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Biodiversity and ecosystem functioning: Regional and local determinants of plant diversity in montane grasslands

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'No single process or theory can explain a phenomenon as complex as biological diversity.'

Huston, 1994

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Chapter 1: General Introduction

One of the most striking features of the Earth's biota is its extraordinary biological diversity, estimated to include three million to 30 million different species (May 1992) from which merely 1.75 million living species are taxonomically described (Groombridge & Jenkins 2002). This biological diversity, or biodiversity, refers not only to the number and variety of species but also encompasses the diversity of ecosystems, and the genetic variation contained within species. In combination with physical and chemical processes, biodiversity largely influences the conditions on Earth including ecosystem processes that provide many services to humanity, including production of food and fibre, purification of water and air, formation of soil, and carbon sequestration. Human beings strongly depend on biodiversity and the well-functioning of ecosystems for their existence.

Threats to biodiversity

At the same time, human activities largely influence the environment worldwide (Pimm et al. 1995; Vitousek et al. 1997; Chapin et al. 2000; Swift 2005) and are changing biodiversity at an unprecedented rate (Pimm et al. 1995). About 83% of the Earth's land surface has been influenced by human beings (Sanderson et al. 2002), including the conversion from natural habitats into agricultural land and urban systems, the destruction and fragmentation of habitats, environmental pollution and global warming. Global mobility has dramatically increased the number of organisms translocated by humans, either deliberately or by accident (Mooney & Drake 1986; Vitousek et al. 1997; Sala et al. 2000). The most dramatic and irreversible human impact is the extinction of species (Chapin 2003).

Although the extinction and formation of species are natural and ongoing processes (Raup 1991), extinctions in recent decades are estimated to be a 100 to 1,000 times greater than the pre-human rates (Pimm et al. 1995; Chapin et al. 1998). This disappearance of species causes concerns not only for ethical and aesthetic reasons, it could also be decried as a loss of organisms with potential agricultural and economic value, as a loss of medical cures not yet discovered and as an irreversible loss of the Earth's genetic diversity. Even though the potential consequences of species extinction have been hotly debated for more than 35 years (May 1974; McNaughton 1977; Doak et al. 1998; Tilman 1999), the knowledge and understanding of the precise link between biodiversity and ecosystem functioning is still limited (Schulze & Mooney 1993; Jones & Lawton 1995).

Biodiversity and ecosystem functioning research

There is long standing interest in how biodiversity may affect ecosystem functioning. The first observations of a positive effect of diversity on productivity were made nearly 150 years ago by Darwin (1859; see Hector & Hooper 2002). Another important ecosystem function, ecosystem stability, has been a central topic in ecology since the nineteen-fifties. Based on observations (Elton 1958) and heuristic models (MacArthur 1955), the diversity-stability hypothesis stated that more diverse communities should be more stable. The current rapid loss of biodiversity and its unknown consequences for ecosystem processes has rekindled the interest in this question. In 1991, at a conference in Bayreuth, Germany, the knowledge to date on the potential consequences of biodiversity on ecosystem processes was compiled (Schulze & Mooney 1993), and 'biodiversity-ecosystem functioning research' as a separate ecological discipline was established.

Since 1991 our understanding of the relationship between biodiversity and ecosystem functioning has been improved by cumulative results from a variety of well-controlled experiments mainly conducted in ecotrons (Naeem et al. 1994), microcosms (McGrady-Steed et al. 1997), and experimental grassland communities like the U.S. Cedar Creek LTER (Tilman et al. 1996), the European BIODEPTH (Spehn et al. 2005) and the German JENA-Experiment (Roscher et al. 2004). All of these experiments have largely focused on the ecosystem-level consequences of changes in plant diversity (e.g., Loreau et al. 2001; Schmid et al. 2001; Loreau et al. 2002b). Results emerging from these direct experimental tests reveal that plant species diversity or plant functional diversity, or even both, are positively correlated with productivity (Hector et al. 1999; Tilman et al. 2001; Roscher et al. 2005), and other ecosystem functions such as resistance to invasion (Tilman 1997; Knops et al. 1999; Naeem et al. 2000), which is defined as one measure of ecosystem stability (McCann 2000; Loreau et al. 2002a).

This positive relationship between diversity and ecosystem processes observed in such experiments is attributed to two general mechanisms: first, to a higher probability of including species with particular traits in species-rich communities, known as sampling or selection effect (Aarssen 1997; Huston 1997); and second to niche complementarity among species leading to an increased use of resources or facilitation among species (Tilman et al. 1997; Loreau & Hector 2001).

However, considerable debate exists about the applicability of the conclusions drawn from diversity experiments with artificial communities to natural ecosystems (Wardle et al. 2000; Naeem 2002; Lepš 2004; Zobel et al. 2006; Grace et al. 2007). Moreover, results from experimental grasslands and from observational studies are often contradictory (Loreau et al. 2001), and resolving these contradictions requires research on biodiversity – ecosystem functioning to be carried out along diversity gradients under natural conditions. In this context, multi-site experiments across larger scales are a valuable tool to elucidate general patterns. Such studies are essential in assessing the consequences of the current loss of diversity and in developing wiser strategies for maintaining and preserving biodiversity.

Determinants of biodiversity

A prerequisite for understanding the functional consequences of the rapid change and loss of biodiversity is a better knowledge of the processes responsible for generating and maintaining diversity in biological communities (Ricklefs 1987). One of the most fundamental issues in ecology remains the question of which factors control species diversity in a community (Hutchinson 1959; Tilman 2000). It is a very complex issue as biodiversity can be influenced by ecological processes acting on different spatial scales (Ricklefs 1987; Huston 1999; Hillebrand & Blenckner 2002) that may also covary with each other or with other extrinsic factors such as habitat heterogeneity, resource availability, or disturbance regime.

Regional factors such as dispersal processes or land use patterns including fragmentation and isolation are increasingly recognized as important determinants of plant diversity (Zobel 1997; Krauss et al. 2004). An extreme position is that the local diversity is just a sample from the regional species pool determined by the dispersal ability of the species (Cornell & Lawton 1992). In particular, many experiments conducted across a wide range of habitats and community types showed that dispersal limitation indeed affected plant species diversity (for review see Turnbull et al. 2000; Cadotte 2006). However, generalization of the effect of dispersal limitation on local diversity is still difficult (Zobel & Kalamees 2005).

At a local scale, abiotic conditions such as productivity strongly affect diversity by defining a fundamental niche for each species. The role of productivity in controlling biodiversity has been a central but contentious issue within ecology (Rosenzweig & Abramsky 1993). On the one hand, studies in experimental communities have found a linear (or asymptotic) positive effect of diversity on productivity (Naeem et al. 1996; Hector et al. 1999; Tilman et al. 2001; Loreau et al. 2001). On the other hand, species richness is hypothesized to first increase and then decrease with productivity or resource availability of the habitat, resulting in a hump-shaped or unimodal relationship (Fig. 1.1, Grime 1973; Huston 1979; Rosenzweig 1992). At low levels of productivity too few resources are available, and at high levels competitive exclusion by dominant species occurs. This hump-shaped relationship has been detected in experimental studies that manipulated site fertility

(Waide et al. 1999; Mittelbach et al. 2001), but its relevance for natural communities is less clear. It has been suggested that the hump-shaped curve might be better viewed as the upper boundary of the diversity-productivity relationship rather than a line of fitted average values (Grace 1999; Schmid 2002).

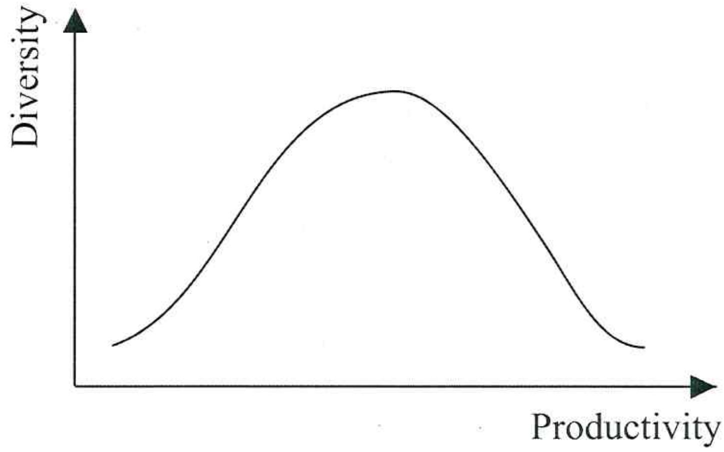

Fig. 1.1: Hump-shaped relationship between species diversity and productivity (after Grime 1973).

Besides abiotic factors influencing diversity at a given site, plants engage in multiple biotic interactions such as intra- and interspecific competition, mutualism and herbivory. In view of these multiple, simultaneous processes between plants and other organisms the diversity of species interactions might be the most important component of biodiversity (Thompson 1996; Price 2002).

A general opinion is that antagonistic interactions such as between plants and their herbivores, pathogens and parasites generate negative feedbacks on species abundance and are therefore important drivers for maintaining plant diversity (Tilman & Pacala 1993; Chesson 2000). A prominent example of plant antagonists, especially in grasslands, are invertebrate herbivores (Wilson 1987). Together with their host plants, invertebrate herbivores make up approximately half of all organisms on Earth (Strong et al. 1984). Recently, their importance for ecosystem functioning and the need for further studies has been stressed (Weisser & Siemann 2004). Theoretical considerations predict that the effect of herbivores at the ecosystem level depend on plant diversity and ecosystem productivity. According to the resource concentration hypothesis (Tahvanainen & Root 1972; Root 1973), the effect of herbivores should decrease with increasing plant diversity. According to the hypothesis of exploitation ecosystems, also known as the Fretwell-Oksanen model (Fretwell

1977; Oksanen et al. 1981; Fretwell 1987; Oksanen 1990), the effect of herbivores should be strongest at intermediate levels of productivity.

In contrast to antagonistic interactions, mutualistic ones have long been thought not to generate negative feedbacks and therefore they were expected to be less important for the maintenance of diversity (May 1974). However, Bever (1999; 2002) recently demonstrated that negative feedback can also result from mutualistic interactions, in particular when plants are associated with arbuscular mycorrhizal fungi (AMF). These fungi form intimate associations with the roots of about 75-80% of all terrestrial plants (Treseder & Cross 2006), and can greatly supply additional nutrients, i.e. phosphorus, to plants (Smith & Read 1997). The plant, in return, provides photosynthetic assimilates to the fungi. This mutualistic interaction has recently been shown to be an important determinant of plant diversity (for review see van der Heijden 2002), it can enhance plant community productivity (van der Heijden et al. 1998b), and it has significant effects on the shape of the diversity-productivity relationship (Klironomos et al. 2000).

New challenges for biodiversity-ecosystem functioning research

The challenge for biodiversity – ecosystem functioning research nowadays is to expand the investigations to ecosystems under natural conditions and to consider the high complexity of natural communities (Duffy 2002). More studies manipulating higher trophic levels are also urgently needed for a comprehensive understanding of the effects of diversity on ecosystem functioning (Raffaelli et al. 2002; Duffy et al. 2007), especially due to the fact that multiple trophic levels are common in natural ecosystems, and extinction threats appear to be higher for species at higher trophic levels (Petchey et al. 1999).

To address these challenges an interdisciplinary research project called DIVA Jena[1] (http://www2.uni-jena.de/biologie/ecology/biolog/english.htm) was initiated in 2001. Six research groups with expertise in entomology, soil ecology, biogeochemical processes and plant ecology have investigated important components of biodiversity and their importance for ecosystem functioning in semi-natural grasslands.

Generally semi-natural grasslands, also referred to as extensively managed grasslands, are characterized by their traditional land-use (e.g. mowing and livestock grazing) that exerts

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[1] DIVA Jena is one of five projects within BIOLOG Europe (Grant 01LC0013), an interdisciplinary research programme funded by the German Federal Ministry of Education and Research (BMBF) that aims to elucidate the impact of human land use and environmental change on biodiversity in Central Europe (http://www.biolog-europe.org/).

a continuous disturbance inhibiting succession and preventing many grassland specialists from being competitively excluded. As a result, these habitats contain an exceptionally high diversity of plants as well as fungi, insects and birds (Curry 1994; Baur et al. 1996; Vessby et al. 2002; Critchley et al. 2004; Treseder & Cross 2006). This high diversity and the fact that experimental manipulation of temperate grasslands is quite feasible make these grasslands excellent model systems for the investigation of effects of biodiversity on ecosystem processes.

The investigations were conducted in hay meadows in the Thüringer Schiefergebirge and Frankenwald, a plateau-like mountain range in central Germany (Fig. 1.2). Based on previous intensive field assays during the first phase of the DIVA Jena project (Perner et al. 2005; Kahmen et al. 2005a), 20 grasslands (Fig. 1.2) were selected to cover a gradient in plant species richness but to be similar in orographic and edaphic parameters, environmental conditions, and management regime (see Chapters 2, 3 & 5 for a detailed description of the study sites). Previous studies revealed that plant diversity was not correlated with productivity in these grasslands (Kahmen et al. 2005a), leading to the question of why there is no relationship and which other factors might be important drivers for plant diversity.

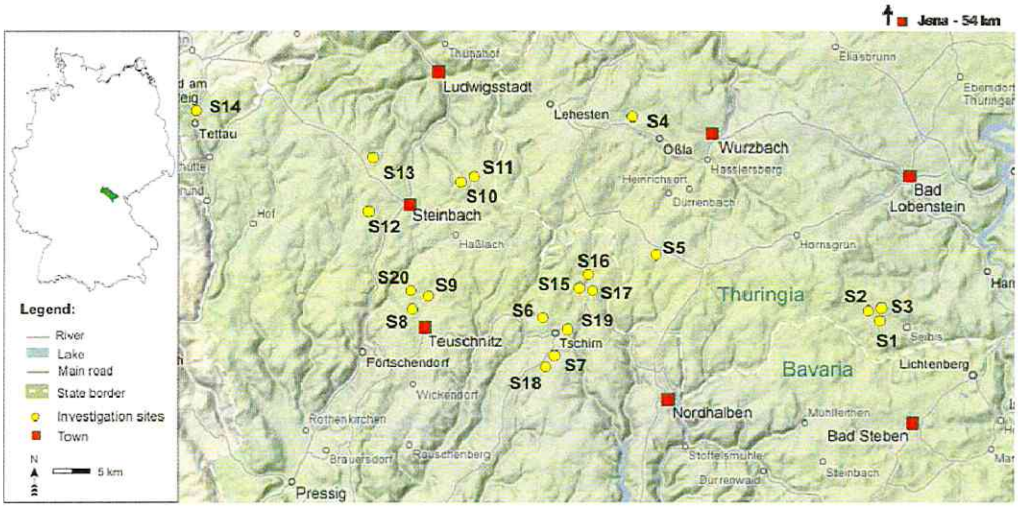

Fig. 1.2: Study area of the DIVA Jena project in the Frankenwald and Thüringer Schiefergebirge with the 20 grassland sites investigated in this thesis.

Objectives and overview of this thesis

The aim of my thesis is to assess the importance of different local and regional factors for the maintenance of local plant diversity in semi-natural grasslands, and to assess how diversity affects ecosystem functioning, i.e. the productivity and, as a measure of ecosystem stability, the invasibility of grassland communities. Specifically, I used several field and greenhouse experiments to address the following objectives:

(i) to determine if the local plant diversity of extensively-managed grasslands is limited by the availability of seeds, that is to test whether dispersal limitation as a regional process and seed limitation as a local process affect local plant species richness; and to elucidate the consequences for the diversity-productivity relationship;
(ii) to test the effects of above- and belowground invertebrate herbivores on the plant community and to test how these effects are determined by site characteristics, i.e. productivity and diversity;
(iii) to investigate interactive effects of arbuscular mycorrhizal fungi and a hemiparasitic plant on the diversity and productivity of experimental grassland communities;
(iv) to test how the diversity of plant species and functional groups affects the invasibility of semi-natural grasslands.

In Chapter 2, results are presented from an experiment in which I tested how dispersal and seed limitation affect diversity and productivity of semi-natural grasslands. This study accounts for the increasing demand for the incorporation of regional factors like dispersal limitation in biodiversity – ecosystem functioning research. By adding seeds of 60 grassland plant species from the regional species pool to plots in 20 extensively managed grasslands, I investigated if local plant species richness is constrained by the availability of seeds. Furthermore, I tested the following hypothesis: if species could overcome their dispersal or seed limitation, the local diversity and the productivity of the sites should increase to approach a hump-shaped relationship as proposed by theory. Plant species diversity and aboveground biomass were recorded for two years after adding seeds to the grasslands.

The study described in Chapter 3 tested the effects of invertebrate herbivory on plant community diversity and productivity. Over a period of five years, pesticides were applied to 14 grasslands that encompass a wide range of plant species richness to exclude aboveground herbivores, belowground herbivores or both. Based on the resource concentration hypothesis we predicted that the effect of herbivory on the plant community decreases with increasing plant diversity. Furthermore, we tested the prediction of the exploitation ecosystem hypothesis that the effect of herbivores is strongest at intermediate levels of productivity.

Chapter 4 reports on two complementary greenhouse experiments that I conducted to investigate the importance of antagonistic and mutualistic interactions for the diversity and the functioning of plant communities. So far, it has been shown that plant communities can be affected both by different types of arbuscular mycorrhizal fungi (AMF) and hemiparasitic plants. However, little is known about the interactive effects of these two biotic factors on the productivity and diversity of plant communities. To address this question, experimental grassland communities were inoculated with different types of AMF and a root hemiparasite (*Rhinanthus minor*) in a fully factorial design. In addition, single plants of each species in the grassland community were grown with the different AMF species to distinguish direct AMF effects from indirect effects *via* plant competition.

The experiments presented in Chapter 5 tested the diversity-invasibility hypothesis which predicts that more diverse communities should be less vulnerable to invasion of non-indigenous plant species. Therefore, I conducted two seed addition experiments using four non-indigenous plant species in the field. At a landscape scale, I added seeds to 20 grassland sites which represented a large gradient of plant species richness. The second experiment was carried out at one site only and seeds were added to plots where either legumes or non-legume forbs were previously excluded by herbicides. Seedling recruitment and establishment of non-indigenous plant species were recorded for two years.

In Chapter 6 I discussed the importance of the results for biodiversity and ecosystem functioning, and deduced implications for future studies as well as for management practices for semi-natural grassland in central Germany.

Chapter 2:

Dispersal and seed limitation affect diversity and productivity of montane grasslands

with Harald Auge, Markus Fischer, Wolfgang W. Weisser & Daniel Prati

Oikos, in press

ABSTRACT

So far, seed limitation as a local process, and dispersal limitation as a regional process have been largely neglected in biodiversity-ecosystem functioning research. However, these processes can influence both local plant species diversity and ecosystem processes, such as biomass production.

We added seeds of 60 species from the regional species pool to grassland communities at 20 montane grassland sites in Germany. In these sites, plant species diversity ranged from 10 to 34 species m^{-2} and, before manipulation, diversity was not related to aboveground biomass, which ranged from 108 to 687 g m^{-2}.

One year after seed addition, local plant species richness had increased on average by 6 species m^{-2} (29%) compared with control plots, and this increase was highest in grasslands with intermediate productivity. The increased diversity after adding seeds was associated with an average increase of aboveground biomass of 36 g m^{-2} (14.8%) compared with control plots. Thus, our results demonstrate that a positive relationship between changes in species richness and productivity, as previously reported from experimental plant communities, also holds for natural grassland ecosystems. Our results show that local plant communities are dispersal limited and a hump-shaped model appears to be the limiting outline of the natural diversity-productivity relationship. Hence, the effects of dispersal on local diversity can substantially affect the functioning of natural ecosystems.

INTRODUCTION

The relationship between plant species diversity and ecosystem processes is of major interest in ecology (Hooper et al. 2005). In natural communities, plant diversity is hypothesized to first increase and then decrease with productivity of the habitat, resulting in a hump-shaped or unimodal relationship (Grime 1973; Huston 1979; Rosenzweig 1992). This hump-shaped relationship has been detected in experimental studies manipulating site fertility (Waide et al. 1999; Mittelbach et al. 2001), but its relevance for natural communities is less clear. It has been suggested that the hump-shaped curve might better be viewed as the upper boundary of the diversity-productivity relationship rather than a line of fitted average values (Grace 1999; Schmid 2002). Whether the maximum possible plant diversity at a given productivity is actually achieved depends on several ecological processes acting at the local and the regional scale (Ricklefs 1987; Huston 1999; Hillebrand & Blenckner 2002). In particular, many experiments conducted across a wide range of habitats and community types showed that plant species diversity is often limited by dispersal (Turnbull et al. 2000; Cadotte 2006). This may obscure any underlying intrinsic relationship between diversity and productivity.

Diversity itself is increasingly recognized as an important determinant of ecosystem properties (Loreau et al. 2002b). In studies manipulating initial species richness by sowing on bare ground and maintaining species richness by subsequent weeding, productivity was found to increase with increasing species richness (Hector et al. 1999; Tilman et al. 2001; Roscher et al. 2005). This positive effect of species richness on productivity observed in such experiments is attributed to niche complementarity among species, to a higher probability of including particularly productive species in species-rich communities, or to facilitation among species (Loreau & Hector 2001). However, considerable debate exists about the applicability of the conclusions drawn from diversity experiments with artificial communities to natural ecosystems (Wardle et al. 2000; Naeem 2002; Lepš 2004; Zobel et al. 2006; Grace et al. 2007).

When diversity is limited by dispersal but ecosystem processes are positively affected by diversity, it is likely that dispersal limitation also limits the functioning of the plant communities (Bengtsson et al. 2002; Zobel et al. 2006). Such tests have rarely been performed (Foster 2001; Wilsey & Polley 2003; Foster & Dickson 2004; Zeiter et al. 2006). Dispersal limitation can be tested experimentally by adding seeds of new species to plant communities of formerly unoccupied habitats, whereas seed addition of species that are already present in a plant community can be used to test for seed limitation. The use of these two terms is often inconsistent (Münzbergová & Herben 2005), and because many plant species have very short dispersal distances, seed and dispersal limitation are not easily delineated as different ecological processes. Seed limitation means that seed availability is limited at a local scale whereas dispersal limitation accounts for regional factors such as landscape fragmentation or isolation.

The success of seed addition depends on plant traits, especially seed size (Burke & Grime 1996), and on site characteristics. Relevant in the diversity-productivity context is that some experiments from grasslands suggest that the success of seed addition is inversely related to diversity (Tilman 1997; Knops et al. 1999) and productivity (Foster & Gross 1998) of a study site. However, the majority of seed addition experiments has been conducted with only a small number of species or field sites, and thus the general importance of seed limitation for the whole set of species of a community and across many sites in a real landscape is still unclear.

To test for dispersal limitation and to investigate its role for the relationship between diversity and productivity we experimentally added seeds of 60 grassland plant species from the regional species pool to plots in 20 semi-natural montane grasslands in central Germany. These grasslands are extensively managed as hay meadows without any fertilizer applications. We addressed the following specific questions: 1) Is the local diversity of the montane grasslands limited by dispersal, and if so, how is dispersal limitation related to current diversity and productivity of sites and to seed mass? 2) Does increasing local species diversity by overcoming dispersal limitation also increase productivity? 3) Does the diversity-productivity relationship across sites after adding seeds to local communities approach the hump-shaped curve?

MATERIALS AND METHODS

Study sites

The study was conducted in 20 grassland sites in the Frankenwald and Thüringer Schiefergebirge (11°00'-11°37'E and 50°21'-50°34'N), a plateau-like mountain range at the border between Bavaria and Thuringia in central Germany. The bedrock material in the investigated area consists mainly of schist and greywacke and produces a carbonate-free, nutrient-poor soil. The natural vegetation is a montane spruce-fir-beech forest but since medieval times much of the forest has been converted into agricultural fields with a high proportion of montane hay meadows (Geranio-Trisetetum Knapp ex Oberd. 1975) (Hundt 1964).

The 20 grasslands are located on plateaus of the mountain range between 500 and 840 m altitude, so that orographic and edaphic factors were rather similar among sites. All study sites have been extensively managed, with no fertilization or grazing for at least the past 15 years, and they were mown once or twice per year (June/July and September). Mowing takes place relatively late in the growing season after seed set of most of the plant species. Some of the sites were used as grasslands for at least 50 years whereas others were probably converted agricultural fields. However, because some sites were close to the inner-German border, we lack detailed information on the historical land-use beyond the last 15 years. Most of the sites were highly scattered in the landscape and isolated from each other by agricultural fields, intensively-used fertilized grassland and forests. The sites represented a gradient in plant species diversity from about 10 species to about 34 species m^{-2} in the most diverse mountain hay meadows. Thus, we assume that differing original plant diversity of these grasslands is due to slight variation in soil conditions (Kahmen et al. 2005a), to different time span since conversion from forest to grassland, combined with isolation effects. A detailed description of 78 sites from which our study sites were selected can be found in Kahmen et al. (2005a).

Plant material

The aim was to collect as many species as possible from the regional species pool, i.e. species being present in at least one of the study sites which collectively represented the species pool. Altogether we were able to collect seeds of 60 species (Appendix 2.1) including 18 perennial graminoids, 36 forbs and 6 legumes, which were used for the seed addition experiment. The seeds were collected in the study area as mixed samples from mature inflorescences of a large number of individuals from June to September 2004. They were cleaned and stored at room temperature until sowing at the end of October 2004.

Seed mass per species was determined for four batches of 100 seeds. Germination ability was tested in germination trials with five replicates of 20 seeds for each species. The seeds were sown in pots which were placed outdoors for cold stratification from November 2004 to January 2005. Afterwards the pots were transferred into a greenhouse (20°C at daytime and 15°C during night). Germination of the seeds was recorded during the next 3 months for all species, except for *Holcus lanatus* and *Hypochaeris radicata* for which no seeds were left after setting up the field experiment.

Experimental design

In October 2004, four 1 x 1 m plots separated by 0.5 m were established in each of the 20 grasslands. Treatments were randomly assigned to these four plots: two plots were used for addition of seeds and two plots served as control. Seeds were added after mowing the meadows to a height of 3 cm above ground and removing the litter. The same seed mixture containing all 60 species was used for all study sites. In accordance with Thompson et al. (2001) we decided to use seed mass as the criterion for the sowing quantity: seeds with a seed mass higher than 1.7 mg were added with a density of 100 seeds m^{-2}, the ones with a mass less than 1.7 mg per seed with a density of 300 seeds m^{-2}. In total 14.800 seeds m^{-2} were sown per plot. The seeds were mixed with 20 ml of sterilized, washed sand and manually distributed over the plot. The vegetation was lightly shaken to ensure that the seeds settled to the soil surface, while the soil was left undisturbed.

Data collection

In June 2005 and 2006, at peak standing biomass, all vascular plant species were identified in the four 1 m^2 plots and the percent cover was visually estimated using a modified Londo scale (Londo 1976). Since we were only interested in successful plant recruitment, it was not necessary to count seedlings in the spring immediately after emergence. Additionally, leaf area index (LAI) of each plot was measured with a LAI-2000 Plant Canopy Analyzer (LI-COR Biosciences, USA) to investigate if emergence of new species was related to vegetation density. Plant biomass was sampled in a 50 x 50 cm square (0.25 m^2) for each plot by cutting the vegetation 3 cm above ground. After drying at 60°C for 72 h, above-ground biomass was determined in g m^{-2}. Thus, we measured three plant community characteristics: plant species richness, aboveground biomass as a measure of productivity, and leaf area index.

To distinguish newly added species from the ones emerging from the seed bank, soil samples were taken in October 2004. One hundred soil samples were taken randomly per study site from the topsoil using a cylindrical soil core with a volume of 49.1 cm^3 (diameter 2.5 cm, depth 0-10 cm) over a 200 m^2 area surrounding the experimental plots. These 100 samples were pooled to produce one sample per study site. Each soil sample was split on four plastic trays of 30 cm x 23 cm x 6 cm filled with a 2 cm layer of sterilized sand. The trays were randomly arranged on a glasshouse bench, and all seedlings were identified to the species and continuously counted until September 2005. Appendix 2.1 shows the occurrence in the soil seed bank of those species which were used in the experiment.

To be able to determine which of the sown species were already present in the grasslands, all plant species in an area of 200 m^2 surrounding the experimental plots were recorded in eleven additional 1 m^2 plots per grassland. Appendix 2.2 shows the occurrence in the soil seed bank and in the present vegetation of all species which were not used in the experiment.

Data analysis

The statistical software R (version 2.6.1.) was used for all analyses. Statistical assumptions, such as normal distribution of residuals and homoscedasticity were checked and seed mass data were log-transformed prior to analysis. A mixed-model analysis of variance (ANOVA, lm functions) was used to evaluate the effect of seed addition on species richness and aboveground biomass per square meter. We used study site as a random factor and seed addition as a fixed factor and tested the effect of seed addition against the interaction with site. To estimate the change in species richness in response to seed addition, we averaged the number of plant species per square meter across the two replicates for each treatment and study site. Thus we had only one value for the change in species richness per site, and we did not consider the site effect.

Multiple regression analyses were used to examine the relationships between recruitment of new species and site characteristics as well as seed mass. Only species that did not occur in the soil seed bank or in the surrounding area of each grassland site were considered as novel species to the sites ("true novelties" in Appendix 2.1). Thereby, we excluded the confounding effect of seed limitation and referred only to dispersal limitation. A contingency table was constructed to evaluate relationships between plant functional groups and seedling recruitment. For the functional groups we simply distinguished between graminoids, non-legume forbs and legumes.

The diversity-productivity relationship for control plots and for seed addition plots was again investigated using regression analyses. We used the ′leave-one-out′ or jackknife resampling technique (Manly 1997) to test the sensitivity of the models of the diversity-productivity relationship to single data points. To quantify the shape of the upper boundary of the large scatter of data points we also calculated 95% quantile regression (Cade et al. 1999; Koenker 2007).

RESULTS

Effects of seed addition on local diversity

In the first year after sowing, seed addition increased the mean number of species m^{-2} by 5 species compared with control plots corresponding to a 25% increase in species richness (Fig. 2.1, $F_{1,19} = 60.37$, $P < 0.001$). This effect was even higher in the second year: the mean number of species m^{-2} in the seed addition plots increased by 6 species corresponding to 29% in species richness compared with control plots (Fig. 2.1, $F_{1,19} = 64.54$, $P < 0.001$). In both years there were significant differences among the study sites (2005: $F_{19,40} = 17.20$, $P < 0.001$; 2006: $F_{19,40} = 19.77$, $P < 0.001$). The sowing x site interaction was significant in the second year after sowing ($F_{19,40} = 2.23$, $P < 0.02$). It should be noted that this analysis does not distinguish between the effects of seed and dispersal limitation.

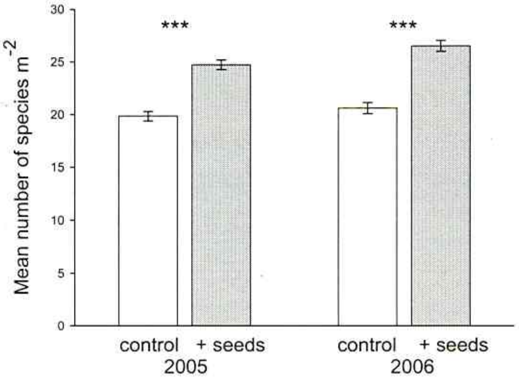

Fig. 2.1: Effect of adding seeds of 60 plant species from the regional species pool to 20 grassland sites in Thuringia and Bavaria on species richness (mean ± SE) m^{-2} one year (2005) and two years (2006) after seed addition. White bars: control plots; grey bars: plots with seed addition (*** $P < 0.001$).

Relationship between species traits and recruitment

Recruitment success of sown species was negatively correlated with their occurrence in the soil seed bank (2005: seedling recruitment [%] = 11.89-0.16 frequency in seedbank [%], $R^2 = 0.11$, $P < 0.011$; 2006: seedling recruitment [%] = 15.73-0.22 frequency in seedbank [%], $R^2 = 0.14$, $P < 0.004$) and the most successful species, such as *Bromus hordeaceus, Chaerophyllum aureum, Heracleum sphondylium, Lotus corniculatus,* and *Medicago lupulina,* were not found in the seed bank at all (Appendix 2.1). Germination ability of the seeds, based on germination rates obtained in the greenhouse, had no effect on plant recruitment (2005: $R^2 = 0.002$, $P > 0.71$; 2006: $R^2 = 0.05$, $P > 0.08$). Grasses, legumes, and non-legume forbs did not differ in recruitment success (2005: $Chi^2 = 4.08$, $df = 2$, $P > 0.05$; 2006: $Chi^2 = 4.38$, $df = 2$, $P > 0.05$), but seedling recruitment increased with mean seed mass of the species (2005: seedling recruitment [%] = 8.60+4.08 log(seedmass[mg]), $R^2 = 0.16$, $P < 0.01$; 2006: seedling recruitment [%] = 11.25+5.72 log(seedmass[mg]), $R^2 = 0.22$, $P < 0.001$).

Relationship between species diversity, productivity and success of recruitment

This analysis includes only those species that did neither occur in the soil seed bank nor in the established vegetation of the respective grassland site ("true novelties" in Appendix 2.1), thus results do only refer to effects of dispersal limitation. In the first year after sowing, the number of newly recruited species was not dependent on the productivity of the grassland sites, even though a tendency to a unimodal relationship could be observed. In the second year, the number of newly recruited species was highest at grassland sites with intermediate productivity, confirming the tendency observed in the first year (Fig. 2.2). In addition, multiple regression analyses revealed no significant effect of leaf area index (as a measure for vegetation density) on recruitment success (Table 2.1). In both years, the number of newly recruited plant species did not depend on the resident species richness of the plots.

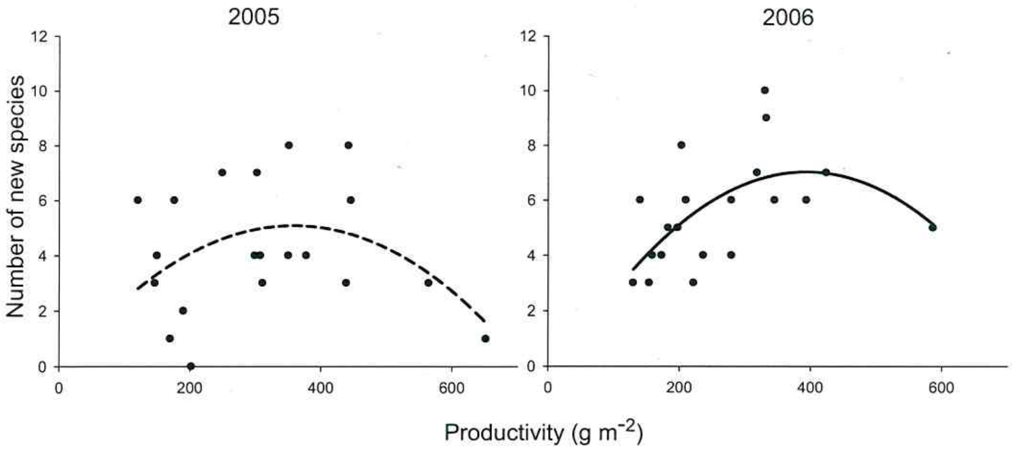

Fig. 2.2: Number of newly recruited species after adding seeds of 60 plant species from the regional species pool to 20 grassland sites in Thuringia and Bavaria. Each data point represents the mean difference between two control plots and two seed addition plots per grassland site. Two years after adding seeds, the number of new species is highest at intermediate productivity. For reasons of illustration, curves are fitted using quadratic regression on productivity only. Left graph: one year after seed addition (2005), $R^2 = 0.17$, $P > 0.05$. Right graph: two years after seed addition (2006), $R^2 = 0.35$, $P < 0.05$.

Table 2.1: Results of multiple regression analyses of the effects of site characteristics on the number of newly recruited species one year (2005) and two years (2006) after seed addition across 20 grassland sites in Thuringia and Bavaria.

2005 ($R^2 = 0.20$)				
Variable	d.f.	Parameter estimate	t	P
Intercept	1	1.469	0.409	0.689
LAI	1	-0.199	-0.504	0.621
Species richness	1	-0.073	-0.632	0.537
Productivity	1	0.033	1.851	0.084
Productivity^2	1	-0.000	-1.916	0.075
2006 ($R^2 = 0.46$)				
Variable	df	Parameter estimate	t	P
Intercept	1	-0.131	-0.049	0.962
LAI	1	-0.526	-1.302	0.213
Species richness	1	-0.046	-0.568	0.578
Productivity	1	0.054	3.106	**0.007**
Productivity^2	1	-0.000	-2.772	**0.014**

Effects of seed addition on productivity and on the diversity-productivity relationship

By adding seeds to 20 grassland sites, the increase in local plant species richness led also to an increase in aboveground biomass. In the first year after sowing (2005), seed addition increased the aboveground biomass by 13.8% compared with control plots (+32 g m^{-2}, $F_{1, 9} = 6.44$, $P = 0.018$, Fig. 2.3). This effect on productivity persisted into the second year when seed addition increased aboveground productivity by 14.8% compared with control plots (+36 g m^{-2}, $F_{1, 19} = 10.51$, $P < 0.0013$, Fig. 2.3). In both years there were significant differences among the study sites (2005: $F_{19, 40} = 25.80$, $P < 0.001$; 2006: $F_{19, 40} = 26.96$, $P < 0.001$) but no significant sowing x site interactions.

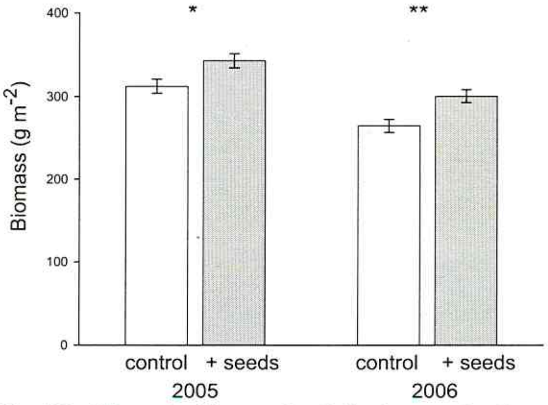

Fig. 2.3: Effect of adding seeds of 60 plant species from the regional species pool to 20 grassland sites in Thuringia and Bavaria on aboveground productivity (mean ± SE) m^{-2} one year (2005) and two years (2006) after seed addition. White bars: control plots; grey bars: plots with seed addition (* $P < 0.05$, ** $P < 0.01$).

As a consequence, seed addition changed the relationship between diversity and productivity. In control plots diversity was independent of productivity, whereas in the seed addition plots, a hump-shaped relationship between diversity and productivity became evident, expressed by significant linear and quadratic terms in regression analyses (Fig. 2.4, Table 2.2). Comparison of models using ANOVA revealed that the quadratic relationships fit the data significantly better than the linear ones do (2005: F = 3.50, P <0.079; 2006: F = 5.23, P < 0.035). Evaluation by jackknife procedure revealed that the unimodal relationship was reliable. No matter which data point was excluded during this procedure the quadratic term remained significant. Furthermore, the unimodal shape of the upper boundary of the data was confirmed by results of 95% quantile regression. This approach revealed a significant unimodal relationship in 2006 ($y = 24.23 + 0.30x - 0.002x^2$, 2nd order term: $P < 0.001$), whereas this was not the case in 2005 ($y = 19.98 + 0.36x - 0.002x^2$, 2nd order term: $P > 0.26$).

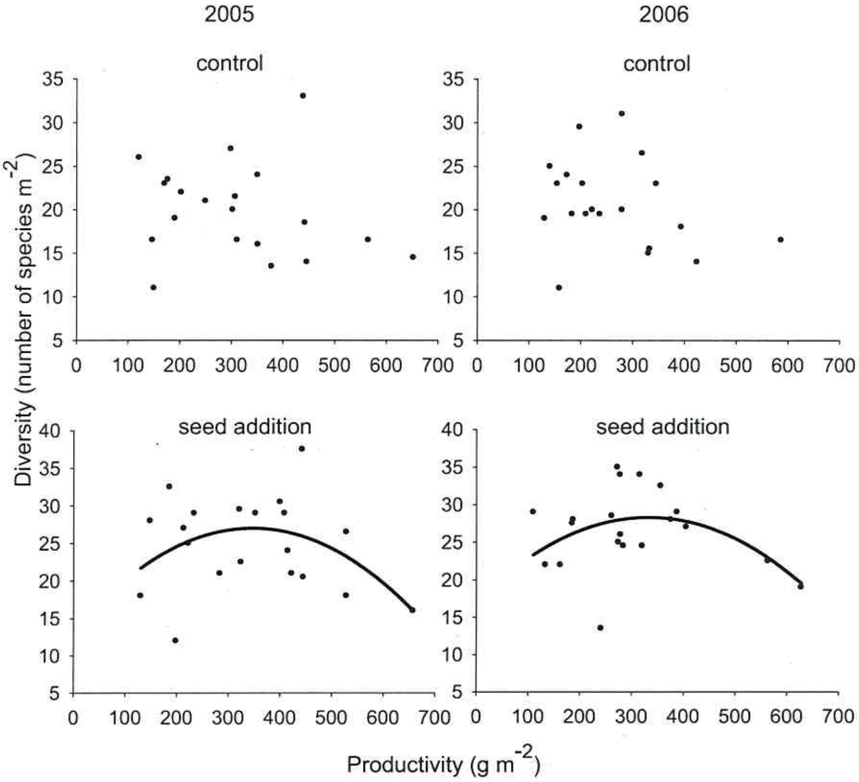

Fig. 2.4: Diversity-productivity relationship measured in the 20 grassland sites in Thuringia and Bavaria without seed addition (control) and after seed addition. Each data point represents the mean of two plots per grassland site. Left side: one year after seed addition (2005). Right graph: two years after seed addition (2006). Control plots show no correlation between diversity and productivity, whereas relationships in the seed addition plots approximate a hump-shaped curve (for statistics see Table 2.2).

Table 2.2: Diversity-productivity relationship in control plots and seed addition plots one year (2005) and two years (2006) after seed addition across 20 grassland sites in Thuringia and Bavaria.

Control plots

2005 (R^2 = 0.07)

Variable	d.f.	Parameter estimate	t	P
Intercept	1	16.749	4.049	**<0.001**
Productivity	1	0.107	1.093	0.281
Productivity^2	1	-0.0007	-1.373	0.178

2006 (R^2 = 0.09)

Variable	d.f.	Parameter estimate	t	P
Intercept	1	19.299	4.312	**<0.001**
Productivity	1	0.0736	0.636	0.529
Productivity^2	1	-0.0007	-1.031	0.309

Seed addition plots

2005 (R^2 = 0.15)

Variable	d.f.	Parameter estimate	t	P
Intercept	1	14.0188	2.496	**0.017**
Productivity	1	0.296	2.2268	**0.029**
Productivity^2	1	-0.00168	-2.459	**0.018**

2006 (R^2 = 0.15)

Variable	d.f.	Parameter estimate	t	P
Intercept	1	18.426	4.106	**<0.001**
Productivity	1	0.236	2.19	**0.034**
Productivity^2	1	-0.0014	-2.462	**0.018**

DISCUSSION

Dispersal limitation

The positive effect of seed addition on diversity observed here is consistent with several studies that demonstrated that local species richness can be constrained by the availability of seeds (Zobel et al. 2006). The positive effect of seed addition on local diversity is supposed to be more common in early successional communities than in grasslands (Turnbull et al. 2000; Cadotte 2006). Our results indicate that even in established grassland communities not all available microsites are saturated with seeds, which could be caused by local processes like seed predation or low seed production (Ehrlén & Eriksson 2000), or by regional processes such as limited seed dispersal. Recruitment in grasslands is known to be limited because many species disperse only over short distances (Cheplick 1998) and they have only a transient or short-term persistent soil seed bank (Thompson et al. 1997).

Even though the effect size of our results did not decrease from the first to the second year after sowing, longer-term observations are required to prove whether the newly recruited species form self-sustaining populations, which is the ultimate criterion of success. However, we have sown the seeds only on one occasion in one single year, which provided a rather conservative test of seed limitation. Furthermore, seeds were sown without artificial disturbance into an intact turf, which could have reduced the recruitment success. Repeated seed addition (Zeiter et al. 2006) and local disturbances (Kotorova & Lepš 1999; Isselstein et al. 2002) were reported to result in an even better recruitment of most grassland species.

We added seeds of novel species and of species that were already present in the grasslands, thus potentially confounding dispersal with seed limitation. However, for the analyses of dispersal limitation we included only species that did not occur in the surrounding area or in the soil seed bank of each grassland site. Species occurring in the soil seed bank were excluded because we suggest that these species are limited by abiotic factors, management practices, competition or predation rather than by the availability of seeds. Thus, in our system dispersal rather than seed limitation was the driving ecological factor restricting local species richness (Fig. 2.2). However, it is important to keep in mind that the effect of seed addition on species diversity, productivity and on the diversity-productivity relationship of the grasslands was partly due to seed limitation because these analyses include all species per plot.

Role of species and habitat characteristics

We tested a number of plant species traits and habitat characteristics which may explain the varying recruitment success of plant species. In accordance with other seed addition experiments, we found that large-seeded species showed a higher recruitment compared with smaller-seeded species (Ehrlén & Eriksson 2000; Thompson et al. 2001; Moles & Westoby 2002). This pattern could be explained (1) by higher germination and seedling survival of larger-seeded species due to better provisioning with resources, or (2) by a stronger limitation by seed availability of larger-seeded species and therefore a higher benefit of these species from seed addition. We think that the second explanation is more likely because large-seeded species produce fewer seeds per unit canopy area than small-seeded species (Moles et al. 2004), they are generally less abundant in soil seed banks (Thompson & Grime 1979; Thompson et al. 1993) and in our study there was no correlation between germination rates tested in the greenhouse and species recruitment in the field.

The question concerning the role of plant diversity of a habitat for the success of sown species remains controversial. In our experiment, the recruitment of sown species was independent of the resident species richness, confirming results from previous studies conducted in a natural grassland (Zobel et al. 2000) and in an artificial sown community (Lavorel et al. 1999). However, in experimental grassland communities it was repeatedly shown that the least diverse plots showed the highest disposition to invasion by other species (Tilman 1997; Knops et al. 1999; Kennedy et al. 2002; Pfisterer et al. 2004). One explanation for these contradicting findings is that the gradient of species richness in our natural grassland communities was not large enough to find a negative influence on species recruitment, or that other variables such as site fertility confounded the effect of diversity. Nevertheless, since our study was conducted in a representative range of natural grasslands as opposed to the experimental plant communities, our results indicate that the effect of diversity on species recruitment is less pronounced than previously assumed.

Most previous sowing studies showed that the success of seed addition decreases with increasing productivity (Burke & Grime 1996; Foster 2001; Foster & Dickson 2004; Foster et al. 2004; Zeiter et al. 2006). In our study the productivity of the habitat had a significant effect on plant recruitment and therefore on diversity at intermediate levels of productivity (Fig. 2.2). These results support the theoretically predicted increasing relevance of competitive exclusion at higher productivity levels (Grime 1979; Huston 1999).

At higher productivity, seedling recruitment is known to be inhibited by competition from established plants and by plant litter (Foster & Gross 1998). However, we did not find such a negative effect of vegetation density on seedling recruitment (Table 2.1). At lower productivity, nutrients may be a limiting factor. As a consequence, the increase in species number through seed addition was maximal at intermediate levels of productivity, and led to the emergence of a hump-shape relationship which was not found in our control plots (Fig. 2.4). Housemann and Gross (2006) found a similar pattern after seed addition along a natural productivity gradient in an old-field, however they only used three different sites. In their study seed addition shifted the productivity-diversity relationship from negative linear to unimodal. We conclude that dispersal limitation (and to some extent seed limitation) not only constrain the local species richness but may prevent the occurrence of one of the most common patterns proposed in plant ecology. This affirms our initial hypothesis, that if plant species overcome dispersal limitation, diversity and productivity would approach the theoretically predicted hump-shaped relationship. Hence, dispersal limitation is one factor that can contribute to the unimodal productivity-diversity relationship – not only in a theoretical situation as shown by Pärtel and Zobel (2007) but also in natural grasslands. Furthermore, our data support the idea that the hump-shaped model describes only the limiting outline of the diversity-productivity relationship (Grace 1999; Schmid 2002).

Effect of seed addition on productivity

From a meta-analysis of 12 observational studies on the relationship between species diversity and productivity of natural grassland ecosystems it was concluded that realised natural small-scale diversity has – at best – little influence on productivity (Grace et al. 2007). However, the role of dispersal limitation for diversity and productivity cannot be detected in observational studies. In our study the increase of species richness through seed addition led also to a considerable increase in aboveground biomass (Fig. 2.2). This pattern is in line with a large number of experimental studies that showed a positive effect of diversity on productivity and other ecosystem processes in artificially assembled experimental plant communities (Tilman et al. 1996; Naeem et al. 1996; Hector et al. 1999; Tilman et al. 2001). In these diversity experiments, the observed increase in aboveground productivity was due to complementarity effects (Roscher et al. 2005) or to positive mutualistic interactions between species (Mulder et al. 2001) rather than to selection effects. Our experiment did not aim to distinguish between these alternatives, but it indicates that diversity does have an important effect on ecosystems across a range of natural grasslands in a real landscape. Thus, seed addition experiments, beyond testing the importance of regional processes on local diversity, are a valuable approach to manipulate diversity and study the ecosystem consequences, thereby overcoming many shortcomings of "classical" diversity experiments (Lepš 2004; Zobel et al. 2006).

Conclusion

Our study shows that enhancing both diversity and productivity by the introduction of indigenous, but dispersal and seed-limited species could be a good tool for management purposes (Schmid 2002). Furthermore, it could be used to enhance agricultural production (Bullock 2001) and thus may be economically profitable. We showed that this is even possible in grasslands with already quite high resident species richness and of low productivity without great efforts such as fertilization. This supports the idea of Tilman et al. (2006) to use high-diversity, low-input mixtures of grassland plants as renewable resources for the production of bioenergy.

We cannot exclude the possibility that the observed results are transient and we want to point out that longer-term observations are required. Nevertheless, our findings stress the importance of dispersal limitation in natural grassland communities for local species diversity as well as for ecosystem processes. To our knowledge this is the first time that the results obtained in experimental grassland communities studying biodiversity-ecosystem functioning could be confirmed experimentally for a wide range of temperate, extensively managed grasslands.

Chapter 3:

Impact of invertebrate herbivory in grasslands depends on productivity and plant species diversity

with Sybille B. Unsicker, Ansgar Kahmen, Markus Wagner, Volker Audorff, Harald Auge, Daniel Prati & Wolfgang W. Weisser

to be submitted

ABSTRACT

Invertebrate herbivores are ubiquitous in most terrestrial ecosystems and theory predicts that their influence on ecosystem functions such as biomass may depend on diversity and productivity of the associated plant communities. While previous studies have confirmed that herbivorous invertebrates and plant communities interact with each other in many different ways, generalization has so far proved difficult.

To elucidate general patterns in the relationship between invertebrate herbivory, plant diversity, and productivity we carried out a long-term herbivore exclusion experiment at multiple semi-natural grassland sites in central Germany. Over a period of five years, we used above-and belowground insecticides as well as a molluscicide to manipulate invertebrate herbivory at 14 grassland sites, covering a wide range of plant species richness (13 – 38 species m^{-2}) and aboveground plant productivity (272 - 1125 g m^{-2} $year^{-1}$), where plant species richness and productivity of the sites were not significantly correlated.

Herbivore exclusion had significant effects on the plant communities: It slightly decreased plant species richness and evenness, and it altered plant community composition. Exclusion of belowground herbivores promoted grasses at the expense of forbs. Herbivore effects on plant community biomass significantly depended on plant diversity and productivity of the grasslands: The effect of herbivory on biomass was negative on sites of high diversity or low productivity and positive on sites of low diversity or high productivity.

In general, the effects of aboveground herbivores were relatively small, while belowground herbivores are more important drivers of plant community composition and biomass. Our study indicates that both, diversity and productivity of the investigated plant communities are important factors influencing the impact of herbivory.

INTRODUCTION

Herbivorous invertebrates outweigh vertebrates as much as 10-fold in temperate terrestrial ecosystems (Pimentel & Andow 1984). Nevertheless, their role in determining plant community dynamics is supposed to be less important compared with vertebrates (Crawley 1989). There is now increasing evidence that invertebrate herbivores, particularly insects and molluscs, can influence plant communities and ecosystem functioning (for overview see Coupe & Cahill 2003; Weisser & Siemann 2004; Schädler et al. 2004; Maron & Crone 2006; Whiles & Charlton 2006).

The most immediate effect of invertebrate herbivores is the consumption of plant tissue (Crawley 1983; Karban & Baldwin 1997; Crawley 1997). In addition, invertebrate herbivores may influence plant species composition and diversity by selective feeding on dominant species and thereby, releasing subordinate species from competition of dominant ones (Crawley 1989; Pacala & Crawley 1992; Carson & Root 1999). These effects, however, are not consistent across different guilds of herbivores, or across different ecosystems. For example, plant diversity in successional plant communities was found to be enhanced by aboveground herbivores (Brown & Gange 1992; Carson & Root 2000), and to be reduced by belowground ones (Brown & Gange 1989b; Brown & Gange 1992). A further source of variation is that the effects of herbivores are also affected by environmental factors and plant community characteristics, such as plant diversity and productivity (Olff & Ritchie 1998).

Ecosystem level effects of herbivores such as on community biomass are predicted to decrease with increasing plant diversity (Pimentel 1961; Root 1973) owing to two different mechanisms: The resource concentration hypothesis (Tahvanainen & Root 1972; Root 1973) states that specialist herbivores should achieve higher abundances when their host plants grow in higher abundances. Alternatively, the natural enemies hypothesis (Root 1973) predicts that herbivore abundances are mediated by the density of natural enemies which should be more abundant in diverse plant stands. However, most studies testing this hypothesis have investigated herbivore abundance rather than load (density per unit mass of the host-plant species) or damage, and their results have been ambiguous. Some studies have confirmed the predicted decrease in herbivore abundance or damage in more diverse plant communities (Mulder et al. 1999; Unsicker et al. 2006), but others have found a positive or negative relationship depending on the type of herbivore studied (Koricheva *et al.* 2000). Yet others have found no such relationship suggesting that particular plant functional groups may be more important than species richness per se (Scherber *et al.* 2006).

In addition to plant diversity, the effects of herbivores on ecosystem function have also been suggested to depend on the primary productivity of an ecosystem (Fretwell 1977; Oksanen et al. 1981; Fretwell 1987; Oksanen 1990) with the strongest herbivore impact expected at intermediate levels of productivity (hypothesis of exploitation ecosystems, also known as the Fretwell-Oksanen model). At low primary productivity, plant biomass may not suffice to sustain appreciable populations of herbivores (bottom-up control of herbivores), whereas at high levels of productivity top-down control of herbivores by predators is expected. Even though this model was originally built for vertebrate herbivores (Oksanen et al. 1981; Oksanen 1990), it has been suggested to apply also to invertebrates (Oksanen & Oksanen 2000).

Although several invertebrate exclusion experiments have been conducted, so far, it is still difficult to draw general conclusions about the importance of invertebrate herbivores for terrestrial plant communities. In particular, it remains unclear how plant community characteristics direct the effect of invertebrates on plant communities and ecosystem functioning (Coupe & Cahill 2003; Schädler et al. 2003b). To improve the general understanding of the importance of invertebrate herbivores for plant community dynamics, long-term field studies in natural ecosystems are therefore urgently needed (Huntly 1991; Crawley 1997; Schädler et al. 2003b). Such studies would also represent an important contribution to the recent debate about the relationship between biodiversity and ecosystem functioning, which has so far mainly focused on the ecosystem-level consequences of changes in plant diversity (Loreau et al. 2001; Schmid et al. 2001; Loreau et al. 2002b), but largely neglected the effects of higher trophic levels (Duffy et al. 2007).

In our study, carried out on 14 extensively managed hay meadows in central Germany, we investigated the effects of above- and belowground invertebrate herbivory on plant community diversity and biomass along gradients of plant species richness and productivity. Using the same community type within one landscape, we tried to minimize the impact of confounding factors. Over a period of five years, we used pesticides to exclude insects and molluscs from experimental plots to test the following predictions: (1) Because invertebrate herbivores remove plant tissue, they generally have a negative effect on plant community biomass. (2) By decreasing competitive ability of dominant plant species, invertebrate herbivores have a positive effect on plant community diversity. (3) The effect of herbivory on plant community biomass decreases with increasing plant diversity and is strongest at intermediate levels of primary productivity.

METHODS

Study sites

The study was conducted in the Frankenwald and Thüringer Schiefergebirge (11°00'-11°37'E and 50°21'-50°34'N), a plateau-like mountain range at the border between Bavaria and Thuringia in central Germany with an elevation ranging from 500 to 870 m a.s.l.. The bedrock material in the area consists mainly of schist and greywacke and produces a carbonate-free, nutrient-poor soil. Mean annual air temperature is 5°C to 7°C, and mean annual precipitation ranges from 840 to 1200 mm with a slight summer maximum (Hiekel et al. 2004). Before human settlement, montane spruce-fir-beech forests formed the natural vegetation. Since human settlement in medieval times much of the forest has been converted into an agricultural landscape with a large proportion of montane hay meadows (Hundt 1964).

Based on a survey of more than 70 grassland sites in 2001 (Perner et al. 2005; Kahmen et al. 2005a), we selected a subset of 14 montane hay meadows (phytosociologically classified as Geranio-Trisetetum, Knapp ex Oberd. 1975) that vary in plant species diversity but are similar with respect to edaphic and orographic conditions and management regime. The size of the total study area was 114 km^2. The minimum distance between two sites was 120 m and the maximum 6.5 km. Across sites, plant species richness as measured in June 2002, ranged from 13 to 38 vascular plants m^{-2}. Mean standing biomass, also measured in June 2002 ranged from 230 to 591 g m^{-2} (dry biomass). All study sites have been managed extensively by cutting for hay for at least the last ten years prior to the onset of the study, with two cuts per year and no grazing or fertilizer application.

Manipulation of invertebrate herbivory

At each of the 14 grassland sites, we established permanent plots (Fig. 3.1) on which pesticides were applied to exclude aboveground herbivores (insects and molluscs), belowground herbivores or both (combined treatment). To exclude aboveground herbivores we sprayed Dimethoate (Perfekthion, BASF, Germany), a broad spectrum quasi-systemic foliar insecticide (Tomlin 2006). Diluted in water, it was applied in biweekly intervals at the recommended rate of 0.15 g of active ingredient m^{-2}. The aboveground treatment also included the application of molluscicide pellets (metaldehyde, 0.6 g of active ingredient m^{-2}) every two or four weeks. When rainfall intensity in the study area was low, monthly application of molluscicide was deemed sufficient. The belowground treatment consisted of the application of Chlorpyrifos, a contact soil pesticide without systemic effect (Tomlin

2006). It was applied monthly at a concentration of 0.9 g m^{-2} of the active compound. From June 2002 to October 2004, we applied a granular form of the belowground pesticide (Hortex, Scotts Celaflor, Germany). Thereafter, Chlorpyrifos (Dow AgroSciences GmbH, Germany) was dissolved in water and sprayed onto the soil surface of the plots (0.05 L m^{-2}). The combined treatment consisted of an application of the molluscicide, the foliar and the soil pesticide. Two control treatments were established: A completely untreated control and a water control. The water control received the same amount of water as used for application of the foliar pesticide (0.04 L m^{-2}). Treatments were always applied at all sites on the same day from May to October in each year. All pesticides used in this study are commonly applied in agriculture and they have been successfully employed in other experimental grassland studies (Brown & Gange 1989a; Carson & Root 2000). Furthermore, direct effects of these pesticides on plant performance are negligible (Hector *et al.* 2004).

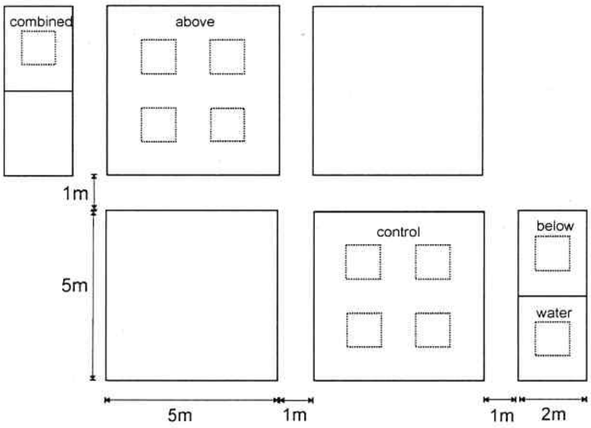

Fig. 3.1: Experimental design for the manipulation of invertebrate herbivory on 14 grassland sites in central Germany by application of foliar pesticide and molluscicide ("above"), soil pesticide ("below") and a combination of foliar and soil pesticide ("combined") to permanent plots. A completely untreated plot ("control") and a plot treated with water only ("water") served as controls. Size of the treatment plots differed due to logistic reasons. Permanent subplots used for cover estimates are depicted as dotted squares. The unlabelled plots were used for a drought experiment (Kahmen et al. 2005b) and not included in the present paper.

Measurement of plant diversity and biomass

In 2003, eleven 1x1m subplots were permanently marked at each grassland site (Fig. 3.1). Every year in late June, at peak standing biomass and directly before mowing, all vascular plants in these subplots were identified to species level and percent cover m^{-2} of each species was visually estimated using a modified Londo scale (Londo 1976). For the aboveground treatment and the control, species richness as well as plant cover were determined for each subplot, and then averaged among subplots in the respective treatment plot.

We used species cover to calculate evenness values for each treatment separately, based on the Shannon index:

$$HE' = \frac{-\sum (p_i)(\ln p_i)}{\ln S},$$

where p_i is the proportion of total abundance of the *i*th species and S is the number of species (Magurran 1988). Cover data were also collected for plant functional groups. Following Roscher *et al.* (2004) we classified all plants into one of four functional types: grasses, small forbs, tall forbs and legumes (Appendix 3.1).

Biomass was harvested twice a year (late June and September) according to the existing mowing regime of the grasslands. Immediately before mowing the complete sites, biomass in each plot was sampled in 20 x 50 cm squares (0.10 m^2) for each plot by cutting the vegetation 2 cm above the ground. Four samples were taken from each plot in June 2002. From 2003 onwards, eight samples were taken in the aboveground treatment and the control and four in the other plots. After drying the biomass samples at 60°C to 70°C for at least 48 h to achieve weight constancy, aboveground dry biomass was determined in g m^{-2}.

Data analysis

The watered control treatment was not included in the analyses, as there were no significant differences compared with the non-watered control treatment with respect to the number of plant species (control: 19.97 ± 0.47 species m^{-2}, water control: 19.79 ± 0.34 species m^{-2}), evenness (control: 0.71 ± 0.03, water control: 0.70 ± 0.02) and biomass (control: 445.93 ± 50.12 g dry mass m^{-2}, water control: 432.60 ± 76.44 g dry mass m^{-2} ; $P > 0.05$ in each case). This suggests that the amount of water used during pesticide application (0.04 L m^{-2}) was too small to have any effects in itself.

We analyzed the effects of 5-years of invertebrate herbivory suppression on plant species richness, evenness, plant functional group cover and aboveground biomass using two-factorial repeated-measures ANOVAs. To account for repeated measurements, we applied a multivariate approach a multivariate approach using MANOVA as implemented in JMP (version 5.1.2, SAS Institute Inc., Cary, NC, USA, 1989-2005). Degrees of freedom were adjusted with Greenhouse–Geisser Epsilon to accommodate for a potential lack of variance–covariance matrix compound symmetry. Biomass data were log-transformed prior to analysis to achieve normality and homoscedasticity.

To evaluate treatment effects on individual plant species, we calculated the log response ratio (Hedges *et al.* 1999) as

(1) $\ln RR_C = \ln(C_{combined}/C_{control})$,

where $C_{combined}$ represents percent cover of plant species$_i$ grown in the combined treatment averaged over all study sites and years and $C_{control}$ its cover of plant species$_i$ in the control treatment averaged over all study sites and years. When a species was absent from a study site over all years, this was treated as a missing value. A positive $\ln RR_C$ indicates that the cover of a plant species increases after pesticide application whereas a negative $\ln RR_C$ indicates the opposite. Only plant species that were present in more than 20% of the study sites (i.e. the 37 most frequent plant species) were included in this analysis.

To analyze whether invertebrate herbivory was dependent on plant diversity and productivity of the study sites we calculated for each site a log response ratio of aboveground biomass:

(2) $\ln RR_B = \ln(B_{combined}/B_{control})$,

where $B_{combined}$ and $B_{control}$ represent total aboveground biomass of the plant community in the combined treatment and the control plot, respectively. The $\ln RR_B$ was calculated for each site and year separately, and the means of each subsequent year were used in linear regression analysis using the number of plant species from the 2002 survey and aboveground biomass from the June 2002 harvest as predictors. To test for a unimodal relationship between herbivore impact and productivity as predicted by the exploitation ecosystem hypothesis, we explicitly included a quadratic term. We used the data of 2002 as explanatory variables, as we needed independent variables for regression analysis to avoid a spurious regression that includes the same biomass in $\ln RR_B$ calculations and on the x-axis (for a detailed discussion see Jackson 1997; Coupe & Cahill 2003). $\mathrm{Ln}RR_B$ of each site and year were used in stepwise multiple regressions to test whether herbivore impact depended on plant species richness or productivity. We used number of plant species, aboveground biomass, and the quadratic term of aboveground biomass as explanatory variables. Year was included as categorical variable. Backward elimination based upon the Akaike Information Criterion (AIC) was used to find the best set of predictor variables (Sokal & Rohlf 1995). The statistical program R (version 2.6.0.) was used for regression analyses.

RESULTS

The response of standing biomass to the pesticide treatments was similar in both June and September, the only difference being that biomass was always higher in June than in September. We therefore only present results based on the annual sums here. There were no significant differences in productivity between plots designated for the different treatments before the long term treatments started (2002 harvest, data not shown). Plant species richness and productivity of the grassland sites were not correlated in any year. When analyzing the untreated control plots of all sites, productivity tended to decrease with increasing species richness, but the correlation was not significant (2002-2006, Pearson's r: $-0.38 < r < -0.23$, $0.20 < P < 0.43$).

Table 3.1: Results of repeated measures ANOVAs (F-values) for the effect of pesticide treatments (above = aboveground pesticide, below = belowground pesticide) on aboveground biomass, diversity (number of species and Shannon evenness), and cover of plant functional groups. Pesticides were applied during four years to experimental plots on 14 grassland sites. Significance levels are denoted with: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, after adjusting the degrees of freedom by Greenhouse–Geisser Epsilon (error degrees of freedom are fewer for biomass because of missing values).

Effects	d.f.	Total plant Biomass	Number of plant species	Evenness	Grasses	Small forbs	Tall forbs	Legumes
Between subjects								
Site	13, 39 (36)	53.25***	28.87***	8.97***	7.98***	18.90***	7.06***	4.15***
Above	1, 39 (36)	2.61	0.71	6.64*	1.12	0.61	0.33	0.23
Below	1, 39 (36)	0.38	4.17*	10.47**	27.77***	20.84***	0.00	1.93
Above*below	1, 39 (36)	1.12	0.57	6.58*	2.73	0.53	0.40	0.45
Within subjects								
Time	3, 117 (108)	26.66***	1.43	18.20***	7.92***	4.61**	3.56*	1.65
Time x site	39, 117 (108)	2.57***	1.62*	2.45***	4.36***	2.92***	3.87	2.10**
Time x above	3, 117 (108)	2.46	0.54	1.04	1.65	1.27	0.34	2.11
Time x below	3, 117 (108)	20.83***	0.28	1.29	1.73	2.31	0.82	1.14
Time x above x below	3, 117 (108)	0.87	0.03	0.95	2.23	1.37	0.30	0.40
Greenhouse-Geisser epsilon		0.92	0.71	0.88	0.89	0.76	0.76	0.76

Effects of herbivory on plant community biomass and diversity

Neither the aboveground treatment nor the belowground treatment had a consistent and significant effect on aboveground biomass of the grassland sites (Table 3.1). However, there was a highly significant interaction between the belowground treatment and time, indicating that the effect of belowground herbivores depended on conditions of the particular year. This is, for instance, reflected by the negative effect of the soil pesticide in 2003 and its positive effect in 2004, while there were no effects in 2005 (Fig. 3.2).

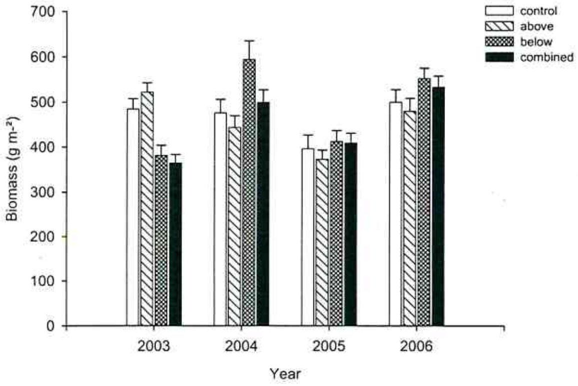

Fig. 3.2: Above-ground biomass of the plant community in untreated plots ("control"), and in plots treated either with aboveground pesticide and molluscicide ("above"), with belowground pesticide ("below"), or with a combination of above- and belowground pesticides ("combined"). Means (+ SE) were calculated using 14 different grassland sites.

By contrast, manipulation of belowground invertebrate herbivores had consistent and significant effects on plant diversity of the grassland sites. Exclusion of belowground herbivory caused a significant decrease in plant species richness. Averaged over all years and study sites, the number of species decreased due to exclusion of belowground herbivory by 1.44 ± 0.29 (mean ± SD) species m^{-2} whereas the aboveground treatment had no significant effect (Table 3.1, Fig. 3.3a). Evenness was significantly reduced by the combined treatment but was not influenced by either of the aboveground or belowground treatments on their own (Fig. 3.3b). Thus, the two pesticides acted synergistically rather than additively as indicated by the significant interaction term (Table 3.1). Contrary to the effects on productivity, the effects on plant diversity were consistent over time (time x above and time x below interactions were not significant, Table 3.1).

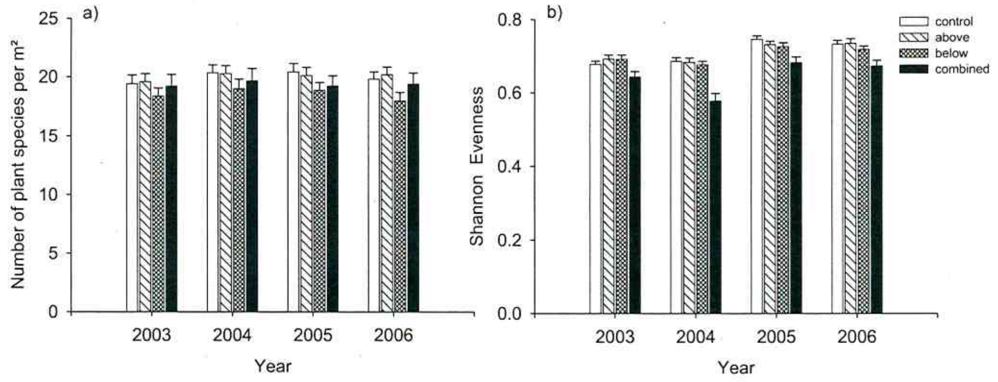

Fig. 3.3: (a) Plant species richness and (b) Shannon evenness in untreated plots ("control"), and in plots treated with aboveground pesticide and molluscicide ("above"), with belowground pesticide ("below"), or with a combination of above- and belowground pesticides ("combined"). Means (+ SE) were calculated using 14 different grassland sites.

Effects of herbivory on plant functional groups and individual plant species

The treatments significantly influenced plant species composition, measured as cover of the different plant functional groups. In particular the exclusion of belowground herbivores caused a significant increase in the cover of grasses by 31.25 ± 5.26% (mean averaged over all study sites and years ± SD) and a significant decrease in the cover of small forbs by 24.86 ± 7.17% (mean averaged over all study sites and years ± SD) while the cover of tall forbs and legumes was not affected (Table 3.1, Fig. 3.4). By contrast, the aboveground treatment as well as the combined above- and belowground treatment had no effect on functional group cover.

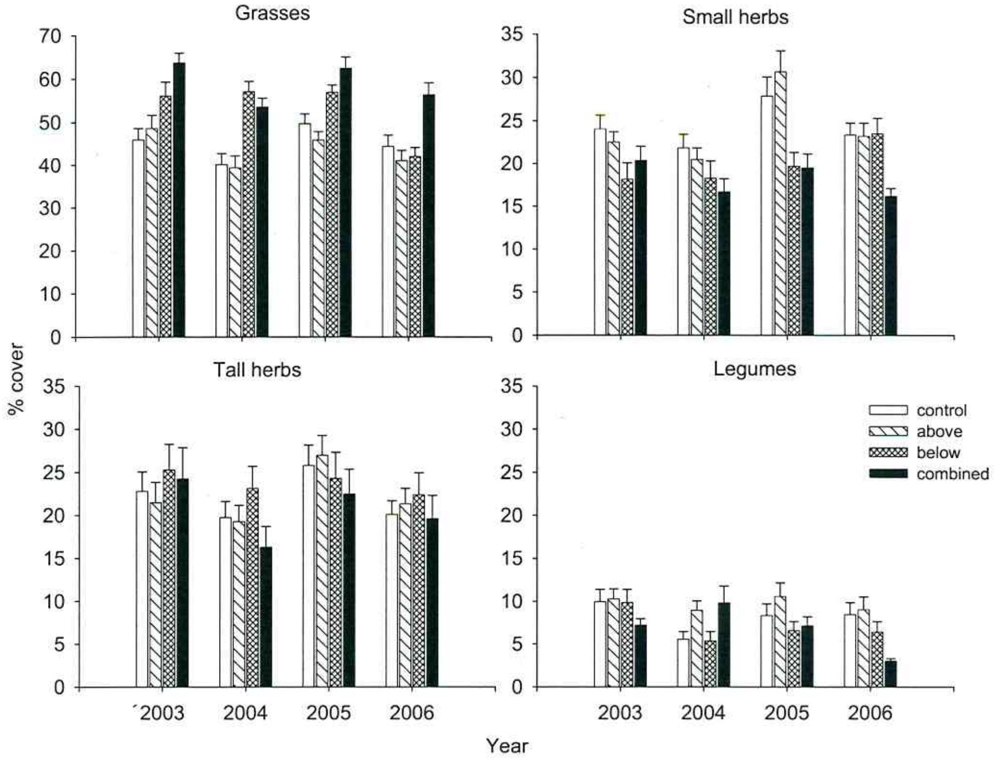

Fig. 3.4: Cover of plant functional groups in untreated plots ("control") and in plots treated with aboveground pesticide and molluscicide ("above"), with belowground pesticide ("below"), or with a combination of above- and belowground pesticides ("combined"). Means (+ SE) were calculated using 14 different grassland sites.

The greatest treatment effect on plant diversity expressed as evenness occurred when the pesticides were applied in combination (Fig. 3.3), and thus we analyzed the response of individual plant species only for the combined treatment. The analysis of the log response ratios ($\ln RR_C$) of the 37 most frequent plant species revealed that, averaged across all study sites and years, two species were significantly positively affected and seven species were significantly negatively affected by the combined treatment (Fig. 3.5). The two positively affected species are the grass *Festuca rubra* and the legume *Vicia cracca*. Two of the seven negatively affected species are graminoids: *Festuca pratensis* and *Luzula campestris*. As shown by the two *Festuca* species even members of the same genus and functional group can differ in their response to invertebrate herbivore exclusion. However, the majority of the species most negatively affected by the pesticides belonged to the group of small forbs, supporting the finding from the cover data of functional groups. The response to the pesticide treatment was not correlated with the mean abundance of plant species (Pearson's $r = 0.14$, $P = 0.41$).

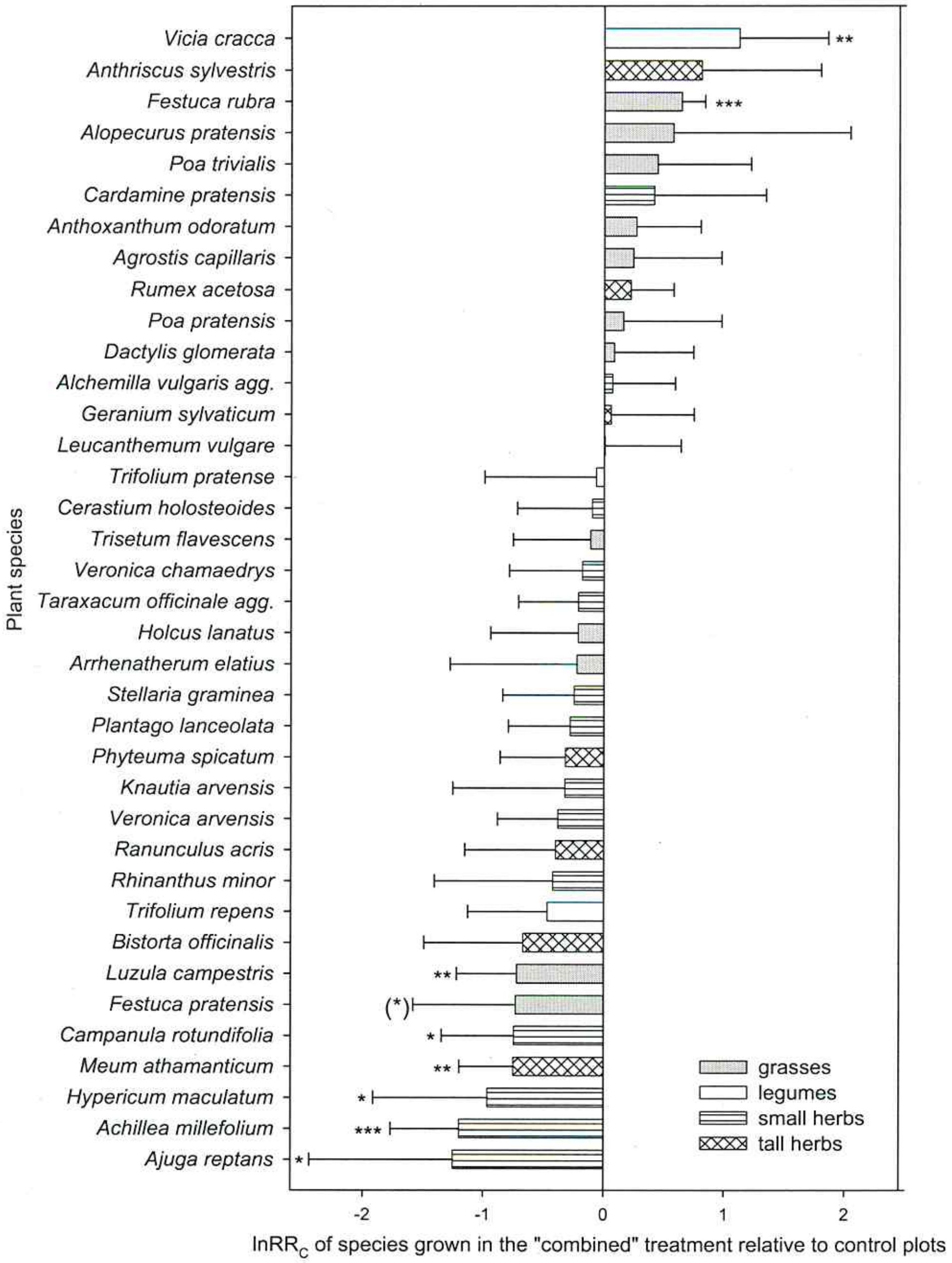

Fig. 3.5: The influence of above- and belowground pesticide application on cover of plant species. Bars represent the response of species cover (± 95% confidence interval) to the "combined" pesticide treatment relative to the control (averaged over 14 study sites and four years). A positive $\ln RR_C$ indicates that the cover of a plant species increases due to pesticide application whereas a negative $\ln RR_C$ indicates a negative effect of pesticide application on the plant species. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; (*) $P < 0.1$.

Dependence of herbivory effects on plant community diversity and productivity

Across all years and sites, the average effect of pesticide application on biomass was close to zero (mean $\ln RR_B = -0.01$) which is in line with the results of the repeated measures ANOVA (Table 3.1). However, as the responses of individual grasslands ranged from negative to positive (-0.25 to 0.32) we used regression analyses to investigate whether this variation in responses could be explained by the diversity and productivity of the grasslands. By using data from 2002 in the regression as predictive values only, spurious correlations were avoided.

The effect of pesticide application depended both on plant species richness and on site productivity. Simple linear regression analyses showed that averaged across all years the response of aboveground biomass to pesticide application was significantly positively correlated with plant species richness, i.e. invertebrate herbivory had a positive effect on biomass in low diversity grasslands and a negative effect in more diverse sites (Fig. 3.6a). Furthermore, the response to pesticides was significantly negatively correlated with site productivity (Fig. 3.6b), i.e. herbivory had a negative effect on biomass at low productivity and a positive effect at high productivity (Fig. 3.6b). To test for a unimodal relationship between herbivore impact and productivity as predicted by the exploitation ecosystem hypothesis, we explicitly included a quadratic term but this was not significant ($P > 0.22$). Stepwise multiple regression analysis confirmed the results from linear regression as it yielded a linear equation without the quadratic term (Table 3.2). The response of aboveground biomass to invertebrate exclusion was influenced by both plant diversity and site productivity.

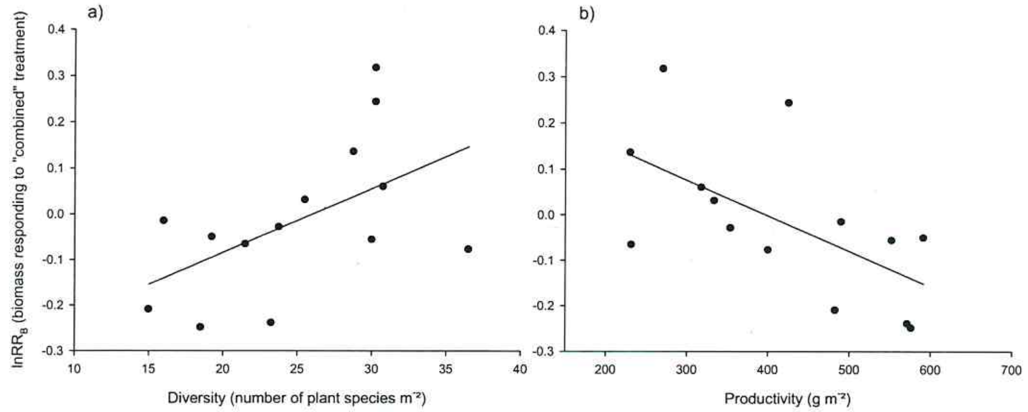

Fig. 3.6: The response of aboveground biomass ($\ln RR_B$) of the 14 grassland sites to application of above- and belowground pesticides ("combined" treatment) is (a) positively correlated with plant diversity ($R^2 = 0.42$, $P < 0.001$) and (b) negatively correlated with productivity of the study sites ($R^2 = 0.43$, $P < 0.001$). A positive $\ln RR_B$ indicates that community biomass increases due to pesticide application whereas a negative $\ln RR_B$ indicates the opposite. For reasons of illustration, curves are fitted using simple regression (averaged over 4 years).

Table 3.2: Results of stepwise multiple regression analyses for the response of aboveground biomass ($\ln RR_B$) to application of above- and belowground pesticides depending on the diversity and productivity of the study sites. Pesticides were applied over four years across 14 grassland sites in Thuringia and Bavaria, Germany.

Source of variation	d.f.	MS	F	P
Year	3	0.504	9.550	<0.001 ***
No. of plant species	1	0.499	9.460	0.003 **
Productivity	1	0.231	4.375	0.042 *
Residuals	49	0.052		
$R^2 = 0.46$				

DISCUSSION

Over a period of five years, we excluded both aboveground and belowground herbivores from 14 extensively managed grasslands along existing gradients of plant diversity and productivity. Our first hypothesis could not be confirmed, as invertebrate herbivory had no consistent negative impact on plant community biomass (Table 3.1, Fig. 3.2). However, in accordance with our second hypothesis, invertebrate herbivores had a positive effect on plant diversity as pesticide application led to a decrease in the number of plant species and in evenness (Table 3.1, Fig. 3.3). The most obvious result was that belowground herbivore exclusion shifted plant functional composition towards grasses at the expense of small forbs. Our third hypothesis, that the effect of herbivory on plant community biomass decreases with increasing plant diversity and is strongest at intermediate levels of primary productivity, was only confirmed in part: Although we found a relationship between effect size of herbivory and site characteristics (diversity and productivity), the specific shape of these relationships was not in accordance with our predictions. In the following, we will discuss experimental restrictions of our study, explore possible mechanisms behind our findings and outline their implications for biodiversity-ecosystem functioning research.

First, we would like to emphasize that invertebrate exclusion experiments should be considered to reveal minimum effects of herbivory, as the use of pesticides under field conditions does not result in a complete reduction of invertebrates (see Coupe & Cahill 2003) - despite frequent spraying and application rates that were at the upper end of the recommended dosage. An additional weakness of using pesticides is that all insects and not just herbivores are killed (Siemann *et al.* 2004). Under natural conditions herbivores are controlled by parasitoids and predators, but pesticides will remove both the herbivore and its natural enemies and thus not necessarily reveal the potential of herbivores to affect the plant population. Furthermore, pesticides may cause hidden effects by affecting decomposer invertebrates and plant nutrient availability (Siemann et al. 2004; Schädler et al. 2004; Fountain et al. 2007). In our case, the changes observed in the cover of plant functional groups could have been the result of a fertilization effect due to the killing of belowground insects. However, in this case we would have also expected an increase in plant community biomass, which was not observed. In addition, such a fertilization effect would have been expected to be strongest in the first year of application and much lower in subsequent years, when the insecticide treatment would have killed eggs and small larvae early in their development. Despite the potential drawbacks and with the appropriate caution in interpreting chains of causation, a number of previous studies have shown that pesticide treatments as applied in the study presented here are valuable tools for ecologists to study herbivore effects in terrestrial ecosystems (Siemann *et al.* 2004).

Invertebrates, in particular the ones living belowground, significantly affected the diversity and functional composition of the investigated 14 grasslands. Upon the exclusion of belowground invertebrates, grasses became more dominant while the abundances of small forbs declined. One potential mechanism underlying this effect could be that belowground herbivores may generally be less specialized than aboveground herbivores (Crawley 1983; Lal 2006 but see also Blossey & Hunt-Joshi 2003), and feed as a consequence on the roots that are most abundant. Since grasses typically have more extensive root systems than forbs (e.g. Kutschera 1960), they are also more exposed to belowground herbivory and would consequently benefit most from the exclusion of belowground herbivory. This would, however, not explain why in our study a few forbs also benefited from exclusion of belowground herbivores (Fig. 3.5). In case of the legume *Vicia cracca*, the most responsive plant species to the pesticide treatment, the effect may be spurious as its mean cover is less than 1%. While many grasses showed an increase in cover, some grasses such as *Holcus lanatus* or *Arrhenaterum elatius* did not show any change, or even a decrease in cover, suggesting at least some specificity in the herbivore effect. In contrast to our study previous investigations have found exclusion of belowground herbivores to improve the performance of forbs (Brown & Gange 1989a; Brown & Gange 1992). However, as the studies of Brown and colleagues were conducted in early successional plant communities with forbs presenting the most abundant group, this could corroborate our hypothesis that belowground herbivores are affecting the most abundant species in a community. Thus, there is a clear need for further studies distinguishing whether the effect of belowground herbivores is also driven by host plant preference.

Effects of invertebrate herbivory on aboveground biomass were small and inconsistent when averaged across the 14 sites. Our findings seem to support a recent meta-analysis (Schädler et al. 2003b) that also demonstrated high variability in effect size when insects and/or molluscs were excluded in field experiments. Much of the variation in the effects of invertebrate herbivory on community biomass across the 14 grassland sites can be attributed to plant diversity and productivity of the study sites. Together, productivity and diversity explained 46% of the variance in the biomass response to herbivory (Table 3.2). In contrast, a meta-analysis of insect effects on primary productivity in different herbaceous communities (Coupe & Cahill 2003), revealed that insects indeed depress primary productivity but that the size of this effect does not depend on plant species diversity or plant productivity.

Our finding that the effect of invertebrate herbivory on aboveground biomass increased significantly with increasing plant species richness (Fig. 3.6a) supports data from a previous study in experimental grassland ecosystems where species richness ranged from one to 32 species (Mulder *et al.* 1999). However, these and our results contradict the prediction that herbivore impact decreases with increasing plant diversity (Pimentel 1961; Root 1973). The resource concentration hypothesis (Root 1973) assumes that specialist herbivores cause most damage on plants, and that their density responds negatively to increasing plant species richness. A previous study carried out in the same grasslands showed that damage caused by aboveground feeding invertebrate herbivores, measured as percent leaf area removed, indeed decreased with increasing plant species richness (Unsicker *et al.* 2006). However, the same study indicated that herbivory was mainly due to generalist herbivores rather than specialists. One reason for this discrepancy between the study of Unsicker *et al.* (2006) and ours could be that the effect of sucking and endophagous insects is not reflected in the measures of leaf damage but might be important. The results might also suggest that in the investigated grasslands, compensation for damage, i.e. plant tolerance, differs between high- and low diversity sites, a result that has also been found with respect to drought-resistance of the same grasslands (Kahmen *et al.* 2005b).

The effect of herbivores on plant community biomass was negatively related to the productivity of the plant communities (Fig. 3.6b). It has been shown that plants are able to compensate damage by herbivores, especially in productive ecosystems such as grasslands, with the consequence that there is no measurable effect of herbivory on plant biomass (Trumble et al. 1993; Strauss & Agrawal 1999; Schädler et al. 2003b). The positive effect of herbivory on biomass in productive grasslands, as indicated by negative log response ratios, could support the notion that plants growing at more productive sites exhibited a stronger capacity for compensatory growth (e.g., Louda et al. 1990; Crawley 1997; but see Hawkes & Sullivan 2001).

Despite the negative relationship between productivity and the effect of herbivory, we argue that our study is in line with previous studies (Fraser & Grime 1997; Fraser 1998) that support the exploitation ecosystem hypothesis (Fretwell 1977; Oksanen et al. 1981; Fretwell 1987; Oksanen 1990). The reason for us not finding the predicted unimodal relationship between herbivore impact and productivity could be that the range of productivities covered by our sites was not wide enough, covering only higher productivity values for which the model predicts declining responses to herbivory. Results of a meta-analysis (Schädler et al. 2003b) showed that a positive effect of invertebrate herbivory on plant productivity occurred only well below 200 g dry above-ground biomass m^{-2}, which is below the least productive of our sites. Furthermore, it remains unclear whether top-down control of herbivores by predators really increases with increasing plant diversity as predicted by the natural enemies hypothesis (Root 1973), or with productivity as predicted by the exploitation ecosystem hypothesis. A recent study of the same grassland sites supports the first presumption but rather contradicts the latter: The abundance of spiders, the most ubiquitous predators in grasslands (Van Hook 1971), was positively related to plant species richness and negatively related to productivity. However, these relationships were only significant for data collected early in the season prior to the first mowing of the grasslands, but not for pooled data from the whole field season (Unsicker *et al.* 2006).

To summarize, our results corroborate that invertebrate herbivores affect ecosystem functioning (Weisser & Siemann 2004). However, these effects strongly depend on specific characteristics of the ecosystem (diversity and productivity), and on the type of herbivory (i.e. aboveground versus belowground herbivory). At our sites, the effects of aboveground herbivores were small in comparison with those of belowground herbivores. Above- and belowground herbivory seemed to act synergistically rather than additively on plant diversity in terms of evenness. This contrasts with results of previous experiments in early successional plant communities where observed effects were additive (Brown & Gange 1989a). Therefore, our results stress the importance of interactions between above- and belowground processes for ecosystem functioning (De Deyn & Van der Putten 2005), and they indicate that belowground herbivores can be important drivers in semi-natural grasslands. Since the main effect of herbivory might be on root biomass (but see McNaughton *et al.* 1998), future studies should in particular examine belowground primary productivity. So far, we have not sampled belowground biomass because of its destructive effect on our plots. Nevertheless, it is aboveground biomass, i.e. hay yield, that is of interest for farmers and that is used in most studies of diversity and ecosystem functioning, which is why we focused on this measure.

In conclusion our results show that invertebrate herbivores can affect ecosystem processes under field conditions, and that the size of these effects depends on diversity as well as on productivity of the plant community. Furthermore, the influence of herbivores on a plant community does not necessarily become evident in plant community biomass. Instead, plant community composition as measured by the contribution of plant functional groups was much more affected by our treatments than total biomass was. Future studies should therefore include more measures of ecosystem structure and functioning, and not just aboveground biomass. They should also actively aim to unravel the more subtle effects of herbivory at the plant community level.

Chapter 4:

Interactive effects of mycorrhizae and a hemiparasite on plant community productivity and diversity

with Cornelia Rißmann, Stefan Hempel, Carsten Renker, François Buscot, Daniel Prati & Harald Auge

Oecologia, in revision

ABSTRACT

Plant communities can be affected both by arbuscular mycorrhizal fungi (AMF) and hemiparasitic plants. However, little is known about the interactive effects of these two biotic factors on the productivity and diversity of plant communities. To address this question, we set up a greenhouse study in which different AMF inocula and a hemiparasitic plant were added to experimental grassland communities in a fully factorial design. In addition, single plants of each species in the grassland community were grown with the same treatments to distinguish direct AMF effects from indirect effects via plant competition.

We found that AMF changed plant community structure by influencing the plant species differently. At the community level, AMF decreased the productivity by 15 to 24%, depending on the particular AMF treatment, mainly because two dominant species, *Holcus lanatus* and *Plantago lanceolata*, showed a negative mycorrhizal dependency. Concomitantly, plant diversity increased and was highest in the treatment with the combination of two commercial AM strains. AMF had a positive effect on growth of the hemiparasite, and thereby induced a negative impact of the hemiparasite on host plant biomass which was not present in non-inoculated communities. However, the hemiparasite did not increase plant diversity.

Our results highlight the importance of interactions with soil microbes for plant community structure and that these indirect effects can vary among AMF treatments. We conclude that mutualistic interactions with mycorrhizal fungi, but not antagonistic interactions with a root hemiparasite promote plant diversity in this grassland community.

INTRODUCTION

Plant diversity and community structure are affected by a large variety of antagonistic and mutualistic interactions. Thus, a comprehensive understanding of the mechanisms determining plant community structure and ecosystem properties requires that interactive effects between different antagonists and mutualists need to be taken into account (Morris et al. 2007). Antagonistic interactions such as between plants and their herbivores, pathogens and parasites may generate negative feedbacks on plant species abundance, and are therefore known as important drivers for maintaining plant diversity (Tilman & Pacala 1993; Chesson 2000). This is particularly true when plant antagonists exhibit a certain degree of host specialization (Huston 1994). In contrast, mutualistic interactions have long been thought not to generate such negative feedbacks and therefore they were not expected to be important for the maintenance of diversity (May 1974). However, Bever (1999; 2002) demonstrated that negative feedbacks can also result from mutualistic interactions, in particular when plants are associated with arbuscular mycorrhizal fungi (AMF). A precondition for mutualism to maintain diversity is that the net benefit for both plants and fungi varies across pairs of interacting species (Bever 1999).

Arbuscular mycorrhiza is one of the most common symbioses worldwide (Smith & Read 1997) and an important player for the dynamics of plant communities (Koide & Dickie 2002; Hartnett & Wilson 2002). Results from several studies showed that AMF may influence structure and diversity (van der Heijden et al. 1998a), productivity (van der Heijden et al. 1998b; Klironomos et al. 2000) and invasibility (Callaway et al. 2004) of plant communities, and may also affect plant-parasite interactions (Sanders et al. 1993). However, the association with mycorrhizal fungi is not always favourable to plants, as the effects of AMF can range from benefit to detriment (Johnson et al. 1997). Several studies indicate that these responses depended on the identity of both the AMF and the plant species (e.g., van der Heijden et al. 1998b; Klironomos 2003). Furthermore, it has been shown that different AMF can exert a functional complementarity (Maherali & Klironomos 2007; Jansa et al. 2008), and therefore an AMF mixture may be more beneficial for host plants than any of the AMF species separately (Koide 2000; Gustafson & Casper 2006).

Not all plants form associations with mycorrhizal fungi, and one group that is often considered to be non-mycorrhizal comprise parasitic plants (Brundrett 2004; but see also Li & Guan 2007). Hemiparasites are common species in many nutrient-poor and species-rich grasslands, in particular root hemiparasites of the family Orobanchaceae. These hemiparasites are photosynthetically active to varying degree and attack their hosts with special contact organs called haustoria. Haustoria are produced by the roots and create vascular continuity of the xylem between host and parasite (Kuijt 1969). Although parasitic plants in grasslands are generally not host-specific (Matthies 1996; Prati et al. 1997), they may exert negative feedbacks by attacking locally abundant plant species and may thereby affect the structure of grassland communities (Press & Phoenix 2005; Bardgett et al. 2006). In addition, as parasitic plants are characterized by high transpiration rates and low water use efficiencies, they also influence the functioning of grassland ecosystems (Press & Phoenix 2005). For instance, the root hemiparasite *Rhinanthus minor* L. has been shown to reduce productivity in grasslands and suppress dominant grasses (Davies et al. 1997; Pywell et al. 2004; Ameloot et al. 2005). As a result, plant diversity may increase because subdominant forbs benefit from competitive release. Therefore, parasitic plants are increasingly used as a tool for the restoration of grassland ecosystems (Bullock & Pywell 2005; Westbury et al. 2006).

Experimental studies have shown that the mycorrhizal status of a host plant can affect the performance of attached parasitic and hemiparasitic plants (Sanders et al. 1993; Davies & Graves 1998; Salonen et al. 2001). However, until now, no study has examined the interactive effects of mycorrhizal fungi and hemiparasitic plants on the structure, diversity and functioning of plant communities. When investigating such complex ecological interactions, it is important to account for indirect effects (Morris et al. 2007), in particular mediated by competition among plants (Schädler et al. 2003a). The aim of our study was to address this question using two complementary greenhouse experiments. Specifically, we asked: (1) How does a hemiparasitic plant affect a grassland community, and how is its impact affected by AMF? (2) How do different AMF treatments influence a grassland community? (3) How is a hemiparasitic plant affected by different AMF treatments? (4) How important are indirect effects when analyzing mycorrhizal effects on a plant community?

In the first experiment, we inoculated experimental grassland plant communities either with two different AMF species (*Glomus intraradices* and *Gigaspora margarita*), with the combination of the two species, or with a field-sampled inoculum. We hypothesized that inoculation with AMF should generally lead to an increased productivity and diversity of the plant community. Because of functional complementarity, the combination of the two AMF species should produce a stronger effect compared with the single species treatments. We expected productivity and diversity of the plant community to be highest when treated with the field-sampled inoculum, which should contain the most complex AMF community. Since AMF usually increase the nutrient status of host plants (Smith & Read 1997) and since hemiparasites of the genus *Rhinanthus* take nutrients from their hosts (Klaren & Janssen 1978), we hypothesized that *R. minor* should benefit from AMF inoculation of host plants in terms of increased growth and reproduction. Such an effect has been shown, so far, only for *Lolium perenne* inoculated with AMF (Davies & Graves 1998) but not for whole plant communities. We also expected that nutrient stress of host plants induced by *R. minor* should be mitigated by the different AMF treatments to a varying degree. To distinguish direct AMF effects from indirect effects via plant competition, we set up a second experiment in which we grew all plant species of the first experiment without competition and applied the same treatments as above.

MATERIALS & METHODS

Study system

The plant material and soil used for the experiments were obtained from a mountain hay meadow in the Frankenwald, a plateau-like mountain range in central Germany. The grassland is situated at an elevation of 606 m a.s.l. (11°26'44''E/50°23'04''N). It has not been fertilized or grazed for at least the past 15 years and was mown once per year in mid-July. The bedrock material consists mainly of schist and graywacke, and produces a carbonate free, nutrient poor soil, categorized as Stagnic Cambisol (siltic). Diversity at this site is about 35 vascular plant species per square meter. Based on species composition the plant community is phytosociologically classified as Geranio-Trisetetum nardetosum Knapp ex Oberd. 1957 (Kahmen et al. 2005b).

The substrate used in the experiments consisted of 50% sieved (1 cm) soil collected from the top 10 cm of the field site and 50% washed silica sand with grain size of 0-2 mm (Mitteldeutsche Baustoffe GmbH, Sennewitz, Germany). To exclude AMF, the substrate was heated for 48 h at 200°C. Part of the sterilized substrate was ground in a mill for chemical analyses. Total carbon and nitrogen contents were measured by high temperature combustion with subsequent gas analysis with an Elementar Vario EL element analyser (Elementar Analysengeräte GmbH, Hanau, Germany). Plant available phosphorus was extracted in double lactate and detected by inductively coupled plasma-atomic emission spectrometry using a Spectro Ciros CCD analyser (SPECTRO Analytical Instruments GmbH, Kleve, Germany). Analyses prior to the experiments showed that the sterilized substrate was generally low in nutrients with a pH (H_2O) of 6.58, 0.48% organic C, 0.10% N and 36.85 $\mu g\ g^{-1}$ plant available P.

Seven perennial grassland species common at the field site were selected for the experiments (Table 4.1). Seeds of two grasses, two legumes, two mycorrhizal forbs and one non-mycorrhizal forb were collected from populations at the field site. For plant species of which we could not collect enough seeds in the field we used commercially available material (Rieger Hofmann GmbH, Germany). All seeds were sown into trays containing heat sterilized substrate and germinated. Seedlings were then grown in a greenhouse for 4 weeks with a 14 h/10 h day/night cycle at 18°C/13°C.

As hemiparasite we choose *Rhinanthus minor* L. (Orobanchaceae), a facultative annual root hemiparasite which grows in natural and semi-natural grasslands throughout Europe and North America (Westbury 2004). It has a wide host range of at least 50 plant species from 18 different families, preferably fast-growing grasses and legumes (Gibson & Watkinson 1989; 1991). *R. minor* is very abundant at the field site where seeds were collected. To break dormancy, *R. minor* seeds were placed on moist filter paper in Petri-dishes and stratified at 5°C for three months in darkness until germination (Gibson & Watkinson 1991).

We selected two species of AMF, *Gigaspora margarita* (isolate Isol UPLB/PH) and *Glomus intraradices* (isolate BEG 140), which occur naturally in the study area (Börstler et al. 2006; Hempel et al. 2007). These fungi were bought from a commercial supplier (SYMbio-M®, Lanškroun, Czech Republic). In addition, we used a field-sampled inoculum, consisting of roots of all plant species used in the experiments. For this purpose, roots from approximately 10 L soil collected at the field site were washed free of soil, cut into pieces of approximately 1 cm length and added as inoculum.

Experiment 1: Multi-species experiment

Experimental set-up and design

To test the interactive effect of AMF and parasitic plants on plant communities, a fully factorial experiment was set up as a randomized block design. The plant community comprised seven species (Table 4.1) which were planted together in mesocosms. The fungal treatments contained five levels: plant communities either inoculated with each of the commercial AM strains (*Gi. margarita* or *G. intraradices*) alone, a mixture of both strains, the field-sampled mycorrhiza or non-mycorrhizal treatment. The parasite treatment consisted of two levels, either with or without eleven individuals of *R. minor* per mesocosm. Each treatment combination was replicated 10 times, resulting in 100 mesocosm. The mesocosms were grouped into ten randomized blocks, each of them containing one replicate of each treatment, and placed in a greenhouse chamber.

Each mesocosm (27 cm x 17 cm x 21.5 cm deep) was filled with a 3 cm layer of expanded slate for drainage, a 3 cm layer of washed sand and a 14 cm layer of the substrate. All materials were heat sterilized at 200°C for 2 days prior to use. Hundred-sixty gram per fungal species of the commercially available AM inoculum was placed 4 cm below the soil surface (80 g per species in the mixture). For the field-sampled treatment the respective pots received 32 g cut roots. To establish a natural microbial community, all mesocosms were irrigated with 35 ml suspensions of the field soil filtered through a filter paper (No. 4, Whatman International Ltd., Kent, UK) to exclude AM propagules.

Two seedlings of each plant species (4 weeks old) were randomly planted into each mesocosm. During the first two weeks dead seedlings were replaced by seedlings of the same age that were grown in sterile substrate. The communities were allowed to establish during six weeks with temperatures between 15°C at daytime and 10°C at night at ambient light conditions. Afterwards temperatures ranged between 20°C (14 h day) and 13°C (night) and additional light was provided by 400 W lamps. The plants were watered three times a week but did not receive any fertilizer. After 15 weeks, all plants were cut 3 cm above the surface and eleven *R. minor* seedlings with established cotyledons and a 1-2 cm long root were transplanted to half of the mesocosms. We followed this procedure in order to consider the importance of well developed host roots for a successful parasitism by *R. minor* (Saona 2002 cit. in Westbury 2004). During the first week, dead hemiparasite seedlings were replaced. After transplanting the hemiparasite, the experiment continued for eight weeks until final harvest.

Data collection

At the final harvest, all plants were cut above the soil surface. Aboveground biomass was sorted by species and dried for two days at 60°C before weighting. We used total biomass (not including the first cut after 15 weeks) as an estimate of aboveground net productivity per mesocosm, but excluded biomass of *R. minor*.

To quantify plant species diversity, expressed as evenness, we calculated the Shannon index using the function: $HE' = \frac{-\sum (p_i)(\ln p_i)}{\ln S}$, where $p_i = m_i/M$, m_i is the aboveground biomass for species i, M is the total aboveground biomass of the community, and S is the number of species of the community. Again *R. minor* was excluded from the calculation.

Mycorrhizal dependency of each plant species was calculated by $(1\text{-}[bn/\sum a]) \times 100\%$, when a > b, and by $(-1+[\sum a/bn]) \times 100\%$, when a < b,

where a is the mean dry mass of a treatment containing AMF, b is the mean dry mass of the non-mycorrhizal treatment, n is the number of treatments containing AMF (van der Heijden 2002). Mycorrhizal dependency was calculated over all AMF treatments.

Dried aboveground biomass of each plant species from five randomly selected mesocosms per treatment that included the hemiparasite was ground in a ball mill. The phosphorus concentrations were determined by the molybdate blue ascorbic acid method (Watanabe & Olsen 1965) after combusting the samples at 550°C and dissolving the ash with 4 N nitric acid. Total nitrogen content was measured with an Elementar Vario EL element analyser (Elementar Analysengeräte GmbH, Hanau, Germany). The sum of individual P and N content of each plant species per mesocosm was calculated and gave the total P and N content per mesocosm.

It was not possible to separate the roots of the different species and therefore root biomass as well as the amount of mycorrhizal colonization was not quantified. Instead, we took a mixed root sample from three soil cores (diameter: 2 cm, depth: 12 cm) per mesocosm, washed it free of soil and stored it in formaldehyde-acetic acid (FAA, 6.0% formaldehyde, 2.3% glacial acetic acid, 45.8% ethanol, 45.9% H_2O (v/v)). We used these root samples to record presence or absence of arbuscules and vesicles.

Experiment 2: Single-species experiment

Experimental set-up and design

The single-species experiment was set up in order to distinguish direct effects of AMF on the plants from indirect effects via plant competition. To investigate the response of the plant species grown without competition, a split-plot experiment was set up consisting of the seven plant species, five fungal treatments and two parasite treatments. For each plant species, single individuals were grown with the same five fungal treatments as described for the multi-species experiment. The parasite treatment consisted of two levels, either with or without one individual of *R. minor*. Additionally, *R. minor* was grown without host plant but with the same fungal treatments. Each treatment combination was replicated ten times resulting in a total of 750 pots. Fifteen pots with the same fungal treatment were placed together in a tray, resulting in ten trays per fungal treatment. Thus, the fungal treatment represented the plot-level treatment whereas plant species and the parasite treatment represented the within-plot treatments. The trays were randomly positioned in ten blocks.

The pots (9 cm diameter, 10 cm height) were filled with slate and sand for drainage as well as soil accordingly to the mesocosms. The pots received 20 g per fungal species of the commercially available AM inoculum, 10 g per AMF species in the mixture, and 6 g cut roots for the field treatment. We proceeded accordingly to the multi-species experiment when growing and transplanting the plant species as well as the hemiparasite.

Ten weeks after transplantation of the hemiparasite seedlings, all plants were harvested and separated in above- and belowground biomass.

Data collection

Aboveground biomass was dried for two days at 60°C and weighed. To quantify belowground biomass, roots were washed free of soil, dried for three days at 60°C and weighed. To quantify the mycorrhizal status, a subsample of 2 g fresh mass of roots per species was stored in FAA. Root samples were dyed using the staining procedure of Vierheilig et al. (1998). Mycorrhizal colonization of host roots was determined for each species ($n = 4$). Percent colonization of root length was determined with a Zeiss Axioplan light microscope (Zeiss, Oberkochen, Germany) using the line intersect method based on at least 300 segments per root sample (Ambler & Young 1977) modified after (Schmitz et al. 1991).

Statistical analysis

The statistical software SAS version 9.1 (SAS Institute, Cary, NC, USA) was used for all analyses. Biomass data were log-transformed prior to analysis. To test the effects of AMF treatment and parasitic plants on aboveground biomass and on evenness of the plant communities we used a three-way analysis of variance (ANOVA), with block, parasite and AMF treatment as factors (PROC GLM). Additionally, the effect of AMF treatment on *R. minor* biomass and on nutrient contents of the plant communities was analyzed using a two-way ANOVA with block and AMF treatment as factors. Data of the single species experiment were analyzed using a three-way, split-plot ANOVA, with fungal treatment as plot factor tested against variation among trays, and host plant and parasite as within-plot factors. Two host plant individuals (*T. pratense*) and 19 individuals of the parasite (*R. minor*) died during the experiment. Because this plant mortality caused our data to be unbalanced, we used type III sum of squares (Shaw & Mitchell-Olds 1993). Differences in arcsine-transformed mycorrhizal colonization rates within each plant species were analyzed by two-way ANOVA and posthoc pairwise comparison of AMF treatments (Tukey´s HSD). In both experiments, separate ANOVAs were performed for each plant species to explicitly characterize fungal effects and to investigate interaction effects in detail. Using orthogonal contrasts, we tested the following four a priori hypotheses: (1) "non-AMF vs. fungi": Plants inoculated with AMF should perform better than plants grown in non-mycorrhizal (non-AMF) soil. (2) "field vs. commercial": Due to niche complementarity and resource partitioning, a more diverse AMF community should provide more host benefit compared with the commercially available treatment. (3) "both vs. single": Same as (2), the mixture of *Gi. margarita* and *G. intraradices* should provide more host benefit compared with each single AMF treatment. (4) "giga vs. glom": The two different AMF species should provide different benefits to host plants. We applied the Benjamini-Hochberg method (Verhoeven et al. 2005) to account for multiple comparisons. In general, belowground biomass and aboveground biomass of host plants grown in the single-species experiment responded similarly to AMF treatments. Thus, we only presented data for aboveground biomass of host plants.

To investigate to what extent the response of individual plant species to AMF was mediated by indirect effects, e.g. competition, we compared the species' response in the mesocosms with their response in the single-species experiment. For this purpose, we calculated the log response ratios (Hedges et al. 1999) of plant species for the two experiments separately as $\ln RR = \ln(B_{fungi}/B_{non\text{-}AMF})$,

where B_{fungi} represents aboveground biomass of a plant species grown in the respective fungal treatment and $B_{non\text{-}AMF}$ represents aboveground biomass of a plant species grown in the non-AMF treatment. This index has recently been used to quantify competitive interactions between plants (Goldberg et al. 1999; Weigelt et al. 2005). We calculated linear regressions of the lnRR of plants in the multi-species experiment versus the lnRR of singly grown plants, and used the coefficient of determination to characterize the relative importance of indirect effects of AMF on plant species grown in mixture. If indirect effects are weak, species' response in mixture should be highly predictable by their response when grown singly, as indicated by a large R^2. If indirect effects are strong, their response should be less predictable and R^2 should be low.

Table 4.1: Investigated plant species and their degree of mycorrhizal colonization. Arbuscules (A) and total colonization rates (T) including colonization by hyphae, vesicles and arbuscules in the roots of plants grown in the single species experiment were recorded (mean ± se, n = 4). Within each plant species, AMF treatments with different letters are significantly different in percent colonization of (A) or (T) by Tukey's post-hoc test results at $P < 0.05$. The non-AMF treatment was excluded from the comparisons as no mycorrhizal structures were observed. Mycorrhizal dependency was calculated for each plant species grown in the multi-species experiment not including the hemiparasite treatment (see Materials and Methods). For calculation of mycorrhizal dependency of *R. minor*, mesocosms without the hemiparasite were excluded.

Plant species (Family)	Origin of seeds	Percent colonization of root length depending on mycorrhizal treatment								Mycorrhizal dependency
		Gigaspora margarita		*Glomus intraradices*		both		field sampled		
		A	T	A	T	A	T	A	T	
Rumex acetosa (Polygonaceae)	purchased	0.8±0.1^{a}	4.4±2.7^{a}	0.6±0.2^{a}	4.5±1.4^{a}	0.7±0.2^{a}	8.3±1.6^{a}	0.0±0.0^{a}	1.6±0.3^{a}	0.00
Veronica chamaedrys (Plantaginaceae)	purchased	13.5±2.3^{a}	37.2±9.0^{a}	23.5±2.3^{a}	60.8±5.3^{a}	22.8±1.9^{a}	51.7±9.6^{a}	20.4±2.0^{a}	44.4±2.4^{a}	0.72
Plantago lanceolata (Plantaginaceae)	field sampled	8.0±0.7^{a}	37.0±2.5^{a}	22.0±2.1^{b}	63.1±6.0^{b}	21.8±1.6^{b}	51.8±5.1^{ab}	10.3±1.1^{ab}	43.8±5.1^{a}	-0.19
Vicia cracca (Fabaceae)	purchased	2.7±0.2^{a}	26.5±2.2^{a}	5.2±0.7^{ab}	48.0±1.2^{b}	20.2±2.9^{b}	50.8±7.2^{b}	5.6±0.6^{a}	17.7±6.2^{a}	0.93
Trifolium pratense (Fabaceae)	field sampled	11.7±0.6^{a}	53.7±3.0^{a}	5.1±0.4^{b}	55.5±6.5^{a}	12.7±1.3^{a}	46.0±10.1^{a}	3.2±0.5^{b}	21.3±2.9^{b}	0.94
Festuca rubra (Poaceae)	field sampled	9.6±1.0^{a}	28.5±2.5^{a}	4.0±0.3^{ab}	28.6±2.9^{a}	6.2±1.0^{ab}	29.7±1.8^{a}	2.3±0.4^{b}	10.4±3.3^{b}	0.25
Holcus lanatus (Poaceae)	purchased	6.4±0.9^{a}	20.9±2.9^{a}	3.6±0.7^{a}	16.8±4.4^{a}	5.1±0.6^{a}	15.4±0.6^{a}	3.3±0.6^{a}	5.1±1.7^{a}	-0.27
Rhinanthus minor (Orobanchaceae)	field-sampled	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.82

RESULTS

Mycorrhizal colonization

At the end of both experiments, no AMF structures were found in the non-AMF treatments, whereas all plants growing in the different AMF treatments were infected by AMF, indicating successful inoculation and absence of contamination.

The degree of total mycorrhizal colonization determined in the single-species experiment varied between the seven plant species ($F_{6, 84} = 53.99$, $P < 0.001$) and the different AMF treatments ($F_{3, 84} = 26.35$, $P < 0.001$, Table 4.1). Highest degree of colonization by arbuscules was found in *V. chamaedrys* inoculated with *G. intraradices* (23.5%). *R. acetosa* was confirmed as a non-mycorrhizal forb with less than 1% mycorrhizal colonization. No mycorrhizal structures were found within the roots of *R. minor*, but only external hyphae attached to the root surface.

Effect of AMF on plant – hemiparasite interactions

The effects of AMF and the hemiparasite on total community biomass were dependent on each other, as indicated by a significant AMF x parasite interaction (Table 4.2). More specifically, *R. minor* did not suppress total community biomass in the non-AMF treatment. In contrast, infection with *R. minor* significantly reduced the total aboveground biomass of AMF inoculated plant communities. The parasitized plant communities produced 22.3% (inoculated with *Gi. margarita*), 21.9% (inoculated with *G. intraradices*), 5.4% (inoculated with both commercial fungi) and 10.3% (inoculated with field-sampled mycorrhiza) less biomass than the respective non-parasitized plant communities (Fig. 4.1a). This reduction was obviously due to a significantly negative impact of *R. minor* on the grass *H. lanatus*, as the biomass of other plant species was not significantly reduced by parasitism of *R. minor* (Table 4.2). Averaged over all AMF treatments, *H. lanatus* parasitized by *R. minor* produced 20.1% less biomass than unparasitized plants. This suggests that AMF induce rather than mitigate the negative impact of the hemiparasite on total community biomass (Fig. 4.1a). Furthermore, total community biomass decreased due to AMF inoculation. In the treatment with *R. minor*, this negative effect of AMF was stronger than without the hemiparasite.

The diversity of the communities, expressed as evenness, was not affected by the hemiparasite, but it was positively affected by AMF inoculation (Table 4.2). Evenness in the non-AMF treatment was significantly lower compared with the AMF treatments and it was highest in the treatment with both commercial AM strains (Fig. 4.1b). In contrast to biomass, this effect was independent of the hemiparasite treatment.

Table 4.2: Results of ANOVAs for the multi-species experiment – Plant community diversity and productivity as well as biomass of each plant species responding to different mycorrhizal treatments and parasitism by *R. minor*. Evenness was calculated as Shannon Index. Biomass data are log-transformed prior to analysis. Orthogonal contrasts are calculated for fungal treatments which are described in detail in the Materials & Methods section. F values and significance levels are given after accounting for multiple comparisons with the Benjamini-Hochberg method (line-by-line): * P < 0.05; ** P < 0.01; *** P < 0.001.

Source of variation	d.f.	Evenness	Total biomass	*R. acetosa*	*V. chamaedrys*	*P. lanceolata*	*V. cracca*	*T. pratense*	*F. rubra*	*H. lanatus*
Block	9	0.88	0.83	1.66	0.26	1.83	0.54	0.88	0.59	2.05
Parasite	1	0.49	12.65 **	0.84	0.02	2.32	0.03	1.31	4.48	6.57 *
Fungi	4	11.73 ***	8.48 ***	1.88	6.85 ***	11.21 ***	18.35 ***	8.75 ***	1.13	9.56 ***
non-AMF vs. fungi	1	34.30***	29.46***	0.14	9.16**	14.41***	65.92***	8.90**	2.15	23.27***
field vs. commercial	1	6.22*	4.17*	5.15	7.62*	25.25***	3.89	2.23	0.01	0.24
both vs. single	1	6.27*	0.01	2.00	1.74	5.13	3.03	10.85*	1.83	2.75
giga vs. glom	1	0.13	0.28	0.21	8.87*	0.05	0.57	5.02	0.55	0.16
Fungi*parasite	4	0.45	2.95 *	0.50	0.74	0.62	1.27	1.91	1.07	1.32
Residuals (MS)	81	(0.005)	(0.03)	(0.21)	(0.07)	(0.38)	(0.83)	(0.22)	(0.37)	(0.13)

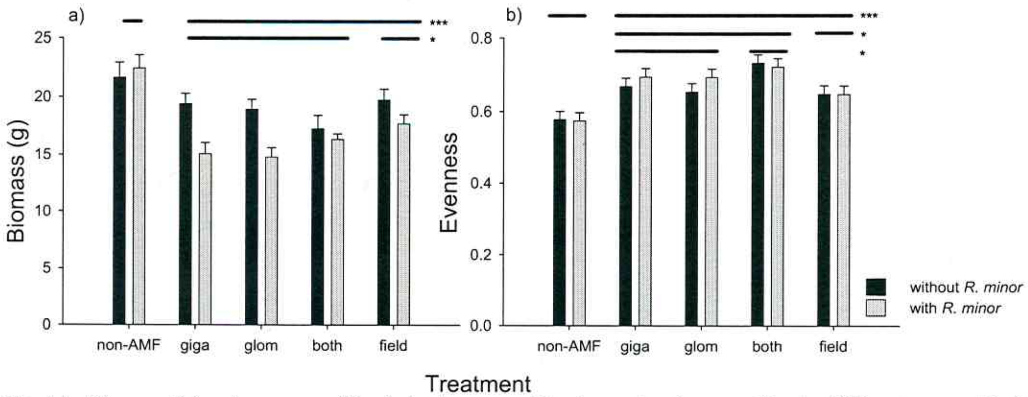

Fig. 4.1: Biomass (a) and evenness (b) of plant communities (mean + se) responding to different mycorrhizal treatments and parasitism by *R. minor*. Community grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza (field). Horizontal lines above bars indicate a significant difference among AMF treatments (averaged over both hemiparasite treatments), according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. ***: $P < 0.001$; *: $P < 0.05$. AMF promote the negative impact of *R. minor* on community biomass (a).

Response of the grassland community to different AMF treatments

Even though changes in plant community biomass were influenced by interactive effects of AMF and *R. minor*, the direction of the response to AMF inoculation was the same (Fig. 4.1a): Total community biomass decreased in the AMF inoculation treatments, whereby the negative effect of the two commercial AM strains was stronger than the one of the field-sampled AMF (Fig. 4.1a). The significant biomass increase in the non-AMF treatment was related to the high biomass of *H. lanatus* and *P. lanceolata* (Fig. 4.2). In all treatments, these two species and *R. acetosa* were the dominant ones in the communities, producing more than 85% of the total biomass. The three species were not dependent on AMF as shown by their low or negative mycorrhizal dependency (Table 4.1), i.e. their growth was not or even negatively influenced by AMF (Fig. 4.2). However, biomass of *P. lanceolata* was significantly higher in the field-sampled inoculum compared with the commercial AMF strains (Fig. 4.2, Table 4.2). Concomitantly, AMF inoculation promoted the growth of the subordinate species *V. chamaedrys, T. pratense* and *V. cracca* which were highly mycorrhizal dependent (Table 4.1). Contrasts showed that *V. chamaedrys* benefited most from inoculation with *Gi. margarita* and the legume *T. pratense* from inoculation with a mixture of *Gi. margarita* and *G. intraradices*, whereas *V. cracca* benefited similarly from all types of AMF inoculations (Fig. 4.2, Table 4.2). In general, association with AMF stimulated mean plant biomass of these three subordinate species across the four AMF treatments compared with the non-AMF treatment. Hence, their contribution to the total biomass of the community increased from 1% to 8% due to AMF inoculation.

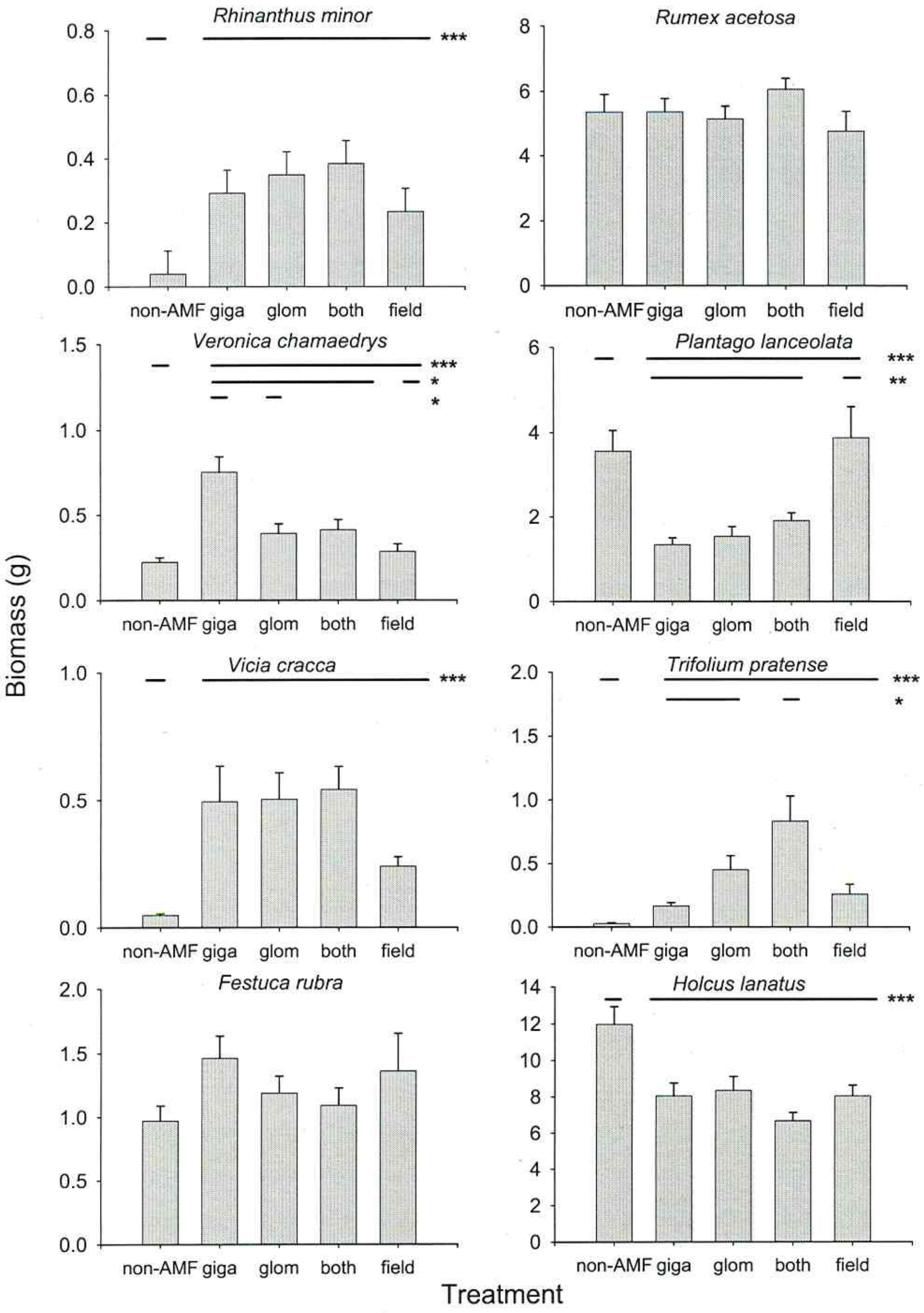

Fig. 4.2: Responses of *R. minor* and host plants grown in the mesocosms (mean + se) responding to different mycorrhizal treatments (multi-species experiment). Results are averaged over both hemiparasite treatments. Plants grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza (field). Horizontal lines above bars indicate a significant difference among AMF treatments, according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$.

Total P content of the community was positively affected by AMF (Fig. 4.3a). Compared with the non-AMF treatment ($P = 10.13$ mg g^{-1}) the P content increased significantly due to AMF inoculation ($F_{4,\ 20} = 7.84$, $P < 0.011$). Noteworthy, no significant differences were found between the different AMF treatments. Total N content significantly decreased due to AMF inoculation (Fig. 4.3b, $F_{4,\ 20} = 14.95$, $P < 0.001$), with again no significant differences between the different AMF inoculates.

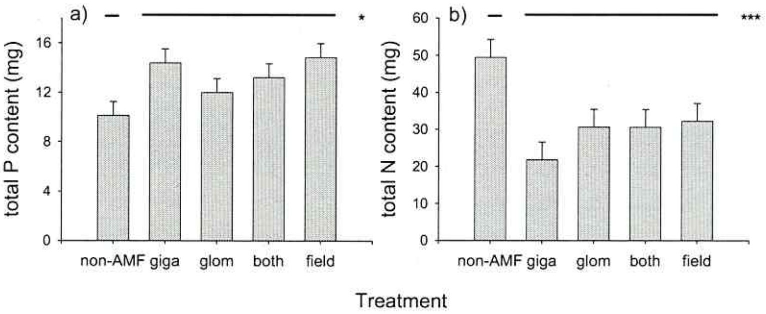

Fig. 4.3: Nutrient contents of plant communities (mean + se) responding to different mycorrhizal treatments. Results are based upon four replicates including the hemiparasite. Plants grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza. Horizontal lines above bars indicate a significant difference among AMF treatments, according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. ***: $P < 0.001$; *: $P < 0.05$.

Response of the hemiparasite to different AMF treatments

Rhinanthus minor was significantly influenced by the AMF treatments (Table 4.3). When grown in the mesocosms, *R. minor* produced five to ten times more aboveground biomass in the AMF treatments compared with the non-AMF treatment (mycorrhizal dependency of 0.82), but no differences were found between the different AMF inoculates (Fig. 4.2). Also total P content of the hemiparasite was influenced by mycorrhizal inoculation ($F_{4,18} = 3.35$, $P < 0.05$), but it showed a distinct pattern compared with the aboveground biomass of *R. minor*. P content was highest when the parasitized plant communities were inoculated with *Gi. margarita* (4.05 ± 0.29 mg g^{-1}), but it was similar among all other treatments – including the non-AMF treatment – and ranged from of 2.80 mg g^{-1} in the *G. intraradices* treatment to 3.25 mg g^{-1} in the mixture of *Gi. margarita* and *G. intraradices*.

When *R. minor* was grown with host plants in the single-species experiment, significant differences between the AMF types were found (Table 4.3). The *R. minor* plants attached to host plants inoculated with AMF produced more biomass than those attached to hosts without inoculation, and the highest biomass was produced in the treatment with both commercial AM strains (Appendix 4.1). Furthermore, *R. minor* benefited from AMF even without host plants, having a mycorrhizal dependency of 0.66 when grown alone.

Only the design of the single-species experiment allowed comparisons between the effects of the different host plant species. The results showed that the growth of *R. minor* was significantly influenced by host plant species (Table 4.3). *R. minor* grew better on its own than with *P. lanceolata* and *R. acetosa* as host plants and it grew better when associated to grasses and legumes as host plants (data not shown).

Table 4.3: Results of ANOVAs on the aboveground biomass of *Rhinanthus minor* (data log transformed prior to analysis) grown in the single-species and in the multi-species experiment. Orthogonal contrasts are calculated for fungal treatments which are described in detail in the Material & Methods section.

Single-species experiment

Source of variation	d.f.	MS	F	P
Fungi	4	3.06	12.35	**<0.001**
non-AMF vs. fungi	1	6.32	25.54	**<0.001**
field vs. commercial	1	0.87	3.52	0.07
both vs. single	1	4.11	16.59	**<0.001**
giga vs. glom	1	0.91	3.68	0.06
Tray [fungi]	45	1.33	1.51	**0.03**
Host plant	7	31.57	35.96	**<0.001**
Fungi x Host plant	28	1.78	2.02	**0.002**
Residuals	294	0.88		

Multi-species experiment

Source of variation	d.f.	MS	F	P
Block	9	0.38	0.34	0.95
Fungi	4	7.75	7.34	**<0.001**
non-AMF vs. fungi	1	30.18	30.34	**<0.001**
field vs. commercial	1	0.08	0.01	0.75
both vs. single	1	0.68	0.67	0.42
giga vs. glom	1	0.05	0.04	0.83
Residuals	35	1.06		

Responses of single grown plant species to AMF and importance of indirect effects

The results of the single-species experiment – when the plants were grown without competition – showed that the AMF treatments had a significant effect on plant biomass ($F_{4, 45} = 5.46$, $P = 0.001$). A significant fungi x host plant interaction ($F_{24, 583} = 8.4$, $P < 0.001$) indicated that different plant species showed distinct responses to the five AMF treatments. Detailed responses of plants grown in the single-species experiment to the different AMF treatments are shown in the Appendix 4.1 (for statistics see Appendix 4.2).

In fact, the single-species experiment was set up in order to distinguish the direct mycorrhizal effects on the plants from indirect effects via plant competition in the community experiment. Using the log response ratio (lnRR) we compared the responses to the different AMF treatments of each plant species in the single-species and in the multi-species experiment. A high coefficient of determination between the lnRR of the two experiments showed that plant growth responses without and with competition were roughly similar for three AMF treatments: *G. intraradices* ($R^2 = 0.70$, $P < 0.02$), the mixture of both AMF strains ($R^2 = 0.68$, $P < 0.02$) and the field-sampled AMF treatment ($R^2 = 0.82$, $P < 0.005$). In contrast, when inoculated with *Gi. margarita*, plant responses in mixtures were poorly predictable by their responses when grown without competition ($R^2 = 0.49$, $P > 0.08$), highlighting the importance of indirect effects in plant community responses to inoculation with this AMF type.

The magnitude of indirect effects was also dependent on plant species. For example *V. cracca* responded stronger to AMF in the multi-species experiment compared with non-competitive conditions (Fig. 4.4). In contrast, response of *P. lanceolata* was weaker in the multi-species experiment when inoculated with *Gi. margarita* or *G. intraradices* compared with its responses without competition.

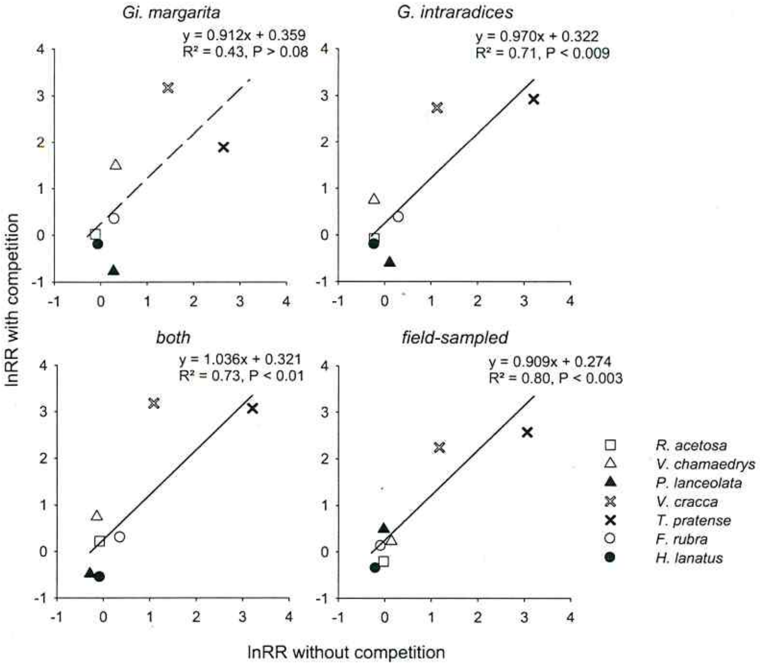

Fig. 4.4: Importance of indirect effects. Log response ratio (lnRR) of plants grown in the single-species experiment and lnRR of plants grown in the multi-species experiment when inoculated with different AMF treatments. Positive residuals are indicating positive indirect effects.

DISCUSSION

Mycorrhizal fungi induce a negative effect of the hemiparasite on plant community

The most important result was that the negative impact of the hemiparasite on plant community biomass was strongly dependent on the presence or absence of AMF. Infection with the hemiparasite *R. minor* did not have a negative effect on total biomass of the non-mycorrhizal plant communities. When plant communities were grown with AMF, *R. minor* reduced total biomass production of the plant communities, irrespective of the different AMF treatments (Fig. 4.1a). This reduction was due to a significant growth suppression of the dominant species *H. lanatus*. This confirms results from other studies in which *R. minor* has been shown to parasitize and to suppress the competitively superior grasses, and therefore reduce total biomass of the plant communities (Davies et al. 1997; Bardgett et al. 2006). However, legumes or other dicotyle forbs have been reported not to be negatively affected by *R. minor* (Cameron et al. 2006), which is consistent with our results. In contrast to our initial hypothesis, AMF generated rather than mitigated the negative impact of the hemiparasite on total aboveground biomass of the plant communities. These findings are not in accordance with results obtained in another pot experiment in which the grass *Lolium perenne* L. was infected both with AMF and *R. minor*, and host responses were not affected by an interaction between AMF and the parasite (Davies & Graves 1998).

Nevertheless, the hemiparasite did not influence the diversity of the plant community. This result is in contrast to previous studies (Bullock & Pywell 2005; Bardgett et al. 2006) showing that parasitism by *R. minor* increases the diversity of grasslands in field and also in mesocosm experiments. This suggests that in our study the AMF are the main driving forces of diversity.

Mycorrhizal dependency of dominant species determines community responses

The investigated plant community was dominated by *R. acetosa*, a non-mycorrhizal forb, and *H. lanatus* and *P. lanceolata*, two species which are negatively affected by AMF inoculation. This may explain that the community structure was significantly affected by AMF inoculation. Total community productivity decreased and concomitantly, diversity increased because AMF promoted the growth of subordinate species with a high mycorrhizal dependency. This response is consistent with AMF effects on plant communities dominated by weakly mycotrophic plants (Grime et al. 1987; van der Heijden et al. 1998a), in which plant diversity increases with little effect on community productivity. Especially dominant species in a community and their mycorrhizal dependency or responsiveness are supposed to play a key role in determining plant community structure (Bergelson & Crawley 1988; van der Heijden 2002). In communities dominated by high mycorrhizal dependent species, suppression of AMF led to an increase in diversity owing to a release of subordinate species from competition (Hartnett & Wilson 1999; O'Connor et al. 2002). Our results demonstrate that in communities dominated by species with no or even a negative mycorrhizal dependency, AMF can lead to an increase in diversity when subordinate or competitive inferior species show a high mycorrhizal dependency.

Hemiparasitic plants profit from mycorrhizal fungi

AMF have been shown to impact parasitic plants both positively (Sanders et al. 1993; Salonen et al. 2001) or negatively (Lendzemo & Kuyper 2001; Gworgwor & Weber 2003). In our experiments the hemiparasite *R. minor* was positively affected by AMF inoculation. On the one hand, *R. minor* had an indirect relationship to AMF by having increased growth and reproductive output (data not shown) when host plants were inoculated with AMF. Those indirect positive effects were also reported by Davies & Graves (1998), who found that *R. minor* was non-mycorrhizal itself, but profited when its host plants (*Lolium perenne* L.) were mycorrhizal. According to these authors, the stimulation of hemiparasite growth by AMF can be attributed to increases in the availability of carbon or mineral nutrients in the host plants. We detected increased phosphorus content in host plant tissues due to AMF inoculation (Fig. 4.3a) but decreased nitrogen content (Fig. 4.3b) and no differences in carbon content (data not shown). This suggests that if there is an indirect promoting effect of AMF on hemiparasites, it could be via improved P allocation to the host plants. However, P content of *R. minor* was significantly increased in the *Gi. margarita* treatment only.

On the other hand, we also found direct effects of AMF on the hemiparasite: *R. minor,* grown without host plants in the single-species experiment, was significantly affected by the different types of AMF inoculates, profiting most from the mixture of both commercial fungi and least from the non-mycorrhizal treatment. Notwithstanding these direct effects of AMF on *R. minor*, the hemiparasite has been listed as a non-mycorrhizal plant (Harley & Harley 1987). Recently, *R. minor* has been listed as weakly mycorrhizal (Wang & Qiu 2006). This was based on a survey of three semi-natural grasslands in Norway (Eriksen et al. 2002), where only a few samples of *R. minor* roots were infected with less than 1% mycorrhizal structures (internal hyphae and vesicles). The only fungal structures we found were external hyphae attached to the root surface of *R. minor*, which are not a definite sign for an AMF colonization (McGonigle et al. 1990) but can be considered to facilitate nutrient and especially phosphate uptake (Jakobsen et al. 2005). Phosphorus is known to increase the photosynthetic rates of unattached *R. minor* plants (Seel et al. 1993), which could explain the increased biomass. However, our experiments were not designed to elucidate these particular mechanisms.

AMF treatments differ in their effects on the plant community

Our hypothesis that a more diverse AMF community leads to higher plant productivity and diversity could not generally be confirmed. Plant community biomass was highest in the non-mycorrhizal control. At least, the field-sampled AMF provided significantly more benefit to the plant community than the commercial AMF treatments did, which might indicate functional complementarity within a diverse field-derived AMF community (Maherali & Klironomos 2007; Jansa et al. 2008). However, diversity was highest for plant communities inoculated with a combination of both commercial AMF strains, but lower in the field-sampled inoculum compared with any single AMF treatment (Fig. 4.1b). The variability in these result indicate that functional complementarity among AMF species may become evident in one particular ecosystem trait (productivity) but not in the other (plant diversity).

In the present study, the effects of the different AMF treatments were larger at the level of individual plant species compared with the effects on the whole community. Each plant species was affected in a different way by the respective AMF treatments (Fig. 4.2). Therefore, we confirm that the direction and magnitude of plant growth responses to mycorrhizal colonization is strongly dependent on the specific combination of AMF species and plant species (Klironomos 2003) and our suggestion that a more diverse or complex AMF community should provide more host benefit could not generally be verified. *P. lanceolata* and *R. acetosa* (when grown without competition) were the only plant species producing the highest biomass in the field-sampled treatment. This result might be interpreted as hint for local adaptation because both, seeds of *P. lanceolata* and AMF, originated from the same grassland site.

Significant differences in plant growth responses to the two AMF species were only detected for *V. chamaedrys* which profited most from the single species inoculation with *Gi. margarita* in both experiments. *P. lanceolata* exhibited the same pattern but only in the single-species experiment. Our study does not elucidate the mechanisms responsible for differences in plant growth responses. One explanation might be that different AMF species display different strategies in their association with host plants (Hart & Reader 2002b) and provide different benefits (Johnson 1993). AMF of the family Gigasporaceae form large extraradical mycelia whereas members of the genus *Glomus* do obviously form a large intraradical mycelium (Hart & Reader 2002b; Hempel et al. 2007). These differences in mycelia size and location are likely to affect host plant responses (Hart & Reader 2002a) as a large extraradical mycelium might be better able to mobilize nutrients over long distances for its host. However, the finding that *Glomus* spp. have a smaller extraradical mycelium has to be viewed with some caution because of the marker system used by Hart and Reader (2002b) (Olsson et al. 2003 but see Hart & Reader 2003).

Importance of indirect effects varies among fungal treatments

Many studies showed that AMF can alter competitive relationships between plants (Fitter 1977; West 1996; Moora & Zobel 1996; Koide & Dickie 2002). A pattern that has been repeatedly observed is that the effects of mycorrhizal fungi on a plant community are not simply the sum of their effects on the individual plant species (Koide & Dickie 2002). Therefore, indirect effects, e.g. mediated by competition between plant species, should be taken into account when analyzing responses of a plant community to AMF inoculation. The design of our experiments allowed us to appraise the magnitude of those indirect effects but not to definitively say that differences between plant growth responses to AMF are only due to competition between plants. A confounding effect in our study is that we used pots of different sizes for the experiments. The fact that plants did not grew in the same volume of soil in both experiments might have influenced their growth responses to AMF inoculation. Nevertheless, our results showed that the response of plant species in a community can differ to some extent from their responses when grown without competitors (Fig. 4.4). Hence, indirect effects are obviously involved in the responses of our plant community to AMF inoculation. Additionally, the importance of these indirect effects varies with the AMF species used for inoculation, suggesting that not only the presence of AMF but also their identity influences species interactions in a community. These findings are in line with the results of Scheublin *et al.* (2007) who have shown that not only the presence but also the identity of AMF can influence, for instance, competitive interactions between plants.

CONCLUSION

These results highlight the importance of interactions between aboveground and belowground organisms determining plant community structure. Mycorrhizal fungi changed the structure of the grassland community by influencing the various plant species differently: the presence of AMF decreased community productivity but promoted plant diversity (expressed as evenness), disregarding which mycorrhizal species was inoculated. Mycorrhizal fungi also promoted the growth of *R. minor*, thereby generating a negative impact of the hemiparasite on host plant biomass. Furthermore, it became apparent that the effect of mycorrhizal fungi on a plant community is not simply the sum of their effects on the individual plant species. Besides direct effects of AMF on plants, our experiments revealed great indirect effects of AMF due to the strong impact on interactions between plants and their antagonists, and due to a change in competitive relations among plant species. The results show that mutualistic interactions with mycorrhizal fungi, but not antagonistic interactions with a root hemiparasite promote plant diversity in this grassland community.

Chapter 5:

Native plant diversity reduces invasibility of montane grasslands

with Harald Auge, Markus Fischer & Daniel Prati

to be submitted

ABSTRACT

Does diversity beget stability in terms of resistance against invasion by non-indigenous species? This question is of on-going debate in ecology and results from theory, observational and experimental studies are equivocal. Well-replicated large-scale field studies are a valuable tool to elucidate the relationship between diversity and invasibility under natural conditions.

To investigate the effects of plant species and functional diversity on ecosystem invasibility we set up two seed addition experiments using four non-indigenous plant species, all known as successful invaders, in montane grasslands in central Germany. In the first experiment, which was carried out at a landscape scale, we added seeds to 20 grassland sites that represented a large gradient of plant species richness ranging from 11 to 31 species m^{-2}. In the second experiment, that was established at one of the sites, we added seeds to plots where plant functional groups (legumes or non-legume forbs) were previously excluded by applying herbicides. Generally, the grasslands were inherently resistant to invasions, as three of the four non-indigenous species were hardly able to establish themselves. Seedling emergence of the only successful invader (*Geranium pyrenaicum*) was independent of species richness across the 20 grassland sites. However, at the local scale it was significantly enhanced in plots where non-legume forbs had been removed. Results from the second year showed that survival and establishment success of *G. pyrenaicum* seedlings decreased with increasing plant diversity. Species richness as well as functional group diversity were important determinants of the invasion success.

Thus, our experimental results confirm the hypothesis that diversity begets stability of montane grasslands in terms of increased resistance to invasion at least for one of the four non-indigenous species. In contrast to other studies, the negative effect of diversity on invasibility became evident at both, the landscape and the local scale.

Chapter 5

INTRODUCTION

Humans are altering the composition of biological communities through a variety of activities that increase rates of species extinctions (Pimm et al. 1995; Vitousek et al. 1997) and species invasions (Mooney & Drake 1986; Vitousek et al. 1997; Sala et al. 2000), at all scales, from local to global. Concern about the potential consequences of these changes has stimulated a renewed interest in understanding the relationship between diversity and ecosystem invasibility, i.e. community susceptibility to invasion (Crawley 1987; Lonsdale 1999).

Already fifty years ago, it was stated by the diversity – invasibility hypothesis that more diverse communities are less vulnerable to invasion because vacant niches are less common and the intensity of interspecific competition is more severe (Elton 1958). However, results from observational and experimental studies addressing Elton's hypothesis revealed controversial results. Some studies have shown positive (Stohlgren et al. 1999; Lonsdale 1999) or negative (Tilman 1997; Knops et al. 1999; Levine 2000; Naeem et al. 2000; Kennedy et al. 2002) relationships between species richness in the community and the number of invading species while other studies have revealed no relationship at all (Crawley et al. 1999; Collins et al. 2007). One possible explanation for these seemingly contradicting results is that several mechanisms, acting on different spatial scales, cause the observed patterns (Shea & Chesson 2002). At a landscape or regional scale, i.e. studies conducted over whole regions comprising many different communities, the positive correlation between invasibility and diversity appears to reflect the effect of covarying extrinsic factors such as habitat heterogeneity, resource availability, or disturbance regime on colonization and diversity, which override any local influence of diversity on invasibility (Stohlgren et al. 1999; Levine & D'Antonio 1999; Naeem et al. 2000). In other words: factors mediating co-existence of native species allow also exotic species to co-exist, resulting in higher diversity of both of them. Productivity, for example, is known to promote the diversity of native communities but it can also facilitate invasions (Davies et al. 2007). At a local scale, the diversity-stability hypothesis is often supported in experimental studies but these were mostly conducted at one single site only (Kennedy et al. 2002), thus the generality of this relationship remains poorly studied (Stohlgren et al. 1999). An alternative approach to test the relationship between invasibility and diversity is therefore to study the same type of community across a range of different sites on a regional scale.

Besides species richness, functional diversity is receiving a renewed interest because the number of functional groups per se, as well as the presence or absence of particular functional groups can influence ecosystem processes, including invasibility (Hooper 1998; Symstad 2000;

Prieur-Richard & Lavorel 2000). In grassland microscosms, for example, the success of invasive *Centaurea solstitialis* was reduced by high plant functional diversity (Dukes 2001). Further studies have shown that species identity matters more than species richness in determining both the number of invading species and the total biomass of invaders (Crawley et al. 1999; Dukes 2002; Fargione et al. 2003).

Manipulative experiments in natural ecosystems are an important contribution to improve our understanding of the diversity-invasibility hypothesis under natural conditions (e.g. Symstad 2000). To investigate the effect of diversity (the number of resident plant species and the number of plant functional groups) on invasibility we set up two seed addition experiments of four non-indigenous plant species to semi-natural montane grasslands in central Germany. We hypothesized that (1) at a landscape scale, ecosystem invasibility decreases with increasing plant species richness across grassland sites; and (2) at a local scale, invasibility decreases with increasing number of plant functional groups within a community. To test hypothesis 1, we conducted a between site comparison and added seeds to 20 grassland sites covering a natural wide range of plant diversity (Experiment 1). To test hypothesis 2, we added seeds to plots within a given site where either legume or non-legume forbs were previously excluded by herbicides (Experiment 2).

MATERIALS AND METHODS

Study sites

The studies were performed in semi-natural grassland sites in the Frankenwald and Thüringer Schiefergebirge (11°15'-11°38'E and 50°22'-50°27'N), a plateau-like mountain range at the border between Bavaria and Thuringia in central Germany. Experiment 1 was conducted across 20 grassland sites which are located within an area of 114 km². These sites represent a gradient of plant species richness from 11 – 31 species m^{-2} and of productivity from 130 to 587 g m^{-2} as measured in 2006. However, plant species richness and productivity were not correlated (Pearson´s $r = -0.24$, $P < 0.32$). Experiment 2 was conducted at one of these 20 sites with a mean of 28 plant species m^{-2}. This grassland is situated at an elevation of 606 m a.s.l. (11°26'44''E/50°23'04''N). Based on a previous intensive field survey (Kahmen et al. 2005a), the 20 sites were selected for different plant diversities. All sites are located on high-elevation plateaus between 500 and 840 m altitude, so that orographic, edaphic and climatic factors were rather similar among sites. Mean annual air temperature in the study area is 5-7°C, and mean annual precipitation ranges from 840 to 1200 mm (Hiekel et al. 2004). They have been extensively managed for at least the past 15 years, with no fertilization or grazing, and one or two cuts per year (June/July and September). The bedrock material in the investigated area consists mainly of schist and greywacke and produces a carbonate-free, nutrient-poor soil, categorized as Stagnic Cambisol (siltic). The natural vegetation is a montane spruce-fir-beech forest but since medieval times much of the forest has been converted into agricultural fields with a high proportion of montane hay meadows (Geranio-Trisetetum Knapp ex Oberd. 1975) (Hundt 1964).

Non-indigenous species

Four plant species that are naturalized neophytes in Germany (http://www.floraweb.de) but which were not present at the study sites were selected for the experiments. Two of them are known as successful invaders of montane grassland systems: *Lupinus polyphyllus* Lindl. (Fabaceae) is a hemicryptophyte from North America, which was introduced as ornamental plant to Europe in 1826 (Schwerin 1913). It is currently invading montane meadows in Germany (Volz 2003). *Geranium pyrenaicum* Burm.f. (Geraniaceae) is a hemicryptophyte from south-western Europe and is spreading mostly at slightly disturbed margins of meadows along corridors like roads and railway tracks (Brandes 2004). Additionally, two species were selected which are known as successful invaders from lower altitudes: *Bunias orientalis* L. (Brassicaceae) is a semi-rosette hemicryptophyte, which presumably originates from south-west Russia (Tutin et al. 1993) and has currently been invading central Europe, where it is spreading in disturbed habitats, arable land, vineyards and meadows (Dietz & Winterhalter 1996). *Senecio inaequidens* DC. (Asteraceae) is a dwarf shrub from eastern South Africa, which was introduced to Europe in around 1896 via sheep wool transport (Ernst 1998). Today it is widespread throughout Western Europe where it mainly colonises railways, roadsides and pastures (Caño et al. 2007).

Seeds of *B. orientalis* and *S. inaequidens* were collected around Halle, Germany, whereas we used commercially available seeds of *G. pyrenaicum* and *L. polyphyllus* (Rieger Hofmann GmbH, Germany). Germinability was tested under natural conditions in the investigated grassland sites. At each site, 50 seeds for each species were sown in 0.25 x 0.25 m plots where all vegetation was removed in autumn 2005. Germination of the seeds was recorded in May 2006 (Appendix1). All species are perennials and were harvested before seed set to avoid spreading of non-native species at the study sites.

Experiment 1: Importance of plant species richness

To investigate the relationship between species richness, site productivity and invasibility at a regional scale eight 0.5 x 0.5 m plots (0.25 m^2) each separated by 0.5 to 1 m and finally forming a square were set up at each of the 20 grassland sites. These plots were randomly assigned to receive seeds of one out of four non-indigenous species, with two replicates per species and grassland. In autumn 2005, the seeds were added immediately after mowing the meadows to a height of 3 cm, removing the litter and scarifying the soil with a rake. One hundred seeds of the large-seeded *B. orientalis* and *L. polyphyllus* and 300 seeds of the small seeded *G. pyrenaicum* and *S. inaequidens* were sown per 0.25 m^2 plot. The seeds were mixed with 20 ml of sterilized, washed sand and manually distributed over the plot. During the whole experimental period, extensive management of the 20 grassland sites continued (one to two cuts per year).

Recruitment success of the non-indigenous plant species was measured by counting number of seedlings and estimating percent cover in June 2006, the first year after seed addition. Establishment success of the non-indigenous plant species was measured by counting number of individuals and their percent cover in June 2007, two years after seed addition.

To determine species richness of the native plant community at each site, two 1 m^2 plots separated by 0.5 m were permanently marked in the centre of the square formed by the seeded plots. Each year in June, immediately before the first mowing occured, percentage cover of all vascular plant species was visually estimated on these plots using a modified Londo scale (Londo 1976). Aboveground plant biomass was then sampled in a 50 x 50 cm square (0.25 m^2) in each of the two 1m^2 plots by cutting the vegetation 3 cm above ground. Biomass of all harvested plots was dried at 60°C for 72 h and weighed.

Experiment 2: Importance of plant functional diversity

To investigate the relationship between plant functional group diversity and invasibility at a local scale, a split plot experiment with ten replicated blocks (Fig. 5.1) was set up in one of the 20 grasslands, in October 2004. We manipulated plant functional diversity by eliminating either legumes or non-legume forbs with a 6% glyphosate solution (non-selective herbicide, Roundup^{TM}). Individuals of either functional group were carefully touched with a herbicide-soaked brush ("selective exclusion") and as a control the same number of touches were applied on randomly selected individuals irrespective their affiliation to a functional group ("random exclusion"). These control plots were adjacent to the plots where plant functional groups were selectively eliminated (Fig. 5.1). Thus, our experiment consisted of two treatments: exclusion of legume forbs versus exclusion of non-legume forbs ("functional group") as the plot-level-treatment and selective exclusion versus random application of herbicide ("exclusion type") as the subplot treatment. These treatments were first applied in autumn 2004, and then continued twice the year throughout the experiment. In spring 2005, after the second exclusion, seeds of the four non-indigenous plant species were added to the plots in the same way as described for experiment 1. An additional pair of control plots was set up, comprising the herbicide application but where no seeds were added (Fig. 5.1). Mowing regime of this grassland consisted of one cut per year and was maintained during the whole study period.

Recruitment and establishment success of the non-indigenous species were measured as described for experiment 1. Percentage cover of open ground was recorded for each plot. In July 2006, plant biomass was sampled for each plot (0.25 m²) by cutting the vegetation 3 cm above ground to mimic mowing and to verify selective exclusion of plant functional groups. Biomass was separated for plant functional groups (not including the non-indigenous species), dried at 60°C for 72 h and weighed.

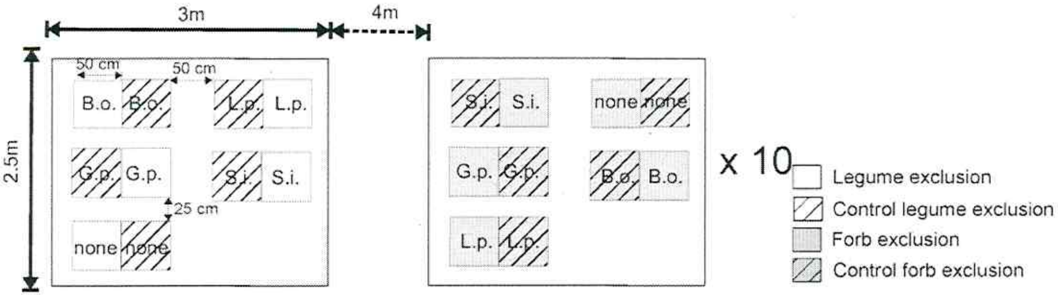

Fig. 5.1: Experimental design for exclusion of plant functional groups (experiment 2). Seeds of *B. orientalis* (B.o.), *G. pyrenaicum* (G.p.), *L. polyphyllus* (L.p.), *S. inaequidens* (S.i.) or no seeds (none) were added to plots where plant functional groups were selectively excluded by herbicide application or to control plots where the same amount of herbicide as used for the adjacent selective exclusion was randomly applied to plants.

Statistical analysis

For experiment 1, multiple regression analyses were used to examine the relationships between recruitment as well as establishment success of the non-indigenous species and site characteristics, i.e. number of resident plant species and aboveground productivity of the study sites. Performances of the non-indigenous species were averaged across the two replicates for each species and study site. We used Pearson's correlation coefficient to explore relationships among the independent variables.

In experiment 2, split-plot ANOVAs were used to examine the success of the non-indigenous plant species in response to removal of plant functional groups. Block and "functional group" was used as main plot levels and "exclusion type" as subplot level. Consequently, it is the "functional group" x "exclusion type" interaction testing our initial hypothesis that plots with selective exclusion of functional plant groups are more susceptible to invasions. Including the percentage of open ground as covariate yielded qualitatively similar results as the model without covariate. Thus, we only presented results from the analysis without covariate.

The success of the selective exclusion of plant functional groups on the biomass of the respective functional group was tested with the same model. Additionally, to test for treatment effects on the resident plant community, differences in percentage cover of open ground per plot were assessed with the same model using data from the plots where no seeds were added (Fig. 5.1).

The statistical software R (version 2.7.0.) was used for all analyses. Biomass data were log-transformed and abundance data were arcsine-square root transformed prior to analysis to meet the assumptions of an ANOVA. The success of the non-indigenous species was analyzed as the proportion of recruited or established individuals in relation to the number of seeds sown using a generalized linear model with binomial error and quasi F-test to correct for overdispersion (Crawley 2007).

RESULTS

Table 5.1: Range and mean percentage per non-indigenous plant species grown in both experiments after seed addition in 2005.

	2006 Recruitment success [%]			2007 Establishment success [%]		
	min	max	mean ± sd	min	max	mean ± sd
Experiment 1						
B. orientalis	0	40	3.0 ± 6.3	0	2	0.1 ± 0.5
G. pyrenaicum	0	67.3	18.4 ± 15.3	0	32.7	4.7 ± 8.3
L. polyphyllus	0	14	3.5 ± 3.3	0	3	0.4 ± 0.7
S. inaequidens	0	14	3.2 ± 3.5	0	0	0.0 ± 0.0
Experiment 2						
B. orientalis	0	11	2.1 ± 2.6	0	1	0.1 ± 0.2
G. pyrenaicum	3	23.7	8.6 ± 5.6	0	8.3	2.9 ± 2.3
L. polyphyllus	0	6	1.3 ± 1.7	0	1	0.2 ± 0.4
S. inaequidens	0	13.7	2.5 ± 2.9	0	2.7	0.4 ± 0.6

Experiment 1: Importance of plant species richness

Seedling establishment of *B. orientalis*, *L. polyphyllus* and *S. inaequidens* was very low and therefore not further analysed statistically (Table 5.1). Seedling recruitment of *G. pyrenaicum*, expressed as proportion of seedlings to seeds sown, and as percentage cover did not depend on species richness (Fig. 5.2a, Table 5.2) or on aboveground biomass of the resident community (Fig. 5.2b, Table 5.2). However, seedling establishment and plant cover of *G. pyrenaicum* in the second year after seed addition was significantly lower the higher was the plant species richness of the community (Table 5.2, Fig. 5.2). Compared with the first year after seed addition, proportion of established individuals across all grassland sites decreased by 74%, but these fewer individuals attained an even higher cover ranging from 0-60% per plot (mean 10.6%) in the second year and from 0-30% per plot (mean 5%) in the first year. In the second year after seed addition, porportion of established individuals and percentage cover of *G. pyrenaicum* was significantly lower the higher plant species richness of the community was (Table 5.2, Fig. 5.2). Aboveground biomass of the resident community, when considered in a simple regression analysis, did not affect the establishment success of *G. pyrenaicum* (Fig. 5.2d). However, when plant species richness and biomass were considered simultaneously using multiple regressions, both characteristics of the resident community did negatively affect establishment of *G. pyrenaicum* (Table 5.2). The significant quadratic term of resident biomass revealed that seedling establishment was highest in grasslands with intermediate productivity (Table 5.2).

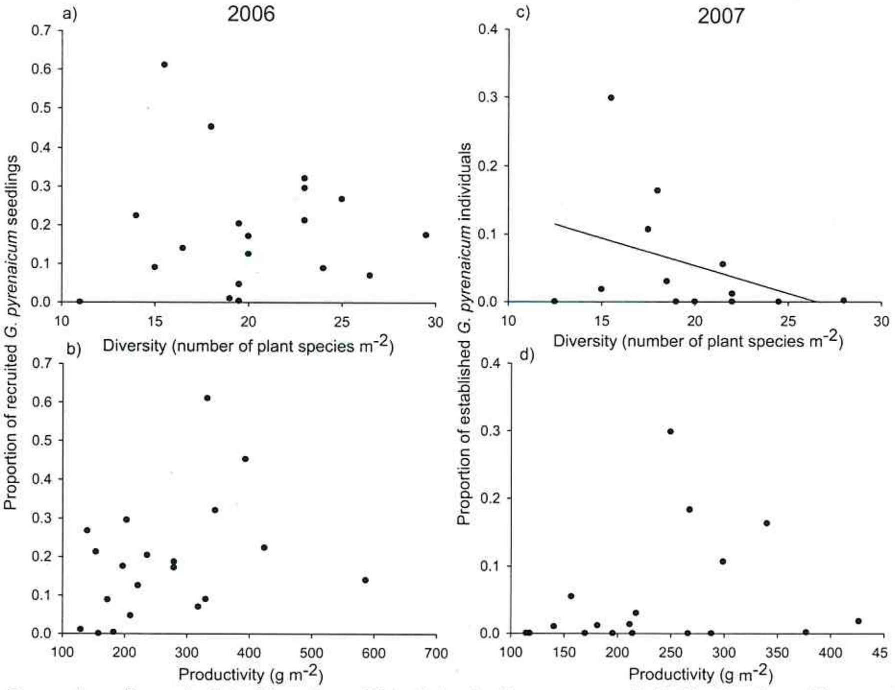

Fig. 5.2: Proportion of recruited (a, b) and established (c, d) *G. pyrenaicum* individuals after adding seeds to 20 grassland sites in Thuringia and Bavaria. Each data point represents the mean of two experimental plots on each grassland site. Two years after adding seeds, the number of established *G. pyrenaicum* individuals is negatively correlated with the resident species richness of the sites. For reasons of illustration, the line is fitted using simple regression on species diversity only ($P < 0.05$).

Table 5.2: Results of multiple regression analyses of the effects of site characteristics on the recruitment success (2006) and establishment success of *G. pyrenaicum* (2007) across 20 grassland sites in Thuringia and Bavaria. Proportion data are analysed as generalised linear model with binomial error (glm function). Arcsine square root transformed percent cover data are analysed as general linear model (aov function).

		Proportion of seeds sown			**Percent cover**		
Variable	d.f.	Parameter estimate	t	P	Parameter estimate	t	P
Recruitment success (2006)							
Intercept	1	-4.67	-2.55	**0.021**	-0.11	-0.61	0.549
Species richness	1	0.01	0.30	0.769	0.00	0.19	0.847
Productivity	1	0.02	1.81	0.088	0.00	1.31	0.207
Productivity^2	1	0.00	-1.51	0.151	0.00	-0.46	0.653
Establishment success (2007)							
Intercept	1	-6.58	-1.48	0.160	-0.36	-0.68	0.508
Species richness	1	-0.33	-2.24	**0.042**	-0.04	-2.46	**0.028**
Productivity	1	0.07	2.49	**0.026**	0.01	2.48	**0.027**
Productivity^2	1	-0.00	-2.42	**0.029**	-0.00	-2.23	**0.043**

Experiment 2: Importance of plant functional diversity

Resident vegetation was dominated by graminoid species with a total cover of 64% while non-legume forbs reached 44% and legumes 10% total cover (averaged across two unmanipulated 1 m^2 control plots as described for experiment 1).

Data collected in July 2006 were used to explore the effects of functional group exclusion on the resident community. Selective exclusion of functional groups reduced biomass of legumes from 0.29 ± 0.20 g to 0.13 ± 0.07 g ($F_{1, 18} = 3.60$, $P < 0.074$) and biomass of forbs from 6.06 ± 5.98 g to 0.81 ± 0.59 g ($F_{1, 18} = 19.62$, $P < 0.001$) compared with the respective control plots, i.e. plots with random application of herbicide. The amount of herbicide used to exclude all non-legume forbs resulted in a larger proportion of open ground (25 ± 15%) than exclusion of legumes (6 ± 4%) ($F_{1, 9} = 112.40$, $P < 0.001$). No significant differences were detectable among the selective and random application of herbicides across both functional group treatments ($F_{1, 18} = 0.62$, $P = 0.44$). The "functional group" x "exclusion type" interaction was also not significant ($F_{1, 18} = 1.72$, $P = 0.21$).

Similar to experiment 1, *B. orientalis*, *L. polyphyllus* and *S. inaequidens* failed to establish (Table 5.1), and therefore only data of *G. pyrenaicum* were further analysed. One year after seed addition, the proportion of recruited seedlings of *G. pyrenaicum* tended to increase in response to selective exclusion of legumes and of forbs (Fig. 5.3a), but this effect was not significant ("functional group" x "exclusion type" interaction not significant, Table 5.3). However, *G. pyrenaicum* cover was significantly higher in plots with selective removal of functional groups, in particular forbs, compared with random exclusion as indicated by a significant "functional group" x "exclusion type" interaction (Fig. 5.3b, Table 5.3).

Two years after seed addition, proportion of established *G. pyrenaicum* individuals was again not significantly affected by selective removal of functional groups (Fig. 5.3d, Table 5.3). However, in accordance to the first year, mean cover of *G. pyrenaicum* was significantly higher in plots with selective exclusion of forbs compared with the corresponding random exclusion control plots (Fig. 5.3d, Table 5.3).

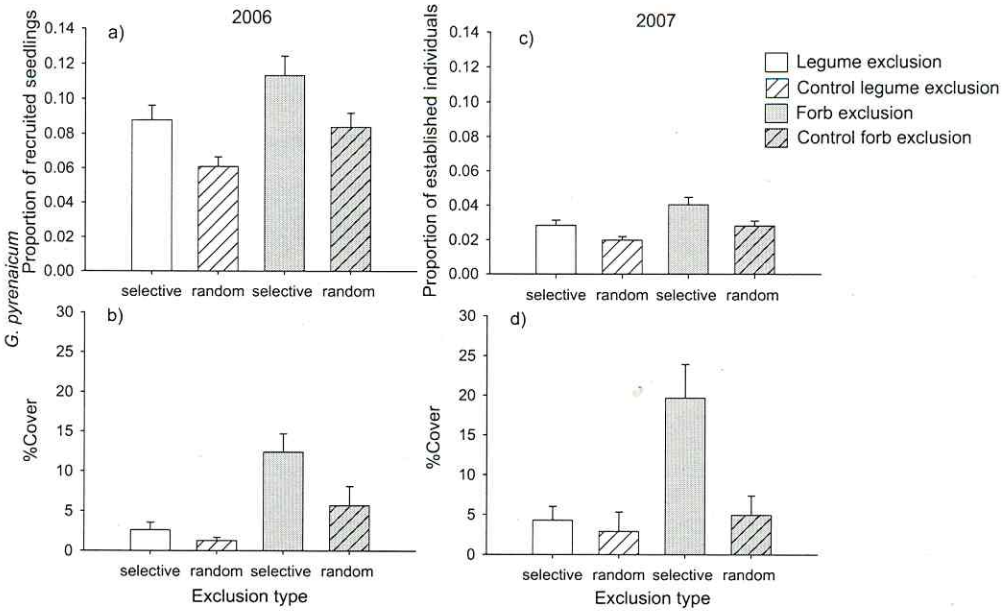

Fig. 5.3: Performance of *G. pyrenaicum* one year (a, b) and two years (c, d) after adding seeds to experimental plots where plant functional groups were previously excluded. For statistics see Table 5.3.

Table 5.3: Split plot analysis of the effects of plant functional group exclusion on recruitment and establishment success of *Geranium pyrenaicum* in a grassland. Proportion data are analysed as generalised linear model with binomial error (glm function). Arcsine square root transformed percent cover data are analysed as a general linear model (aov function).

		Proportion of seeds sown			**Percent cover**		
Variable	d.f.	Deviance	quasi-F	P	MS	F	P
Recruitment success (2006)							
Block	9	167.77	2.46	0.098	0.01	0.81	0.619
Functional group	1	18.42	2.44	0.153	0.24	14.26	**0.004**
Exclusion type	1	27.22	2.80	0.112	0.09	17.60	**<0.001**
Block * functional group	9	68.07	0.78	0.640	0.02	3.34	**0.014**
Functional group * exclusion type	1	2.03	0.21	0.653	0.03	5.36	**0.033**
Residuals	18	190.44			0.005		
Establishment success (2007)							
Block	9	106.98	4.73	**0.015**	0.05	2.98	0.060
Functional group	1	16.77	6.67	**0.029**	0.29	16.04	**0.003**
Exclusion type	1	15.91	6.63	**0.019**	0.25	14.82	**0.001**
Block * functional group	9	22.63	1.05	0.442	0.02	1.05	0.443
Functional group * exclusion type	1	2.18	0.91	0.353	0.10	6.11	**0.024**
Residuals	18	46.26			0.017		

DISCUSSION

In order to investigate the effects of plant diversity on invasibility of temperate grassland ecosystems, we conducted seed addition experiments at a landscape and a local scale using four non-indigenous plant species. The results of our study show that the grasslands are inherently resistant to biological invasions, and confirm that this resistance is provided by plant species richness and functional diversity, at least for the most successful non-indigenous plant species in our study, *G. pyrenaicum*. Moreover, we revealed a negative relationship between plant diversity and invasion resistance at both spatial scales, i.e. among different grasslands at the landscape scale, and within a grassland community at a local scale.

Montane grasslands are inherently resistant to invasion

Three of the four sown non-indigenous plant species could not establish in either of the two experiments presented here. A simple explanation could be that seed quality was poor or that the species are not pre-adapted to the study area. However, germination rates on open ground under natural conditions yielded reasonable germination rates (Appendix 5.1) and each of the species reached high numbers of seedlings at least at one grassland site (Table 5.1). This clearly demonstrates that all four species were able to germinate under the given environmental conditions. In addition, two of the species are very common in mountain regions of central Europe: *G. pyrenaicum* (Brandes 2004) and *L. polyphyllus*. The latter is regarded as a threat for mountain meadows (Kowarik 2002) and currently invades montane grassland sites in Germany (Volz 2003). Similarly, *S. inaequidens* can build up viable populations in grassland ecosystems in western Europe (Caño et al. 2007) and was shown experimentally to establish in grasslands in the UK (Scherber et al. 2003). This species was introduced from mountainous regions from southern Africa and is therefore to a certain degree pre-adapted to higher altitudes (Bossdorf et al. 2008). The same could be assumed for *B. orientalis*, which presumably originates from highlands of south-west Russia (Meusel et al. 1965).

Consequently, we argue that the mediocre performance of three of the four non-indigenous plant species indicate that the investigated grasslands are inherently resistant to invasion of exotic species. In contrast to the three unsuccessful exotic species, native species from the regional species pool were able establish and to increase local diversity of the same grasslands after seed addition (Stein et al. 2008), indicating that the investigated grasslands are not saturated in species number. In consequence, a high immigration rate of non-indigenous species might be expected as reported from a study in Scandinavian semi-natural grasslands (Eriksson et al. 2006). The reason why we found quite the opposite result remains unclear. A possible explanation is that due to the extensive management regime of these grasslands disturbances are too small to promote invasions.

Importance of plant species richness

One of the four species sown, *G. pyrenaicum*, established in sufficiently high numbers that merited further analyses. We tested the effect of plant species richness on invasion success of this species using a natural gradient of plant diversity across 20 grasslands. Across the study sites, establishment of *G. pyrenaicum* significantly decreased with increasing plant species richness. Thus, our data are confirming theoretical predictions that species richness offers invasion resistance (Elton 1958). However, empirical evidence provided by other studies is inconsistent and does not allow generalizations: Native diversity and invasibility are often positively correlated at landscape or regional scales (Stohlgren et al. 1999; Lonsdale 1999), but negatively at the local scale (Tilman 1997). An explanation for these contrasting results is that under field conditions there is often a lack of control of extrinsic factors that covary with biodiversity and that can simultaneously influence invasibility (Rejmánek et al. 2005). Such covarying extrinsic factors are suggested to produce the positive diversity-invasibility correlation usually observed at the landscape scale (Levine & D'Antonio 1999; Naeem et al. 2000). Why then did we found evidence for a negative effect of native plant diversity on invasibility in our study?

We suggest that one reason for this finding is that our grassland sites are located within a comparably small area of 144 km², and are rather similar in environmental conditions and management regime. Therefore, variability of extrinsic factors should be rather low compared to the other studies mentioned above. Nevertheless, there is still some variation in site conditions as reflected by the different productivity of grasslands. Indeed, productivity did influence establishment success of the non-indigenous species in our study: After controlling for the effect of species richness, establishment success of invaders was highest at intermediate levels of productivity. In a previous study, we found a similar pattern when sowing native plant species into the same grasslands (Stein et al. 2008) indicating that extrinsic factors (such as site productivity) promote both, coexistence among native species and establishment of invaders. In consequence, this should produce a positive correlation between native diversity and invasibility among our grasslands rather than the observed negative relation. However, we also revealed in our previous study that due to dispersal limitation, not all native species which could potentially coexist were actually present at each site. In addition, dispersal limitation prevented a covariation between native plant diversity and site productivity (Stein et al. 2008). Obviously, the more native species are absent from a site due to dispersal limitation, the higher establishment of *G. pyrenaicum* can be. Hence, we were able to confirm that susceptibility to invasions decreases with increasing species diversity at a landscape scale because environmental conditions are rather uniform across our grasslands, and because extrinsic factors (such as site productivity) are not affecting native diversity and invasibility simultaneously.

Importance of plant functional diversity

We investigated the effect of plant functional diversity on ecosystem invasibility by excluding non-legume forbs or legumes from experimental plots within a grassland site, i.e. on a local scale. Recruitment success of *G. pyrenaicum*, measured as percentage cover of seedlings one year after seed addition, and establishment success of *G. pyrenaicum* significantly increased in plots where all non-legume forbs had been excluded (Fig. 5.3). This is in accordance with results from other recent grassland studies. In Scandinavian semi-natural grasslands a tendency was observed that removal of non-legume forbs resulted in an increased invasion success of four different forb species (Eriksson et al. 2006). Pokorny et al. (2005) reported that seedling establishment of the invasive forb *Centaurea maculosa* increased in sites where all native forbs had been removed. These results support the prediction that the relative dearth of certain functional groups in a community facilitate invasion by species of that particular functional group (Fox 1987).

The challenge of removal experiments is to disentangle real diversity from side effects of manipulations. Removal experiments are, in general, a valuable tool to improve our understanding of the importance of diversity for ecosystem processes (Diaz et al. 2003), nevertheless they hold several disadvantages. One of them is that removal treatments are resulting in physical disturbances, which is known to promote invasion (Hobbs & Huenneke 1992; Burke & Grime 1996). In our experiment we started with removal treatments one year before adding seeds of the non-indigenous plant species, which should allow plants of other functional groups to "fill in" the gaps created by the treatments. Another disadvantage of removals is the inability to remove belowground biomass and control for nutrient release from decomposing roots (Pokorny et al. 2005). Based on our direct "herbicide treatment" control, i.e. the random treatment irrespective of functional group, we could demonstrate that it was really the exclusion of non-legume forbs which facilitated the invasion by *G. pyrenaicum* in our study, and not only the percentage of bare ground produced by herbicide application. Even though excluding legume species caused significant less open ground compared with exclusion of forbs, simply because legumes were less abundant at this site, covering 10% per plot, whereas all non-legume forbs covered 44% per plot. Hence, our results are a more precise proof of the importance of forbs in preventing plant communities from invasions.

CONCLUSIONS

Our results are supporting Elton's hypothesis that biodiversity is a barrier to invasions (Elton 1958). The investigated semi-natural grasslands are inherently resistant to biological invasions, and this resistance is provided by plant species richness and functional group diversity, at least for the most successful non-indigenous plant species in our study, *G. pyrenaicum*. The negative effect of diversity on invasibility became evident at both, the landscape and the local scale. These results suggest that, if species or functional diversity of semi-natural grasslands declines owing to fragmentation or intensification of land use (Groombridge & Jenkins 2002; Poschlod et al. 2005), susceptibility to invasions by non-indigenous species will increase. As resistance against biological invasions is defined as one aspect of ecosystem stability (McCann 2000; Loreau et al. 2002a) we consider our results also as an important contribution to the current debate about the relationship between biodiversity and ecosystem functioning in natural ecosystems.

Chapter 6: General discussion

In the last decades, ecologists have focussed on determining the nature of the relationship between biodiversity and ecosystem functioning, mainly by using experimental communities. The results, however, are controversial and difficult to verify in the field (Kaiser 2000). Consequently, the applicability of conclusions drawn from diversity experiments with artificial communities to natural ecosystems is hotly debated (Wardle et al. 2000; Naeem 2002; Lepš 2004; Zobel et al. 2006; Grace et al. 2007). To gain deeper understanding more biodiversity – ecosystem functioning studies along natural diversity gradients are required. In the framework of this dissertation, I conducted such studies across a range of montane grasslands in central Germany.

An advantage of the conducted multi-site experiments is that although these grasslands were selected to represent a large gradient in plant species richness, they were still located within a comparably small area of 144 km² and were characterized by rather similar environmental conditions and management regimes (Perner et al. 2005; Kahmen et al. 2005a). This caused the variability of confounding extrinsic factors to be rather low and helped elucidate general patterns of the relationship between diversity and ecosystem functioning of grassland ecosystems.

Determinants of biodiversity

The results of the multi-site seed addition experiment show that, in accordance with recent suggestions (Zobel & Kalamees 2005; Zobel et al. 2006) local plant diversity of the grasslands is limited by the availability of seeds, highlighting the importance of dispersal and seed limitation for plant diversity in natural communities (Chapter 2). The establishment success of species added from the regional species pool was highest at intermediate levels of productivity (Chapter 2), as predicted by Huston (1999). A similar pattern was observed when sowing non-indigenous plant species into the same grasslands, but only after controlling for the effect of species richness (Chapter 5). This indicates that extrinsic factors such as site productivity determine how many native species can coexist and to a certain extent how many invaders may establish. Overcoming dispersal and seed limitation by seed addition resulted in a hump-shaped relationship between diversity and productivity as predicted by theory (Grime 1973; Huston 1979; Rosenzweig 1992). This relationship was not obvious without seed addition nor was it detected in other studies of the same grasslands (Chapters 3 & 5; Kahmen et al. 2005a). This indicates that reduced seed availability obscured any relationship between diversity and productivity (Pärtel & Zobel 2007).

A further relationship revealed was that land use history had a significant impact on the realized plant diversity of the study sites. In the framework of another subproject of the DIVA-Jena project, the age of the grasslands was estimated using aerial photograph analysis and questionnaires of land owners (Stockmann 2005). On average, the sites have been used as hay meadows for 43 years, ranging from 14 to 100 years. The number of plant species that established after experimental seed addition (Chapter 2) was negatively correlated with the age of the meadows (number of new species = 8.20 - 0.06 × age, $R^2 = 0.34$, $P < 0.05$). That is, the younger the meadows are the more new species could establish after seed addition indicating that older meadows are more "saturated" than younger ones. This again points to the importance of dispersal as a regional process affecting local diversity. Unfortunately, detailed information on historical land-use beyond the last 15 years is missing for some sites because they were close to the former inner-German border (Fig. 1.2). Nevertheless, the observed relationship demonstrates that future studies on grassland biodiversity should explicitly consider the role of land use history. An important conclusion from this result is that changes in land-use are not easily revertible and that old hay meadows have therefore particularly high conservation value.

At a local scale, biotic interactions are important determinants of biodiversity. A general opinion is that antagonistic interactions generate negative feedbacks on species abundance and are therefore important drivers of plant diversity (Tilman & Pacala 1993; Chesson 2000). In contrast mutualistic interactions have long been assumed not to result in negative feedbacks and, therefore, to be less important for the maintenance of diversity (May 1974). Indeed, plant antagonists, in particular belowground herbivores, positively affected plant diversity across the grassland sites (Chapter 3). However, my greenhouse experiments revealed quite the opposite: a hemiparasitic plant (*R. minor*) had no impact on the diversity of experimental plant communities but belowground mutualists, namely arbuscular mycorrhizal fungi (AMF), increased diversity of experimental plant communities (Chapter 4). Several other studies have reported both positive effects (e.g. Grime et al. 1987; van der Heijden et al. 1998b) and negative effects of AMF on plant diversity (Hartnett & Wilson 1999; O'Connor et al. 2002). One explanation is that in plant communities differences in mycorrhizal dependency of plant species can influence competitive interactions between species. In communities dominated by mycorrhizal independent species, AMF should increase plant diversity by promoting mycorrhizal dependent but competitively weaker species (Moora & Zobel 1996; Hartnett & Wilson 1999).

My experimental results clearly show that ecological processes acting on different spatial scales are influencing biodiversity. Hence, it is not sufficient to consider only local processes. Studies addressing processes at larger scales and even combining different spatial scales are urgently needed to understand which factors influence diversity and assembly of biological communities.

Effects of biodiversity on ecosystem functioning

Studies using experimental plant communities generally support the hypothesis that biodiversity positively affects ecosystem functioning (Hooper et al. 2005; Balvanera et al. 2006). Results of the multi-site experiments conducted in the framework of the DIVA-Jena project provide clear evidence that such positive diversity effects also exist in natural or near-natural ecosystems. First, adding seeds to the grasslands enhanced not only plant diversity, but also aboveground biomass (Chapter 2), indicating that seed and dispersal limitation influences both diversity and ecosystem functioning (Bengtsson et al. 2002; Zobel et al. 2006). These results show for the first time that a positive effect of diversity on productivity, as previously revealed in experimental plant communities (Loreau et al. 2001), also holds for real grasslands. Second, invasibility by a non-indigenous plant species was higher in grasslands of low diversity compared with more diverse grasslands (Chapter 5), as predicted by the diversity-invasibility hypothesis (Elton 1958). Third, increasing plant species richness enhanced below-ground biomass during drought, thus maintaining more stable productivity of the respective community (Kahmen et al. 2005b). Finally, damage imposed by aboveground feeding invertebrate herbivores, measured as percent leaf area removed, decreased with increasing plant species richness (Unsicker et al. 2006), which is in accordance with the resource concentration hypothesis (Tahvanainen & Root 1972; Root 1973). The effect of herbivores on plant community biomass, however, was positively correlated with plant diversity, as more diverse grasslands were more vulnerable to herbivory (Chapter 3). One explanation for this contradiction might be that compensatory growth after damage by herbivores (McNaughton 1983) is higher in plants from less diverse sites. Such a scenario presumes that plant traits associated with competitive ability, and as a result with lower diversity, are correlated with tolerance to herbivory (Strauss & Agrawal 1999; Agrawal et al. 2006). Alternatively, species rich communities may harbor a higher diversity of specialized herbivores that passed unnoticed by assessing leaf area removal, such as miner or gall builders. However, Perner et al. (2005) generally found little evidence that plant species richness was related to herbivore abundance in several taxonomic groups of insect herbivores. Thus, disentangling the relationship between herbivore and plant diversity, and how this relationship is modified by site conditions such as productivity, probably requires an even larger number of and more variable sites than I had in my study.

Furthermore, the seed addition experiment of native plant species (Chapter 2) represents a useful tool to resolve the apparent conflict between the unimodal effect of productivity on plant diversity, and the linear (or asymptotic) effect of diversity on productivity (Rosenzweig & Abramsky 1993; Schmid 2002). In accordance with previous suggestions (Schmid 2002), I showed that the hump-shaped curve represents the effect of varying site productivity on plant diversity, while biomass of the plant community at a given site is influenced by a change in diversity (Chapter 2). Thus, seed addition experiments, beyond testing the importance of dispersal on local diversity, are a valuable approach to manipulate plant diversity and study its consequences for ecosystem functioning, thereby overcoming shortcomings of "classical" diversity experiments (Lepš 2004; Zobel et al. 2006).

In addition to diversity, productivity of the grassland sites *per se* influenced ecosystem processes. Site productivity did not only influence the establishment success of native and of invasive plant species (Chapters 2 & 5), but also modified the effect of invertebrate herbivores on plant community biomass (Chapter 3). The negative relationship between the effect of herbivory and productivity does not, at first sight, correspond with the predictions of the exploitation ecosystem hypothesis (Fretwell 1977; Oksanen et al. 1981; Fretwell 1987; Oksanen 1990). One explanation why only the decreasing part of the proposed unimodal relationship between herbivory and productivity became evident, is that the productivity gradient represented by our grasslands (270 to 1125 g m^{-2}, based on two cuts per year) was still too narrow. This suggestion is also supported by a recent meta-analysis, which showed that a positive effect of invertebrate herbivory on plant productivity occurred only well below 200 g m^{-2} above-ground dry mass (Schädler et al. 2003b). Studies conducted at multiple sites across even larger productivity gradients than covered by our study are necessary to elucidate the full pattern of the relationship.

As indicated by the greenhouse experiments (Chapter 4), interactive effects between plant antagonists and mutualists need to be considered in biodiversity-ecosystem functioning research (Morris et al. 2007). The presence of AMF as mutualists induced rather than mitigated a negative impact of a hemiparasite on total community biomass. Besides direct effects on plant growth, AMF have great indirect effects on plants due to a strong impact on interactions between plants and their antagonists. Interestingly, the non-mycorrhizal hemiparasite *Rhinanthus minor* benefited from AMF when grown without host plants, even though no mycorrhizal structures could be detected in its roots. This clearly indicates the need for further studies elucidating the mechanisms of how AMF can affect plant species.

One underlying process for positive effects of diversity on ecosystem functioning is an increased use of resources due to niche complementarity (Tilman et al. 1997; Loreau & Hector 2001). However, it is a contentious topic whether each species is necessary to maintain the functioning of ecosystems. It is often assumed that species exert a certain functional redundancy (Walker 1992; Naeem 1998) and that ecosystem functioning is assured as long as each functional group is presented in a community. The results of my thesis emphasise, on the one hand, that the loss of complete functional groups makes an ecosystem more vulnerable to invasions by non-indigenous species (Chapter 5). On the other hand, species-specific effects are important as well: for instance, although plant functional groups responded, on average, differently to herbivore exclusion, there was still much variation among species within functional groups (Chapter 3). Similar variation could be detected in plant responses to inoculation with arbuscular mycorrhizal fungi (Chapter 4). Although grasses are generally mycorrhizal plants (Harley & Harley 1987), the most dominant grass species in my experiment responded negatively to mycorrhization and thus caused an overall decrease in productivity of the community. Furthermore, species that are functionally redundant for one trait can be functionally complementary for another (Hector & Bagchi 2007), as shown in previous studies of the same grasslands with respect to nitrogen use (Kahmen et al. 2006) and drought resistance of plant species (Kahmen et al. 2005b). Soil microbes including AMF might also exert a certain functional redundancy within an ecosystem (van der Heijden et al. 2008 but see Fitter 2005). Results of the presented greenhouse experiments (Chapter 4), however, suggest functional complementarity between AMF species (Maherali & Klironomos 2007; Jansa et al. 2008) as a field-sampled inoculum resulted in higher plant community biomass than inoculation with only one or two AMF species.

It should be noted that functional redundancy in our grassland communities has to be considered with caution because defining functional groups itself is challenging. In my thesis, I distinguished three functional groups, i.e. grasses, legumes, and non-legume forbs, which is a relatively coarse classification, even though it is often applied (e.g. Tilman et al. 1997; Hector et al. 1999). A more detailed quantification of functional diversity requires that many functional traits concerning phenology, physiology or morphology need to be combined (Petchey et al. 2004). However, even sophisticated procedures considering many traits have led to functional groups of grassland plants which are rather similar to those used in the experiments of my thesis (Roscher et al. 2004).

Future challenges

Our understanding of the relationship between biodiversity and ecosystem functioning could be significantly enhanced if more multi-site experiments across landscape and regional scales were conducted. Including factors such as land use history and management regime will lead to a more comprehensive understanding of this relationship. Furthermore, a larger range of ecosystem types should be addressed because studies conducted in grassland ecosystems are overrepresented so far (Balvanera et al. 2006).

Another shortcoming of many studies is that they have focused on the ecosystem-level consequences of changes in plant diversity only. Studies addressing also the effects of higher trophic levels (Raffaelli et al. 2002; Duffy et al. 2007) as well as interactive effects between plants, their antagonists and their mutualists for ecosystem functioning are needed (Morris et al. 2007). The results of my thesis (Chapters 3 & 4) emphasize in particular the importance of interactions between the belowground and the aboveground subsystem (Wardle et al. 2004; De Deyn & Van der Putten 2005; van der Heijden et al. 2008). However, a major obstacle to a better understanding of these processes is the limited potential to reliably and selectively manipulate the diversity of other organisms than plants. This is particularly acute for belowground organisms, including not only mycorrhizal fungi but soil pathogens, herbivores, or decomposers. Mesocosm experiments such as presented in here (Chapter 4) probably represent the best approach, but increasing both spatial and temporal scale of these experiments would be highly desirable. The current lack of knowledge is alarming especially in light of the fact that agricultural land use (Helgason et al. 1998; Douds & Millner 1999) and other anthropogenic disturbances (Egerton-Warburton & Allen 2000) can reduce soil diversity which in turn may have unanticipated negative consequences on plant communities.

The conclusions of this study also raise implications for management strategies, which should aim at conserving these endangered semi-natural montane grasslands. Due to intensified land use, e.g. increased stocking rates and fertilization, or due to abandonment, these semi-natural grasslands in Europe and the biodiversity they contain have been dramatically reduced during the past century (Groombridge & Jenkins 2002; Poschlod et al. 2005). An additional threat to these ecosystems is that a decline in species or functional diversity increases ecosystem susceptibility to invasions by non-indigenous species (Chapter 5). More extensive land use is probably not sufficient to restore the diversity of formerly intensively used grasslands. As suggested by Schmid (2002), re-colonization by species that have gone locally extinct, e.g. due to intensive land use, may be hindered by dispersal limitation. As I demonstrated, restoration of species diversity, and thereby of functioning of semi-natural grasslands, can be facilitated by actively sowing seeds of the respective species into these sites (Chapter 2).

To summarize, all findings of my thesis clearly support the statement of Huston that "no single process or theory can explain a phenomenon as complex as biological diversity" (Huston 1994). Several regional and local processes, as well as their interactions need to be considered if the relationship between biodiversity and ecosystem functioning under natural conditions is to be understood. So far we are just at the beginning of studying different and interacting components of biodiversity. Experimental studies under controlled conditions, e.g. in the greenhouse and laboratory, are a fundamental first step in understanding the effects of biodiversity on ecosystem functioning. However, it is important to expand these studies to natural ecosystems and across larger spatial and temporal scales in order to elucidate underlying general processes. Such approaches are essential in assessing the consequences of the current human-driven loss of biodiversity for ecosystem functioning. A better understanding of the relationship between biodiversity and ecosystem functioning in natural communities will help to develop wiser strategies for maintaining and preserving biodiversity, and eventually humanity.

Summary

The current loss of biodiversity mainly caused by human activities seriously threatens the services that well-functioning ecosystems provide to humanity. Therefore, the question if, and how, biodiversity matters for the functioning of ecosystems is one of the most important questions in ecology today.

In the last decades, one approach to answer this question has been manipulative studies mainly in experimental plant communities. The results, however, varied and considerable debate exists about the applicability of the conclusions drawn from artificial communities to natural ecosystems. Multi-site experiments across larger scales under natural conditions are needed to improve our understanding of the ecological consequences of changes in biodiversity, especially to the loss of biodiversity.

The aim of my PhD thesis was to assess (1) how ecological factors acting at different spatial scales influence local plant diversity in semi-natural grasslands, and (2) how diversity in turn affects ecosystem functioning, in particular productivity of grassland ecosystems and their invasibility as a measure of ecosystem stability. Therefore, I conducted various field experiments in extensively managed montane grasslands in central Germany and additional greenhouse studies to address the following specific questions:

(i) How do seed and dispersal limitation affect the local plant diversity of montane grasslands, and what are the consequences for ecosystem functioning?
(ii) How do invertebrate herbivores affect plant communities, and how are these effects modified by characteristics of the plant community?
(iii) How do interactions between arbuscular mycorrhizal fungi and a root hemiparasitic plant affect plant diversity and productivity of experimental grassland communities?
(iv) How does the diversity of plant species and of plant functional groups affect the invasibility of montane grasslands?

(i) To study the effects of seed and dispersal limitation, I conducted a seed addition experiment across 20 grassland sites. The results suggest that the actual plant diversity of these grassland ecosystems is lower than their potential diversity. Local plant species richness increased when seeds representing the regional species pool were added to the grasslands, pointing both to dispersal limitation and to seed limitation. Simultaneously, productivity increased with increasing diversity. Thus, this study shows for the first time that a positive

effect of diversity on productivity – which has previously found only in experimental plant communities – also holds for real grassland ecosystems. Furthermore, overcoming dispersal and seed limitation by seed addition changed the relationship between diversity and productivity in such a way that it approached the theoretically proposed hump-shaped curve that was not evident in unmanipulated plots.

(ii) Over a period of five years, pesticides were applied to 14 extensively managed grasslands in a factorial design to exclude aboveground invertebrate herbivores, belowground herbivores, or both. The most obvious effect of invertebrate herbivores was a change in plant community composition and diversity: Exclusion of belowground herbivores resulted in a significant increase in the cover of grasses, a decrease in the cover of herbaceous species and a decrease in plant species richness. In contrast, effects of herbivores on plant community biomass were rather inconsistent. The size and direction of this effect depended on plant species richness and productivity. With increasing plant species richness the effect of herbivory on biomass increased significantly and it was negatively related to the productivity of the communities. In general, the effects of aboveground herbivores were relatively small, suggesting that belowground herbivores are more important drivers of plant community composition and biomass. Future studies should therefore focus more on soil herbivores and include belowground responses of plant communities.

(iii) The results of two complementary greenhouse experiments demonstrate that interactive effects between plant mutualists, i.e. arbuscular mycorrhizal fungi (AMF), and plant antagonists, i.e. a hemiparasitic plant (*Rhinanthus minor*), may significantly affect ecosystem functioning. Contrary to the initial hypothesis, mutualists induced a negative impact of the hemiparasitic plant on biomass of the experimental grassland community rather than mitigated a negative impact of the hemiparasite. Furthermore, the experiments revealed that indirect effects play an important role because plant responses to AMF differed to some extent when plants were grown with or without competition. Evenness of the experimental plant community was increased by mycorrhizal fungi but not by the hemiparasite. Hence, mutualistic interactions with mycorrhizal fungi, but not antagonistic interactions with a root hemiparasite were main drivers of plant diversity in this study system.

(iv) To investigate the effects of native plant diversity on invasibility of plant communities, I added seeds of four non-indigenous plant species across 20 grassland sites that represented a large gradient of plant species richness. In addition, I added seeds of the same non-indigenous species to plots at one site where different plant functional groups were previously excluded by applying herbicides. The results of both experiments suggest that the grasslands are inherently resistant to biological invasions, because three of the four species sown were hardly able to establish. For the most successful non-indigenous plant species in this study, invasibility of the semi-natural grasslands increased with decreasing plant species richness and with reduced functional diversity. Hence, in accordance with the results from (i), plant diversity had a positive effect on ecosystem functioning, i.e. resistance of the ecosystem to invasions.

The results of my thesis show that ecological factors acting at a regional scale, i.e. dispersal limitation, and at a local scale, i.e. site productivity or antagonistic and mutualistic interactions, are important determinants of local plant diversity. Furthermore, plant diversity also exerted positive effects on ecosystem functioning in natural communities, including productivity of the plant community and stability in terms of resistance to invasions.

It is evident that experimental studies under controlled conditions, e.g. in the greenhouse, laboratory or in experimental communities, are a fundamental first step in understanding the effects of biodiversity on ecosystem functions. It is imperative to verify the results obtained in these studies under natural conditions. In this context, multi-site comparisons across larger spatial and temporal scales are important to distinguish general effects of diversity from site-specific ones and are therefore valuable tools to assess general patterns in the relationship between biodiversity and ecosystem functioning. Such approaches are essential in assessing the consequences of the current human-driven loss of diversity and in developing wiser strategies for maintaining and preserving biodiversity.

Zusammenfassung

Derzeitig erleben wir einen drastischen Verlust an globaler Biodiversität, der zu großen Teilen von anthropogenen Einflüssen angetrieben wird. Dieser Verlust gefährdet auch die Dienste, die gut funktionierende Ökosysteme der Menschheit erweisen. Aus diesem Grund ist heutzutage die Frage, welche Bedeutung Diversität für das Funktionieren von Ökosystemen hat, eine der wichtigsten in der ökologischen Forschung.

Um diese Frage beantworten zu können, wurden in den letzten Jahrzehnten viele Untersuchungen vornehmlich in experimentellen Pflanzengemeinschaften durchgeführt. Die Ergebnisse sind jedoch sehr vielfältig. Es ist zudem umstritten, ob die Schlussfolgerungen, die aus Studien in künstlichen Gemeinschaften gezogen werden, auch auf natürliche Ökosysteme übertragbar sind. Um die ökologischen Konsequenzen des Wandels und besonders des Verlustes von Biodiversität besser verstehen zu können, sind Experimente, die auf mehreren Flächen über großräumige Skalen unter natürlichen Bedingungen durchgeführt werden, unabdingbar.

Ziel meiner Doktorarbeit war es, zu untersuchen (1) wie verschiedene regionale und lokale Prozesse die lokale Pflanzendiversität in extensiv genutzten Grasländern beeinflussen; und (2) welchen Einfluss Diversität auf Ökosystemfunktionen hat, insbesondere auf die Produktivität der Grasland-Ökosysteme und deren Invasibilität, als ein Maß für die Stabilität eines Ökosystems. Ich führte verschiedene Freilanduntersuchungen in extensiv genutzten montanen Grasländern in Mitteldeutschland sowie zusätzliche Gewächshausexperimente durch, um die folgenden speziellen Fragestellungen zu bearbeiten:

(i) Wie beeinflussen Samen- und Ausbreitungslimitierung die lokale Pflanzendiversität in montanen Grasländern, und welche Auswirkungen hat dies auf die Ökosystemfunktionen?

(ii) Wie beeinflusst Herbivorie durch Invertebraten die Pflanzengemeinschaft, und wird dieser Einfluss durch Eigenschaften der Pflanzengemeinschaft modifiziert?

(iii) Wie beeinflussen Interaktionen zwischen arbuskulären Mykorrhiza-Pilzen und einem pflanzlichen Wurzelhemiparasiten die Diversität und die Produktivität einer experimentellen Pflanzengemeinschaft?

(iv) Wie beeinflusst die pflanzliche Artenvielfalt und Vielfalt an funktionellen Pflanzengruppen die Invasibilität montaner Grasländer?

(i) Um die Effekte von Samen- und Ausbreitungslimitierung zu untersuchen, habe ich ein Einsaatexperiment in 20 Grasländern durchgeführt. Die Ergebnisse zeigen, dass die aktuelle Pflanzendiversität dieser Grasland-Ökosysteme niedriger ist als die potentiell mögliche Diversität. Durch die Einsaat von Pflanzenarten aus dem regionalen Artenpool konnte die lokale Artenanzahl erhöht werden. Dies beweist, dass die lokale Diversität durch Samen- und Ausbreitungslimitierung beeinflusst wird. Mit dem Anstieg der pflanzlichen Diversität erhöhte sich auch die Produktivität der Grasländer. Somit zeigt diese Studie zum ersten Mal, dass Diversität auch in realen Grasland-Ökosystemen einen positiven Einfluss auf die Produktivität hat – dies konnte bisher nur in experimentellen Pflanzengemeinschaften nachgewiesen werden. Desweiteren führte die Überwindung der Ausbreitungs- und Samenlimitierung dazu, dass sich eine unimodale Beziehung zwischen Diversität und Produktivität einstellt, so wie es zwar theoretisch vorhergesagt wird, in nicht manipulierten Flächen aber nicht auftrat.

(ii) Über einen Zeitraum von fünf Jahren wurden Pestizide auf 14 extensiv genutzten Grasländern in einem faktoriellen Design ausgebracht, um oberirdische Herbivorie durch Invertebrate, unterirdische Herbivore durch Invertebrate oder beides auszuschließen. Der deutlichste Effekt der Invertebratenherbivorie zeigte sich in der Veränderung der Zusammensetzung der Pflanzengemeinschaft und deren Diversität: Der Ausschluss unterirdischer Herbivorie führte zu einem signifikanten Anstieg der Deckung der Gräser, einem Rückgang der Deckung krautiger Arten und zu einem Rückgang der pflanzlichen Artenanzahl. Im Gegensatz dazu gab es keinen konsistenten Herbivorieeffekt auf die Biomasse der Pflanzengemeinschaft. Die Größe und die Richtung des Effektes auf die Biomasse waren von dem pflanzlichen Artenreichtum und der Produktivität der Flächen abhängig. Mit steigender pflanzlicher Diversität nahm der Herbivorieeffekt auf die Biomasse signifikant zu und er nahm mit steigender Produktivität der Gemeinschaft ab. Generell war der Effekt oberirdischer Herbivorer relativ gering, was zu der Vermutung führt, dass unterirdische Herbivore einen größeren Einfluss auf die Zusammensetzung und Biomasse von Pflanzengemeinschaften haben als oberirdisch fressende Invertebraten. Zukünftige Studien sollten sich daher mehr auf unterirdische Herbivore fokussieren und auch Merkmale und Reaktionen von Pflanzengemeinschaften aufnehmen, die sich unterhalb der Erdoberfläche abspielen.

(iii) Die Ergebnisse von zwei komplementären Gewächshausexperimenten zeigen, dass Wechselwirkungen zwischen pflanzlichen Mutualisten, d.h. arbuskulären Mykorrhiza-Pilzen (AMF) und pflanzlichen Antagonisten, d.h. einer hemiparasitischen Pflanze (*Rhinanthus minor*), einen signifikanten Einfluss auf Ökosystemfunktionen haben können. Im Gegensatz zu der ursprünglichen Hypothese, induzierten die Mutualisten allerdings einen negativen Effekt des Hemiparasiten auf die pflanzliche Biomasse der experimentellen Pflanzengemeinschaft, anstatt einen negativen Effekt des Hemiparasiten abzuschwächen. Desweiteren zeigten die Experimente, dass indirekte Effekte eine wichtige Rolle spielen, da die pflanzlichen Reaktionen auf die AMF zu einem gewissen Grad davon abhängig waren, ob die Pflanzen mit oder ohne Konkurrenz wuchsen. Die Evenness der experimentellen Pflanzengemeinschaften wurde durch die mykorrhizalen Pilze erhöht, allerdings nicht durch den Hemiparasiten. Somit kann für dieses experimentelle System geschlussfolgert werden, dass die pflanzliche Diversität vor allem von mutualistischen Wechselwirkungen mit mykorrhizalen Pilzen beeinflusst wird und nicht von antagonistischen Interaktionen mit einem Wurzel-Hemiparasiten.

(iv) Um die Bedeutung der einheimischen Pflanzendiversität für die Invasibilität von Pflanzengemeinschaften zu untersuchen, habe ich Samen von vier nicht einheimischen Pflanzenarten in 20 Grasländer eingesät. Diese Wiesen deckten einen großen Gradienten pflanzlichen Artenreichtums ab. Zusätzlich habe ich die Samen derselben Arten nur auf einer Wiese eingesät, auf der zuvor verschiedene funktionelle Pflanzengruppen durch das Auftragen von Herbiziden ausgeschlossen wurden. Die Ergebnisse der beiden Experimente zeigen, dass die Grasländer in sich sehr resistent gegenüber biologischen Invasionen sind, da drei der vier eingesäten Arten sich kaum etablieren konnten. Für die erfolgreichste nicht einheimische Art in diesen Studien zeigte sich, dass die Invasibilität der extensiv genutzten Grasländer mit abnehmendem Artenreichtum und reduzierter funktioneller Diversität zunahm. Somit bestätigen sich die Ergebnisse aus (i), dass pflanzliche Diversität einen positiven Einfluß auf Ökosystemfunktionen hat, hier also auf die Resistenz des Ökosystems gegenüber Invasionen.

Die Ergebnisse meiner Doktorarbeit zeigen, dass ökologische Faktoren, die auf regionaler Skala wirken, wie Ausbreitungslimitierung, und solche die auf lokaler Skale wirken, wie Produktivität eines Standortes oder antagonistische und mutualistische Interaktionen, wichtige Steuerungsgrößen lokaler pflanzlicher Diversität sind. Desweiteren zeigte sich, dass

Pflanzendiversität einen positiven Einfluss auf Ökosystemfunktionen in natürlichen Gemeinschaften hat, dies umfasst die Produktivität der Pflanzengemeinschaft und deren Stabilität im Sinne von Resistenz gegenüber Invasionen.

Es ist offensichtlich, dass experimentelle Studien unter kontrollierten Bedingungen, wie z.B. im Gewächshaus, im Labor oder in experimentellen Gemeinschaften, ein grundlegender erster Schritt sind, um Biodiversitätseffekte auf Ökosystemfunktionen verstehen zu können. Es ist jedoch zwingend notwendig, die Ergebnisse, die mit solchen Studien erzielt werden, auch unter natürlichen Bedingungen zu überprüfen. In diesem Kontext sind Studien, die vergleichend auf mehreren Flächen über größere räumliche und zeitliche Skalen durchgeführt werden, sehr wichtig. Solche Studien ermöglichen es, allgemeine Diversitätseffekte von Flächen-spezifischen Effekten zu unterscheiden. Außerdem sind diese Vergleiche wichtige Instrumente, um allgemeingültige Muster in der Beziehung zwischen Biodiversität und Ökosystemfunktionen feststellen zu können. Solche Ansätze sind unentbehrlich für die Abschätzung möglicher Folgen des gegenwärtigen anthropogen verursachten Diversitätsverlustes. Sie helfen zudem, bessere Strategien für den Erhalt und Schutz der Biodiversität entwickeln zu können.

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Appendix

Appendix 2.1: Species from the regional species pool used for the seed addition experiment (Chapter 2), their seed mass, germination rate in the greenhouse and frequency of occurrence in the soil seed bank as well as in the present vegetation of the 20 study sites (x out of 20). Species were regarded as true novelties in the 20 study sites when they did not occur in the soil seed bank analysis and in 13 x 1 m² vegetation surveys of the surrounding vegetation. NA = data not available.

Species	Seed mass [mg/seed]	Germination rate [%]	Frequency in soil seed bank	Frequency in present vegetation	True novelties	
					2005	2006
Graminoids						
Agrostis capillaris	0.107	12	18	13	2	0
Alopecurus pratensis	0.728	15	3	3	0	0
Anthoxanthum odoratum	0.729	45.33	8	15	0	2
Arrhenatherum elatius	3.289	31	6	11	0	0
Avenella flexuosa	0.356	30	10	4	0	0
Bromus hordeaceus	6.082	85	0	3	12	4
Dactylis glomerata	1.073	28	6	16	1	1
Festuca pratensis	0.944	27	2	4	0	0
Festuca rubra	0.916	27	17	16	0	0
Holcus lanatus	0.423	NA	9	4	1	0
Holcus mollis	0.404	33	6	7	0	1
Lolium perenne	1.775	43	3	5	0	0
Luzula campestris	0.792	76	12	14	0	0
Nardus stricta	0.808	34	0	1	1	1
Phleum pratense	0.587	63	1	8	1	1
Poa pratensis	0.332	41	14	16	0	0
Poa trivialis	0.160	48	0	6	1	0
Trisetum flavescens	0.292	30	5	13	0	0
Non-legume forbs						
Achillea millefolium	0.210	72	6	17	2	0
Anthriscus sylvestris	1.852	33	0	9	5	6
Bistorta officinalis	5.371	4	0	3	1	1
Campanula patula	0.022	64	9	3	0	0
Campanula rotundifolia	0.054	59	15	13	1	1
Centaurea pseudophrygia	2.718	68.89	0	3	1	4
Cerastium holosteoides	0.159	96	16	16	0	2
Chaerophyllum aureum	6.927	15	0	1	14	14
Cirsium arvense	0.932	37	1	0	0	0
Crepis biennis	0.805	39	1	0	0	0
Epilobium angustifolium	0.073	74	4	0	0	0

Appendix 2.1: continued

Species	Seed mass [mg/seed]	Germination rate [%]	Frequency in soil seed bank	Frequency in present vegetation	True novelties	
					2005	2006
Geranium pusillum	0.853	12	1	1	2	1
Heracleum sphondylium	5.063	15	0	2	4	11
Hieracium lachenalii	0.472	36	0	0	3	2
Hieracium pilosella	0.222	31	1	1	0	0
Hypericum maculatum	0.054	16	17	7	0	0
Hypochaeris radicata	0.852	NA	1	5	0	3
Leontodon autumnalis	0.748	56	2	2	0	2
Leontodon hispidus	1.857	35	1	3	1	0
Leucanthemum vulgare	0.460	81	13	12	2	1
Lychnis flos-cuculi	0.106	66	1	1	0	0
Meum athamanticum	8.049	40	4	13	6	7
Myosotis arvensis	0.290	6	8	1	0	1
Phyteuma spicatum	0.158	24	10	9	0	1
Plantago lanceolata	2.016	83	12	15	1	0
Plantago major	0.244	68	6	1	0	0
Potentilla erecta	0.464	16	2	1	0	0
Rumex acetosa	1.273	26	11	20	0	0
Rumex crispus	2.315	42	1	1	0	0
Rumex obtusifolius	2.304	45	2	2	0	0
Stellaria graminea	0.306	6	18	15	0	0
Taraxacum officinale agg.	0.714	58	9	15	0	0
Tragopogon pratensis	10.488	28	0	1	0	0
Veronica arvensis	0.107	39	13	10	1	1
Veronica chamaedrys	0.270	73	13	16	1	0
Viola arvensis	0.602	37	12	2	0	0
Legumes						
Lathyrus linifolius	13.019	4	1	4	2	8
Lotus corniculatus	1.444	37	0	1	7	14
Medicago lupulina	1.857	14	0	0	10	13
Trifolium dubium	0.295	3	3	2	2	3
Trifolium repens	0.662	24	19	16	0	1
Vicia cracca	13.680	4	4	4	1	4

Appendix 2.2: Species not used for the seed addition experiment (Chapter 2), but which also occurred in the present vegetation or in the soil seed bank of the 20 study sites (x out of 20).

Species	Frequency in soil seed bank	Frequency in present vegetation
Graminoids		
Agrostis stolonifera ssp. *prorepens*	1	0
Carex spec.	8	0
Cynosurus cristatus	0	4
Deschampsia caespitosa	1	1
Elytrigia repens	2	3
Helicotrichon pubescens	1	2
Juncus effusus	1	0
Lolium multiflorum	2	11
Poa annua	10	1
Poa nemoralis	1	0
Non-legume forbs		
Aegopodium podagraria	0	2
Ajuga reptans	4	2
Alchemilla montana	18	15
Anemone nemorosa	0	3
Arabidopsis thaliana	1	1
Arnica montana	0	2
Athyrium felix-femina	4	0
Bellis perennis	2	5
Calluna vulgaris	2	0
Capsella bursa-pastoris	7	1
Cardamine pratensis	4	6
Cardaminopsis arenosa	0	1
Cardaminopsis halleri	1	0
Centaurea jacea	0	1
Cerastium arvense	1	4
Chenopodium album	5	0
Cirsium palustre	1	1

Appendix 2.2: continued

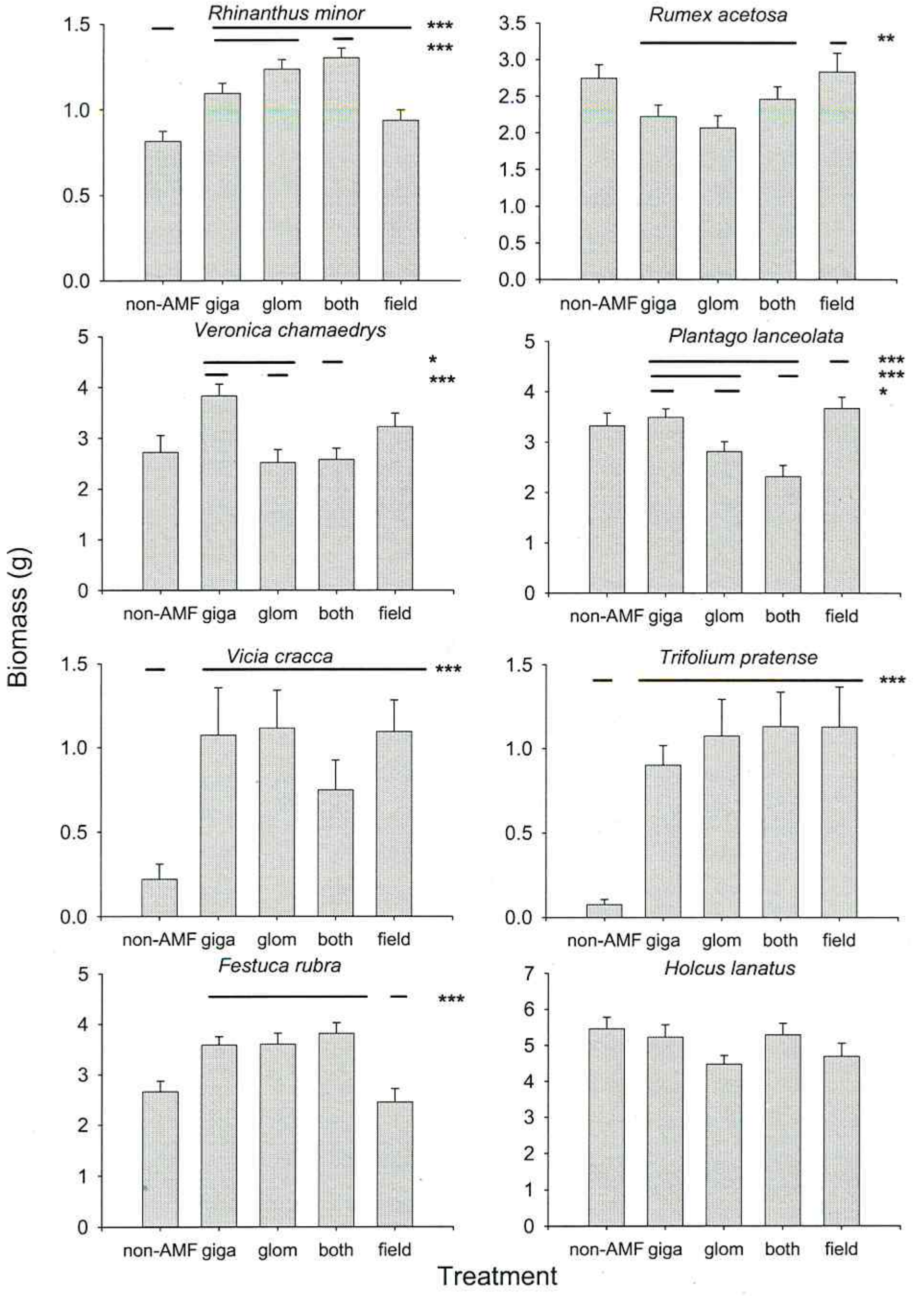
Species	Frequency in soil seed bank	Frequency in present vegetation
Crepis mollis	0	1
Dryopteris felix-mas	11	0
Epilobium ciliatum	4	0
Erophila spathulata	1	0
Erophila verna	2	2
Euphorbia helioscopa	1	0
Euphrasia rostkoviana	0	1
Fallopia convolvulus	3	0
Galeopsis tetrahit	6	1
Galium mollugo agg.	2	15
Galium saxatile	1	6
Geranium sylvaticum	1	5
Glechoma hedera	1	0
Gnaphalium sylvaticum	4	5
Hypericum humifusum	5	0
Lamium album	0	1
Lamium amplexicaule	1	0
Lamium purpureum	1	0
Lysimachia numularia	18	0
Mentha arvensis ssp.	2	0
Oxalis acetosella	2	0
Pimpinella saxifraga	0	8
Plantago media	3	0
Polygala vulgaris	1	1
Polygonum aviculare	4	1
Prunella vulgaris	1	1
Ranunculus acris	7	13
Ranunculus auricomus	2	1
Ranunculus repens	8	1
Rhinanthus minor	1	8
Rubus spec.	2	0

Appendix 2.2: continued

Species	Frequency in soil seed bank	Frequency in present vegetation
Senecio jacobaea	0	1
Senecio sylvaticus	1	1
Sergina procumbens	6	1
Silene vulgaris	1	0
Solidago canadensis	7	0
Sonchus arvensis	2	0
Spergula arvensis	2	0
Stellaria media	4	1
Thlaspi caerulescens	0	2
Thymus pulegioides	0	1
Torilis japonica	1	0
Urtica dioica	0	2
Veronica hederifolia	0	1
Veronica officinalis	14	3
Veronica persica	0	1
Veronica serpyllifolia	13	5
Legumes		
Lathryus pratensis	0	1
Trifolium hybridum	0	1
Trifolium pratense	4	13
Vicia angustifolia	2	1
Vicia sepium	0	1

Appendix 3.1: Assignment of all plant species growing in the 14 grassland sites to plant functional groups.

Grasses	**Tall herbs**	**Small herbs**	**Small herbs**
Agrostis capillaris	*Aegopodium podagraria*	*Achillea millefolium*	*Plantago lanceolata*
Alopecurus pratensis	*Aethusa cynapium*	*Ajuga reptans*	*Plantago major*
Anthoxanthum odoratum	*Anthriscus sylvestris*	*Alchemilla vulgaris agg.*	*Plantago media*
Arrhenatherum elatius	*Bistorta officinalis*	*Anemone nemorosa*	*Potentilla erecta*
Avenella flexuosa	*Centaurea pseudophrygia*	*Arabidopsis thaliana*	*Prunella vulgaris*
Briza media	*Chaerophyllum aureum*	*Arnica montana*	*Ranunculus aurecomus*
Bromus hordeaceus agg.	*Cirsium arvense*	*Bellis perennis*	*Ranunculus repens*
Carex pilulifera	*Cirsium helenioides*	*Campanula patula*	*Rhinanthus minor*
Cynosurus cristatus	*Cirsium palustre*	*Campanula rotundifolia*	*Rumex acetosella*
Dactylis glomerata	*Crepis biennis*	*Capsella bursa-pastoris*	*Saxifraga granulata*
Deschampsia cespitosa	*Crepis capillaris*	*Cardamine pratensis*	*Spergula arvensis*
Elytrigia repens	*Crepis mollis*	*Cardaminopsis arenosa*	*Stellaria graminea*
Festuca pratensis	*Epilobium angustifolium*	*Cardaminopsis halleri*	*Taraxacum officinale agg.*
Festuca rubra	*Geranium sylvaticum*	*Carum carvi*	*Thesium pyrenaicum*
Helictotrichon pubescens	*Heracleum sphondylium*	*Cerastium arvense*	*Thlaspi arvense*
Holcus lanatus	*Hieracium laevigatum*	*Cerastium holosteoides*	*Thlaspi cf. caerulescens*
Holcus mollis	*Hieracium pilosella*	*Erophila verna*	*Thymus pulegioides*
Lolium multiflorum	*Meum athamanticum*	*Fallopia convolvulus*	*Tripleurospermum inodorum*
Lolium perenne	*Phyteuma spicatum*	*Fragaria vesca*	*Veronica arvensis*
Luzula campestris	*Ranunculus acris*	*Galeopsis tetrahit*	*Veronica chamaedrys*
Nardus stricta	*Ranunculus nemorosus*	*Galium mollugo agg.*	*Veronica filiformis*
Phleum pratense	*Rumex acetosa*	*Galium saxatile*	*Veronica hederifolia*
Poa annua	*Rumex crispus*	*Galium uliginosum*	*Veronica officinalis*
Poa pratensis	*Rumex obtusifolius*	*Genister tinctoria*	*Veronica serpyllifolia*
Poa trivialis	*Sanguisorba officinalis*	*Geranium pusillum*	*Viola tricolor*
Trisetum flavescens	*Senecio sylvaticus*	*Glechoma hederacea*	
Legumes	*Tanacetum vulgare*	*Hieracium lachenalii*	
Lathyrus linifolius	*Tragopogon pratensis*	*Hypericum maculatum*	
Lotus corniculatus	*Urtica dioica*	*Hypochaeris radicata*	
Trifolium dubium		*Knautia arvensis*	
Trifolium pratense		*Lamium album*	
Trifolium repens		*Leontodon autumnalis*	
Vicia angustifolia		*Leontodon hispidus*	
Vicia cracca		*Leucanthemum vulgare*	
Vicia sepium		*Listeria ovata*	
		Lychnis flos-cuculi	
		Myosotis arvensis	
		Pimpinella saxifraga	



Appendix 4.1: Responses of *Rhinanthus minor* and host plants (mean + se) grown in the single-species experiment responding to different mycorrhizal treatments. Biomass of host plant species was averaged across the two hemiparasite treatments. Plants grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza. Horizontal lines above bars indicate a significant difference among AMF treatments, according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Appendix 4.2 Results of ANOVAs for the single-species experiment. Biomass data are log-transformed prior to analysis. Orthogonal contrasts are calculated for fungal treatments which are described in detail in the Material & Methods section. F values and significance levels are given after accounting for multiple comparisons with the Benjamini-Hochberg method (line-by-line): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Source of variation	d.f.	*R. acetosa*	*V. chamaedrys*	*P. lanceolata*	*V. cracca*	*T. pratense*	*F. rubra*	*H. lanatus*
Fungi	4	4.66 **	8.43 ***	11.92 ***	6.50 ***	12.54 ***	8.20 ***	2.89 *
non-AMF vs. fungi	1	4.20	2.19	1.92	21.95***	47.66***	5.13	3.80
field vs. commercial	1	11.00**	1.26	18.58***	0.44	0.58	27.18***	1.14
both vs. single	1	2.84	6.66*	18.29***	3.56	1.19	0.39	2.08
giga vs. glom	1	0.59	23.62***	8.89*	0.04	0.62	0.10	4.55
Tray [fungi]	45	1.72	0.73	1.30	1.36	0.86	1.91	1.46
Parasite	1	0.10	0.67	1.61	3.34	0.11	0.003	1.13
Fungi x parasite	4	1.53	0.65	1.70	1.84	1.44	0.95	0.32
Residuals (MS)	45	(0.27)	(0.62)	(0.39)	(0.33)	43 (0.33)	(0.35)	(0.85)

Appendix 5.1: Germination rates of the non-indigenous plant species under field conditions. In autumn 2005 seeds were sown on bare ground at each study site, and in May 2006, number of germinated seedlings was recorded. For *Lupinus polyphyllus* not enough seeds were available to perform this test. Species richness and biomass of the sites were measured in June 2006.

Site characteristics					2006 Germination rate [%]		
Site	longitude (E) 11°	latitude (N) 50°	species richness [m^2]	aboveground biomass [g/m^2]	*Bunias orientalis*	*Geranium pyrenaicum*	*Senecio inaequidens*
S1	37'43°	24'25°	14.0	423.98	4	16	0
S2	37'35°	24'33°	15.5	332.48	8	12	2
S3	37'42°	24'33°	16.5	586.62	20	20	0
S4	29'42°	28'03°	18.0	393.66	2	38	4
S5	30'28°	25'28°	24.0	173.06	8	32	0
S6	26'41°	24'11°	29.5	197.38	6	6	20
S7	26'44°	23'04°	23.0	154.14	24	8	2
S8	22'51°	24'16°	19.5	182.82	4	6	9
S9	23'15°	24'43°	26.5	318.38	0	0	0
S10	24'23°	26'54°	23.0	203.20	2	0	4
S11	24'29°	26'56°	31.0	279.42	12	8	15
S12	20'16°	26'21°	15.0	330.24	0	0	0
S13	20'51°	27'28°	20.0	221.68	2	0	1
S14	15'45°	28'42°	19.0	129.86	2	4	9
S15	27'29°	24'31°	25.0	140.30	0	2	8
S16	27'33°	24'33°	19.5	209.82	0	0	0
S17	27'33°	24'31°	11.0	158.64	0	0	0
S18	26'33°	22'58°	20.0	279.24	2	12	0
S19	27'24°	23'45°	23.0	345.30	10	0	0
S20	20'50°	24'49°	19.5	236.48	0	10	10

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Hempel S., **Stein C.**, Unsicker S., Renker C., Auge H., Weisser W. W., Buscot F.: Differing effects of arbuscular mycorrhizal fungi across three interacting trophic levels. *Oecologia* (in revision)

Stein C., Unsicker S.B., Kahmen, A., Wagner, M., Audorff, V., Auge H., Prati D. and Weisser W.W.: Impact of invertebrate herbivory in grasslands depends on productivity and species diversity. (to be submitted)

Stein C., Auge H., Fischer, M. & Prati D.: Impact of resident plant diversity on invasibility of montane European grasslands. (to be submitted)

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Oral presentations

Stein C., Auge H., Fischer M. and Prati D. (2006): Dispersal limitation of plant diversity in grassland ecosystems and its consequences for productivity. 36th Annual Conference, Ecological Society of Germany, Austria and Switzerland, Bremen, Germany.

Stein C., Rißmann, C., Hempel S., Renker C., Buscot F., Prati D. and Auge H. (2007): The effect of plant parasites and mycorrhizal fungi on plant communities. 20th Annual Conference, Progress in Plant Population Biology, Basel, Switzerland.

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Poster presentations

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Stein C., Auge H., Fischer M. and Prati D. (2007): Does plant diversity influence invasibility in montane grasslands? 37th Annual Conference, Ecological Society of Germany, Austria and Switzerland, Marburg, Germany.

AUTHORS' CONTRIBUTIONS TO THE MANUSCRIPTS

Chapter 2: Dispersal and seed limitation affect diversity and productivity of montane grasslands.

Together with HARALD AUGE, MARKUS FISCHER, WOLFGANG W. WEISSER & DANIEL PRATI.
Oikos, in press.

I performed the experiment, analyzed and discussed the data, and wrote the manuscript.
H. Auge, M. Fischer and D. Prati gave statistical advice, discussed the data, and commented on earlier versions of the manuscript. W. W. Weisser was responsible for the implementation of the overall DIVA Jena research project, and commented on earlier versions of the manuscript.

Chapter 3: Impact of invertebrate herbivory in grasslands depends on productivity and plant species diversity.

Together with SYBILLE UNSICKER, ANSGAR KAHMEN, MARKUS WAGNER, VOLKER AUDORFF, HARALD AUGE, DANIEL PRATI & WOLFGANG W. WEISSER.
Manuscript to be submitted.

I performed parts of the experiment from 2004 to 2006 (including pesticide application and vegetation censuses), analyzed and discussed the data across the whole experimental period, and wrote the manuscript.
S. Unsicker performed parts of the experiment from 2002-2006 (including pesticide application and biomass sampling), discussed the data, and commented on earlier versions of the manuscript. A. Kahmen, M. Wagner and V. Audorff provided vegetation data for the initial phase of this experiment, and commented on earlier versions of the manuscript. A. Kahmen provided also biomass data from 2002 to 2003. H. Auge and D. Prati gave statistical advice, discussed the data, and commented on earlier versions of the manuscript. W. W. Weisser was responsible for the implementation of the overall DIVA Jena research project. He planned and initiated the experiment, discussed statistical analyses and the data, and took part in the preparation of the manuscript.

Chapter 4: Interactive effects of mycorrhizae and a root hemiparasite on plant community productivity and diversity.

Together with CORNELIA RIßMANN, STEFAN HEMPEL, CARSTEN RENKER, FRANÇOIS BUSCOT, DANIEL PRATI & HARALD AUGE.

Oecologia, in revision

I performed the multi-species experiment, analyzed and discussed the data, supervised C. Rißmann as a diploma student, and wrote the manuscript.

C. Rißmann worked as a diploma student on the single-species experiment and determined mycorrhizal colonization rates. S. Hempel and C. Renker discussed the experimental design, gave advice in mycorrhizal handling, helped in the greenhouse and in the lab, and commented on earlier versions of the manuscript. F. Buscot, H. Auge and D. Prati gave statistical advice, discussed the data, and commented on earlier versions of the manuscript.

Chapter 5: Native plant diversity reduces invasibility in montane grasslands.

Together with HARALD AUGE, MARKUS FISCHER & DANIEL PRATI.

Manuscript to be submitted.

I performed the experiments, analyzed and discussed the data, and wrote the manuscript.

H. Auge, M. Fischer and D. Prati gave statistical advice, discussed the data, and commented on earlier versions of the manuscript.

EHRENWÖRTLICHE ERKLÄRUNG

Die geltende Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Potsdam ist mir bekannt.

Ich habe die Dissertation selbst angefertigt. Sämtliche von mir benutzten Hilfsmittel, persönliche Mitteilungen oder Quellen sind in der vorliegenden Arbeit angegeben. Die Hilfe eines Promotionsberaters habe ich nicht in Anspruch genommen.

Mein Beitrag sowie der Anteil der Ko-Autoren an den einzelnen Manuskripten sind im Kapitel "Authors' contributions to the manuscripts" dargestellt.

Die vorgelegte Dissertation habe ich weder in dieser noch in einer abgewandelten Form als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht.

Potsdam, 07.07.2008

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