion. The increase in the mean proximity of stands of all cover classes and the decrease in the average nearest neighbor distance between lupine stands presents further evidence for this argument.

The study area has been characterized by large stands with low *L. polyphyllus* cover (Fig. 2.1). Lack of appropriate management may have led to the rapid increase of lupine cover on invaded meadows (Volz 2003). Similar processes were observed in other habitats, such as gravel beds of the Patagonian Paloma river (Meier et al. 2013). In 2016, more than half of the invaded area consisted of lupine stands of medium or high cover, and many grassland patches were completely invaded by *L. polyphyllus*. Low cover lupine stands may have only minor impact on native species richness (Thiele et al. 2010; Hejda 2013); however, an increase in lupine cover is problematic, because the richness of butterfly (Valtonen et al. 2006) and vascular plant species (Valtonen et al. 2006; Hejda et al. 2017) decreases with high cover of *L. polyphyllus*. Particularly large, dense stands ($> 1000 \text{ m}^2$; Ramula and Pihlaja 2012) may strongly affect the species richness of meadows (Otte and Maul 2005; Thiele et al. 2010) by shading the underlying vegetation and increasing plant-available nitrogen (Thiele et al. 2010; Hiltbrunner et al. 2014). Species-rich mountain meadows are particularly threatened by *L. polyphyllus* invasion, as they provide habitat to many small-growing species (Otte and Maul 2005; Thiele et al. 2010; Ramula and Pihlaja 2012). Moreover, the negative effects of *L. polyphyllus* on biodiversity have been observed even in its native range (Hejda et al. 2017), but not for other lupine species, such as the invasive *L. nootkatensis* in Icelandic grasslands (Vetter et al. 2018). Compared to the negative effects of large stands, small

L. polyphyllus stands are less problematic, although they can represent early invasion stages (Volz 2003; Otte and Maul 2005).

Overall, sites with decreasing *L. polyphyllus* cover and disappearing lupine stands were scarce (Fig. 2.4). The main reasons for decreases in *L. polyphyllus* cover are successional processes (mainly colonization of fallow grassland by shrubs), extinction of smaller populations, and eradication measures. The latter two can hardly be disentangled, as there is little information on where lupine control measures were undertaken, especially during the first years of our investigation period.

2. Which grassland types does *L. polyphyllus* invade most heavily? Did the level of invasion among different grassland types change during the last 20 years?

The level of invasion between the different grasslands changed substantially during our investigation period. In 1998, mesic and wet grasslands were equally invaded, whereas in 2016, mesic grasslands were more heavily invaded than wet grasslands (Fig. 2.6). Most likely, habitat characteristics, such as water availability or plant community composition, may explain this pattern. Even though *L. polyphyllus* can tolerate wet soil conditions (Otte and Maul 2005; Meier et al. 2013), it has an Ellenberg indicator value of 5 (Ellenberg et al. 1992), indicating an ecological optimum on mesic sites. Consequently, its competitive ability and vitality are most likely reduced under wet or waterlogged conditions, because N₂-fixation and root growth and nodulation are very sensitive to reduced O₂ supply in the *Lupinus* genus (Dracup et al. 1998; Malik et al. 2015). This may impede the spread or establishment of *L. polyphyllus* in wet habitats.

3. Do the size and shape of grasslands, and their proximity to linear habitats lead to a more frequent occurrence of *L. polyphyllus*?

In addition to habitat and site characteristics, landscape structure and landscape composition play a pivotal role in explaining plant invasions (With 2002). Landscapes are complex patch-corridor-matrix mosaics, consisting of suitable habitats (patches), unsuitable habitats (matrices), and corridors for the spread of invasive species (Thiele et al. 2008). Thus, both landscape composition and land-use patterns affect the success and speed of invasions (Mooney and Hobbs 2000; With 2002). Recent studies have shown that landscape structure can be as important as local factors for the spread of invasive species and also for subsequent habitat restoration (Leite et al. 2013). High availability of potential, open habitat and the lack of natural barriers in landscapes may facilitate the spread of invaders and hamper restoration (Rigot et al. 2014). This argument is clearly reflected by the case of *L. polyphyllus* in the Rhön Biosphere Reserve. The high proportion of suitable habitat, well-connected landscape consisting of mainly meadows, and a

change in management regime favorable for *L. polyphyllus* made the area particularly susceptible to the invasion. Differences in the degree of invasions across grassland types are also well-explained by landscape metrics, as they clearly show that patches of mesic mountain hay meadows and mesic *Nardus* grasslands were much larger and located closer to roads (i.e., the localities of initial introduction) than their wet counterparts (Fig. 2.5). Therefore, our results support the finding that relatively homogenous landscapes with a high percentage of large and compact patches of suitable habitat are highly prone to invasions (Rigot et al. 2014; Lustig et al. 2017). This is especially true when areas of introduction are well-connected to other patches of suitable habitat in the landscape (Morel-Journel et al. 2016), in the way that the probability of population increase and spread of an invasive insect decreases with higher habitat shape complexity (Lustig et al. 2017).

Linear landscape elements serve as corridors for invasive species to spread across landscapes (Christen and Matlack 2006; Thiele et al. 2008; Rodewald and Arcese 2016). In particular, roads provide potential pathways (Meunier and Lavoie 2012; Ansong and Pickering 2013), leading to higher propagule pressure and more frequent introduction events on adjacent habitat patches, both of which benefit invasion success (Lockwood et al. 2009; Thomas and Moloney 2015). We found that the proximity of grasslands to linear landscape elements has a significant effect on the proportion of area invaded and the number of *L. polyphyllus* stands (Tab. 2.A1). Mesic grasslands were on average located closer to the road network, and they contained more clearance cairns than wet grasslands. *L. polyphyllus* is frequent on roadsides both inside and outside our study area. During most of our investigation period, roadsides were mown after the main flowering period of *L. polyphyllus* in early July (Kirchner, pers. comm.). This allowed the seeds to be dispersed from the roadsides to the adjacent (mostly mesic) meadows, i.e., via ballistic dispersal (Fenner and Thompson 2005; Ramula and Pihlaja 2012), which is characterized by dispersal distances of more than 6 m (Volz 2003). The mechanisms underlying the mid- to long-distance dispersal of *L. polyphyllus* seeds are unknown, but transport via mowing machinery and migratory sheep herds (Auffret and Cousins 2013), both of which utilize roads within the study area, seem most probable. We conclude that, overall, the characteristic grasslands of our study region are highly susceptible to the invasion by *L. polyphyllus*, because they are large and well-connected, and the roads can be considered important corridors for its ability to spread.

Management implications

The effectiveness of control measures for habitat restoration strongly depends on surrounding land use (Thomas and Moloney 2015) and landscape composition (Jonsson et al. 2015), thus management should set spatial priorities (Lustig et al. 2017). As the major share of our study area consists of non-intensively used grasslands that are mown late in the season and provide suitable habitat for *L. polyphyllus*, the effectiveness of eradication measures on invaded sites may be undermined by simultaneous recolonization of treated sites or colonization of new sites. Between 1998 and 2016, the colonization of formerly uninvaded areas was the predominant pattern. Therefore, we suggest that the primary focus of landscape managers should be the protection of meadows that have not yet been invaded by *L. polyphyllus*. Inhibiting the spread of the species may be difficult, as the vectors for its spread are largely unknown. Early mowing dates (in this case, in non-intensively used grasslands in central Germany typically before July 1st) may inhibit the establishment of *L. polyphyllus* (Volz 2003). In our study area, mowing dates are determined by the local government, but also depend on other factors, such as weather conditions and farmer's decisions. Consequently, management for each grassland patch may differ among years, and mowing dates may range from mid-June to late October. Despite these differences, our study clearly shows that, overall, management has not been adequate to inhibit the spread of *L. polyphyllus* in the study area during the last two decades. In the case of the Rhön Biosphere Reserve, we make the following suggestions for landscape management, in order of priority:

1. Inhibit further spread of *L. polyphyllus* by eradicating small stands
2. Manage stands on road verges
3. Set early mowing dates on meadows with large, high cover stands

As long as there are still uninvaded grasslands, landscape managers should first target meadows containing small, pioneer lupine stands, as these stands can still be completely eradicated before they continue to grow, reproduce, and rapidly increase in cover (Volz 2003; Otte and Maul 2005; Meier et al. 2013). While the prevention and early eradication of invasives can be complicated, managing areas that are heavily invaded is even more difficult and costly (Lodge et al. 2006), especially when different conservation goals have to be considered at the same time (Rinella et al. 2009). Furthermore, recolonization can reduce the efficacy of control measures in heavily invaded landscapes (Banks et al. 2018), and identifying important source populations on the landscape level becomes much more difficult during later stage invasions. In the case of *L. polyphyllus*, we therefore suggest carefully monitoring uninvaded sites to inhibit its further spread. Aerial mapping provides an easy, cost-effective, and highly accurate method to monitor the distribution of invasives on smaller scales. The application of landscape metrics can

give landscape managers valuable information about where control measures are most effective by giving information about size, cover, and connectivity of invasive stands.

Our results underline the importance of roads as corridors for the spread of *L. polyphyllus* through the landscape. Therefore, our second suggestion is that lupine stands on roadsides should be a main target of control. Thirdly, meadows with large low cover stands should be managed, as the native species composition might not have been heavily affected yet (Thiele et al. 2010), but an increase in *L. polyphyllus* cover can be expected for low cover sites in the future. This is especially true if the mowing scheme of invaded meadows is not adapted to inhibit the further spread of *L. polyphyllus*. On meadows where species diversity is already reduced, restoration measures, such as seed bank activation and the transfer of seed containing plant material, should be undertaken as soon as lupine cover is reduced.

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Appendix

Appendix 2.1 Model tables for the glms and Post-hoc-tests performed in Chapter 2. GLMs follow the formula: `glm(formula = Lup_per~ Grassland+ Dist_Road +Dist_cairn,family = quasibinomial)`.

1998

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1,35	-0,51912	-0,33275	0,07192	20,40932

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	-1,903988	-0,118276	16,098	< 2e-16	***
Nardus	0,201589	0,17253	1,168	0,243	
Wet Hay meadow	-0,292917	0,348875	-0,84	0,401	
Wet Nardus	-0,05529	0,198797	-0,278	0,781	
Dist_Road	-0,006147	0,001324	-4,645	4,01E-06	***
Dist_Cairn	0,007916	0,001355	5,843	7,62E-09	***

Signif. codes: 0 '***' 0.001
'**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasibinomial family taken to be 0.4433857)

Null deviance: 310.92 on 763 degrees of freedom

Residual deviance: 286.33 on 758 degrees of freedom

Number of Fisher Scoring iterations: 5

2016

Deviance Residuals:

1Q	Median	3Q	Max
-0,6335	-0,3086	0,3713	2,0516

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	-0,670501	0,087186	-7,69	4,49E-14	***
Nardus	-0,001539	0,148378	-0,01	0,99173	
Wet Hay meadow	-0,88107	0,322625	-2,731	0,00646	**
Wet Nardus	-0,694391	0,173655	-3,999	6,98E-05	***
Dist_Road	-0,0092	0,001251	-7,353	4,98E-13	***
Dist_Cairn	0,004345	0,001328	3,273	0,00111	**

Signif. codes: 0 '***' 0.001
'**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasibinomial family taken to be 0.489995)

Null deviance: 452.22 on 774 degrees of freedom

Residual deviance: 390.72 on 769 degrees of freedom

Number of Fisher Scoring iterations: 5

2016: Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:	Estimate	Std. Error	z value	Pr(> z)	
Nardus - Hay meadow == 0	-0,001539	0,148378	-0,01	1	
Wet Hay meadow - Hay meadow == 0	-0,88107	0,322625	-2,731	0,02871	*
Wet Nardus - Hay meadow == 0	-0,694391	0,173655	-3,999	< 0.001	***
Wet Hay meadow - Nardus == 0	-0,879531	0,336098	-2,617	0,03973	*
Wet Nardus - Nardus == 0	-0,692852	0,196427	-3,527	0,00217	**
Wet Nardus - Wet Hay meadow == 0	0,186679	0,340547	0,548	0,94328	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

Chapter 3

Germination of the invasive legume *Lupinus polyphyllus* depends on cutting date and seed morphology

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Abstract

In semi-natural grasslands, mowing leads to the dispersal of species that have viable seeds at the right time. For invasive plant species in grasslands, dispersal by mowing should be avoided, and information on the effect of cutting date on the germination of invasive species is needed. We investigated the germination of seeds of the invasive legume *Lupinus polyphyllus* Lindl. depending on the cutting date. We measured seed traits associated with successful germination that can be assessed by managers for an improved timing of control measures. To this end, we sampled seeds of *L. polyphyllus* on six cutting dates and analyzed the germination of these seeds in climate chambers and under ambient weather conditions. We collected information on seed morphology (color/size/hardseededness) for each cutting date to identify seed traits associated with successful germination. Observed germination patterns were highly asynchronous and differed between seeds cut at different dates. Seeds cut early, being green and soft, tended to germinate in autumn. Seeds cut late, being dark and hard, were more prone to germinate the following spring, after winter stratification. This allows the species to utilize germination niches throughout the year, thus indicating a bet-hedging strategy. Seed color and the percentage of hard seeds were good predictors of germination percentage, but not of mean germination time and synchrony. Managers should prevent the species producing black and hard seeds, while cutting plants carrying green and soft seeds is less problematic. Furthermore, germination patterns differed between climate chambers and the common garden, mainly because germination of dormant seeds was lower in climate chambers. More germination experiments under ambient weather conditions should be carried out, as they can give information on the germination dynamics of invasive species.

Keywords

dormancy, grassland management, lupine, phenology, seed traits

Introduction

The timing of germination determines which environmental conditions the seedling will experience and thereby influences a variety of plant characteristics (Casas et al. 2012). Consequently, the germination ecology of a species largely decides in which habitats and under which climates it may establish. The introduction of species to new ranges often leads to new germination conditions (Kudoh et al. 2007), and the ability to germinate successfully under a variety of environmental conditions is a characteristic of many successful and widespread invasive species (Baker 1974; Wainwright and Cleland 2013). Whether seeds are viable depends largely on their development stage, which is influenced by the timing of seed set and seed ripening.

In semi-natural grasslands, the mowing date is the environmental factor that most strongly determines the timing of seed release. Furthermore, mowing is a way of seed dispersal for species that have viable seeds at the right time. In most cases, the dispersal of mature seeds after mowing is a desirable process, as it is responsible for sustaining a high plant diversity in semi-natural grasslands (Auffret 2011; Humbert et al. 2012). In other cases, such as weeds or non-native invasive species, dispersal of ripe seeds by mowing is not wanted (Wilson et al. 2009) and shifts in grassland management and the time of cutting may create opportunities for invasives to establish in these ecosystems. Consequently, understanding the germination ecology of invasive plants is essential for their management and control and for limiting their spread to new sites.

Established invasive species are often more challenging to manage than newly arrived species (Simberloff 2003). Ideally, control measures would take place before seed formation, but time windows for adequate management can be short in areas where different conservation goals have to be matched. In the case of species invading mountain grasslands, e.g. mowing of areas critical for the protection of ground-nesting birds has to be postponed until nesting is finished, which means that invasive species may have produced viable seeds by the time of mowing. Consequently, managers are looking for information on the relationships between cutting dates, seed morphology, and seed germinability. *Lupinus polyphyllus* Lindl. is a widespread perennial legume originating from North-America. It is widely found as an ornamental plant (Fremstad 2010) and commonly used for soil stabilization and soil melioration (Rehfuess et al. 1991). Due to its many uses, the species is naturalized in different regions all over the world, e.g. in Europe (Fremstad 2010; Hejda 2013), New Zealand (Holdaway and Sparrow 2006) and Chile (Meier et al. 2013). Invaded habitats include road verges (Valtonen et al. 2006), riparian terraces (Meier et al. 2013), and mountain grasslands (Klinger et al. 2019). Due to its ability to fix nitrogen, it is considered an ecosystem engineer and may cause unwanted ecosystem effects (Hiltbrunner et al. 2014). In invaded habitats, *L. polyphyllus* is capable of overgrowing and shading the underlying

vegetation and may cause a considerable decline in the richness of small species (Thiele et al. 2010; Hiltbrunner et al. 2014), while promoting the spread of tall-growing, nitrogen-demanding vegetation (Otte and Maul 2005). Meadows invaded by this species provide hay of low fodder quality, because of its high water-content and the presence of alkaloids in *L. polyphyllus* (Hensgen and Wachendorf 2016).

Despite the importance of seed ecology for the spread and establishment of species, there is often insufficient knowledge concerning germination and ripening characteristics of invasive species (Gallinat et al. 2018). The capability of seeds to after-ripe and germinate, which depends on the interaction between phenology and cutting date, may have important implications for the management of invasive species in grasslands. Therefore, we investigated the germination of the invasive legume *L. polyphyllus* in relation to the cutting date. Over the course of the vegetation period, i.e., weekly from the beginning to the end of fruiting, we sampled seeds from five locations invaded by *L. polyphyllus*. We combined two experiments to investigate the germination of *L. polyphyllus*: A common garden experiment to analyze the germination patterns under ambient weather conditions and a climate chamber experiment under standardized conditions. We aim to provide management recommendations based on seed traits such as seed color and hardseededness that may help to decide when fruiting lupine stands should be cut and when plant material has to be removed from the sites after mowing.

Specifically, our research hypotheses were:

- 1) The germination ability of *L. polyphyllus* seeds increases with later cutting date. Consequently, we expect a higher germination percentage, a shorter mean germination time, and a higher synchrony of germination with later cutting date.
- 2) Seed traits such as seed size, seed color, and the percentage of hard seeds provide reliable information about the germination ability of seeds sampled at different dates. We expect larger seeds, seeds with darker color and harder seeds to show higher germination percentage, shorter mean germination time and higher synchrony compared to small, green, and soft seeds.

Methods

Seed sampling, seed handling, and experimental design

Seeds were collected in the Rhön UNESCO Biosphere Reserve, in central Germany. The study area (from 50°26'N to 50°32'N and from 09°54'E to 10°05'E), a part of the Biosphere Reserve, is situated between 600 m and 950 m a.s.l. It is characterized by large and coherent semi-natural grasslands of high conservational value that are non-intensively used as meadows and pastures

(e.g. Habitats Directive 92/43/EEC, habitat types 6520: mountain hay meadows, and 6230: species-rich *Nardus* grasslands). These grasslands have a centuries-long land-use history of mowing and pasturing with low nitrogen-inputs. In the 1990s, the traditional mowing date in early July was postponed to August and September, in order to safeguard the populations of protected ground-nesting birds and because the meadows decreased in importance for local farmers. This allowed *L. polyphyllus*, already present along roadsides in the area, to produce seeds before mowing and to spread extensively into the meadows. During the past 20 years, parts of the region were heavily invaded, with the area covered by *L. polyphyllus* doubling in some localities (Klinger et al. 2019). This invasion is considered a major threat to the biodiversity of the mountain grasslands in the study region. Depending on site conditions, *L. polyphyllus* can reach a height of 60 to 150 cm. In June and July inflorescences are formed, each consisting of 50 to 80 single flowers (Fremstad 2010; Bundesamt für Naturschutz 2017). *L. polyphyllus* develops seed pods with four to twelve seeds, which burst at seed maturity and spread the seeds ballistically up to several meters (Otte et al. 2002; Volz 2003). Per plant, up to 2500 seeds can be produced (Aniszewski 2001).

Seeds of *L. polyphyllus* were manually collected from five meadows (*sampling locations*) over six weeks (July–August 2015; *cutting dates*). The distance between sampling locations ranged between 1500 and 5000 meters. For each cutting date and location, we sampled one inflorescence each from ten plants for the germination experiments. From each inflorescence, we randomly took one pod and determined seed size, seed color, and the proportion of hard seeds. For seed color, we distinguished between four colors: green, dark green, brown and black. Seeds with different pigmentations and puncturing (see Aniszewski 2001) were integrated to the different classes according to the predominant color, seeds were assigned the color “black” when they were considerably darker than brown seeds. Usually, seeds of several colors were found on the same location or even within the same seed pod. To determine the average color for each replicate, we gave ranks from one (green) to four (black) to each color and calculated the median. For seed hardness, we classified the seeds into five classes, from undeveloped and very soft to very hard. Based on these data, we calculated mean seed size, average seed color and the proportion of hard seeds for each replicate. For the germination experiments, we pooled the seeds within each *sampling location*. Seeds were manually cleaned, air-dried and stored in darkness at room temperature (app. 20 °C) until the start of the germination experiments on September 28th, 2015.

Laboratory experiments are a standardized tool to investigate germination in a controlled environment and can provide information on germination cues, dormancy, and other factors (Baskin and Baskin 2014). Nonetheless, germination in the laboratory often differs from

germination under (semi-)natural conditions (Grime et al. 1981; Hölzel and Otte 2004) and thus gives only a limited representation of germination patterns that can be observed in the field. We combined a climate chamber experiment and a common garden experiment to study the germination of *L. polyphyllus* both under standardized and ambient weather conditions. A factorial experimental design was used to analyze the effects of *cutting date* (6 dates), *sampling location* (5 locations), and *temperature* (day/night: 20/10 °C and 15/5 °C; only in the climate chamber experiment) on seedling emergence. Germination was defined as protrusion of the radicle.

In the climate chamber experiment (from September 28th, 2015 to July 28th, 2016), seeds were placed into petri dishes with distilled water (25 seeds per replicate) in climate chambers (Rumed type 3401, Rubarth Apparate GmbH). Each treatment combination (*cutting date* × *sampling location* × temperature) was replicated five times, resulting in 300 petri dishes. For incubation in climate chambers, we exposed the seeds to 12 h light and 12 h darkness and two diurnally fluctuating temperatures (15/5 °C and 20/10 °C) that represent spring and early summer temperature conditions. Similar fluctuating temperature conditions have been applied by Elliott et al. (2011). Moisture content of the Petri dish was controlled during the experiment. For seeds in the climate chambers, germination was checked once a week and seedlings were removed.

In the common garden experiment, germination was observed under ambient weather conditions from September 17th, 2015 to July 14th, 2016. The seeds were placed on a 1:1 mixture of sand and commercial potting soil (Fruhstorfer Erde, Type P, Industrie-Erdenwerke Archut GmbH, Lauterbach/Germany) in trays (18 × 28 cm) in a common garden at the research station Linden-Leihgestern of the Justus-Liebig University (50°32'N, 8°41'E). Per tray, 25 seeds were used ($n = 5$ for each *cutting date* × *sampling location* combination, resulting in 150 trays). Seeds were protected from predation using wire cages. For seeds in the common garden, germination was checked once every seven to fourteen days. After three months of incubation, germination decreased in both experiments and thus was checked every other week. After ten months of incubation, the experiments ended since no further germination was observed. By the end of the experiments, the remaining seeds were covered by mold and collapsed when pinched by hand. Thus, the remaining seeds were considered dead (following Baskin and Baskin 2014).

Germination variables and statistical analyses

As response variables, we calculated the germination percentage (%), mean germination time (days) and synchrony of germination (unitless) per replicate (according to Ranal and Santana 2006; Ranal et al. 2009). The germination percentage is the proportion of germinated seeds of the total number of seeds. Mean germination time and synchrony of germination were calculated

based on seedling counts over time (Ranal et al. 2009). Mean germination time is a measurement of the weighted average time required for germination (Ranal and Santana 2006). The synchrony index is a measure for the overlapping of germination that ranges from 0 (when no two seeds germinated at the same time) to 1 (when all germinating seeds germinated at the same time; for details see Ranal et al. 2009).

Seeds from the climate chamber experiment and from the common garden experiment were analyzed separately. The effects of the experimental variables *cutting date*, *sampling location* and *temperature* on the response variables germination percentage and germination time were analyzed using linear mixed-effect models (LMM) and synchrony of germination using generalized linear mixed-effect models (GLMM) for binomial distributions. The factors *cutting date* and *temperature* were included as fixed factors in the first models. As there was no effect of the *temperature*, the final models only included cutting date or seed color fixed factors. We added an error term for repeated measures to the models to account for variation within each *sampling location*. Furthermore, we added a general linear hypothesis and multiple comparisons (glht) to determine significant differences between groups.

To identify seed traits associated with germination success, we checked for correlation of seed traits with the factor *cutting date* using Pearson's R^2 . This was the case for *seed size*, *seed color*, and *proportion of hard seeds*. We then fitted models with these traits as fixed factors (both in combinations and as single-factor models) and *sampling location* as random factor. To choose the best seed traits or trait combination to explain germination success of *L. polyphyllus*, we compared these models via AIC and pairwise model ANOVA. To assess model quality, we calculated Nagakawa and Schielzeth's R^2 for linear mixed-effect models (Nakagawa and Schielzeth 2013). We visually checked for normality of residuals and homogeneity of variances using diagnostic plots (Zuur et al. 2010). Mixed-effect models were carried out using the 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017) packages, post-hoc-tests were calculated using the 'multcomp' package (Hothorn et al. 2008), graphs were created using the 'ggplot2' package (Wickham 2016) in R (R Core Team 2016).

Results

During the sampling period, seed color became darker (changing from green via dark green and brown, to black) and the proportion of hard seeds increased gradually. Mean seed size ranged from 3.9 mm (date six, August 11th) to 6.4 mm (date three, July 21st). It increased during the first three weeks of cutting and then decreased thereafter as seeds became drier. Seed color and the proportion of hard seeds were correlated, as hard seeds usually were darker than soft seeds.

There were no differences in the total germination percentages between different *sampling locations*, although the germination peaks shifted by up to two weeks between different locations.

In climate chambers, 16.3% of all collected lupine seeds germinated (Fig. 3.1a, b). Germination percentage was lowest after the first cutting date (July 7th, 8.6%) and increased until the third date (July 21st) where it peaked at 26% (Fig. 3.1a). Afterwards, we observed a significant decrease from week three (July 21st) to four (July 28th; to 13.4%; Table 3.1). Mean germination time was 114 days and varied from 3 days to 303 days in climate chambers (Fig. 3.1c, d), with seeds collected on the first date having the longest mean germination time (141 d; Fig. 3.2d). Mean germination time decreased until week three (98 d), then increased again and had its overall minimum in week six (74 d). Synchrony of germination was quite low with an average of 0.08 over all cutting dates (Fig. 3.1e, f).

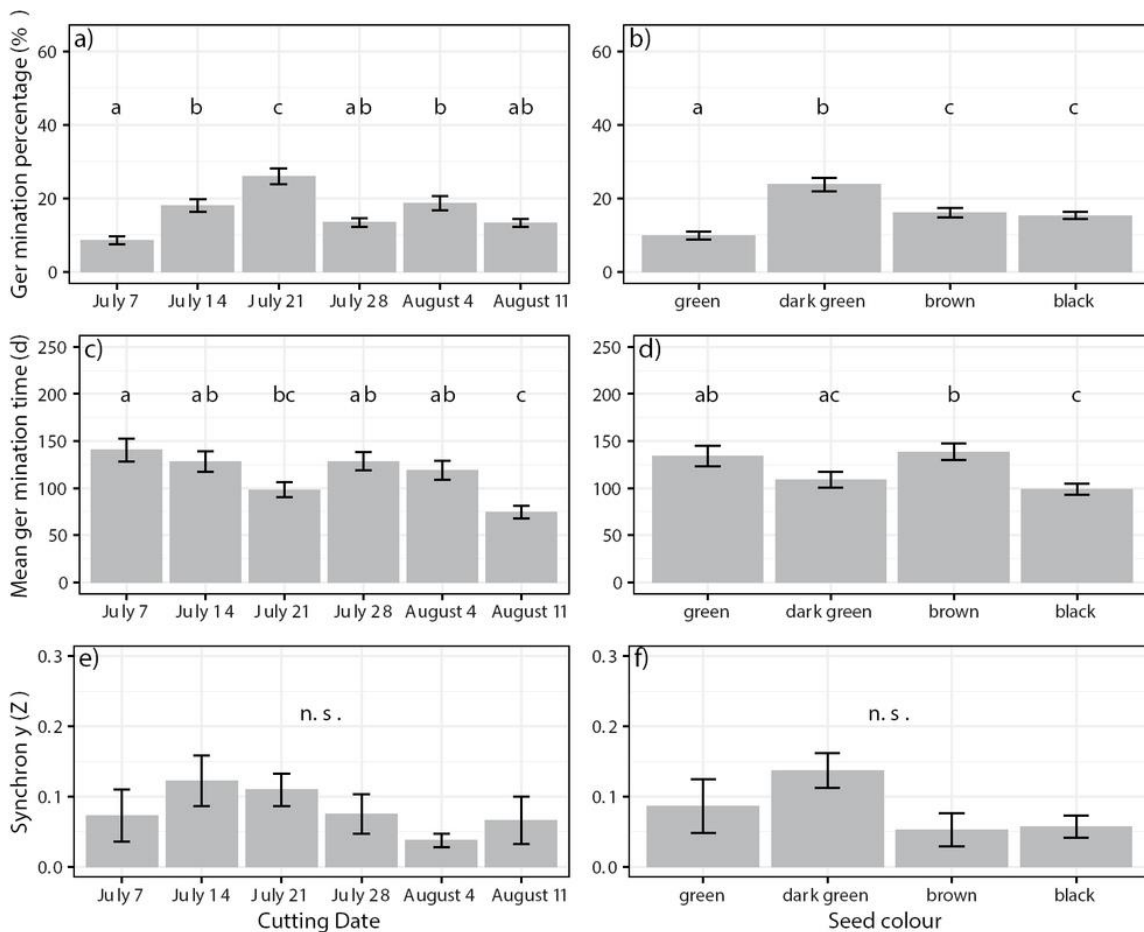


Figure 3.1 The effect of the factors cutting date (weekly from July 7th to August 11th) and seed color on germination percentage (**a**, **b**), mean germination time (**c**, **d**), and synchrony of germination (**e**, **f**) in seeds stored in climate chambers averaged over the two temperature regimes. Bars show mean values \pm standard errors.

Table 3.1 Differences in germination percentages of *L. polyphyllus* seeds between six cutting dates assessed in two germination experiments (climate chamber and common garden). Differences were assessed using mixed effect models for each experiment separately with sampling location as random factor (formula: Germination percentage ~ Cutting Date + (1 | Sampling location)).

Climate chamber	n = 300 R ² _{marginal} = 0.20 R ² _{conditional} = 0.25				
	Estimate	Std. Error	Df	t Value	p Value
Date 1 (July 7; Intercept)	8.64	1.86	22.56	4.65	< 0.001
Date 2 (July 14)	9.36	2.11	295	4.45	< 0.001
Date 3 (July 21)	17.36	2.11	295	8.25	< 0.001
Date 4 (July 28)	4.8	2.11	295	2.28	0.023
Date 5 (August 4)	10	2.11	295	4.75	< 0.001
Date 6 (August 11)	4.64	2.11	295	2.20	0.028
Common garden	n = 150 R ² _{marginal} = 0.63 R ² _{conditional} = 0.71				
	Estimate	Std. Error	Df	t Value	p Value
Date 1 (July 7; Intercept)	16.96	3.88	14.21	4.37	< 0.001
Date 2 (July 14)	13.6	3.86	145	3.52	< 0.001
Date 3 (July 21)	46.24	3.86	145	11.99	< 0.001
Date 4 (July 28)	47.68	3.86	145	12.37	< 0.001
Date 5 (August 4)	52	3.86	145	13.49	< 0.001
Date 6 (August 11)	48.8	3.86	145	12.66	< 0.001

In the common garden, 51.7% of seeds germinated and mean germination time was 153.6 days (Fig. 3.2). Thus, seeds in the common garden germinated to a higher degree compared to seeds in climate chambers, but slower. Germination percentages in the common garden were lowest in seeds sampled during the first two weeks (17.0% on July 7th and 30.6% on 14th), reached the highest level in week three (63.2% on July 21st) and stayed high afterwards (Fig. 3.2a, Table 3.1). In the common garden, mean germination time was similar for all cutting dates and averaged 153.6 days. Synchrony of germination in the common garden was quite low with an average of 0.12 over all treatments and on all cutting dates (Fig. 3.2e, f).

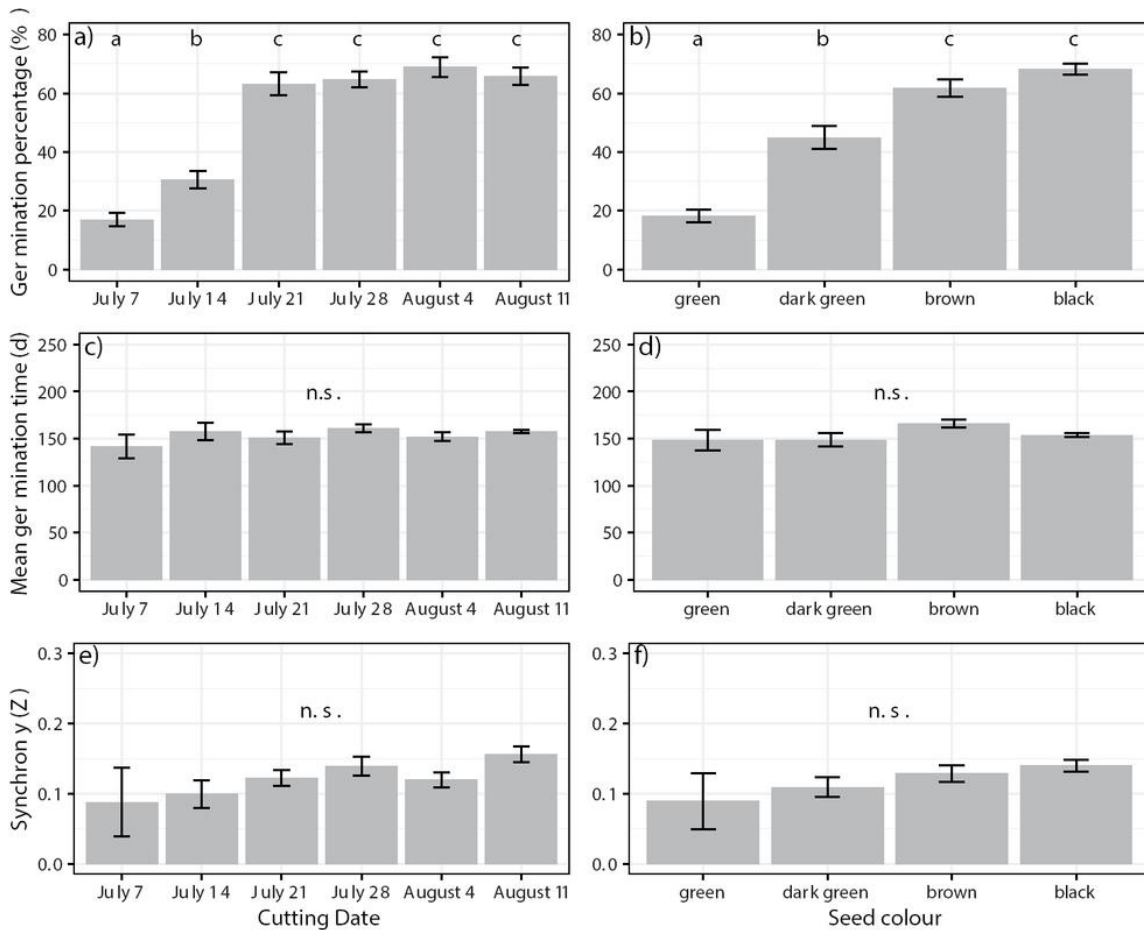


Figure 3.2 The effect of the factors cutting date (weekly from July 7th to August 11th) and seed color on germination percentage (**a**, **b**), mean germination time (**c**, **d**), and synchrony of germination (**e**, **f**) in seeds stored under ambient weather conditions. Bars show mean values \pm standard errors.

There were significant differences in germination percentages between seeds of different color (Figs 3.1b, 3.2b, Table 3.2). In climate chambers, dark green seeds showed the highest germination while in the common garden, germination percentages increased steadily as seeds darkened (Figs 3.1b, 3.2b). In climate chambers, germination percentage peaked when 60% of collected seeds were hard and decreased when the amount of hard seeds was lower or higher while in the common garden, germination percentage increased continuously with the amount of hard seeds. In both experiments, seeds of different color had relatively similar germination times with black (99 d) and dark green (109 d) seeds germinating most rapidly in climate chambers (Fig. 3.1d). In the common garden, there were no significant differences in mean germination time between seeds of different colors (Fig. 3.2d).

Table 3.2 Differences in germination percentages of *L. polyphyllus* seeds between four seed colors (median seed color per sample with four levels: green, dark green, brown, and black) assessed in two germination experiments (climate chamber and common garden). Differences were assessed using mixed effect models for each experiment separately with sampling location as random factor (formula: Germination percentage ~ Seed color + (1 | Sampling location)).

Climate chamber	n = 300 R ² _{marginal} = 0.15 R ² _{conditional} = 0.22				
	Estimate	Std. Error	Df	t Value	p Value
Green (Intercept)	9.45	1.88	16.2	5.03	< 0.001
Dark green	14.34	1.94	298.48	7.4	< 0.001
Brown	7.71	2.12	299.21	3.64	< 0.001
Black	5.64	1.74	297.85	3.25	< 0.01
Common garden	n = 150 R ² _{marginal} = 0.58 R ² _{conditional} = 0.65				
	Estimate	Std. Error	Df	t Value	p Value
Green (Intercept)	17.29	4.11	12.24	4.21	< 0.01
Dark green	27.78	3.82	146.4	7.27	< 0.001
Brown	47.13	4.18	146.84	11.27	< 0.001
Black	50.14	3.42	146.09	14.65	< 0.001

While in climate chambers, germination peaked early and decreased afterwards (Fig. 3.3), two peaks (in autumn and spring) characterized germination in the common garden (Fig. 3.4). There were no significant differences between colors in climate chamber, while synchrony in the common garden increased slightly with the increase in the percentage of hard seeds.

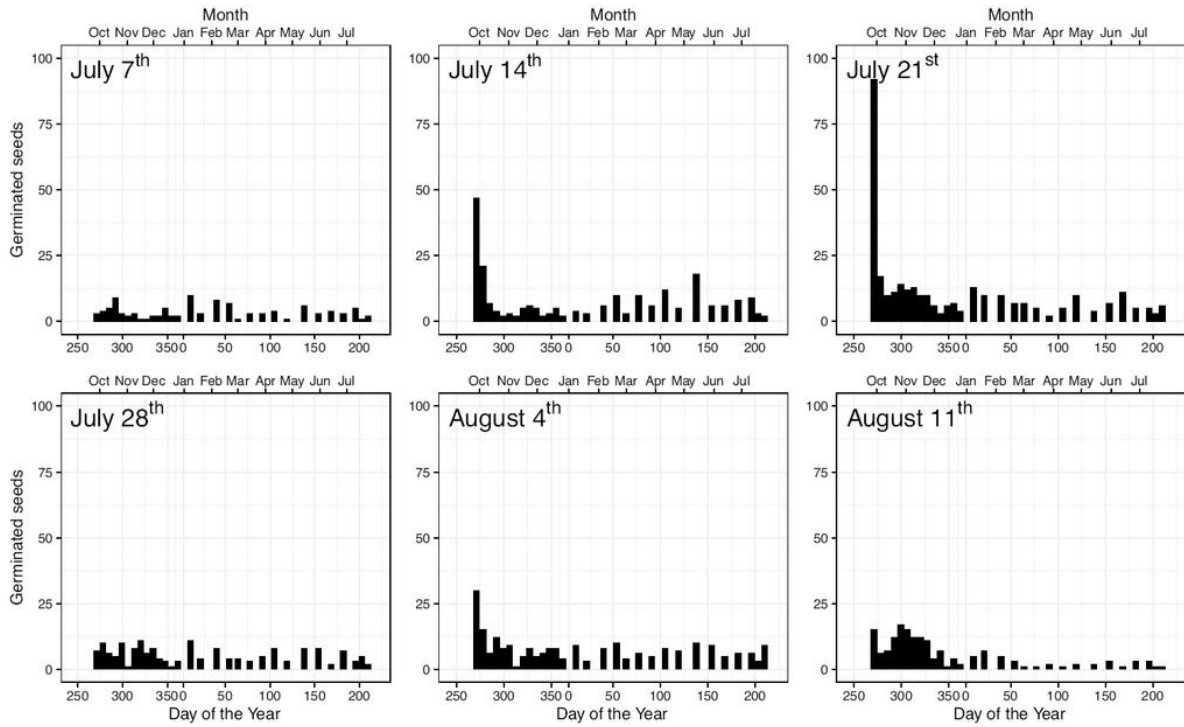


Figure 3.3 Germination patterns of *L. polyphyllus* in climate chambers conditions (15/5 °C and 20/10 °C diurnally fluctuating temperatures) sampled weekly on six cutting dates (July 7th to August 11th) after seed set.

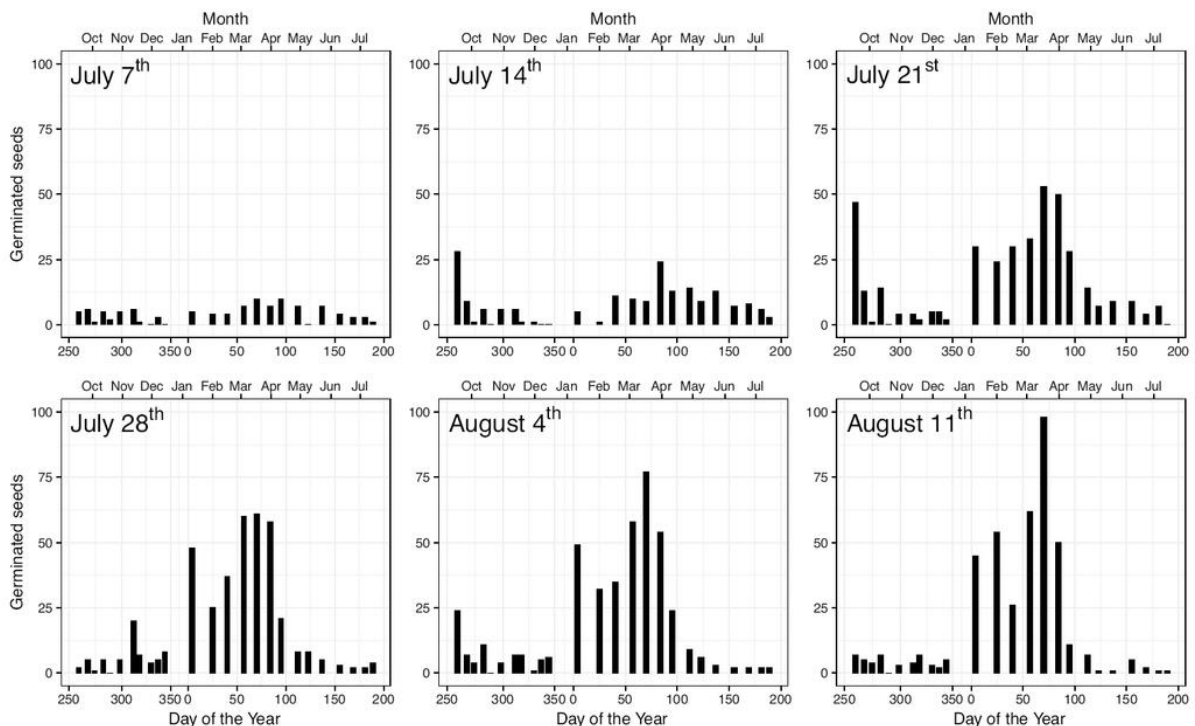


Figure 3.4 Germination patterns of *L. polyphyllus* under ambient weather conditions sampled weekly on six cutting dates (July 7th to August 11th) after seed set.

Germination percentage (in both experiments) and mean germination time (only in climate chambers) responded significantly to *cutting date*, while there was no effect of the different

temperature regimes. For germination percentage and mean germination time, the best explanatory models (see Suppl. material 3.1: Model Tables) each contained solely one fixed factor, mainly due to high correlations between explaining factors. Germination percentage in climate chambers was best explained by seed color and showed highly significant differences between colors ($R^2 = 0.15$). Germination percentage in the common garden was well explained by both seed color ($R^2 = 0.58$) and proportion of hard seeds. For mean germination time, the best explaining factors were either color or proportion of hard seeds, while both models performed poorly overall. Synchrony was not affected significantly by any factor and there was no model of significant explanatory value.

Discussion

The germination patterns of the invasive legume *L. polyphyllus* differed between different cutting dates, partially confirming our first hypothesis. Seeds collected early, while being green and soft, germinated to a lower degree and more slowly compared to seeds collected later. While seeds of early-cut *L. polyphyllus* plants germinated in autumn, seeds of late-cut plants were more prone to germinate in spring. This relationship may be associated with their progression through different phases of seed development. During morphogenesis the embryo develops, then during maturation, storage compounds are synthesized in the growing endosperm and thereafter, seeds may go through a phase of desiccation, in which they dry and eventually enter dormancy (Angelovici et al. 2010). Consequently, the different cutting dates of our experiment covered the phases of maturation and desiccation. Until late July, *L. polyphyllus* seeds were in the phase of maturation. Afterwards (end-July to mid-August), seeds were in the desiccation phase. Although dormancy per se was not tested in our study, the observed germination patterns and differences between climate chambers and the common garden strongly indicate that seeds from late cut *L. polyphyllus* plants expressed dormancy, which is also supported by our observation that seeds decreased in size and became harder. Physical dormancy is common in legumes (Russi et al. 1992a), but whether an individual plant produces dormant seeds at a given point in time depends on a variety of factors, such as temperature and moisture conditions during seed ripening (Masaka and Yamada 2009; Bolingue et al. 2010; D'hondt et al. 2010). Thus, the expression of dormancy can vary strongly in legume seeds, even within plants of the same population (D'hondt et al. 2010), which may consequently lead to asynchronous germination patterns.

Despite pronounced peaks of germination in autumn and spring, germination of *L. polyphyllus* seeds was highly asynchronous. In both experiments and under all cutting dates, some seeds germinated over the whole duration of the experiments, over 300 days. The timing of

germination determines which environmental conditions the seedling will experience and may influence plant characteristics, such as growth and reproduction (Donohue 2002; Casas et al. 2012). The timing of germination itself may be influenced by plant life-history traits, e.g. the phenology of flowering, seed maturation, and seed dispersal (Galloway 2001; Donohue 2002). Variations in germination depending on the time of seed collection have been observed by other authors (e.g. Greipsson and El-Mayas 2003; Samarah 2005; El-Keblawy and Al-Rawai 2006; Brobäck 2015), but there is little information on the long-term germination patterns of species and seasonal effects that are associated with this factor. In invasive species, asynchronous germination can lead to the exploitation of open germination niches throughout the year, which might contribute to their invasion success (Wolkovich and Cleland 2011; Gioria et al. 2016). In the case of *L. polyphyllus*, this effect may be amplified by its high seed production (Volz 2003), its long-lasting flowering, by its ability to resprout and produce seeds after early cutting (Brobäck 2015), and by the observation that the ballistic seed dispersal of the species takes place over many weeks if stands are left untouched (Klinger et al., unpublished data). The observed germination patterns of *L. polyphyllus* thus suggest a bet-hedging strategy (Cohen 1966), which may partly explain its invasion success and its capability to colonize many different habitats.

Our second hypothesis can be verified, as seed color and the percentage of hard seeds were good predictors of germination percentage and give information on the germination patterns that can be expected. Soft and green seeds germinated to the lowest degree and in autumn. However, germination percentages of these seed batches were relatively high, given their early developmental phase. High germination rates in immature seeds have been found in some legumes, e.g. in *Lotus* and *Scorpiurus* (Cristaudo et al. 2008), and *Vicia* (Samarah 2005), but germination failed in others, such as in green seeds of *Lupinus nootkatensis* (Greipsson and El-Mayas 2003). Black and hard seeds germinated to a high degree and in spring. In temperate climates, seedlings germinating in autumn face harsh environmental conditions during winter combined with low competition, while spring germination is associated with more favorable environmental conditions, but higher competition (Masuda and Washitani 1992). Since soft and green seeds mostly germinated in autumn, the winter survival of the emerging seedlings may be low, as *L. polyphyllus* seedlings seem to be sensitive to freezing and showed high mortality when exposed to $-10\text{ }^{\circ}\text{C}$ (Arfin- Khan et al. 2018). Furthermore, unripe seeds of roadside *L. polyphyllus* stands in Sweden were prone to mold infection that led to very low germination rates (Brobäck 2015). The last cutting date represents the state in which seeds are shed by the plant. Both ballistic seed dispersal as well as the expression of physical dormancy go along with the drying of the pods and the seed coat. Black and hard seeds are more prone to germinate in spring and may

thus have higher survival rates compared to green seeds. Furthermore, as *L. polyphyllus* follows a c-strategy (Grime et al. 1988), it may be able to cope with higher competition in spring, especially in habitats with weak competitors, such as semi-natural grasslands. Additionally, water impermeable/hard seeds are more prone to being carried over into the seed bank (Russi et al. 1992b) or dispersed via endozoochory (Otte et al. 2002; D'hondt and Hoffmann 2011). Although *L. polyphyllus* may not have invaded the seed bank of meadows in our study region yet (Ludewig et al., unpublished data), a carry-over of seeds should be avoided, as it makes invasive species management lengthier and more expensive. Consequently, managers should target plants that still have green and soft seeds, which can be considered less problematic despite germination percentages being relatively high.

Germination patterns differed between climate chambers and the common garden, particularly after seeds darkened and became harder. Overall, germination percentages in the climate chamber experiment (ca. 16%) were similar to the emergence rates found by Söber and Ramula (2013) (21.5%), but relatively low compared to other studies on *L. polyphyllus* (Elliott et al. 2011; Arfin-Khan et al. 2018; over 60%). We suggest that this is at least partly due to the fact that seeds were not scarified and that dormancy was probably not broken by imbibition in the climate chamber experiment. This is also supported by the results of the common garden experiment, in which germination percentages were considerably higher than under laboratory conditions, mainly due to a second germination peak in spring after winter-stratification *in situ*. However, germination of *L. polyphyllus* only slightly increased when seeds were pre-treated by cold in another study (Elliott et al. 2011). Our results show that, while laboratory experiments give valuable information on the environmental factors influencing germination, the germination patterns observed under artificial conditions may diverge from germination dynamics under ambient weather conditions (Hölzel and Otte 2004). A better understanding of invasive species germination under natural conditions is necessary, as it can potentially reveal windows of opportunity for invasive species management. We thus recommend to complement germination experiments in climate chambers with common garden or field experiments.

Conclusions

Seeds of *L. polyphyllus* are capable of after-ripening and germinating even if plants are cut while most seeds are still green and soft. Germination capability increased strongly during the first weeks after seed set with a maximum when most seeds were brown to black and not fully hardened. Therefore, *L. polyphyllus* stands should be cut before seed set, if possible. If this is not feasible due to different limitations, we recommend cutting while plants carry green and soft

seeds. When stands with black and hard seeds are cut, the plant material should be removed immediately to reduce propagule pressure on site.

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Appendix

Appendix 3.1 Model tables for linear mixed models performed in Chapter 3. Model formulas are indicated before each analysis.

Formula: Germination percentage ~ Cutting Date + (1| Sampling location)

a) Climate chamber experiment

AIC	BIC	logLik	deviance	df.resid
2287	2316,6	-1135,5	2271	292

Scaled residuals:

Min	1Q	Median	3Q	Max
-2,0274	-0,7277	-0,0832	0,4419	3,2932

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location	(Intercept)	6,198	2,49
Residual		110,78	10,53

Number of obs: 300, groups: Sampling location, 5

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t)	
(Intercept)	8,64	1,859	22,557	4,648	0,000117	***
week2	9,36	2,105	295	4,446	1,24E-05	***
week3	17,36	2,105	295	8,247	5,43E-15	***
week4	4,8	2,105	295	2,28	0,023307	*
week5	10	2,105	295	4,75	3,17E-06	***
week6	4,64	2,105	295	2,204	0,02828	*

b) Common garden experiment

AIC	BIC	logLik	deviance	df.resid
1235,2	1259,3	-609,6	1219,2	142

Scaled residuals:

Min	1Q	Median	3Q	Max
-4,741	-0,6038	0,0395	0,6734	2,2581

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location	(Intercept)	38,04	6,168
Residual		185,79	13,63

Number of obs: 150, groups: Sampling location, 5

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t)	
(Intercept)	16,96	3,878	14,21	4,373	0,000616	***
week2	13,6	3,855	145	3,528	0,000562	***
week3	46,24	3,855	145	11,994	< 2e-16	***
week4	47,68	3,855	145	12,368	< 2e-16	***
week5	52	3,855	145	13,488	< 2e-16	***
week6	48,8	3,855	145	12,658	< 2e-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	week2	week3	week4	week5	week6
week2	-0,566					
week3	-0,566	0,5				
week4	-0,566	0,5	0,5			
week5	-0,566	0,5	0,5	0,5		
week6	-0,566	0,5	0,5	0,5	0,5	

Schielzeth's and Nagasaka's R²

R ² m	R ² c
0,1979829	0,2522441

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)	
2 - 1 == 0	9,36	2,123	4,409	< 0.001	***
3 - 1 == 0	17,36	2,123	8,177	< 0.001	***
4 - 1 == 0	4,8	2,123	2,261	0,21051	
5 - 1 == 0	10	2,123	4,71	< 0.001	***
6 - 1 == 0	4,64	2,123	2,185	0,24445	
3 - 2 == 0	8	2,123	3,768	0,00239	**
4 - 2 == 0	-4,56	2,123	-2,148	0,26286	
5 - 2 == 0	0,64	2,123	0,301	0,99967	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	week2	week3	week4	week5	week6
week2	-0,497					
week3	-0,497	0,5				
week4	-0,497	0,5	0,5			
week5	-0,497	0,5	0,5	0,5		
week6	-0,497	0,5	0,5	0,5	0,5	

Schielzeth's and Nagasaka's R²

R ² m	R ² c
0,6300274	0,7049721

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)	
2 - 1 == 0	13,6	3,923	3,466	0,00701	**
3 - 1 == 0	46,24	3,923	11,785	< 1e-04	***
4 - 1 == 0	47,68	3,923	12,152	< 1e-04	***
5 - 1 == 0	52	3,923	13,254	< 1e-04	***
6 - 1 == 0	48,8	3,923	12,438	< 1e-04	***
3 - 2 == 0	32,64	3,923	8,319	< 1e-04	***
4 - 2 == 0	34,08	3,923	8,686	< 1e-04	***
5 - 2 == 0	38,4	3,923	9,787	< 1e-04	***

6 - 2 == 0	-4,72	2,123	-2,223	0,22699	
4 - 3 == 0	-12,56	2,123	-5,916	< 0.001	***
5 - 3 == 0	-7,36	2,123	-3,467	0,00693	**
6 - 3 == 0	-12,72	2,123	-5,991	< 0.001	***
5 - 4 == 0	5,2	2,123	2,449	0,13965	
6 - 4 == 0	-0,16	2,123	-0,075	1	
6 - 5 == 0	-5,36	2,123	-2,525	0,11707	

 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 (Adjusted p values reported -- single-step method)

6 - 2 == 0	35,2	3,923	8,972	< 1e-04	***
4 - 3 == 0	1,44	3,923	0,367	0,99913	
5 - 3 == 0	5,76	3,923	1,468	0,68474	
6 - 3 == 0	2,56	3,923	0,652	0,98688	
5 - 4 == 0	4,32	3,923	1,101	0,88114	
6 - 4 == 0	1,12	3,923	0,285	0,99975	
6 - 5 == 0	-3,2	3,923	-0,816	0,96478	

 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 (Adjusted p values reported -- single-step method)

Formula: Germination percentage ~ Seed color + (1 | Sampling location)

a) Climate chamber experiment

AIC	BIC	logLik	deviance	df.resid
2301,8	2324	-1144,9	2289,8	294

Scaled residuals:

Min	1Q	Median	3Q	Max
-2,4578	-0,6746	-0,185	0,5087	3,4411

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location	(Intercept)	7,669	2,769
Residual		117,71	10,849

Number of obs: 300, groups: Sampling location, 5

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t)	
(Intercept)	9,447	1,879	16,204	5,027	0,000119	***
Dark green	14,336	1,937	298,475	7,403	1,37E-12	***
Brown	7,707	2,118	299,206	3,639	0,000322	***
Black	5,642	1,735	297,853	3,252	0,001277	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	Dark green	Brown
Dark green		-0,553	
Brown		-0,502	0,479
Black		-0,615	0,603 0,538

b) Common garden experiment

AIC	BIC	logLik	deviance	df.resid
1262,3	1280,4	-625,2	1250,3	144

Scaled residuals:

Min	1Q	Median	3Q	Max
-3,3677	-0,5677	-0,0853	0,7059	2,3559

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location	(Intercept)	45,62	6,755
Residual		228,77	15,125

Number of obs: 150, groups: Sampling location, 5

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t)	
(Intercept)	17,289	4,111	12,241	4,206	0,00117	**
Dark green	27,78	3,822	146,398	7,268	2,02E-11	***
Brown	47,131	4,181	146,837	11,272	< 0.001	***
Black	50,135	3,423	146,089	14,645	< 0.001	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	Dark green	Brown
Dark green		-0,499	
Brown		-0,452	0,478
Black		-0,555	0,603 0,537

Schielzeth's and Nagasaka's R²

R ² m	R ² c
0,153127	0,2198253

Simultaneous Tests for General Linear Hypotheses**Multiple Comparisons of Means: Tukey Contrasts**

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)	
Dark green - Green == 0	14,361	1,948	7,373	< 0.001	***
Brown - Green == 0	7,786	2,13	3,654	0,00151	**
Black - Green == 0	5,658	1,745	3,243	0,00627	**
Brown - Dark green == 0	-6,575	2,089	-3,147	0,00877	**
Black - Dark green == 0	-8,704	1,654	-5,261	< 0.001	***
Black - Brown == 0	-2,128	1,894	-1,124	0,67186	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

Schielzeth's and Nagasaka's R²

R ² m	R ² c
0,5777551	0,647965

Simultaneous Tests for General Linear Hypotheses**Multiple Comparisons of Means: Tukey Contrasts**

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)	
Dark green - Green == 0	27,78	3,822	7,268	<1e-04	***
Brown - Green == 0	47,131	4,181	11,272	<1e-04	***
Black - Green == 0	50,135	3,423	14,645	<1e-04	***
Brown - Dark green == 0	19,351	4,102	4,717	<1e-04	***
Black - Dark green == 0	22,354	3,246	6,887	<1e-04	***
Black - Brown == 0	3,003	3,718	0,808	0,849	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

Formula: Mean germination time ~ Cutting Date + (1|Sampling location)

a) Climate chamber experiment

AIC	BIC	logLik	deviance	df.resid
3173.6	3202.8	-1578.8	3157.6	274

Scaled residuals:

Min	1Q	Median	3Q	Max
-2.920	-0.6605	-0.1182	0.5657	26.533

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location (Intercept)		118.1	10.87
Residual		4198.8	64.80

Number of obs: 282 groups: Sampling location, 5

Fixed effects:

	Estimate	Std.Error	df	t value	Pr(> t)	
(Intercept)	139.90	49.14	49.14	12.448	<0,001	***
week2	-12.12	13.86	277.73	-0.874	0.38264	
week3	-41.56	13.66	277.58	-3.042	0.00258	**
week4	-11.35	13.80	277.82	-0.823	0.41141	
week5	-20.99	13.73	277.75	-1.529	0.12743	
week6	-65.40	13.87	277.96	-4.716	3.81e-06	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

b) Common garden experiment

AIC	BIC	logLik	deviance	df.resid
1493.4	1517.4	-738.7	1477.4	140

Scaled residuals:

Min	1Q	Median	3Q	Max
-3.5705	-0.3910	0.0025	0.5285	3.1506

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location (Intercept)		0	0.00
Residual		1321	36.34

Number of obs: 148, groups: Sampling location, 5

Fixed effects:

	Estimate	Std.Error	df	t value	Pr(> t)	
(Intercept)	141.755	7.418	142.000	19.109	<2e-16	***
week2	16.044	10.385	142.000	1.545	0.125	
week3	9.315	10.491	142.000	0.888	0.376	
week4	19.027	10.385	142.000	1.832	0.069	.
week5	10.328	10.385	142.000	0.994	0.322	
week6	15.831	10.385	142.000	1.524	0.130	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	week2	week3	week4	week5		(Intr)	week2	week3	week4	week5
week2	-0.659					week2	-0.714				
week3	-0.669	0.542				week3	-0.707	0.505			
week4	-0.663	0.538	0.545			week4	-0.714	0.510	0.505		
week5	-0.666	0.540	0.548	0.543		week5	-0.714	0.510	0.505	0.510	
week6	-0.660	0.535	0.543	0.538	0.540	week6	-0.714	0.510	0.505	0.510	0.510

Schielzeth's and Nagasaka's R²

R ² m	R ² c
0.09637376	0.1298687

Schielzeth's and Nagasaka's R²

R ² m	R ² c
0.02855211	0.02855211

Simultaneous Tests for General Linear Hypotheses**Multiple Comparisons of Means: Tukey Contrasts**

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)	
2 - 1 == 0	-120.588	139.898	-0.862	0.95539	
3 - 1 == 0	-414.832	137.895	-3.008	0.03138	*
4 - 1 == 0	-112.705	139.241	-0.809	0.96589	
5 - 1 == 0	-209.311	138.567	-1.511	0.65725	
6 - 1 == 0	-652.982	139.955	-4.666	< 0.001	***
3 - 2 == 0	-294.245	132.864	-2.215	0.23065	
4 - 2 == 0	0.7883	134.195	0.059	100.000	
5 - 2 == 0	-88.724	133.519	-0.665	0.98574	
6 - 2 == 0	-532.394	134.910	-3.946	0.00111	**
4 - 3 == 0	302.127	132.149	2.286	0.19940	
5 - 3 == 0	205.521	131.455	1.563	0.62265	
6 - 3 == 0	-238.149	132.882	-1.792	0.47059	
5 - 4 == 0	-96.606	132.804	-0.727	0.97861	
6 - 4 == 0	-540.276	134.196	-4.026	< 0.001	***

6 - 5 == 0 | -443.670 133.537 -3.322 0.01158 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

Formula: Mean germination time ~ Seed color + (1 | Sampling location)

a) Climate chamber experiment

AIC	BIC	logLik	deviance	df.resid
3180.5	3202.3	-1584.2	3168.5	276

Scaled residuals:

Min	1Q	Median	3Q	Max
	-2.348	-0.7524	-0.1944	0.5619
				2.7159

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location	(Intercept)	174	13.19
Residual		4346	65.92

Number of obs: 282, groups: point, 5

Fixed effects:

	Estimate	Std.Error	df	t value	Pr(> t)
(Intercept)	135.461	11.000	27.463	12.314	1.07e-12 ***
Dark green	-27.966	12.397	281.273	-2.256	0.024854 *
Brown	5.077	13.406	281.116	0.379	0.705211
Black	-38.094	11.184	280.747	-3.406	0.000756 ***

b) Common garden experiment

AIC	BIC	logLik	deviance	df.resid
1489.5	1507.5	-738.8	1477.5	142

Scaled residuals:

Min	1Q	Median	3Q	Max
	-3.773	-0.4317	0.0967	0.5024
				2.981

Random effects:

Groups	Name	Variance	Std.Dev.
Sampling location	(Intercept)	1.741	1.319
Residual		1.301	36.082

Number of obs: 148, groups: Sampling location, 5

Fixed effects:

	Estimate	Std.Error	df	t value	Pr(> t)
(Intercept)	148.3	6.728	58.95	22.050	<2e-16 ***
Dark green	0.2117	9.127	143.64	0.023	0.9815
Brown	17.82	9.855	142.74	1.809	0.0726 .
Black	5.402	8.163	143.79	0.662	0.5092

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	Brown	Black
Dark green	-0.637		
Brown	-0.579	0.507	
Black	-0.704	0.630	0.565

Schielzeth's and Nagasaka's R²

R2m	R2c
0.06704349	0.1151886

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z)	
Dark green - Green == 0	-28.227	12.478	-2.262	0.10528	
Brown - Green == 0	5.111	13.492	0.379	0.98123	
Black - Green == 0	-38.349	11.255	-3.407	0.00358	**
Brown - Dark green == 0	33.338	12.945	2.575	0.04847	*
Black - Dark green == 0	-10.122	10.258	-0.987	0.75447	
Black - Brown == 0	-43.460	11.731	-3.705	0.00117	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	Dark Green	Brown
Dark green	-0.732		
Brown	-0.677	0.499	
Black	-0.818	0.603	0.558

Schielzeth's and Nagasaka's R²

R2m	R2c
0.02848064	0.02977783

Chapter 4

Mowing machinery and migratory sheep herds are complementary dispersal vectors for grassland species

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Abstract

Aim: We assessed the role of mowing machinery and endozoochory by migratory sheep as dispersal vectors in semi-natural grasslands by comparing the species compositions and traits of species found in the vectors to the regional above-ground vegetation and soil seed bank. Furthermore, we discuss how their interplay may affect the conservation of semi-natural grasslands.

Location: Rhön Mountains, central Europe.

Methods: Plant material from mowers (n = 12 from one date) and dung samples from migratory sheep (n = 39 from 13 dates) were collected and the dispersed plant species were determined using the emergence method. We compared the species compositions to the regional above-ground vegetation and seed bank using non-metric multidimensional scaling (NMDS) and indicator species analysis. Furthermore, we compared functional traits of the dispersed species to traits of non-dispersed species of the regional species pools by calculating log-response ratios and performing metaregressions.

Results: While 43 species were shared between the vectors, the vegetation compositions differed from each other. Mower samples were more similar to the above-ground vegetation whereas dung samples were more similar to the seed bank. Mowers and sheep endozoochory favoured the dispersal of species with different traits and phenologies. Species with small seed sizes were prevalent in both vectors. Mowers were less selective concerning most traits, but favoured high-growing grasses such as *Alopecurus pratensis* and *Trisetum flavescens*. Sheep dung samples contained less grasses and more palatable species, such as *Urtica dioica*. Mowers were most selective concerning phenology, whereas endozoochory by migratory sheep also included late-flowering species.

Conclusion: Sheep endozoochory and mowing machinery are complementary dispersal vectors favouring species with differing functional traits. Sheep endozoochory enables dispersal of species that have unfavourable traits (e.g. low releasing heights) or phenologies for dispersal by mowing machinery. To ensure the dispersal of a high number of plant species in semi-natural grasslands, the interplay of different vectors should be considered.

Keywords

Dispersal vector, endozoochory, grassland conservation, mowing machinery, plant traits, seed bank, seed dispersal, semi-natural grasslands

1 INTRODUCTION

Semi-natural grasslands are among the most species-rich ecosystems in the world at small spatial scales (Wilson et al., 2012). They are severely threatened by both land abandonment and intensification (Bakker & Berendse, 1999) and the current distribution of high-nature-value grasslands in Europe is mostly restricted to remote landscapes or protected areas. Often, some remnants of species-rich grasslands occur within intensively used landscapes and are thus prone to the negative effects of isolation. This is why, despite considerable efforts, both the amount of well-preserved grasslands and the number of species associated with these habitats are continuously decreasing in many places (Dahlström et al., 2008).

The conservation of semi-natural grasslands strongly relies on the continuation of traditional low-intensity land use practices that originally led to the formation of these ecosystems over centuries (Pärtel et al., 2005), such as mowing and grazing. While site conditions and disturbance regimes induced by these practices are typically influenced by their timing and intensity (Vogt et al., 2019), the long-term conservation depends on additional factors that cannot be influenced at the local scale. These include for example airborne nitrogen deposition or the supply with seeds of characteristic grassland species from other sites (Bakker & Berendse, 1999). In particular, seed dispersal was strongly facilitated by traditional land use, e.g. by migratory sheep herding or application of hayseed (Babai, 2014), and is nowadays strongly restricted in many modern landscapes (e.g. Poschlod et al., 2005). Both the lower number and quality of seed sources and the discontinuation of dispersal vectors have led to seed dispersal limitation, which severely jeopardizes the success of conservation efforts (Eriksson, 2000).

Late mowing (typically after July 1) and low-intensity grazing are two of the most widely applied traditional land use practices in semi-natural grasslands (Kapfer, 2010). Both mowing

machinery and grazing animals act as dispersal vectors that transport species within and between grasslands, although species with different traits are expected to benefit from each vector. Mowing machinery, i.e. a mowing unit mounted on the rear of a tractor, can carry plant material that remains attached after mowing between grassland patches managed by the same farmer. Mowing machinery favours the dispersal of plants that grow high, carry viable seeds at the time of mowing, and are abundant at the mown sites (Strykstra et al., 1997). Grazing leads to the dispersal of seeds by either epizoochory or endozoochory and can favour different species depending on grazing preferences and size of the respective animal. Especially endozoochory is a rather selective mechanism favouring highly palatable species that carry high numbers of small seeds that resist decomposition in the gut (Janzen, 1984; Hattermann et al., 2019). This has led to the assumption that similar plant traits favour endozoochoric dispersal and allow the survival of seeds in the soil seed bank (Janzen, 1984; Kuiters & Huiskes, 2010), although it is unclear to what degree these species pools overlap in grasslands. For the planning of successful conservation management schemes in semi-natural grasslands, an improved understanding of the role of different dispersal vectors compared to the above-ground vegetation and the soil seed bank is needed (Török et al., 2018). However, comparative assessments of different dispersal vectors that include the regional soil seed bank are rare.

In our study, we assessed the role of two crucial dispersal vectors by comparing them to the local grassland species pool in the above-ground and seed bank vegetation of semi-natural grasslands in the nature reserve ‘Lange Rhön’, a protected central-European landscape. To this end, we analyzed the species composition of seeds attached to mowers and in dung of migratory sheep. We performed a combination of a compositional and a trait-based analysis to assess which species of the local grassland species pool (hereafter referred to ‘above-ground vegetation’) and the local grassland seed bank species pool (hereafter referred to ‘seed bank vegetation’) are dispersed and discuss how the interplay of both vectors affects the conservation of grasslands. Overall, our study aims at investigating the compositional and functional differences between the four investigated groups.

Additionally, we tested the following hypotheses:

1. Plants dispersed through endozoochory by migratory sheep are functionally more similar with plants prevalent in the local seed bank than with plants in the above-ground vegetation. Namely, plants with a high number of small seeds, a longer flowering duration, a high seed bank longevity and plants that are easily palatable (herbs with low

leaf dry matter content [LDMC] and high Ellenberg indicator value for nutrients) have an increased probability to be dispersed by sheep.

2. Plants dispersed by mowing machinery are less constrained by their seed or dispersability traits compared to species dispersed by sheep endozoochory. However, species that have a higher abundance in the above-ground species pool and/or grow higher are more prone to be dispersed by mowing machinery.

2 METHODS

2.1 Study area

The study was conducted in the nature reserve “Lange Rhön” (50°26′–50°32′ N, 09°54′–10°05′ E) in central Germany. The nature reserve comprises an area of 32 km² and is situated between 600 m and 950 m a.s.l. The climate of the study area is characterized by a short growing season, with a mean annual temperature of 5.4°C and an annual precipitation of approx. 1,176 mm (means of 1980–2010 of Mt Wasserkuppe, 950 m a.s.l. ; DWD, 2016). Basalt rocks form the bedrock in the study area. Although soils that develop on basaltic bedrocks are well supplied with base cations, high precipitation as well as land-use-induced nutrient removal have led to low nutrient availability and very low soil pH values in most of the area (Puffe & Zerr, 1988). Furthermore, calcareous soils can be found in small parts of the study area. Two thirds of the nature reserve (ca. 21 km²) is covered by species-rich semi-natural grasslands (e.g. Habitats Directive 92/43/EEC, habitat types 6520: mountain hay meadows, and 6230: species-rich *Nardus* grasslands) that are non-intensively used as meadows and pastures. These grasslands have a centuries-long land use history of mowing and pasturing with low nutrient inputs. Therefore, the nature reserve is of supraregional importance for the conservation of these habitat types in central Europe (Grebe, 1995).

In the study area, migratory sheep herding is carried out between April and October. Mostly Rhön sheep and Merino sheep are used in the area. Grazing may be carried out longer or shorter, depending on the weather conditions. Until August 15, sheep graze mainly on pastures that are not mown by machinery due to steep terrain or stoniness of the surface (although sheep herds move between different pastures, thus some grazing on other areas/roadsides may happen). After August 15, all of the study area may be used for pasturing, and aftermath grazing is carried out in mown areas. Different shepherds focus on different parts of the study area, but some overlap in grazed areas cannot be ruled out. Overall, which areas are grazed exactly depends on the decisions of the shepherds and local management may thus differ between years.

The mowing regime in the study area is based on contractual nature conservation, meaning farmers are subsidized to manage meadows in the study area. For the protection of ground-nesting birds, mowing is staggered based on different mowing dates between June 15 and August 1 and is not carried out on the respective areas before these dates. Apart from the mowing units, other haymaking machinery is employed in the area, such as tractor-mounted rakes (e.g. rotary rakes and wheel rakes) and a mobile baling press.

2.2 Sampling

To analyze the endozoochoric dispersal, we took dung samples from three flocks of sheep (*Ovis aries*) weekly from July 4 to September 30, 2017. This resulted in 39 samples (three samples per week over the course of 13 weeks). For each sample, 500 ml of several fresh droppings were randomly collected. Sheep of all sampled flocks are herded in the study area throughout the summer. While the shepherds of three flocks of sheep focus on managing different parts of the study area (Flock 1, consisting of 800 Merino sheep, in the north, Flock 2, consisting of 350 Merino sheep, in the centre, and Flock 3, 800 Rhön sheep, in the south), the flocks often move several kilometres per day and both pastured areas and pens are frequently relocated. Due to this and as the retention times in the sheep gut can vary depending on the digested biomass and seed traits (Cosyns et al., 2005b), the collected dung samples could not be linked directly to grazing sites.

On July 4, 2017, under dry weather conditions, 12 samples of mowing machinery were taken. The chosen mowing date represented the most common mowing date in the study area, with ~60% of the area being mown after this date. The sampling was performed in the central part of the study area, and samples were taken from nine disc mowers and from three sickle bar mowers. Before the first sampling, we cleaned the mowers from adherent plant material. Afterwards, the farmers mowed the corresponding meadows as they would normally do. After mowing, when leaving the meadow, they usually elevate the mowing unit on-site (“transport mode”) and move to the next meadow. To not overestimate the plant material that could potentially be transported, sampling was carried out after the mower was put into transport mode once and lowered again. For each of the 12 meadows, we then collected all plant material from all parts of the mowers. The amount of sampled plant material varied between meadows and ranged from 1 to 5 L per sample. Seeds were extracted from excess plant material by threshing before further handling.

All samples were stored in a refrigerator at 4°C until germination in the greenhouse from the end of September 2017. The amount of germinable seeds was determined using the emergence method (Roberts, 1981). To this end, samples were spread in a layer of 1–2 mm on a 3–4 cm

layer of a 2:1 sterile garden soil (Fruhstorfer Erde LD80 Archut®)–sand mixture in styrofoam trays of 18 cm × 28 cm size. In the greenhouse, the trays were exposed to controlled diurnally alternating temperatures (day: 18–24°C, night: 12–18°C), light (>10,000 lx from 6:00 a.m. to 10:00 p.m.), and humidity (<70%) conditions and were watered every three days. From December 2017 to March 2018, the samples were cold-wet-stratified under outdoor conditions. After stratification, the trays were moved to the greenhouse, where germination was observed until August 2018. We added ten control trays containing sterile garden soil only to account for wind-borne seeds. Species germinating from these trays were excluded from both groups (mowers and sheep dung).

To represent the local grassland species pool, we carried out vegetation surveys on 72 study plots (size: 5 m × 5 m) from the three most abundant grassland types (mesic and wet mountain hay meadows as well as species-rich *Nardus* grasslands) in 2016. We estimated plant species abundance following the approach of Braun-Blanquet (1964) and transferred the classes to percentage values (r = 1%, + = 2%, 1 = 3%, 2 = 13%, 3 = 38%, 4 = 68%, 5 = 88%). The invasive legume *Lupinus polyphyllus* can be found frequently in the above-ground vegetation of the study area and was present in most of the plots (Otte & Maul, 2005; Klinger et al., 2019).

For the local grassland seed bank species pool, soil samples were taken from the same plots used for the vegetation surveys in September 2015. For each plot, we pooled nine soil cores (0–10 cm depth, 2.8 cm diameter) resulting in 0.554 L of soil volume for each of the 72 plots. We removed plant remains, litter and roots immediately after sampling. Seed bank samples were kept in the refrigerator under similar conditions as the dung and mower samples. In the greenhouse, samples were spread on the same styrofoam trays and were kept under the same controlled conditions as the dung and mower samples. We identified all emerging seedlings from October to December 2015. From December 2015 to March 2016, the samples were cold-wet-stratified under outdoor conditions. After stratification, samples were transferred to the greenhouse again and germination was observed until July 2016, when no more seeds germinated. More information on the vegetation and seed bank sampling can be found in Ludewig et al. (2021). Plant nomenclature follows Jäger (2017). A full list of all species found in the four groups and their abundances can be found in Appendix S1.

We focused on seed and plant traits that have been identified as relevant for the dispersal through the two vectors by other studies (e.g. Strykstra et al., 1997 for mowers; Albert et al., 2015a for sheep endozoochory). Explicitly, we looked at the traits LDMC as indicator for palatability and plant resource use, maximum releasing height (RHmax) as trait relevant for the

seed uptake both by animals and mowers, and seed longevity as indicator for how long a seed can survive in the soil seed bank or in the animal gut (as taken from the LEDA Database; Kleyer et al., 2008). Furthermore, we included seed volume (seed length * width * height) as proxy for seed size, as smaller seed sizes are associated with high seed production and high seed dispersability, and flowering duration as phenological parameter (from the Biolflor Database; Kühn et al., 2004). For missing trait values, we calculated the mean trait value of the genus. For 5.7% of trait values, there were no data available, mainly concerning seed longevity. Furthermore, as the most prevalent functional groups, we included the proportion of herbs and grasses (including grasses, sedges, and rushes) of each sample. Additionally, we included the Ellenberg indicator value for nutrients (EIV-N; Ellenberg, 1991) as indicator for palatability as well as plant resource accumulation capability. The effective number of species, calculated as the exponent of the Shannon entropy (Jost, 2006), was included as diversity index in the analysis. Additionally, we considered sampling week as explanatory variable in the analysis of the sheep samples.

2.3 Data analysis

For the statistical analyses, we calculated relative species abundances. To this end, we set the sum of all species abundances/seedling numbers for each sample to 100% and calculated the relative abundance of each species in each sample. We examined species-sampling relationships for the four groups separately using species accumulation curves (see Appendix 4.2). All data analyses were carried out using R 3.6.1 (R Core Team, 2019).

To identify species indicative for each of the four groups (above-ground, seed bank, sheep, and mowers), we performed an indicator species analysis (ISA) for the single groups and all possible two-way and three-way group combinations using the package *indicspecies* (Cáceres et al., 2010; Dufrene & Legendre, 1997). The ISA combines both abundance and frequency of tested species independently for each species in the assemblage and creates an indicator value (IV) ranging from 0 to 100.

To compare the species compositions of dung and mower samples to the local above- and below-ground grassland species pools, we performed a NMDS ordination using the *vegan* package (Oksanen et al., 2019). We applied Bray–Curtis distances to create a dissimilarity matrix and calculated the NMDS based on 20 random starts and three dimensions (determined by a stress plot). We grouped the sample points according to the four groups: sheep, mowers, above-ground vegetation, seed bank vegetation. The same ordination method was applied to assess compositional differences between sheep samples; in this case the samples were grouped by

sheep flock. We fitted the average, abundance-weighted trait values to the ordination plot using the `envfit` function. Differences in the vegetation composition of the different groups were tested using a PerMANOVA with 999 permutations (`adonis` function) followed by pairwise group comparisons (results see Appendix 4.3).

We compared the dispersed species' traits to the traits of the above- and below-ground species pools. We focused on the relative trait differences between the groups by calculating the log ratios (according to Hedges et al., 1999) of the unweighted mean trait values or the proportions of herbs and grasses. For the species' traits, we compared the mean trait values of each dung and mower sample (dispersed species) to the mean trait values of the species that were present in the respective species pool, but not in the dispersal vector (non-dispersed species). Log-response ratios greater than zero indicate higher trait values or higher percentages of herbs and grasses in the two vectors (sheep, mower) compared to the respective species pool, while values below zero indicate lower trait values or lower percentages of herbs and grasses. We used fixed-effects metaregressions (Viechtbauer, 2010) to test for significant differences in trait values between dispersed and non-dispersed species. In the metaregression, we treated the three sheep flocks as single studies while the mower samples were treated as one study. The metaregressions were performed using the `metafor` package (Viechtbauer, 2010).

3 RESULTS

3.1 Species composition

We found a total of 3,041 seedlings of 52 species in the sheep dung. The number of species per sample ranged between 2 and 11 species and mean effective species richness per litre dung was six. The species with the highest seedling numbers in the dung samples was *Urtica dioica*, which made up 56% of the total number of seedlings and was present in 28 of 39 samples, followed by *Juncus effusus* (13% of seedlings) and *Poa trivialis* (13% of seedlings). Furthermore, *Stellaria media* and *Scirpus sylvaticus* were strongly associated with sheep dung samples according to the ISA (Table 4.1). The sheep samples shared 34 species (66%) with the above-ground vegetation while 37 species (71%) found in the sheep dung were also present in the seed bank.

Table 4.1 Indicator species for the groups aboveground, seed bank, sheep dung, and mower as well as for group combinations ‘sheep dung + mower’ and ‘sheep dung + mower + seed bank’ with indicator value (IV), Frequency, and p-value.

Aboveground				Seed bank			
Indicator species	IV	Freq.	p-value	Indicator species	IV	Freq.	p-value
<i>Bistorta officinalis</i>	89.5	90.2	0.001	<i>Luzula luzuloides</i>	73.3	75.8	0.001
<i>Lupinus polyphyllus</i>	88.9	96.4	0.001	<i>Carex pilulifera</i>	64.9	97.7	0.001
<i>Sanguisorba officinalis</i>	86.4	84	0.001	<i>Stellaria alsine</i>	52.5	99.4	0.002
<i>Rhinantus minor</i>	85.8	100	0.001	<i>Calluna vulgaris</i>	45.8	94.5	0.014
<i>Festuca ovina</i>	66.7	100	0.001	<i>Rumex acetosella</i>	45.5	99.4	0.004
Sheep				Mower			
Indicator species	IV	Freq.	p-value	Indicator species	IV	Freq.	p-value
<i>Urtica dioica</i>	83.1	96.2	0.001	<i>Alopecurus pratensis</i>	97.1	94.3	0.001
<i>Stellaria media</i>	39.1	99.6	0.001	<i>Trisetum flavescens</i>	95.6	91.3	0.001
<i>Scirpus sylvaticus</i>	32	100	0.021	<i>Cerastium holosteoides</i>	91.9	84.5	0.001
				<i>Festuca pratensis</i>	76.7	70.6	0.001
				<i>Silene flos-cuculi</i>	72.1	56.7	0.001
Sheep dung + mower				Sheep dung + mower + seed bank			
Indicator species	IV	Freq.	p-value	Indicator species	IV	Freq.	p-value
<i>Poa trivialis</i>	77.3	95.3	0.001	<i>Agrostis capillaris</i>	80.3	96.7	0.001
<i>Plantago media</i>	37.0	100	0.001	<i>Juncus effusus</i>	77.3	94.3	0.001

In the mower samples, we found 19,175 seedlings of 102 species, ranging between 35 and 61 species and averaging 15 effective species per sample. *Cerastium holosteoides* (16.8% of seedlings), *Holcus lanatus* (8%) and *Poa trivialis* (6.4%) had the highest number of seedlings in the mower samples. Furthermore, there were several indicator species for mowers, e.g. a number of grasses such as *Alopecurus pratensis*, *Trisetum flavescens* or *Festuca pratensis*, or herbs like *Veronica arvensis*, *Silene flos-cuculi*, and *Taraxacum* spp. (Table 4.1). The mower samples shared 72 species (71%) with the above-ground vegetation and 63 species (62%) with the seed bank vegetation. Two species were strongly associated with both dispersal vectors: *Juncus effusus* and *Plantago media* (Table 4.1).

The above-ground vegetation consisted of 146 species. Species associated with the above-ground vegetation, but not with the dispersal vectors, were for example *Bistorta officinalis*, *Sanguisorba officinalis* or *Rhinanthus minor*, but also the non-native *Lupinus polyphyllus* (Table 4.1). The seed bank consisted of 106 species, and typical species associated with the seed bank were e.g. *Luzula luzuloides*, *Carex pilulifera*, and *Stellaria alsine*.

The NMDS of the dung samples revealed no major differences between the three flocks (Figure 4.1). The first dimension mainly showed differences associated with sampling week, LDMC, and flowering duration, while the second dimension was mainly associated with differences in the effective number of species and EIV-N. This indicates a phenological shift in species composition during the sampling period. Samples collected later during the vegetation period contained species with lower LDMC (Figure 4.1). Compared to the other groups, sheep dung samples had higher community weighted EIV-N, higher longevity and longer flowering durations. Furthermore, species compositions of seed bank samples and dung samples were more similar than those of above-ground vegetation and sheep dung (Figure 4.2).

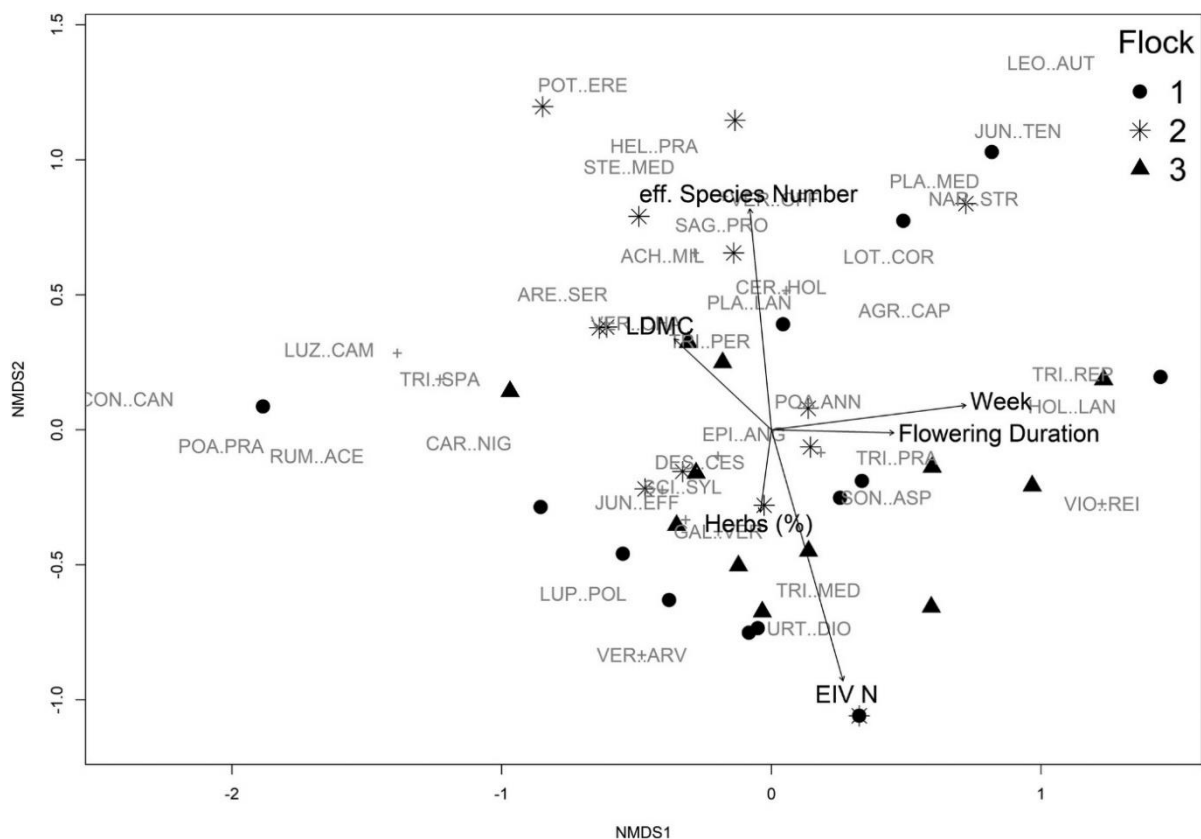


Figure 4.1 Non-metric multidimensional scaling (stress level: 0.131) of the vegetation composition found in sheep dung of three sheep herds between July and September, 2017. We observed a shift in species composition during the sampling period

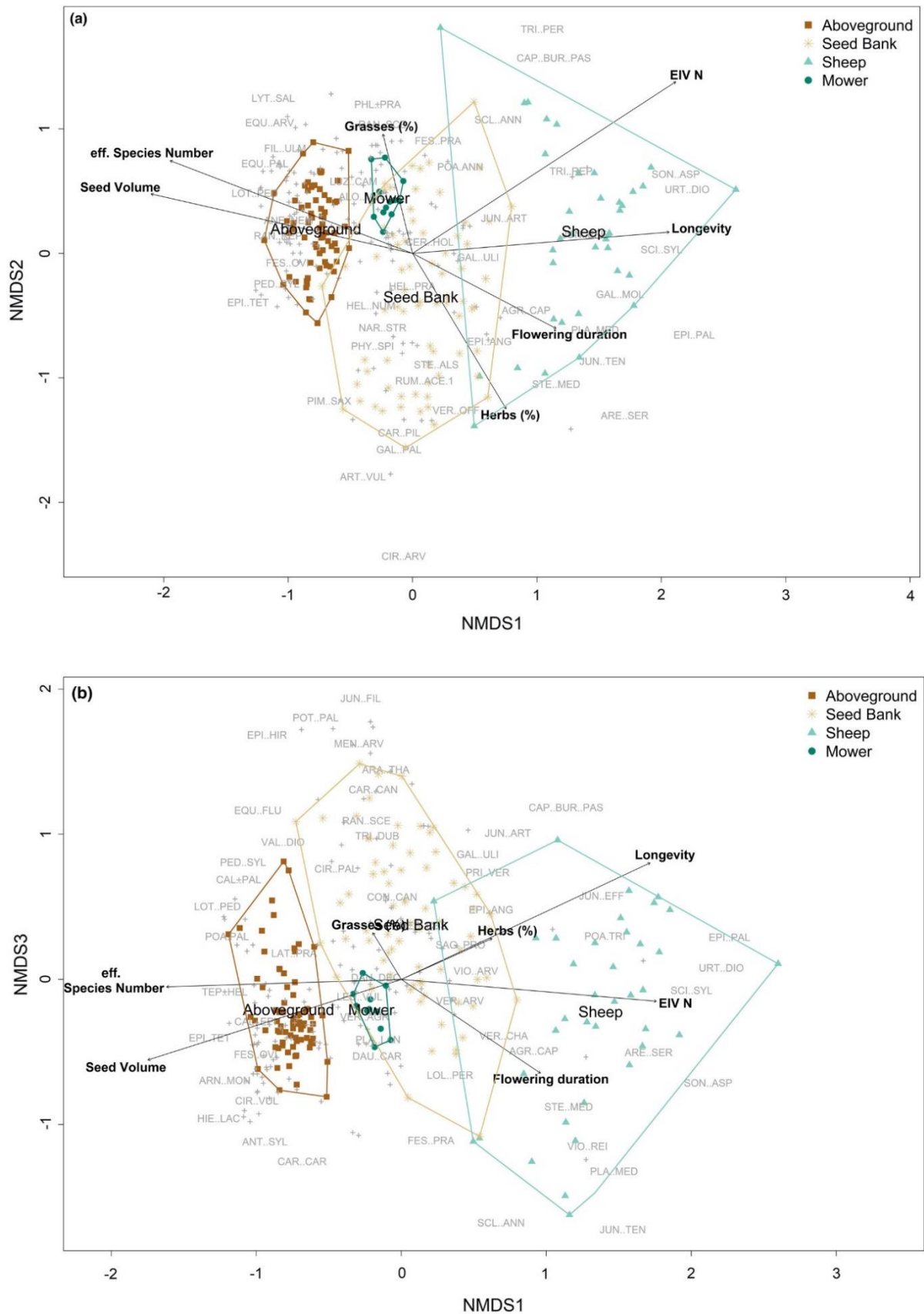


Figure 4.2 Non-metric multidimensional scaling (stress level: 0.151) axes (a) one and two and (b) one and three of the above-ground vegetation and seed bank vegetation compared to species compositions found in the dispersal vectors sheep dung and mowing machinery

While sheep dung and mowers shared a high number of species (43), the vegetation composition of samples of both vectors differed strongly from each other (as indicated by a mean Bray–Curtis dissimilarity of 0.821, Figure 4.2). Along the first dimension, the NMDS clearly differentiated the samples according to the four groups (Figure 4.2). The above-ground vegetation, seed bank, and mower samples were located relatively close to each other. In the above-ground vegetation, effective species numbers were highest of all samples and species with larger seed volumes were more prevalent. The soil seed bank consisted of species with smaller seeds and higher longevity compared to the above-ground vegetation. While above-ground vegetation and mower samples showed little dispersion along the first two dimensions, seed bank and sheep dung samples were more scattered. Looking at the first and third dimension of the ordination, there was an overlap between the mower samples and the seed bank, while sheep samples overlapped marginally with the seed bank (Figure 4.2b).

3.2 Trait comparison

Sheep dung samples contained 44% less grasses; sheep-dispersed species had slightly (8%) longer flowering durations than the above-ground vegetation and had approx. 90% lower seed sizes than non-dispersed species from both species pools. Furthermore, the species dispersed by sheep usually had high longevity values (+103% compared to the above-ground vegetation and +47% compared to the seed bank; Figure 4.3). Overall, sheep-dung-dispersed species had similar maximum releasing heights compared to non-dispersed species of the above-ground vegetation (Figure 4.3a). They contained 25% less herbs and similar percentages of grasses compared to the seed bank (Figure 4.3b). Concerning palatability, there were no large differences in mean LDMC values of dispersed and non-dispersed species, but EIV-N was significantly higher in sheep-dispersed species compared to non-dispersed species of both species pools.

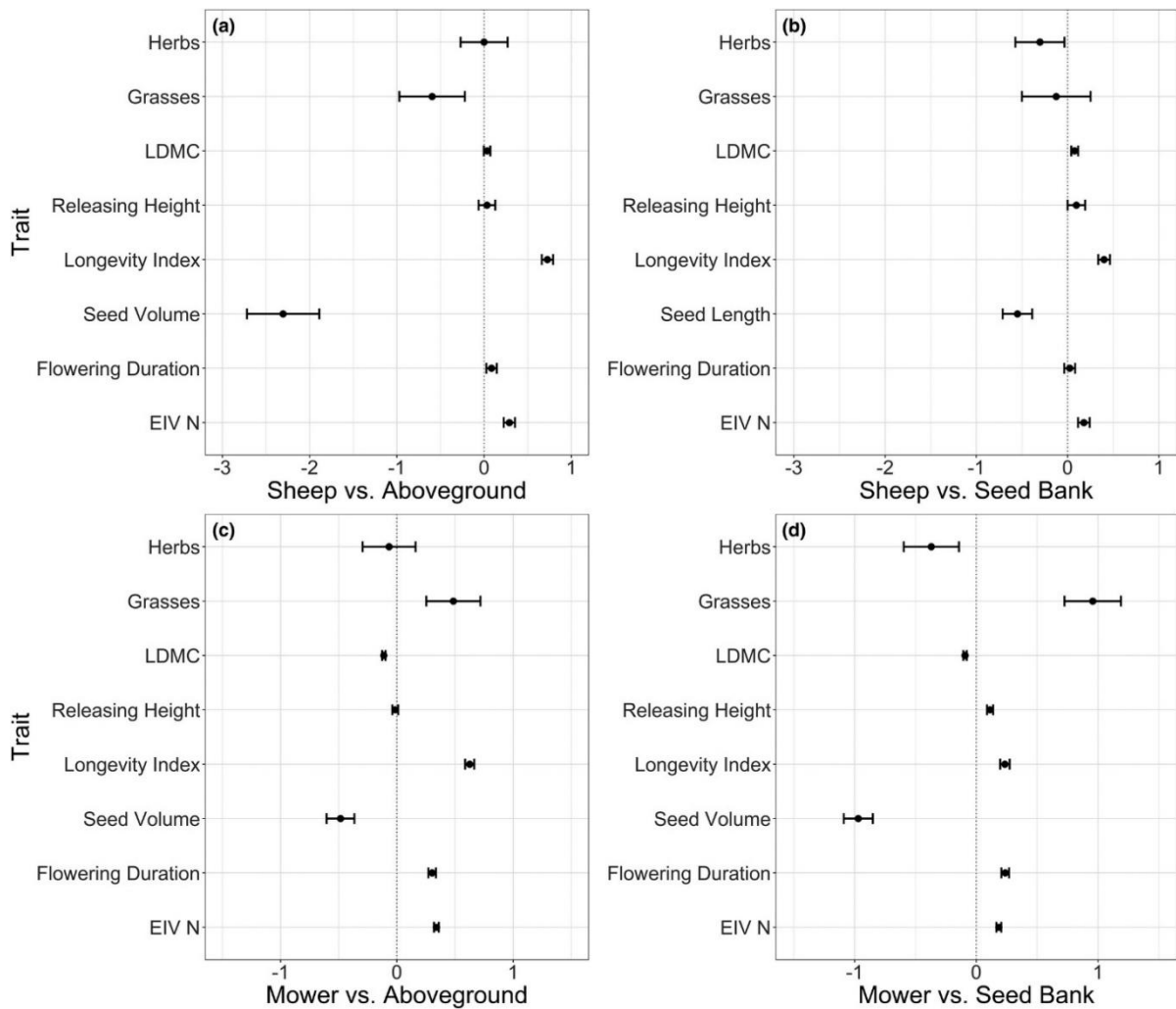


Figure 4.3 Log-response ratios dispersal vectors vs species pools (a) sheep vs above-ground, (b) sheep vs seed bank, (c) mower vs above-ground, (d) mower vs seed bank. Zero indicates the mean value of the non-dispersed species from the respective species pool, bars show mean log-response ratios \pm confidence intervals. No overlap between bars and zero line thus indicates significant differences in mean trait value between the respective dispersal vector and species pool. Note the different scaling of the x-axes.

Mower samples were less clearly differentiated from the other groups concerning their traits, although they had lower seed sizes and contained more grasses than both species pools. Compared to the above-ground vegetation, they were characterized by 38% smaller seeds and 56% more grass species per sample (Figure 4.3c). Contrastingly, releasing heights and percentages of herbs in the samples were similar to those of non-dispersed species of the above-ground vegetation. Compared to the seed bank vegetation, species found in mower samples had 62% smaller seeds and 11% higher releasing heights (Figure 4.3d). Furthermore, the percentage of herbs was 25% lower than in seed bank samples and the percentage of grasses was strongly increased by 130% in mower samples compared to seed bank samples. Longevity of species was

80% higher in the mower samples compared to the non-dispersed species of the above-ground vegetation.

4 DISCUSSION

The first hypothesis could be partially confirmed: while the number of shared species was only marginally higher between sheep and seed bank than between sheep and above-ground vegetation, samples of sheep and seed bank were more similar concerning species composition and some of the observed traits. Many different species were found in sheep dung; however, most species were found in very low frequencies. The most common sheep-dispersed species were typical roadside or field border species. A large fraction of seedlings emerging from sheep dung consisted of *Urtica dioica*, which is rarely found in the grasslands of our study area, but which is prevalent on road verges or field borders. This is most likely due to the species' high palatability (Cosyns et al., 2005a; Kuiters & Huiskes, 2010). We found relatively low abundances of typical grassland species in sheep dung, which is contradictory to other findings on ungulate endozoochory (Auffret & Cousins, 2013). This may be due to the grazing management in the study area, where from mid-August on, aftermath grazing is carried out on meadows that have been mown when most typical grassland species carry viable seeds. In the course of the sampled period (July to September), we observed a shift in the species dispersed via endozoochory. As expected, this shift was mainly associated with phenological traits, such as flowering time.

Seeds dispersed by endozoochory were on average one order of magnitude smaller than seeds of non-dispersed species of both species pools. This is probably due to the fact that small seed sizes are related to shorter retention times of seeds in the animal's gut, which strongly increases the probability of the seed surviving the gut passage (Janzen, 1984). Furthermore, small-seeded species are often characterized by high seed production, which increases the number of ingested seeds and thus the probability that some seeds survive the gut passage (Bruun & Poschlod, 2006). Contrary to these results, species with large and round seeds had the highest survival rates when ingested by Kazakh sheep in another study (Wang et al., 2017). Sheep dung contained less grasses than the above-ground vegetation. Low survival rates of grasses in sheep dung have been reported by other authors (Wang et al., 2017) and this finding may partially be explained by sheep preferring herbs over grasses. Furthermore, the relatively high releasing heights of typical grasses in our species pools may lead to reduced seed ingestion, as low releasing heights (<20 cm) may increase the seed intake of grazers (Albert et al., 2015b) and plants with lower releasing heights may be more resistant to grazing pressure due to an increased ability to resprout (Díaz et al.,

2001). However, in our study, species dispersed by sheep had similar releasing heights compared to non-dispersed species of both species pools. This was due to the fact that the high-growing *Urtica dioica* and *Juncus effusus* were found in most sheep dung samples. Legume seeds were not more common than herbs or grasses in sheep dung (being absent from half of the dung samples and thus not considered in the trait analysis). This was unexpected, as hardseededness, a trait common in legumes, was found to increase the probability of the seeds surviving the gut passage (Russi et al., 1992; Wang et al., 2017), but is in line with findings by other studies (e.g. Karimi et al., 2018). Additionally, species found in sheep dung had higher longevity values, which may be associated with small seed sizes. Sheep preferred species with higher EIV-N, which indicates selective feeding on plants of higher palatability. However, there were no major differences concerning leaf dry matter content compared to both species pools, probably due to the fact that LDMC decreases during the grazing season (Kleinebecker et al., 2011). Compared to non-dispersed species of the above-ground vegetation, species dispersed by sheep had longer flowering durations. This may be due to migratory sheep herding taking place during all of the vegetation period and thus enabling the dispersal of species with late/long flowering periods. Overall, sheep endozoochory dispersed many different species, but nonetheless seems to be rather selective concerning some traits. While some species and traits are indeed similar to traits of species commonly found in the seed bank, the grazing preferences of the animals and herd management lead to differing species compositions. Furthermore, some of the differences may be observed because the soil seed bank represents the past vegetation rather than the present one (Bakker et al., 1996). Additionally, epizoochory, i.e. seed dispersal via animal fur or hoofs, is estimated to transport high numbers of seeds and may favour species with different traits compared to endozoochory, such as higher-growing plants or species producing seeds with appendages (Albert et al., 2015a). Furthermore, seed dispersal by epizoochory also depends on animal characteristics, such as hair length (Couvreux et al., 2005). Thus, epizoochory complements endozoochory and enables more species with different traits to be dispersed by sheep.

Our second hypothesis could be confirmed: overall, the species composition of mower samples was similar to the composition of the above-ground vegetation (with a shift towards small-seeded species that may also be found in the seed bank) and mowers contained a high number of typical grassland species. This is probably due to the mowing taking place around July 1, which allows most species in central-European semi-natural grasslands to have produced (and still carry) viable seeds, but may disadvantage some species with unfitting phenology (Leng et al.,

2011). Obviously, only species that carry viable seeds by the time of mowing can be dispersed, and mowing is thus strongly selective concerning phenological traits. In our data set, the underrepresentation of some of the more common grassland species, such as *Bistorta officinalis*, *Sanguisorba officinalis* or *Filipendula ulmaria* gives evidence of this, as they are characterized by late flowering and fruiting compared to the analyzed mowing date around July 1. However, as there are different mowing dates in our study area, these species may be dispersed between meadows that were not sampled in our study. In the mower samples, species that were more abundant or frequent in the above-ground vegetation had a higher probability to be dispersed than rare species. Similar results have been reported by Strykstra et al. (1997), but in a study on seed dispersal by manure and motor vehicles, typical grassland species were less abundant in mud attached to motor vehicles (Auffret & Cousins, 2013). The favouring of locally abundant species by mowing machinery may lead to the homogenization of mown grassland patches (Lepš, 2014) and mowing may have negative effects both on the richness of the above-ground vegetation and the seed bank (Klaus et al., 2018). In our study, mowers were selective towards several traits, although to a lesser degree than sheep, and favoured small-seeded species, in particular grasses. This favouring of smaller seeds was also found by other authors, e.g. for *Panicum miliaceum* in Canada (McCanny & Cavers, 1988). Furthermore, smaller seeds are potentially dispersed over longer distances by mowing machinery (Bullock et al., 2003). Compared to the non-dispersed species found in the seed bank, tall-growing species had a higher probability to be dispersed, as they are more prone to getting caught in the mowing unit (Strykstra et al., 1997). However, there were no differences between releasing heights of mower-dispersed and non-dispersed species of the above-ground vegetation in our study. Overall, our results suggest that mower samples are functionally more similar to the above-ground vegetation than sheep dung samples.

Our study revealed that mowing machinery and sheep endozoochory are complementary dispersal vectors for grassland species, while some of the observed differences among the four groups may be due to the samples being related to different areas: the above-ground vegetation and seed bank were bound to the same plots, species composition in mower samples depends on the size of the respective meadows, whereas the composition of sheep samples is related to the area grazed by the respective flock. However, we showed that many different species can be transported and species with different traits are favoured by each vector. Thus, the interplay of both vectors may be crucial for sustaining high plant diversity. Overall, sheep endozoochory is a more selective ecological filter, favouring easily palatable species with very small seeds. Mowers, although also favouring small-seeded species, were less selective concerning most of our analyzed

traits, but in general favoured high-growing grasses and species that were abundant in the above-ground vegetation. As tall-growing vegetation may be able to autonomously disperse seeds further than small-growing plants (Thomson et al., 2011), these species may be less reliant on dispersal vectors to sustain populations. On the one hand, due to their grazing preferences, sheep may disperse some unwanted species, such as *Urtica dioica*. On the other hand, migratory sheep herding enables the dispersal of species that are underrepresented in mower samples due to their phenology, or may allow dispersal in years that are characterized by early or late fruiting compared to the mowing date. Both vectors provide “directed dispersal” (Fischer et al., 1996), as mowing machinery and sheep herds move between suitable habitats, lowering competition by reducing the biomass and creating microsites with open soil. In the case of sheep dung, these microsites are nutrient-rich, but may expose the seed to drought (Eichberg et al., 2007). Thus, germination or seedling survival in sheep dung may be increased, as found in legumes by Russi et al., (1992), or decreased, as found in species of the *Koelerion glaucae* association (Eichberg et al., 2007).

Overall, our results show that the long-term conservation of the high species diversity in semi-natural grassland is reliant on the interplay of different dispersal vectors and the seed bank, as the availability of several modes of dispersal facilitates the seed dispersal of species (Ozinga et al., 2004). Thus, the combination of mowing and migratory herding that has traditionally been carried out in many meadows of central Europe (Kapfer, 2010) is highly desirable to ensure the long-term conservation of semi-natural grasslands, particularly in fragmented landscapes. Furthermore, spatial and temporal variations in management, such as different mowing dates and migratory sheep herding throughout the vegetation period, enable the dispersal of a higher number of species and are thus to be recommended.

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AUTHOR CONTRIBUTIONS

KL, AO, and RLE conceived of the research idea; YK, KL and WH collected data, YK and WH with help from TK and RLE performed statistical analyses; YK with contributions from KL, RLE and TK wrote the paper; all authors discussed the results and commented on the manuscript.

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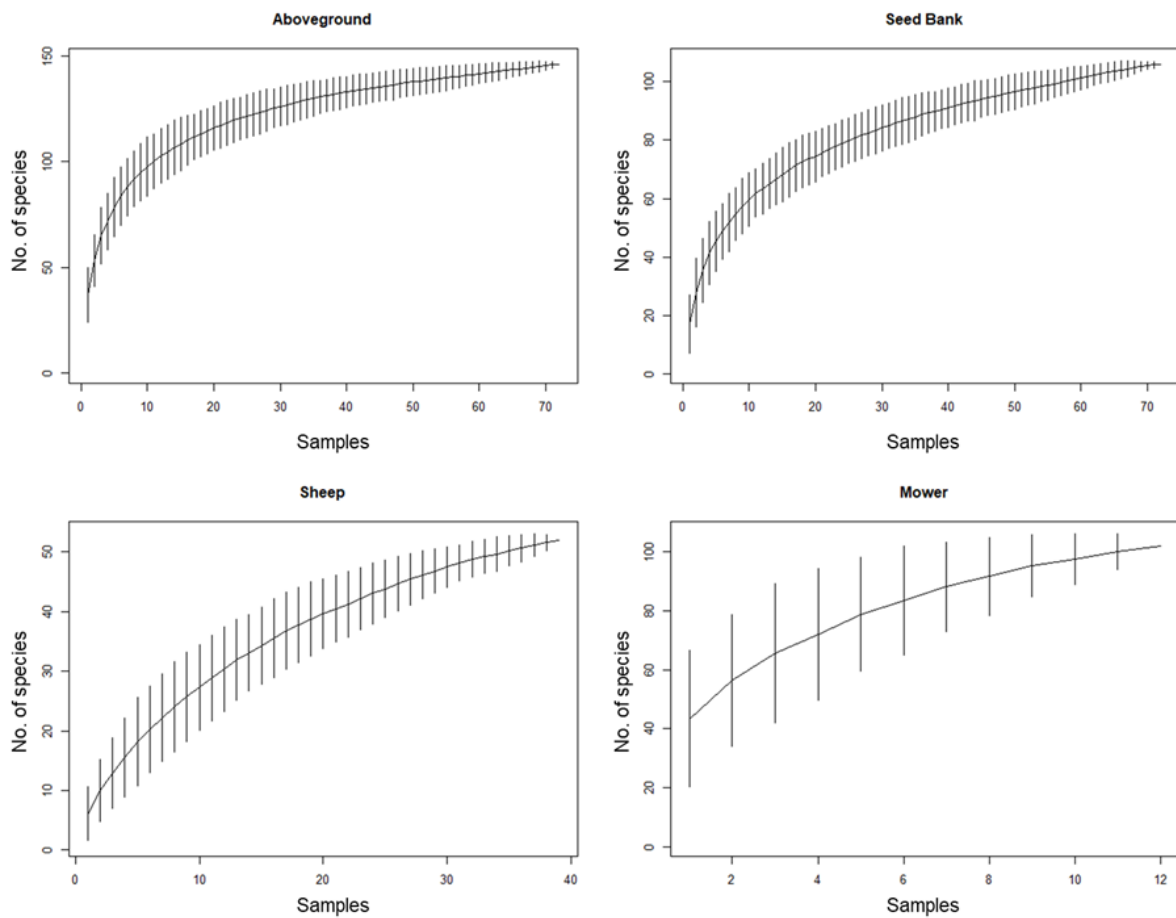
Appendix

Appendix 4.1 Relative species abundances per sample used for the analyses in chapter 4.

The table can be found under the following URL:

<https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Favsc.12579&file=avsc12579-sup-0001-AppendixS1.xlsx>

Appendix 4.2 Species Accumulation curves of the four observed groups Aboveground, Seed Bank, Mowers, and Sheep.



Appendix 4.3 Results of the PERMANOVA analysis and Post-hoc pairwise group comparisons of the four observed groups Aboveground, Seed Bank, Mowers, and Sheep

PERMANOVA

Permutation: free

Number of permutations: 999

Terms added sequentially (first to last)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	sign. level
Group	3	17.609	58.697	18.945	0.22933	0.001	***
Residuals	191	59.177	0.3098	0.77067			
Total	194	76.786	100.000				

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Post-Hoc pairwise comparisons

	diff	lwr	upr	p adj	sign. level
Seed Bank - Aboveground	0.18994207	0.1450320	0.23485216	0.0000000	***
Sheep - Aboveground	0.09358529	0.0400108	0.14715978	0.0000617	***
Mower - Aboveground	-0.04225172	-0.1262708	0.04176735	0.5619148	
Sheep -Seed Bank	-0.09635679	-0.1499313	-0.04278229	0.0000347	***
Mower - Seed Bank	-0.23219380	-0.3162129	-0.14817472	0.0000000	***
Mower - Sheep	-0.13583701	-0.2247893	-0.04688469	0.0006127	***

Abstract

Semi-natural grasslands are one of the most prominent remnants of the historical cultural landscapes of Central Europe. Due to their century-long land-use history, they are remarkably diverse ecosystems. However, they are threatened by different aspects of global change, such as land abandonment, agricultural intensification, and the spread of invasive species. One species of particular concern for grassland managers is the invasive garden lupine (*Lupinus polyphyllus* Lindl.), which is among the most widespread invaders in European semi-natural grasslands. This thesis assesses the ecology of *L. polyphyllus* concerning its distribution, germination, and dispersal in semi-natural grasslands of the UNESCO Biosphere Reserve Rhön against the background of a more efficient management of the species.

To this end, the spatio-temporal distribution of *L. polyphyllus* was assessed by combining mapping via aerial photographs and field mapping. A germination experiment under laboratory and common garden conditions was carried out to assess the potential of *L. polyphyllus* seeds of different development stages to be dispersed by management. Finally, a field experiment was carried out to explore seed dispersal by mowing machinery and migratory sheep, concerning both the invader and typical grassland species. Data analysis was performed using different univariate and multivariate statistical approaches.

This thesis documented a considerable spread of *L. polyphyllus* in the study area and supported the finding that landscape structure is highly relevant for plant invasions. Particularly, the size of a grassland patch and its distance to the next road were deciding factors for the *L. polyphyllus* invasion. Concerning the germination of *L. polyphyllus*, it became evident that both germination capability and timing of germination depend on management, as late cut black seeds showed high germination percentages and germinated under favorable spring conditions. Thus, the production of these seeds should be avoided. Furthermore, seed dispersal by mowing machinery and sheep endozoochory was explored in the heavily invaded grasslands of the study area. It could be shown that both dispersal vectors transport different parts of the regional species pool, and different functional traits are favored by each vector. For the long-term conservation of semi-natural grasslands, several dispersal vectors should be made available by grassland managers. Dispersal of *L. polyphyllus* can be mitigated given adequate management (i.e. before ripe seeds are produced). In invaded grasslands, an adaptation of the management to the phenology of *L. polyphyllus* is to be advised. Additionally, management in semi-natural grasslands should consider the landscape context more thoroughly. To this end, the development of holistic land-use concepts that include the management of patches of semi-natural grassland, neighboring land-use and edge habitats such as roadsides are necessary.

Zusammenfassung

Naturnahe Grünlandflächen gehören zu den markantesten Relikten der historischen Kulturlandschaften Mitteleuropas und stellen außergewöhnlich vielfältige Ökosysteme dar. Sie sind jedoch durch Auswirkungen des globalen Wandels wie Nutzungsaufgabe, Intensivierung landwirtschaftlicher Nutzflächen und die dadurch bedingte Ausbreitung von Neobiota stark bedroht. Die invasive Stauden-Lupine (*Lupinus polyphyllus* Lindl.) zählt zu den am weitesten verbreiteten und problematischsten invasiven Arten in mitteleuropäischen Grünlandbeständen. Vor dem Hintergrund einer effektiveren Bekämpfung der Art wird in dieser Arbeit die Ökologie von *L. polyphyllus* hinsichtlich ihrer Verbreitung, Keimung und Ausbreitung im Berggrünland des UNESCO-Biosphärenreservats Rhön untersucht.

Dabei wurde die räumlich-zeitliche Verbreitung von *L. polyphyllus* durch eine Kombination von Luftbildkartierung und Feldkartierung erfasst. Zudem wurde ein Keimungsexperiment unter Labor- und Freilandbedingungen durchgeführt, um das Ausbreitungspotenzial von *L. polyphyllus*-Samen verschiedener Entwicklungsstadien zu bewerten. Weiterhin wurde ein Feldexperiment durchgeführt, um die Samenausbreitung in Bezug auf *L. polyphyllus* und auf typische Grünlandarten über Mähmaschinen und Wanderschäferie zu erforschen. Die Datenanalyse wurde mit einer Reihe von univariaten und multivariaten statistischen Ansätzen durchgeführt.

Insgesamt konnte eine starke Zunahme der Verbreitung von *L. polyphyllus* nachgewiesen werden. Zudem wurde deutlich, dass die Landschaftsstruktur eine zentrale Rolle für die Verbreitung der Art spielt. Insbesondere waren Größe und die Entfernung einer Grünlandfläche zur nächsten Straße entscheidende Faktoren zur Erklärung der Verbreitungsmuster von *L. polyphyllus*. Hinsichtlich der Keimung der Stauden-Lupine zeigte sich, dass sowohl die Keimfähigkeit als auch der Zeitpunkt der Keimung von der Bewirtschaftung abhängen. Dabei wiesen spät geschnittene schwarze Samen höhere Keimraten und eine günstige Frühjahrskeimung im Vergleich zu früh geschnittenen grünen Samen auf, die durch niedrige Keimraten und eine ungünstigere Herbstkeimung gekennzeichnet waren. Somit ist das Potenzial von spät geschnittenen *L. polyphyllus*-Samen zur Ausbreitung oder zur Verschleppung in die Bodensamenbank hoch und sollte vermieden werden. Darüber hinaus wurde die Rolle von Mähmaschinen und Schaf-Endozoochorie für die Samenausbreitung in den stark invadierten Grünlandflächen des Untersuchungsgebiets untersucht. Durch den Vergleich von Artenzusammensetzung und funktioneller Eigenschaften der ausgebreiteten Arten konnte gezeigt werden, dass Mähmaschinen und Endozoochorie unterschiedliche Teile des regionalen Artenpools transportieren und dass unterschiedliche funktionelle Art-Eigenschaften die Ausbreitung durch die beiden untersuchten

Vektoren begünstigen. Demnach sollten für die langfristige Erhaltung von naturnahem Grünland unterschiedliche Ausbreitungsvektoren zur Verfügung gestellt werden. Im Fall der invasiven Stauden-Lupine zeigen die Ergebnisse, dass die Ausbreitung durch Bewirtschaftungsmaßnahmen minimiert werden kann, wenn eine angemessene Bewirtschaftung erfolgt (d. h. bei einer Bekämpfung, bevor reife *Lupinus*-Samen produziert werden). Daher ist in invadiertem Grünland eine Anpassung der Bewirtschaftung an den phänologischen Status von *L. polyphyllus* zu empfehlen. Zudem sollte die Grünlandbewirtschaftung beim Vorkommen invasiver Arten den Landschaftskontext berücksichtigen. Dafür sind holistische Nutzungskonzepte notwendig, die neben den Grünlandflächen auch benachbarte Habitate und Randbereiche wie Straßen und Lesesteinriegel berücksichtigen.

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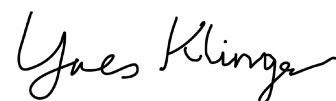
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Declaration

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 the dissertation.

Giessen, July 20, 2021



Yves Philippe Klinger