

**Biodiversity loss in grasslands: consequences  
for ecosystem functioning and interactions  
with above- and belowground organisms**

**De afname van biodiversiteit in graslanden: consequenties  
voor het functioneren van het ecosysteem en interacties met  
boven- en ondergrondse organismen**

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# **Biodiversity loss in grasslands: consequences for ecosystem functioning and interactions with above- and belowground organisms**

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Es gibt nur einen Idioten  
und durch irgendeine Lücke  
durch irgendeine Lücke  
dringt er immer wieder ein

Ich kann die Tür verrammeln  
tagelang im Dunkeln auf der Lauer sein  
Irgendwie kriegt er mich immer  
Irgendwie kommt er immer rein  
Er macht sich schwer wenn ich ihn rauswerf´  
Er stellt sich taub wenn ich ihn anschrei´  
Er ähnelt mir irgendwie im Spiegel  
er lässt mich einfach nicht allein

Ich kann ganz klar sagen: Ich fang von vorne an  
nehm´ alle Konsequenzen dabei mit in Kauf  
Schluss mit dem Unsinn, ich räum´ jetzt auf  
ein Blick nur aus seinen schönen Augen:  
Alles wie gehabt und ich bin dran  
Er gärt irgendwo in meinem, seinem Innern  
und irgendwie kenn´ ich sein Gesicht

Er macht immer wieder dieselben Fehler  
bis auf die fünfte Stelle hinterm Komma  
Er macht mich alt und leicht berechenbar  
Er kennt mich offenbar

Wenn ich versuch´ zu schlafen  
quatscht er mich ständig voll  
Ich denk´ noch: Die Stimme kenn´ ich  
erinnert sie mich doch irgendwie an mich

Der hat irgendwie das Loch kreierte  
Ich verstecke mich in meiner Nische  
und warte völlig ungeniert  
dass er den einzigen Idioten endlich mitnimmt  
und will der nicht von alleine gehn´  
dann helf´ ich ein bisschen nach und geh´ am Ende mit



## Abstract

Van Ruijven, J. 2005. Biodiversity losses in grasslands: consequences for ecosystem functioning and interactions with above- and below-ground organisms. PhD thesis, Wageningen University.

Considering the current rate of extinctions, it is crucial to understand the consequences of these losses of biodiversity for the functioning of ecosystems. Grasslands proved a very suitable ecosystem for biodiversity-ecosystem functioning research.

In earlier experiments, nitrogen-fixing legumes dominated the effects of plant diversity, but in our experiment without legumes we also found that diverse plant communities have higher productivity than species-poor ones. These results could not be explained by sampling effects. Instead, complementarity in nutrient uptake and more efficient nutrient use at high diversity are the most important driving forces behind the positive effects of diversity. We also found that the negative effect of plant diversity on the invasibility of the community may be a sampling effect, as the increased presence of two particular plant species were the most important factors affecting invasion success.

Plant diversity may also affect many other organisms in grasslands. We found that nematodes responded positively to plant diversity. Specific associations between plants and nematodes resulted in increased nematode diversity at high plant diversity.

Of course, higher trophic level organisms do not only respond to plant diversity, they may also regulate plant diversity. We found that arbuscular mycorrhizal fungi (AMF) alter competition between plants and contribute to plant diversity by enhancing the establishment of plants.

Earlier studies also reported strong effects of herbivores, pathogens and mutualists on plant communities, but most of these studies investigated a single group of organisms in isolation from other driving forces. We show that the separate effects of these organisms on plant diversity may change dramatically when studied in combination. A neutral effect of an aboveground insect herbivore on plant diversity and a positive effect of a belowground insect herbivore turned strongly negative when both herbivores were present in the plant community.

It is argued that a combined approach, using interactions between changes in plant diversity and multiple 'drivers of plant diversity' (both biotic and abiotic) is needed to fully understand plant community dynamics. To be able to predict the consequences of biodiversity loss worldwide it is necessary to integrate the results of this approach over different ecosystems and ecosystem processes across large spatial and temporal scales.



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**General introduction**

Jasper van Ruijven



## Biodiversity and ecosystem functioning

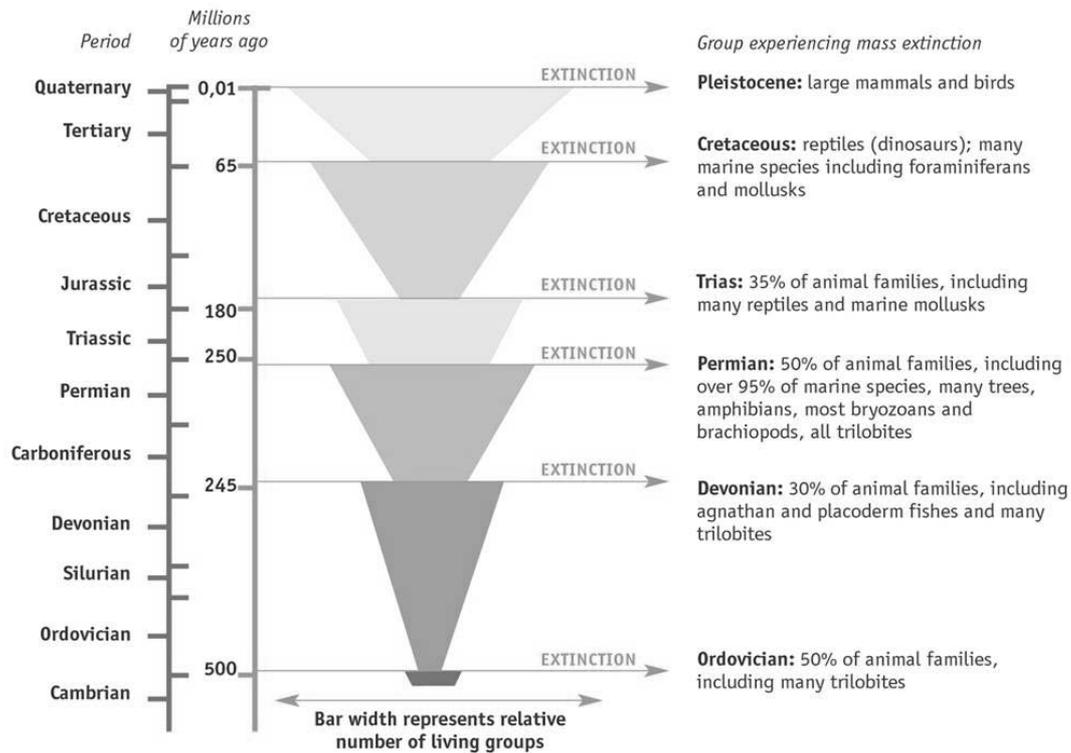
The earth harbours an amazing number of species. A widely quoted number, based on a large number of studies, is one of around 13.5 million (Hawksworth & Kalin-Arroyo 1995; Table 1.1).

**Table 1.1** Approximate numbers (in thousands) of currently described species and estimates of species richness for large groups of organisms. Reliability of estimates is likely to vary greatly, and a crude indication of the accuracy of estimates is given (From Hawksworth & Kalin-Arroyo 1995)

	Described species	Estimated number of species			Working figure	Accuracy of figure
		High	Low			
Viruses	4	1000	50	400	Very poor	
Bacteria	4	3000	50	1000	Very poor	
Fungi	72	2700	200	1500	Moderate	
Protozoa	40	200	60	200	Very poor	
Algae	40	1000	150	400	Very poor	
Plants	270	500	300	320	Good	
Nematodes	25	1000	100	400	Poor	
Arthropods:						
Crustaceans	40	200	75	150	Moderate	
Arachnids	75	1000	300	750	Moderate	
Insects	950	100,000	2000	8000	Moderate	
Molluscs	70	200	100	200	Moderate	
Chordates	45	55	50	50	Good	
Others	115	800	200	250	Moderate	
<b>Totals</b>	<b>1750</b>	<b>111,655</b>	<b>3635</b>	<b>13,620</b>	<b>Very poor</b>	

Together, these organisms move hundreds of thousands of tons of elements and compounds between the hydrosphere, atmosphere, and lithosphere every year through their collective metabolic and growth activities. These activities determine soil fertility, water and air quality, the habitability of ecosystems, and ultimately makes Earth a unique planet in the solar system (Ernst 2000; Schlesinger 1997). While the functional significance of all organisms together for the functioning of the Earth-system is well established, the significance of the diversity of organisms remains unknown. There is a well-developed understanding of primary productivity on a global scale (Roy *et al.* 2001), but we have little understanding of how plant diversity in a meadow or forest affects production at the scale of the ecosystem (Loreau *et al.* 2002).

Biodiversity (defined as the extent of genetic, taxonomic and ecological diversity across all spatial and temporal scales (Harper & Hawksworth 1994)), however, is currently undergoing dramatic changes (Wilson 1993). Life on earth has experienced periods of massive extinctions before, and it has been argued that we are currently facing a sixth extinction crisis (Fig. 1.1).



**Figure 1.1** Periods of mass extinction on a geological timescale. A mass extinction is defined as a relatively brief period in which more species become extinct than at other times. Apart from the current extinction wave, which started with the extinction of large mammals and birds in the Pleistocene, five periods of mass extinctions are recognized. © the Regents of the University of Michigan

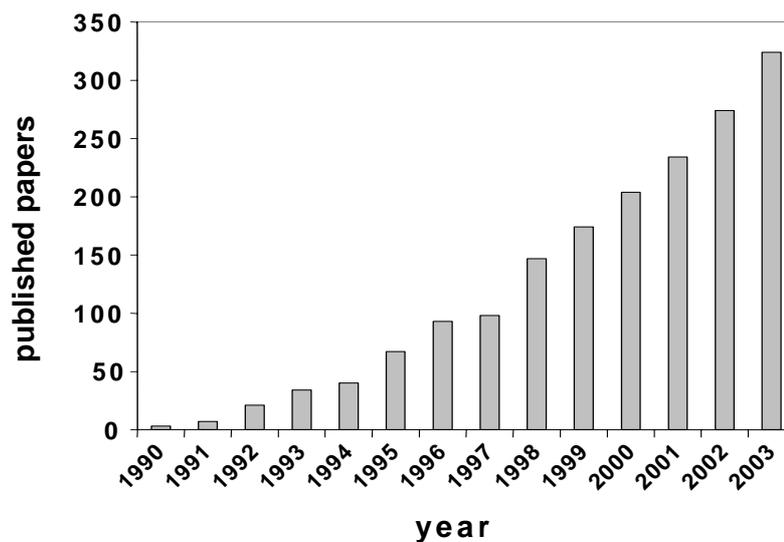
In contrast to earlier mass extinctions, which are thought to have arisen from climatic changes and sea-level fluctuations (possibly related to extraterrestrial impact, volcanism and other abrupt events), the current biodiversity loss is caused by human activities. The main causes of extinction are direct exploitation and habitat loss, degradation and fragmentation (Gaston & Spicer 2004). The fossil record shows that overall levels of biodiversity may recover from mass extinction events very rapidly on an evolutionary time scale, but the recovery typically requires 2 – 10 million years (Erwin 1998). If substantial extinctions occur in the near future, which seems likely if not inevitable, then the species will not be replaced in short order.

Considering the fact that we depend on a range of ecosystem services (primary production, water quality, prevention of soil erosion, pollination, etc.), predicting the ecosystem consequences of these losses of biodiversity has become a critical issue.

### Early hypotheses

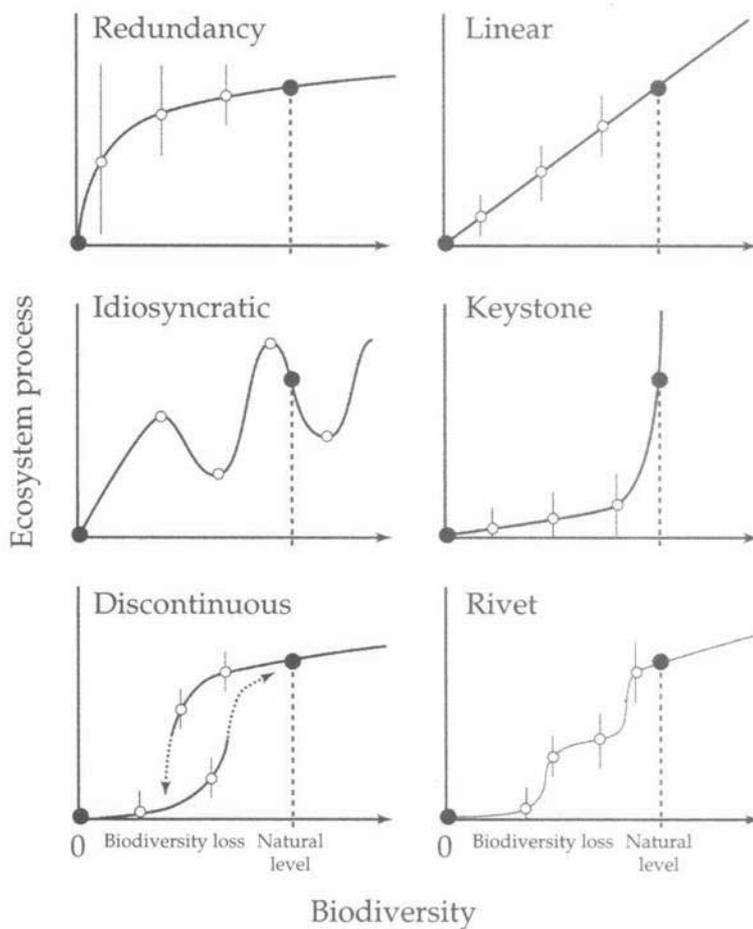
Charles Darwin and the experiment he cited predate current biodiversity research by 150 years (Hector & Hooper 2002). Earlier research, including that which inspired Darwin, focussed on agro-ecological efforts to improve yield through intercropping (de Wit 1960; Trenbath 1974; Vandermeer 1988).

It was not until the last decade, however, that insights in the ecological consequences of changes in biodiversity were derived from empirical and theoretical studies. Since then, studies that consider biodiversity and ecosystem functioning have become an integral part of ecology (Fig. 1.2).



**Figure 1.2** The emergence of biodiversity-ecosystem functioning research. Bars represent the number of papers that included 'biodiversity' and 'ecosystem' in their title, keywords and/or abstract. SCI EXPANDED of ISI Web of Science was the database used for this figure. Adapted from Loreau *et al.* (2002)

At a conference in Germany in 1991, ecologists reviewed what was known about the relationship between biodiversity and ecosystem functioning (Schulze & Mooney 1993). They formally identified the central idea of a relationship between biodiversity and ecosystem functioning. When ecologists were asked what the trajectory of the relationship could be, a range of hypotheses emerged (Schläpfer &



Schmid 1999). A number of early hypotheses are shown in figure 1.3.

**Figure 1.3** Graphs of early hypotheses considering the relationship between biodiversity and ecosystem functioning. Taken from Loreau *et al.* (2002)

In general, three main categories of hypotheses can be identified. 1) Species are redundant, which means that they are at least partially substitutable. This implies that the loss of species is compensated for by other species. 2) Species are singular. This means that each species contributes to ecosystem functioning in a unique way. Their loss causes detectable changes. Keystone species are examples of singular species. 3) Species impacts are context-dependent and therefore idiosyncratic or unpredictable. It implies that a species makes different contributions depending on extrinsic and intrinsic factors such as site fertility, community composition, and disturbance regime (Loreau *et al.* 2002).

### Experimental research

One of the first experiments was conducted in the Ecotron, a controlled environmental facility designed for ecological research (Lawton *et al.* 1993). It established replicated terrestrial ecosystems of high, intermediate, and low diversity. The study demonstrated that the relationship between diversity and ecosystem functioning was not flat (Naeem *et al.* 1994). However, due to its design new issues that emerged could not be addressed (Allison 1999; Lawton *et al.* 1998). Two other early studies focussed the biodiversity-ecosystem functioning debate upon the relationship between plant diversity and productivity in grasslands (Tilman *et al.* 1996; Tilman & Downing 1994) and an array of studies would follow (Hooper & Vitousek 1997; Tilman *et al.* 1997a; Hooper 1998; Hector *et al.* 1999; Van der Putten *et al.* 2000; Caldeira *et al.* 2001; Dukes 2001; Tilman *et al.* 2001; Engelhardt & Ritchie 2002; Fridley 2002, 2003; Dimitrakopoulos & Schmid 2004; Hooper & Dukes 2004). Many, but not all, reported positive effects of diversity on productivity.

### **Interpretation of the results**

The early experiments hypothesized that the observed patterns were caused by niche complementarity for light, nitrogen or water use (Naeem *et al.* 1994; Tilman *et al.* 1996). Niche complementarity refers to the greater efficiency of resource use by a community of species whose niches complement each other (i.e. they do not completely overlap). A well known example is that of a community of shallow- and deep-rooted species, which acquires mineral nutrients in a fixed soil volume better than either group could achieve alone (see Dimitrakopoulos & Schmid 2004).

Controversy, however, arose on the interpretation of these experiments. According to a statement in *Nature*, this particular debate “has erupted into a full-blown war. Opposing camps are duelling over the quality of key experiments, and some are flinging barbs at meetings and in journals” (Kaiser 2000). The debate focussed on two fundamental issues. The first one centred on the fact that the results could also be explained by a so-called sampling effect (Aarssen 1997; Huston 1997; Tilman *et al.* 1997b). It states that high-diversity plots have a higher probability of containing species with major impacts on whatever ecosystem process measured. Initially, it proved difficult to distinguish complementarity from sampling effects, but Loreau & Hector (2001) proposed a new partitioning method that enabled a distinction to be made between complementarity and sampling effects.

The second point involved the role of legumes. Legumes can strongly increase both productivity and complementarity because they do not compete with other plants for soil nitrogen, but fix atmospheric N<sub>2</sub>. In addition, the fixed N can be transferred to co-occurring plants by root exudation (Paynel *et al.* 2001) and decomposition of dead roots (Høgh-Jensen & Schjoerring 2000; Trannin *et al.* 2000). As a consequence, the greater probability of including legume species at greater species numbers will increase both the productivity and the degree of complementarity in species-rich mixtures. Several studies show an important role of legumes in the positive relationship between diversity and productivity (Hector *et al.* 1999; Tilman *et al.* 2001; Mulder *et al.* 2002; Hille Ris Lambers *et al.* 2004; Hooper & Dukes 2004;). These effects of legumes are difficult to detect using the additive partitioning method. Moreover, the two major sets of biodiversity experiments, BIODDEPTH (Hector *et al.* 1999) and Cedar Creek (Tilman *et al.* 1997a; Tilman *et al.* 2001) were not designed to adequately disentangle the effects of legumes from other diversity effects.

### **Effects on other ecosystem processes**

If species richness has detectable effects on primary productivity, this could promote other components of the ecosystem and the processes they regulate. One of the most important processes is decomposition, but the relationship with plant diversity is not straightforward. At the Silwood Park site of the BIODDEPTH experiment, decomposition rates increased with increasing species richness (Hector *et al.* 2000), but at the Swiss site there was no relationship between decomposition and plant diversity (Spehn *et al.* 2000). Additional data come from litter mix experiments. Bardgett & Shine (1999) found that increasing litter diversity from one to six species increased decomposition rates, but Wardle *et al.* (1997) found idiosyncratic effects of plant diversity on decomposition. It is clear that a range of responses to plant diversity is possible, depending on the component species and the responses of decomposer biota (Wardle 2002).

In plant diversity experiments, invasibility was significantly reduced at high diversity (Knops *et al.* 1999; Hector *et al.* 2001; Kennedy *et al.* 2002; Fargione *et al.* 2003). This is an important issue, because the invasion of exotic species into natural communities is a pervasive and widespread phenomenon, which can have serious ecological consequences (Williamson 1999; Mack *et al.* 2000; Pimental *et al.* 2000). Many hypotheses have been put forward to

explain why susceptibility to invasion differs between communities. One of the earliest theories was Elton's hypothesis that high resident diversity reduces invasion (Elton 1958). Although several experimental studies presented evidence that diversity reduces invasibility, most observational studies failed to identify diversity as an important barrier against invasion. In contrast, many of these observational studies showed a positive correlation between diversity and invasibility (Levine *et al.* 2002). Fluctuations in resource availability due to e.g. disturbance, herbivory, eutrophication were identified as the key factor controlling invasibility (Davis *et al.* 2000). The most likely explanation for these conflicting results is the covariance between extrinsic factors (i.e. disturbance, grazing, nutrient levels) and resident diversity in observational studies (Naeem *et al.* 2000). This covariance is removed in most experimental studies (Levine & D'Antonio 1999). Similar to the effects of diversity on productivity, complementarity in resource use has traditionally been proposed as the mechanism responsible for the negative impact of diversity on invasibility. The utilization of resources (mainly light and nutrients) increases with diversity, resulting in fewer resources being available for invaders (Elton 1958; Knops *et al.* 1999; Naeem *et al.* 2000). The sampling effect (Aarssen 1997; Huston 1997) was proposed as an alternative explanation for the results obtained in the experimental studies on invasibility. It was argued that the observed decrease of invasion success with increasing diversity could be caused by an increasing occurrence of a highly suppressive species (Wardle 2001).

### **Effects of plant diversity on higher trophic-levels...**

Whereas the effects of plant diversity on biomass production and invasion resistance received considerable attention, the effects on higher trophic-level organisms received less attention. Initially, studies focussed on aboveground invertebrates. Plant diversity may increase arthropod herbivore, predator and parasite diversity without altering their abundance, but total arthropod diversity could also be reduced due to the loss of predator and parasite species (Siemann 1998; Siemann *et al.* 1998; Knops *et al.* 1999). These contrasting results were explained by differences in specialization and mobility between the arthropod species studied. Other studies pointed to the importance of plant nutritional quality (Symstad *et al.* 2000) and other specific traits related to plant functional groups (Koricheva *et al.* 2000).

The interest in belowground organisms and the soil food web is rapidly increasing, and some studies reported positive effects of plant diversity (Spehn *et al.* 2000a; Stephan *et al.* 2000; Porazinska *et al.* 2003; Wardle *et al.* 2003). Other studies, however, showed no or inconsistent effects of plant diversity (Wardle & Nicholson 1996; Wardle *et al.* 1997; Malý *et al.* 2000; Korthals *et al.* 2001; Hedlund *et al.* 2003). Lack of effects were explained by plant specific traits overruling plant diversity effects (Wardle *et al.* 1999; Wardle *et al.* 2000) and the poor dispersal and colonization abilities of many soil organisms (Korthals *et al.* 2001).

### **... and vice versa**

Many studies pointed to important roles of aboveground and belowground organisms in regulating plant diversity. The Janzen-Connell hypothesis (Connell 1978; Janzen 1970) predicts greater tree diversity in tropical forests when specialist herbivores are present. Recently, a field test confirmed that herbivores affect tree diversity by enforcing habitat specialization among species (Fine *et al.* 2004). Aboveground vertebrate herbivores can promote grassland diversity by selectively feeding on dominant plant species (Crawley 1997; Olff & Ritchie 1998). Root herbivores can enhance plant diversity in a similar way (Van der Putten *et al.* 2001; De Deyn *et al.* 2003), but root feeding by insect herbivores was also found to reduce plant diversity by reducing the establishment of forbs (Brown & Gange 1989). Specialist pathogens enhanced the establishment of other species under adult *Prunus serotina* trees by preventing the establishment of *P. serotina* seedlings (Packer & Clay 2000). Positive effects of mycorrhizal fungi (AMF) on plant diversity have been demonstrated in several mesocosm studies (Grime *et al.* 1987; Van der Heijden *et al.* 1998a,b). These experiments were performed on calcareous soils, which have a very low phosphate-availability. As the supply of phosphorus to plants is the main mechanism by which AMF promote plant growth, the strong responses reported in these studies are perhaps not surprising. The role of AMF on non-calcareous soils, however, remains poorly understood. Moreover, AMF may also reduce plant diversity. Studies in tallgrass prairie showed that AMF promoted the competitive exclusion of C3 species by obligatory mycorrhizal C4 grasses (Hartnett & Wilson 1999; Smith *et al.* 1999).

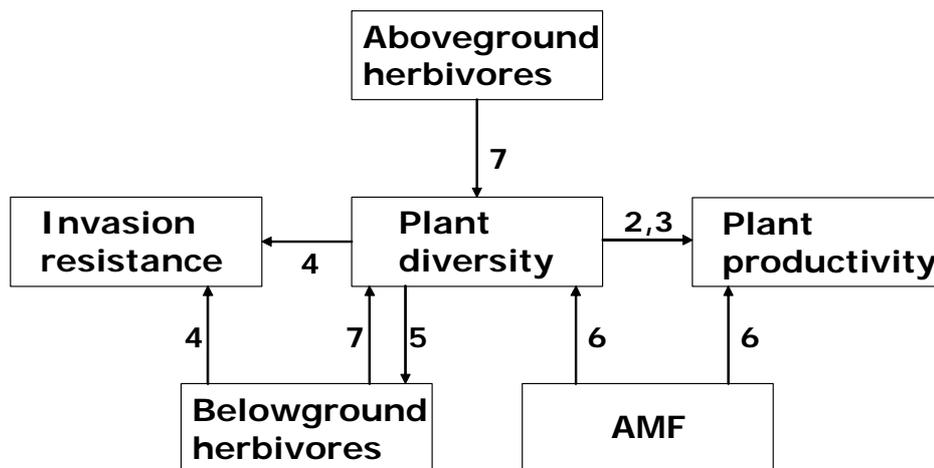
It is important to note that many of these experiments studied the effect of a particular group of organisms in isolation from possible effects of other organisms. Most changes in ecosystem processes

occur through impacts of above- and belowground organisms on plant community structure and composition (Wardle *et al.* 2004), but their joint effects on plant communities rarely have been studied (Bardgett & Wardle 2003).

### Outline of the thesis

Major aims of this thesis are to investigate:

- 1) The importance of plant diversity for ecosystem functioning in grasslands, particularly in the absence of nitrogen-fixing legumes.
- 2) The relationship between diversity and invasion resistance. Emphasis lies on the role of diversity *per se* versus the effect of particular plant species.
- 3) The effects of plant diversity at higher trophic levels.
- 4) The role of AMF in determining plant diversity and community composition in grasslands on sandy soil
- 5) The importance of interactions between different above- and



**Figure 1.6** Graphical presentation of the thesis. Numbers next to arrows refer to chapters.

belowground organisms that affect plant diversity.

These aims will feature prominently in the following chapters (see Fig. 1.6).

In the coming chapter, the largest of our experiments is introduced. The relationship between plant diversity and productivity is evaluated after the first full growing season (16 months after the start of the experiment) and the observed effects are partitioned in complementarity and sampling effects.

This approach is elaborated in chapter 3. Here we analyse the 'long-term' (four years) behaviour of the relationship between plant diversity and productivity. This is important, because the community under investigation is a perennial community. Omitting the legumes may answer the question which other species or group of species are most important for positive effects of plant diversity. This is also addressed in chapter 3.

In chapter 4, the importance of plant diversity for reducing invasibility is investigated. This research was carried out in the experiment introduced in chapters 2 and 3. Chapter 5 is included as an example of the effects of plant diversity on higher trophic levels. It previously appeared in the thesis of Gerlinde De Deyn (De Deyn 2004), and deals with the impact of plant diversity on the nematode community. It was investigated in the experiment described in chapters 2 and 3.

In chapter 6 the importance of AMF for plant diversity is determined. In this chapter we investigate the effects of AMF on sandy soils with a moderate P-availability. On calcareous soils, AMF appear to be a prerequisite for the growth and survival of many plant species, but this is not the case on the sandy soils we studied. Nevertheless, AMF may alter plant community composition by affecting plant competition and seedling establishment.

Chapter 7 then reports the results of the second large experiment. Here, we present a unique experimental approach to tackle the simultaneous impacts of different above- and belowground organisms affecting plant diversity. The thesis is concluded by a short general discussion of the findings reported in these chapters.

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**Positive effects of plant species diversity on  
productivity in the absence of legumes**

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## **ABSTRACT**

We investigated the effect of species richness on productivity in randomly assembled grassland communities without legumes. Aboveground biomass increased with increasing species richness and different measures of complementarity showed strong increases with plant species richness. Increasing productivity could not be attributed to a relative increase of highly productive species. Instead, the increase appeared to be caused by the increased performance of several low-productive species. Our results provide evidence that niche complementarity can strongly increase productivity in grasslands, even though the communities contained only grasses and forbs.

**Keywords:** *productivity; species richness; niche differentiation; legumes; complementarity effect; facilitation; selection effect; grassland*

## INTRODUCTION

Natural ecosystems provide important services to humanity that often cannot be provided by agricultural systems. Examples are the production and preservation of soils, the mitigation of droughts and floods, the purification of water and air and the control of physical characteristics of the atmosphere. Many of these ecosystem services ultimately depend on plant productivity and soil organic matter decomposition. The crucial question is whether the dramatic loss of biodiversity that we are facing today will affect such ecosystem processes.

Most studies that have tackled this question focussed on the effects of biodiversity on grassland productivity as an example that is experimentally tractable. Recent experimental evidence that the productivity of grassland ecosystems increases with increasing species richness (Tilman *et al.* 1996; Tilman *et al.* 1997a; Hector *et al.* 1999; Tilman *et al.* 2001) has however, sparked an intense debate (Aarssen 1997; Huston 1997; Huston *et al.* 2000; Wardle *et al.* 2000; Loreau *et al.* 2001). The main point of controversy is whether the observed increase should be attributed to increased niche complementarity (species-rich communities exploit the available resources more completely) or to the sampling effect. In this sampling or selection effect, the observed increase is caused by chance: randomly assembled communities with many species have a higher probability of containing a highly productive species. Recently, a new approach was presented to separate these two effects (Loreau & Hector 2001). They showed that the complementarity effect prevailed in the European BIODEPTH experiment.

There is, however, a second point of controversy. In the two sets of experiments (Hector *et al.* 1999; Tilman *et al.* 2001) often cited in this debate, a major part of the demonstrated productivity-diversity relationship appears to depend on whether legumes were present or absent (Huston *et al.* 2000). Legumes can strongly increase both productivity and complementarity because they do not compete with other plants for soil nitrogen, but fix atmospheric N<sub>2</sub>. In addition, the fixed N can be transferred to co-occurring plants by root exudation (Paynel *et al.* 2001) and decomposition of dead roots (Høgh-Jensen & Schjoerring 2000; Trannin *et al.* 2000). As a consequence, the greater probability of including legume species at greater species numbers will increase both the productivity and the degree of complementarity in species-rich mixtures. We wanted to test if niche complementarity does occur with just the two functional groups grasses and forbs present and if it increases with increasing species richness in grasslands. To do so, we performed a new experiment without any legume species.

## MATERIAL & METHODS

Plots were established on an arable field in early spring 2000. The topsoil was removed to a depth of 45 cm. At this depth, the sand layer below the arable soil was reached. Square wooden frames measuring 1 by 1 by 0.5 m (l x w x h) were placed into each hole and filled with an arable soil diluted with pure sand (1:3). We used the diluted soil because the arable soil was far too nutrient-rich in comparison to sandy soils on which these plant communities normally occur. The area outside the frames was filled with the arable soil. 144 plants per plot were planted as seedlings following a substitutive design (i.e. total seedling density was equal in all plots). In total, the experiment comprised 102 plots of 1 m<sup>2</sup> distributed over 6 replicated blocks. Each block contained monocultures of all species, and mixtures of two, four, and eight species. Species were selected from a pool of 4 grass species (*Holcus lanatus* (L.), *Agrostis capillaris* (L.), *Festuca rubra* (L.), and *Anthoxanthum odoratum* (L.)) and 4 forb species (*Plantago lanceolata* (L.), *Rumex acetosa* (L.), *Leucanthemum vulgare* (Lamk.), and *Centaurea jacea* (L.)), that commonly coexist in European hay-meadows. We assembled the communities of 2 and 4 species by constrained random selection from the species pool. Selecting a certain composition twice was not allowed in the procedure, in order to include as many different species combinations as possible. The composition was maintained by removing seedlings of all other species at monthly intervals during the growing season.

Our analysis is based on data on aboveground plant biomass in August 2001, which was at the end of the first full growing season, 16 months after the start of the experiment. All plants were cut to 2.5 cm above ground level. Plants growing in the centre (60 by 60 cm) were separated from plants growing in the edges of the plot. Only data from the centres were used for this analysis. Plant material was sorted to species before determining dry weight. All analyses were done with the planned number of species since all species planted were present after two years. We calculated different measures to test for the effect of diversity on productivity. The Relative Yield Total (RYT), originally proposed as the most appropriate measure of niche complementarity (de Wit & Bergh 1965), is the sum of relative yields of all species in a mixture. The relative yield (RY) compares the performance of each species in a mixture to its monoculture yield and is calculated as

$$RY_i = O_i / M_i$$

in which  $O_i$  is the yield of species  $i$  in the mixture and  $M_i$  is its monoculture yield. If the RYT exceeds unity, this indicates complementarity. Recently, this approach was elaborated further to separate the complementarity and selection effects. In this

approach, a positive complementarity effect occurs if species yields in a mixture are on average higher than expected on the basis of the weighted average monoculture yield of the component species. The selection effect is measured by the covariance between the monoculture yield of species and the deviation from expected relative yield in the mixture (Loreau & Hector 2001). Biomass per individual was calculated by dividing the yield of a species in a plot by the number of planted individuals. We used the planted number of individuals because mortality had been very low from April to August 2000 (less than 1% except for *Anthoxanthum* and *Rumex*, which suffered mortality of 1.4 and 4.2%, respectively) and did not differ between diversity levels. Mortality at a later stage of the experiment was hard to determine, because many plants had started spreading vegetatively, but seemed to be low.

Effects of diversity were tested using a GLM with block and species number as factors. Values of biomass and RYT were log-transformed prior to analysis. Values of the biodiversity effects were square-root transformed but preserved their original positive and negative signs (after Loreau & Hector 2001). A GLM with block as factor and species richness or  $\log_2$  of species richness as a continuous predictor was used to determine the relationship with diversity. For RYT, complementarity effect and selection effect, the observed value at each diversity level was compared to the value of the null hypothesis, i.e. no effect of diversity (1 for RYT, 0 for complementarity and selection effect) using t-tests. A GLM with block and species as factors and a Tukey-HSD test were used to determine differences between the yields of species in monoculture. The relationship of individual biomass with diversity was determined per species by using a GLM with block as factor and species richness or  $\log_2$  of species richness as a continuous predictor.

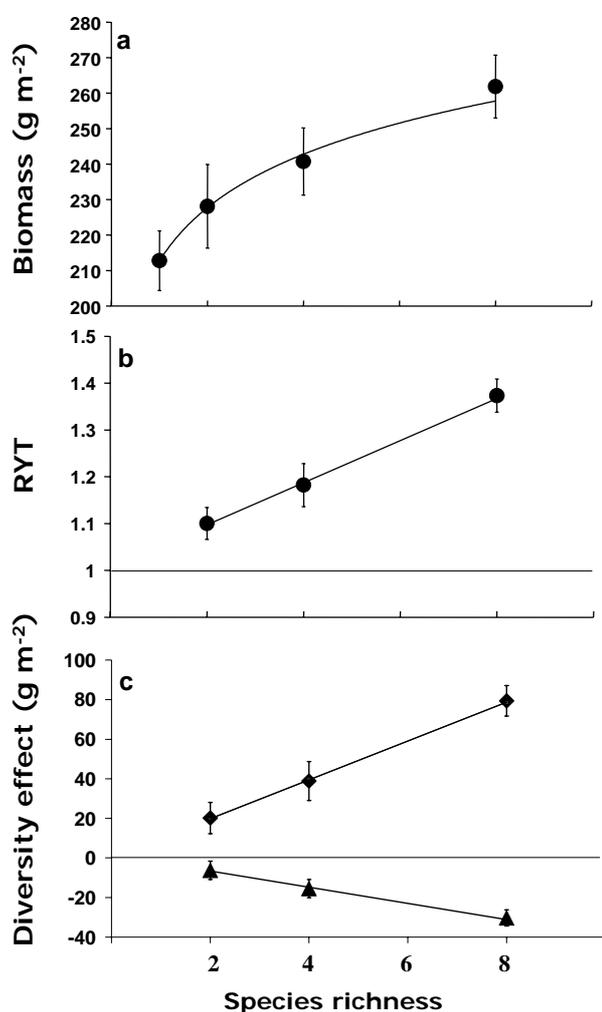
**Table 2.1** Analysis of variance, using a general linear model for effects of block and species richness on biomass, RYT, complementarity effect and selection effect. Significant values ( $P < 0.05$ ) are highlighted in bold.

Variable	Source of variation	Df	SS	MS	F	P
Biomass	Block	5	$1.6 \times 10^{-2}$	$3.2 \times 10^{-3}$	0.28	0.923
	Species richness	3	$9.9 \times 10^{-2}$	$3.3 \times 10^{-2}$	2.88	<b>0.040</b>
RYT	Block	5	0.16	$3.2 \times 10^{-2}$	1.22	0.315
	Species richness	2	0.26	0.13	5.05	<b>0.011</b>
Complementarity effect	Block	5	137.36	27.47	1.16	0.345
	Species richness	2	169.59	84.79	3.58	<b>0.037</b>
Selection effect	Block	5	49.88	9.98	0.70	0.626
	Species richness	2	90.12	45.06	3.16	0.052

## RESULTS

Diversity had a significant effect on aboveground biomass (table 2.1). Assemblages with lower diversity were less productive on average. The relationship between species richness and aboveground biomass was best described by the typical loglinear increase of aboveground production with increasing diversity (figure 2.1a).

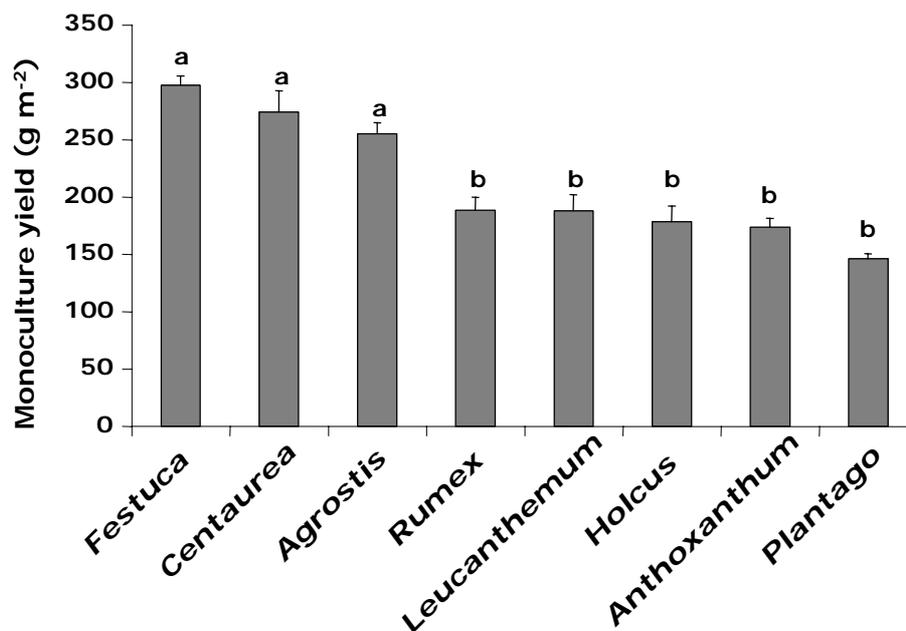
The Relative Yield Total was greatly affected by species richness (table 2.1) and was higher than one at all diversity levels (table 2.2). The RYT showed a linear increase with diversity (figure 2.1b). The complementarity effect showed similar results (table 2.1; figure 2.1c; table 2.2). The sampling effect was only marginally affected



**Figure 2.1** Effects of plant species number on (a) aboveground biomass (g m<sup>-2</sup>) clipped at 2.5 cm above soil surface (log 2 of richness as continuous predictor:  $F = 8.8$ ,  $P < 3.9E-03$ ). (b) Relative Yield Total (richness:  $F = 10.3$ ,  $P = 2.5E-03$ ). (c) The complementarity effect (circles; richness:  $F = 7.3$ ,  $P < 9.8E-03$ ) and the sampling effect (triangles; richness:  $F = 6.5$ ,  $P = 0.015$ ). Data points show means  $\pm$  se.

**Table 2.2** Tests of deviations from the null hypothesis for RYT, complementarity and selection effect at each diversity level. Values of RYT are tested for significant deviation from unity, values of the diversity effects are tested for significant deviation from zero (by t-tests). T and P values are shown. Significant values ( $P < 0.05$ ) are highlighted in bold.

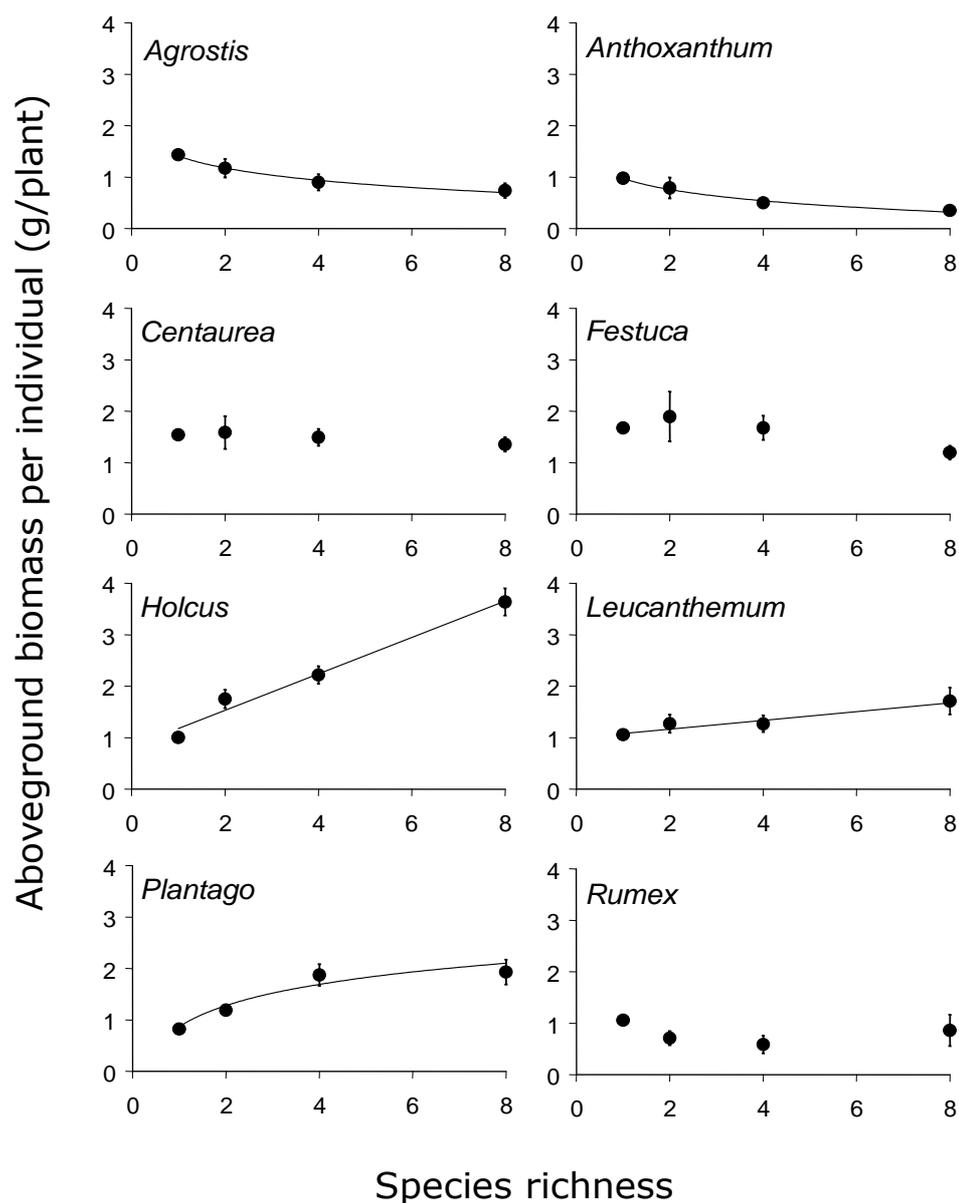
Variable	Species richness	<i>t</i>	<i>P</i>
RYT	2	2.60	<b>0.016</b>
	4	3.76	<b>0.001</b>
	8	12.28	<b>6.3 x 10<sup>-5</sup></b>
Complementarity	2	2.90	<b>0.008</b>
	4	4.00	<b>0.001</b>
	8	20.80	<b>4.8 x 10<sup>-6</sup></b>
Selection	2	-1.46	0.157
	4	-3.36	<b>0.003</b>
	8	-13.36	<b>4.2 x 10<sup>-5</sup></b>



**Figure 2.2** Aboveground biomass ( $\text{g m}^{-2}$ ) of the eight species grown in monoculture. Data are means  $\pm$  se. Different letters indicate significant differences between species ( $P < 0.05$ )

by species richness (table 2.1), but in contrast showed a negative linear relationship (figure 2.1c) and was different from zero only for four and eight-species mixtures (table 2.2). Analysis of the performance of the individual species confirmed that there is no high-productive species that determines mixture productivity. *Agrostis*, *Festuca* and *Centaurea* produced significantly more

biomass in monoculture than the five other species (figure 2.2). However, the biomass per individual plant of these species did not change (*Festuca* and *Centaurea*) or even decreased (*Agrostis*) with increasing species richness (figure 2.3), while explanation of the observed productivity-species richness relation by the sampling effect would require an increase. Three species that were less productive in monoculture (*Holcus*, *Leucanthemum* and *Plantago*) showed a significant increase of plant weight with increasing diversity (figure 2.3).



**Figure 2.3** Yield per individual (gram per plant) as a function of plant diversity for each species. Species richness as continuous predictor: *Agrostis*  $\log_2$  of species richness:  $F = 13.4$ ,  $P = 1.4E-03$ . *Anthoxanthum*  $\log_2$  of species richness:  $F = 21.7$ ,  $P = 1.5E-03$ . *Centaurea* non-significant (ns). *Festuca* ns. *Holcus* species richness:  $F=82.8$ ,  $P = 6.6E-09$ . *Leucanthemum* species richness:  $F=6.8$ ,  $P = 0.016$ . *Plantago*  $\log_2$  of species richness:  $F=20.5$ ,  $P = 1.8E-04$ . *Rumex* ns

## DISCUSSION

Our results show that productivity increases with increasing species-richness when only grasses and forbs are present. Analysis revealed that the increase of productivity should be attributed to an increased complementarity effect. A minor selection effect did occur, but it was negative. This indicates that the increased productivity of mixtures is mainly caused by species that were less productive in monoculture. Analysis of the performance of individual species showed there were at least three species contributing to this increase. Apparently, monoculture biomass was not a good predictor of success in mixtures, in contrast to the results from other studies (Spackova & Leps 2001; Hector *et al.* 2002). However, dominance of low-yielding species is a common phenomenon at low productivity levels. It is known as the Montgomery effect, after Montgomery (1912) who was the first to notice that in some experiments the species or variety yielding best in monoculture did not survive when repeatedly sown in competition with a lower-yielding species or variety (De Wit 1960). We focus on niche complementarity in our discussion, but the positive effects may also be the result of facilitation. Direct positive interactions between species have been demonstrated in many experiments (Callaway 1995). Distinguishing complementarity and facilitation, however, is difficult and the term complementarity effect actually refers to an effect that arises from niche differentiation and/or facilitation (Loreau & Hector 2001).

An increase of the complementarity effect indicates that species-rich communities are able to exploit the available resources more completely (Naeem *et al.* 1994; Tilman *et al.* 1996; Hooper & Vitousek 1997; Tilman *et al.* 1997b; Loreau 1998; Bond & Chase 2002), but the mechanisms by which this is achieved remain unclear. In grasslands where productivity is limited by water availability, facilitation or complementarity in water use may be caused by reduced evaporation from the soil due to increased cover at higher diversity (Caldeira *et al.* 2001). Considering the temperate climate of the Netherlands and the low productivity levels, nutrients such as nitrogen and phosphate probably are the main resources limiting plant growth in our experiment. Complementarity between *Anthoxanthum* and *Plantago* can occur because of differences in rooting depth, probably reflecting spatial differences in nutrient uptake (Berendse 1981). In addition, it has been shown that species can differ not only spatially but also temporally in nutrient uptake (McKane *et al.* 1990). They may also differ in the uptake of different forms of nitrogen (McKane *et al.* 2002). Not every combination of species can be expected to show this kind of differentiation. At higher diversity levels, however, a

species may have a higher chance of growing next to a 'suitable' neighbour.

Complementarity may not only enhance ecosystem functioning. It is also a mechanism that facilitates long-term species coexistence (Berendse 1979; Braakhekke 1980; Lehman & Tilman 2000; Mouquet *et al.* 2002). Identifying the most important mechanisms of complementarity, and their constraints, is essential, not only to understand the effect of diversity on productivity but also to predict the conditions required for the maintenance of the biological diversity of ecosystems.

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**Relationships between diversity and productivity in  
experimental plant communities without legumes:  
initial effects, long-term patterns and underlying  
mechanisms**

Jasper van Ruijven & Frank Berendse

## ABSTRACT

The general pattern emerging from studies on the relationship between plant diversity and ecosystem functioning is that productivity increases with diversity. Most of these studies have been carried out in perennial grasslands, but many lasted only two growing seasons or reported data from a single year. Especially for perennial plant communities, however, the long-term effects of diversity are important. The question whether interactions between few species or among many species lead to increased productivity remained largely unanswered. So far, the main mechanism addressed is the increased input of nitrogen by nitrogen-fixing legumes. We report that other mechanisms can also generate strong increases of productivity with diversity. Results from four consecutive years of a plant diversity experiment without legumes show that a positive relationship between diversity and productivity emerged in the second year and strengthened with time. Different indices reveal overyielding occurred, and also increased with time. Overyielding was caused by niche complementarity. Sampling effects generally were small and often non-significant. This is illustrated by the percentage of species contributing to increased productivity at high diversity increasing from 12.5% in 2000 to more than 60% in 2003. One of the underlying mechanisms is more efficient nutrient use at high diversity. Furthermore, our results suggest that complementary nutrient uptake in space and time is important. Together these mechanisms sustain consistently high productivity at high diversity.

**Keywords:** *biodiversity, ecosystem functioning, niche complementarity, species effects, nutrient-use, productivity*

## INTRODUCTION

The notion that the current loss of biodiversity may be detrimental to ecosystem functioning has led to major experiments in the last decade. Studies investigating the relationship between biodiversity and ecosystem functioning focused on the effects of losses of plant diversity on productivity (as a measure of ecosystem functioning) in grasslands. The dominant view that emerged is that productivity declines with diversity loss (Loreau *et al.* 2002), although several different patterns have been reported, including no response and idiosyncratic differences as plant diversity decreases (see Schmid *et al.* 2002 for a review).

Both the patterns and the underlying mechanisms have been hotly debated (Aarssen 1997; Huston 1997; Huston *et al.* 2000; Hector *et al.* 2000; Loreau *et al.* 2001). A positive relationship between diversity and productivity could arise through causal mechanisms such as facilitation or complementary resource use (Tilman *et al.* 1997; Loreau 2000). Distinguishing between complementary resource use and facilitation is difficult and the term complementarity actually refers to an effect caused by differentiation in resource use and/or facilitative interactions (Loreau & Hector 2001). The same relationship between productivity and diversity, however, could also be generated by chance, through a sampling or selection effect. More diverse plant communities have a higher chance of including a highly productive species that dominates the community (Aarssen 1997; Huston 1997; Tilman *et al.* 1997). Complementarity and sampling effects may operate simultaneously, but can be separated using the additive partitioning equation (Loreau & Hector 2001).

A positive effect of diversity on productivity was reported by several experiments, but most of these studies have been short term (less than three years) or reported results from a single growing season (Hooper 1998; Hector *et al.* 1999; Spehn *et al.* 2000; Leps *et al.* 2001; Dukes 2001; Špaèková & Lepš 2001; Hector *et al.* 2002; Fridley 2002; van Ruijven & Berendse 2003; Fridley 2003). In perennial grasslands interactions between species occur over multiple years, but only three experiments reported results from a period longer than 3 years. They showed that the positive effects of diversity increased several years after the start of the experiment (Mulder *et al.* 2002; Hooper & Dukes 2004). These experiments, however, included legumes, which played an important role in overyielding (Tilman *et al.* 2001; Mulder *et al.* 2002; Hooper & Dukes 2004; Hille Ris Lambers *et al.* 2004). It is not clear if a positive relationship between diversity and productivity is a long-term property of perennial communities without the positive effects of legumes.

It is also largely unknown how many and which species contribute to this phenomenon. Resource partitioning may occur in time, space, and resource type (Berendse 1981a; McKane *et al.* 1990; McKane *et al.* 2002). It has been argued, however, that opportunities for resource complementarity are relatively limited, because plants all need the same set of basic resources (Hooper & Dukes 2004). If so, facilitation may also be important. Direct positive interactions have been demonstrated in many experiments (Callaway 1995), but the main mechanism addressed in biodiversity-productivity experiments so far, is nutrient enrichment by nitrogen fixers (Fridley 2001). Assessing the performance of individual species is crucial for understanding which mechanisms are responsible for the positive effect of diversity on productivity. Interspecific interactions like niche differentiation, facilitation, and frequency-dependent growth promote high diversity by increasing the performance of rare species (Chesson 2000; Bruno *et al.* 2003). Under these mechanisms, a range of species (including rare ones) may show increased performance with increasing diversity, thus increasing total productivity (Petchey 2000; Mouquet *et al.* 2002). A positive diversity-productivity relationship may also arise due to interspecific interactions that lead to competitive exclusion. This occurs when high-productive species, which have a higher chance of being present at high diversity (the sampling effect), out-compete other species. In contrast to the mechanisms that promote diversity, however, only a limited number of productive species will perform better at high diversity under nutrient-limitation.

Few studies have determined the performance of individual species within long-term diversity-ecosystem functioning experiments, and these studies included legumes (Hille Ris Lambers *et al.* 2004; Craine *et al.* 2003). We investigated overyielding among eight plant species. These species include two functional types: grasses and dicots. Legumes were not included. Each species was present in monoculture and at each level of diversity. This experimental set up provided an ideal opportunity to determine the performance of individual species in relation to diversity and their contribution to the relationship between productivity and diversity over time.

## **MATERIAL & METHODS**

### **Experimental design**

Plots were established on an arable field in early spring 2000. In each plot, the topsoil was removed to a depth of 45cm. At this depth the mineral sand layer below the arable soil was reached. Wooden frames measuring 1 x 1 x 0.5m (l x w x h) were placed in each hole and filled with a mixture of pure sand and soil from an old field (3:1). Seeds were laid to germinate in the greenhouse and

after three weeks seedlings were planted. In total, 144 seedlings were planted per plot in a substitutive design (i.e. each plot had the same total seedling density). During the first three months, plots were watered regularly to avoid desiccation of the seedlings. The experiment constituted of 102 plots of 1 m<sup>2</sup> distributed over six replicated blocks. Distance between plots was one meter, and blocks were two meters apart. Each block contained monocultures of all species, four mixtures of two and four species, and an eight species mixture. The mixtures of two and four species were assembled by constraint random selection from the species pool. Selecting a certain composition twice was not allowed in this procedure (van Ruijven & Berendse 2003). Composition was maintained throughout the experiment by removing seedlings of all other species at monthly intervals during each growing season. See van Ruijven *et al.* (2003) for further details about weeding.

Species were selected from a pool of four grass species (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., *Holcus lanatus* L.) and four dicot species (*Centaurea jacea* L., *Leucanthemum vulgare* Lamk., *Plantago lanceolata* L., *Rumex acetosa* L.). Nomenclature follows Van der Meijden (van der Meijden 1990). Species will further be referred to by their genus names. All species are C<sub>3</sub> perennials and commonly coexist in European hay meadows. Each species flowered and produced seeds each year, and established from seed during the experiment. Species are capable of clonal growth, mostly by short stolons growing close to the plant, either above or belowground. These structures often are long-lived (> 2 years) and also serve as storage organs. Only *Agrostis capillaris* and *Festuca rubra* can spread further than 10cm per year away from the parent plant via extensive belowground stems (Klimeš & Klimešová 1999). Rooting depth of grasses is generally less than that of dicots (Berendse 1982; Wardle & Peltzer 2003), but accurate information about individual species is scarce. *Centaurea jacea* is known to form a large perennial rootstock that is important for clonal expansion (Hartemink *et al.* 2004).

### Data collection

The analysis is based on data of aboveground biomass. Total aboveground net primary productivity was measured by harvesting all plant material after the vegetation had reached peak-standing biomass. As all aboveground tissue is new each year and every species is present throughout the growing season, aboveground biomass at the end of the growing season gives a reasonable estimate of total plot production. In August 2000, 2001, 2002 and 2003, plants were clipped to 2.5 cm above the soil surface, sorted to species, and dried for at least 48 hrs at 70° C prior to weighing. To avoid confounding edge effects, plots were divided into a center

of 60 by 60 cm and a surrounding edge. Only data from the centers were used for the analysis. For comparison, biomass per species at different diversity levels was standardized per plot by multiplying species yield with the number of species present.

### Measures of overyielding

Most measures of complementarity actually measure overyielding. It is defined as the production of mixtures exceeding expectations based on monoculture performance. We used several indices that address overyielding. For each species, we used monoculture biomass within blocks instead of using mean monoculture biomass to generate expected values (Hooper 1998; Hooper & Duker 2004). The relative yield total (RYT) measures overyielding by summing the relative yields (RY) of all species in a mixture. The relative yield of a species is calculated as

$$RY_i = Y_i / M_i$$

in which  $Y_i$  is the yield of species  $i$  in mixture and  $M_i$  is its monoculture yield.  $RYT > 1$  indicates complementarity. RYT was originally proposed as the most appropriate measure of niche complementarity (de Wit & van den Bergh 1965) and is one of the most common metrics for assessing overyielding (Fridley 2001). It is a robust measure when planting densities give constant final yield, sufficient time has been allowed for interactions to develop, and indices are calculated on a yield per area basis rather than as yield per individual (de Wit 1960; Jolliffe 2000). These criteria were met for all data throughout our experiment.

RYT, however, does not incorporate the productivity of the component species. Overyielding of a low-productive species is given the same weight as that of a very productive one, which may result in a discrepancy between RYT and the observed effect of diversity on productivity when differences in productivity between species are large. Moreover, RYT does not identify a relationship between biomass in monoculture and mixture performance (the sampling effect). For these purposes, the additive partitioning method can be used (Loreau & Hector 2001). In this method, the net difference in yield for a mixture ( $\Delta Y$ ; the net effect), is equal to the observed yield ( $Y_O$ ) minus the expected yield ( $Y_E$ ):

$$\Delta Y = Y_O - Y_E$$

$Y_E$  is defined as the average monoculture yield of the component species. The net effect is assumed to be the sum of a complementarity and a sampling effect. A positive complementarity effect occurs if species yields in a mixture are on average higher than expected on the basis of the weighted average monoculture yield of the component species:

$N \times \text{mean } (\Delta RY_i) \times \text{mean } (M_i)$

in which  $N$  is the number of species and  $\Delta RY_i$  is the difference between the observed and the expected relative yield for species  $i$ . The expected relative yield of a species is calculated as  $1/N$ . Note that the mean  $(\Delta RY_i)$  is equal to  $\text{RYT} - 1$  and that the productivity of the component species is also incorporated. The sampling effect, which assumes there is a relationship between biomass in monoculture and performance in mixture is calculated as:

$N \times \text{covariance } (\Delta RY_i, M_i)$

To determine the contribution of individual species, we also determined a net effect per species ( $\Delta Y_i$ ). Its calculation is similar to the net effect in the additive partitioning method described above:

$$\Delta Y_i = Y_{O_i} - Y_{E_i}$$

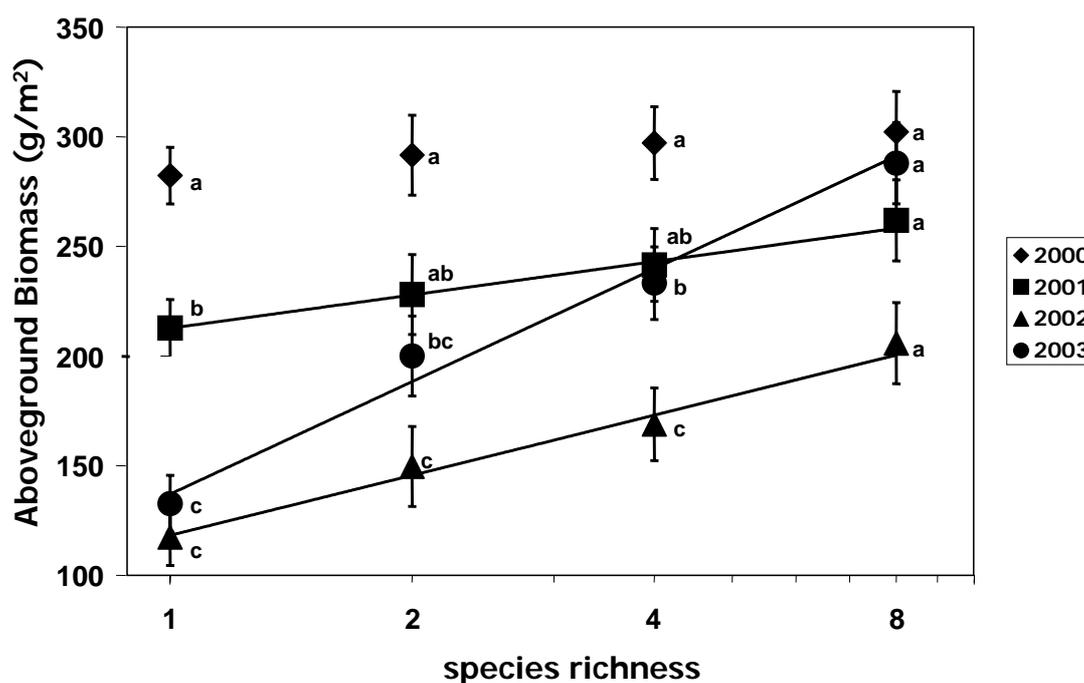
$Y_{O_i}$  is the observed yield of species  $i$  in the mixture.  $Y_{E_i}$  is the expected yield of a species in a mixture, which is determined by dividing its monoculture yield by the number of species in the mixture. Note that dividing  $\Delta Y_i$  by  $E_i$  gives the deviation from expected value  $D_i$  (Loreau 1998). The latter has the advantage that it can easily be compared across diversity treatments, but we prefer  $\Delta Y_i$  because it shows the actual contribution (in  $\text{g/m}^2$ ) to mixture performance.

### **Nutrient analysis**

In 2002, all aboveground plant samples were ground and digested with sulphuric acid, salicylic acid, hydrogen peroxide and selenium (Novozamsky *et al.* 1983). Nitrogen and phosphorus concentrations were measured colorimetrically, using a segmented flow analyzer (SKALAR SAN Plus System, the Netherlands). Potassium was measured by flame atomic emission spectroscopy (Varion SpectrAA-600, the Netherlands). Aboveground nutrient pools were determined per species by multiplying nutrient concentration to aboveground biomass. Summing over species gives the total amount of aboveground nutrients per plot. To allow comparison between different levels of diversity, the total amount of aboveground nutrients of each species was multiplied to the number of species in the plot. These figures were used to calculate RYT values for aboveground nitrogen instead of biomass. RYT-N indicates complementarity in nitrogen uptake (Berendse 1981). We determined the amount of aboveground biomass per unit aboveground nitrogen as a measure of nutrient use efficiency.

## Statistics

All relationships with diversity were determined for each year using a univariate General Linear Model (GLM) with block as random factor and  $\log^2$  of species richness as a covariate. The effect of time was determined at each diversity level using GLM Repeated Measures with block as factor. In this procedure, we used the Huynh-Feldt degrees of freedom adjustment when the sphericity assumption was violated (SPSS 10.0). Differences between years were determined using pair-wise comparisons or linear contrasts for time within this procedure. Biomass and RYT data were  $\ln$ -transformed when necessary. Values from the additive partitioning method were square root transformed when necessary but preserved their original positive and negative signs (Loreau & Hector 2001). Differences from expected values (one for RYT, zero for the other measures) were determined using t-tests at each diversity level for each year.



**Figure 3.1** Annual aboveground biomass ( $\text{g/m}^2$ ) as a function of diversity from 2000-2003. The loglinear relationship with diversity was significant for 2001-2003 (2001:  $F_{1,95} = 9.54$ ,  $P = 0.003$ ; 2002:  $F_{1,95} = 21.72$ ,  $P = 1.03\text{E-}05$ ; 2003:  $F_{1,95} = 28.60$ ,  $P = 6.12\text{E-}07$ ). Different letters denote significant ( $P < 0.05$ ) differences between years at that diversity level (LSD multiple pair-wise comparisons within GLM Repeated Measures). Data show means  $\pm$  se.

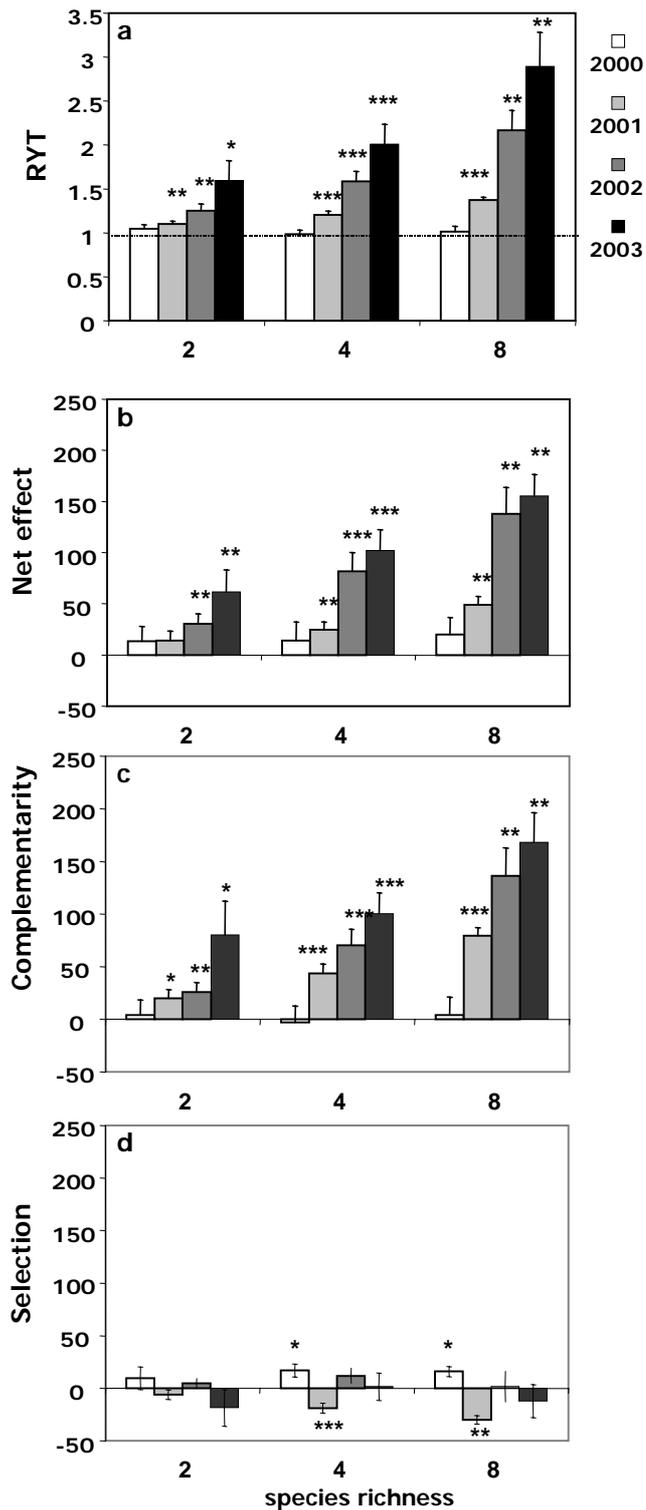
## RESULTS

There was no relationship between diversity and aboveground biomass in the first year, but from the second year (2001) onwards, species richness enhanced productivity. Overall, aboveground productivity decreased with time from 2000 until 2002, although this decrease was not significant at the highest level of diversity. In 2003, especially four and eight species mixtures showed an increase of productivity compared to 2002 (Fig. 3.1). This resulted in a stronger increase of productivity with diversity, as indicated by significant interaction between year and species richness (Repeated measures 2001-2003: time  $\times$   $\log^2$ richness,  $F_{2,93} = 11.59$ ;  $P = 3.2E-05$ ).

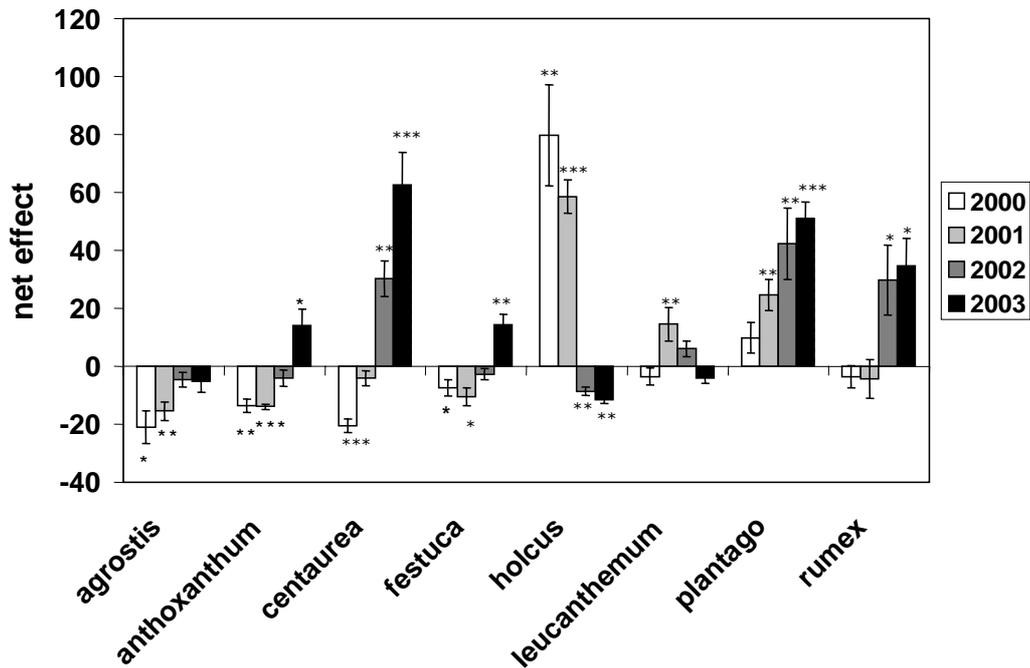
RYT was not different from one in 2000, but was significantly higher than one at each diversity level in the following years. In each of these years, RYT increased log-linearly with diversity and increased linearly with time at each diversity level (Fig. 3.2a). The net effect and the complementarity effect, calculated using the additive partition method, showed similar patterns as RYT. The net effect increased with diversity in 2001, 2002 and 2003, and the complementarity effect increased with diversity in 2001 and 2002. Both effects increased with time at each diversity level (Fig. 3.2b,c). This already indicates that the complementarity effect prevailed. Sampling effects were generally small. A positive sampling effect occurred in four and eight species mixtures in 2000, whereas the same mixtures showed a negative sampling effect in 2001. The sampling effect decreased with diversity in 2001. No relationship with time could be detected (Fig. 3.2d).

The net effects per species shifted with time. In 2000, only one species (*Holcus*) performed better than expected at the highest level of diversity, whereas four species (*Agrostis*, *Anthoxanthum*, *Centaurea* and *Festuca*) performed worse. The contribution of *Holcus* declined with time, however, and turned negative in the last two years. Simultaneously, all species, except *Leucanthemum*, increased their contribution. In 2003, five species contributed significantly more to mixture biomass than expected based on their monoculture performance (Fig. 3.3).

During four years, large shifts in biomass of individual species occurred. All species except *Centaurea* and *Rumex* showed a decrease of monoculture biomass with time (data not shown). The identity of the most productive monoculture depended on the year considered. In 2000 *Agrostis* showed significantly higher biomass than all other species except *Plantago* and *Holcus*, whereas in 2001 *Festuca*, *Centaurea* and *Agrostis* had higher biomass than the other species. In 2002, monoculture biomass of *Centaurea* was higher than that of all other species except *Agrostis* and *Festuca*.



**Figure 3.2** Results of different measures of complementarity from 2000 to 2003. **a)** RYT increased with diversity in 2001, 2002 and 2003 and increased with time at each level of diversity ( $P < 0.05$ ). **b)** The net effect increased with diversity in 2002 and 2003, and increased with time at each level of diversity ( $P < 0.05$ ). **c)** The complementarity effect increased with diversity in 2001 and 2002, and increased with time at each level of diversity ( $P < 0.05$ ). **d)** The sampling effect decreased with diversity in 2001 ( $P < 0.05$ ). Asterisks indicate significant difference from zero. \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ . Data show means  $\pm$  se.



**Figure 3.3** Net effect of each species separately, shown for the 8 species mixtures from 2000 to 2003. Asterisks indicate significant differences from zero. \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ . Data show means  $\pm$  se.

In 2003, *Centaurea* showed significantly higher biomass than any other species (Tukey tests;  $P < 0.05$ ).

Mixtures were initially dominated by the fast-growing *Holcus*, which showed a strong increase of aboveground biomass with diversity in 2000. In 2001, it still showed this positive relationship, but its biomass had decreased at all levels of diversity. In 2002 and 2003, the biomass of *Holcus* suddenly decreased with diversity (Table 3.1), although its monoculture performance did not decrease further. Initially, *Holcus* seemed to out-compete the other species. Two species (*Agrostis* and *Anthoxanthum*) showed a negative relationship between diversity and aboveground biomass in 2000 and 2001. In 2002, however, both species performed equally well at each level of diversity and in 2003, *Agrostis* biomass stabilized at its level of 2002, whereas the performance of *Anthoxanthum* increased with diversity (Table 3.1). The other species that decreased with diversity in 2000 (*Centaurea*) also showed a strong shift: its biomass in mixtures increased with time. As a result, the relationship between diversity and biomass of *Centaurea* was neutral in 2001 and became increasingly positive in 2002 and 2003 (Table 3.1). This species dominated the mixtures in the last two years.

**Table 3.1** Relationships between plant biomass and diversity. Calculated for each species and year separately, from 2000 to 2003.  $\text{Log}^2(\text{species richness})$  was included as covariate in univariate GLM with block as random factor. s = sign of the linear relationship. F and P values for diversity are shown. Block effects were non-significant and are not shown. Significant P values are in bold.

species	2000			2001		
	s	F	P	s	F	P
<i>Agrostis</i>	-	6.15	<b>0.022</b>	-	13.43	<b>0.001</b>
<i>Anthoxanthum</i>	-	25.98	<b>5.5E-05</b>	-	21.71	<b>1.5E-04</b>
<i>Centaurea</i>	-	23.04	<b>8.6E-05</b>		0.32	0.575
<i>Festuca</i>		0.82	0.375		1.17	0.290
<i>Holcus</i>	+	27.33	<b>3.0E-04</b>	+	64.73	<b>5.4E-05</b>
<i>Leucanthemum</i>		0.63	0.436	+	6.60	<b>0.017</b>
<i>Plantago</i>		0.85	0.368	+	20.54	<b>1.8E-04</b>
<i>Rumex</i>		1.16	0.295		2.29	0.085

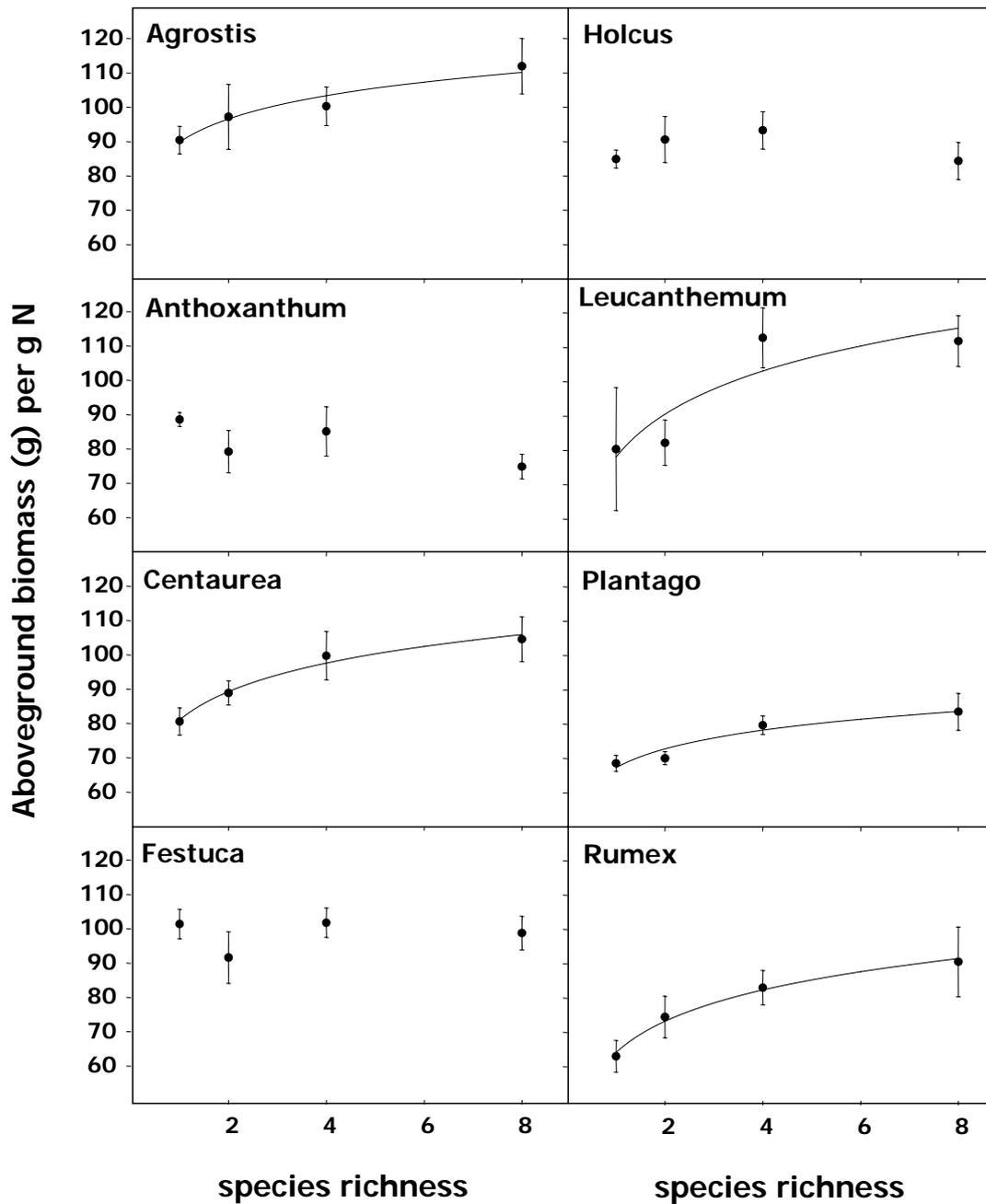
  

	2002			2003		
	s	F	P	s	F	P
<i>Agrostis</i>		0.50	0.489		0.27	0.609
<i>Anthoxanthum</i>		1.21	0.284	+	4.86	<b>0.038</b>
<i>Centaurea</i>	+	12.72	<b>0.002</b>	+	12.60	<b>0.002</b>
<i>Festuca</i>		0.22	0.646	+	6.06	<b>0.021</b>
<i>Holcus</i>	-	9.79	<b>0.005</b>	-	42.17	<b>1.3E-06</b>
<i>Leucanthemum</i>		3.70	0.067	-	10.75	<b>0.003</b>
<i>Plantago</i>	+	16.71	<b>0.001</b>	+	86.17	<b>3.1E-09</b>
<i>Rumex</i>	+	9.54	<b>0.006</b>	+	6.54	<b>0.018</b>

Two other dicot species (*Leucanthemum* and *Plantago*) showed no relationship between diversity and aboveground biomass in the first year, but their biomass increased with diversity in 2001.

The last two years, however, their patterns diverged. *Leucanthemum* biomass dropped greatly at all levels of diversity in 2002 and further decreased with diversity in 2003 (Table 3.1). Biomass of *Plantago*, however, remained fairly constant: in both years it showed an increase with diversity similar to 2001 (Table 3.1). Two species (*Festuca* and *Rumex*) showed no obvious patterns, although both species showed weak but significant increases of aboveground biomass with diversity in the last years (in 2003, and in 2002 and 2003, respectively; Table 3.1).

The ratio of nitrogen (N) to phosphorus (P) in aboveground biomass is considered a useful predictor of N or P limitation. Values of N:P < 14 are generally considered to indicate N-limitation (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003). In 2002, values ranged from  $3.54 \pm 0.09$  (*Leucanthemum*) to  $6.05 \pm 0.15$  for *Rumex* in our experiment. Considering these low values, plants are assumed to be N-limited. Therefore, we focus on nitrogen in our analysis. Total aboveground nitrogen showed patterns similar to plant biomass in 2002: *Centaurea* and *Plantago* showed a log-linear increase of total aboveground N with diversity ( $P < 0.05$  and  $P <$



**Figure 3.4** Amount of aboveground biomass (gram) per gram nitrogen, shown for each species in 2002. *Agrostis*:  $F = 4.92$ ,  $P < 0.05$ , *Centaurea*:  $F = 8.00$ ,  $P < 0.01$ . *Leucanthemum*:  $F = 6.17$ ,  $P < 0.05$ . *Plantago*:  $F = 12.31$ ,  $P < 0.01$ . *Rumex*:  $F = 10.30$ ,  $P < 0.01$ . *Anthoxanthum*, *Festuca* and *Holcus*: non-significant. Data show means  $\pm$  se.

0.001, respectively) whereas *Holcus* showed a log-linear decrease ( $P < 0.01$ ). The other species showed no relationship between aboveground N and diversity. As a result, the total amount of aboveground nitrogen per plot increased with diversity ( $P < 0.01$ ). This was confirmed by applying the RYT approach to aboveground amounts of N. At each level of diversity, RYT-N values were significantly ( $P < 0.05$ ) higher than one.

Interestingly, the amount of aboveground biomass produced per unit aboveground nitrogen also increased with diversity for several species. All dicot species and one grass species (*Agrostis*) showed this pattern. The other three grass species showed a neutral relationship between the amount of biomass per unit nitrogen and species richness (Fig. 3.4). Phosphorus and potassium budgets showed similar patterns, although less pronounced.

## DISCUSSION

In our experiment, a positive relationship between diversity and plant productivity appeared in the second year and became increasingly positive as the experiment continued. This pattern has also been shown in other experiments (Tilman *et al.* 2001; Mulder *et al.* 2002; Hooper & Duker 2004), but in these experiments legumes appeared to be the most important group responsible for this increase. Our results show that similar patterns arise in the absence of legumes.

Different indices revealed that complementarity in resource use and/or facilitative interactions were the main driver(s) of increased productivity at higher levels of diversity. RYT values increased with diversity and with time. In 2003, two-species mixtures had an average value of 1.60. An extensive review found that more than 95% of two and three species mixtures without legumes had RYT < 1.3 (Trenbath 1974). RYT of eight-species mixtures approached unusual values of 3.0 in our experiment. Complementarity effects also increased both with diversity and with time, and were significantly greater than zero since 2001. Sampling effects were small and often non-significant.

In the first year, competitive exclusion dominated the interactions in the experimental plant communities. *Holcus* appeared to out-compete the other species as its strong increase of aboveground biomass with diversity coincided with decreases in biomass of several other species. Probably, its higher growth rate (Grime & Hunt 1975) allowed *Holcus* to effectively forage for nutrients in large parts of the initially unoccupied soil volume, at least in the upper layer. The depletion of nutrients in the upper layer probably forced especially the dicot species to increase their rooting depth (Jumpponen *et al.* 2002; Wardle & Peltzer 2003). From 2001, interspecific interactions that promote diversity became important. Several dicot species started to show an increase of biomass with diversity, which may be the result of complementarity in rooting depth between the dominant grass species and these dicots (Berendse 1981a; Fitter 1986; McKane *et al.* 1990). This is confirmed by the increase of aboveground N with diversity shown by two of these dicot species (*Centaurea* and *Plantago*) and the positive RYT-N values in 2002. Importantly, several species used nutrients more efficiently at high diversity, as shown by the increase of aboveground biomass per unit nitrogen with diversity. In the last year, five species showed an increase of aboveground biomass with diversity, which strongly suggests that the mechanisms described above continued to determine plot productivity. We propose that the productivity of mixtures is increased by complementarity in rooting depth and by the increased production of aboveground biomass per unit nitrogen at high

diversity. Apart from complementarity in rooting depth, temporal differences in nutrient uptake may also be important (McKane *et al.* 1990; Hooper & Vitousek 1998). For two species present in our experiment (*Anthoxanthum* and *Plantago*), it has been shown that they differ in rooting depth, and also in their main periods of nitrogen uptake (Berendse 1981b). Plants may also facilitate each other, both directly by ameliorating harsh environmental conditions, altering substrate characteristics, or increasing resource availability, and indirectly by stimulating beneficial organisms such as mycorrhizal fungi, or by providing protection from herbivores (Callaway 1995). However, we found no evidence for these types of facilitative interactions. In the unusually warm and dry summer of 2003, decreased evaporation from the soil due to increased plant cover with increasing diversity may have been important (Caldeira *et al.* 2001).

Similar to an earlier experiment (Niklaus *et al.* 2001), the importance of the individual species changed during the development of the experimental plots. Initially, plots were dominated by a fast-growing species (*Holcus*) which appeared to outcompete the other species. In 2003, *Centaurea* dominated mixtures, but the aboveground biomass of several other species also increased with diversity, thus contributing to increased productivity of mixtures. Because of its high monoculture biomass, the contribution of *Centaurea* was mainly attributed to the sampling effect. Still, its increase may be caused by increased complementarity in resource use. The sampling effect is not independent of complementarity: if resource partitioning also facilitates more productive species, some complementarity may be attributed to the sampling effect (Petchey 2003). As such, the complementarity effect we report may be conservative as a measure of complementarity.

Two species (*Holcus* and *Leucanthemum*) showed a positive relationship between aboveground biomass and diversity in the first years, but they appeared to be competitively excluded from the mixtures in the last years. *Holcus* has a relatively high turnover rate, which requires high nutrient availability. This availability, however, has probably declined because plant roots have occupied and depleted most of the soil after two years. Moreover, nutrients allocated aboveground are removed from the plot by harvesting aboveground biomass each year. We propose that *Holcus* is out-competed by other species that invested in roots adapted to low nutrient availability (i.e. long-lived roots; van der Krift & Berendse 2002). It can only survive in monoculture because all other species are continuously removed from those plots. Biomass of *Leucanthemum* abruptly declined independent of diversity from 2001 to 2002. Similar to *Holcus*, it maintained low biomass in monoculture, but almost disappeared from mixtures in 2003. In

contrast to *Holcus*, however, its growth and turnover rates are not different from the other species. Its decline may be linked to plant-parasitic nematodes. We found high numbers of two taxa of these nematodes in plots with *Leucanthemum* at each level of diversity in 2002 (van Ruijven *et al.* 2003). The pattern for one of these taxa (*Trichodoridae*) was already present in 2001, and is therefore unlikely to have caused the sudden decline observed in 2002. The other taxon (*Rotylenchus*), however, could only be detected in very low numbers in monocultures of *Leucanthemum* in 2001. We found on average four individuals per 100g of soil in monocultures in 2001, but more than 900 individuals in 2002. Accumulation of pathogens may severely limit plant growth (Klironomos 2002), resulting in low aboveground biomass. In mixtures, interactions with other plant species may cause plants that suffer from pathogens and parasites - like *Leucanthemum* in our experiment - to decline (De Deyn *et al.* 2003). At spatial scales greater than in our experiment, however, *Holcus* and *Leucanthemum* may have persisted in the community and perhaps have continued to contribute to increased productivity. Increased spatial heterogeneity may allow these species to re-colonize patches with high nitrogen availability and low pathogen pressure, respectively.

## Conclusions

In the year of establishment, interspecific interactions were dominated by competitive exclusion, as shown by the strong expansion of one fast-growing species and the decline of many other species. Already in the second year, however, productivity increased with diversity. Our results show that this positive relationship between diversity and productivity strengthens with time in perennial communities. In contrast to other experiments, this pattern cannot be attributed to increased nitrogen input due to the presence of legumes. The patterns observed were caused by increased niche differentiation and/or facilitation, as shown by increasing values of RYT, the complementarity effect and the number of plant species that contributed to increased productivity at higher levels of diversity. The main mechanisms we detected were increased nutrient-use efficiency of several species at higher levels of diversity and probably complementarity in rooting depth, which enabled the diverse communities to acquire greater amounts of nutrients. Thus, the interspecific interactions that promote diversity by increasing the population growth rate of rare species, also sustain high productivity at high plant diversity.

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van Ruijven J., De Deyn G.B. & Berendse F. (2003) Diversity reduces invasibility in experimental plant communities: the role of plant species. *Ecology Letters* 6, 910-918

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**Diversity reduces invasibility in experimental plant  
communities: the role of plant species**

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## ABSTRACT

Several studies have presented experimental evidence that diversity reduces invasibility in grassland communities. The interpretation of these results has been disputed recently and it was proposed that sampling effects were responsible for the observed decrease of invasibility with diversity. The experiments performed to date were not designed to adequately separate sampling from diversity effects. Using the establishment of native plant species in experimental plant communities as a model of invasibility, we show that the number of invaders decreased with increasing diversity. When the presence of particular species is included, their effects are dominant. *Centaurea jacea* showed a strong effect at low diversity, whereas *Leucanthemum vulgare* showed a very strong negative impact at each diversity level. The negative effect of the latter might be related to root-feeding nematodes that showed far higher abundance in plots with *Leucanthemum*. However, diversity remained a significant factor in determining the number of invading species and the numbers of an abundant invader.

**Keywords:** *species richness, invasion resistance, sampling effect, plant-specific effects, negative interactions, Leucanthemum vulgare, Centaurea jacea, plant-feeding nematodes*

## INTRODUCTION

The invasion of exotic species into natural communities is a pervasive and widespread phenomenon, which can have serious ecological consequences (Williamson 1999; Mack *et al.* 2000; Pimental *et al.* 2000). Many hypotheses have been put forward to explain why susceptibility to invasion differs between communities. One of the earliest theories was Elton's hypothesis that lower resident diversity favours invasion (Elton 1958). Recently, several studies have claimed to present experimental evidence that diversity reduces invasibility (Naeem *et al.* 2000; Levine 2000; Prieur-Richard *et al.* 2000; Hector *et al.* 2001; Kennedy *et al.* 2002). Most observational studies, however, failed to identify diversity as an important barrier against invasion. In contrast, many studies showed a positive correlation between diversity and invasibility (see Levine *et al.* 2002). Fluctuations in resource availability due to e.g. disturbance, herbivory, eutrophication were identified as the key factor controlling invasibility (Davis *et al.* 2000). The most likely explanation for these conflicting results is the covariance between extrinsic factors (i.e. disturbance, grazing, nutrient levels) and resident diversity in observational studies (Naeem *et al.* 2000). This covariance is removed in most experimental studies (Levine & D'Antonio 1999).

In the experimental studies, resource use complementarity is often proposed as the mechanism responsible for the negative impact of diversity on invasibility. The utilization of resources (mainly light and nutrients) increases with diversity, resulting in fewer resources being available for invaders (Knops *et al.* 1999; Naeem *et al.* 2000). The sampling effect (Aarssen 1997; Huston 1997) was proposed as an alternative explanation for the results obtained in the experimental studies on invasibility. It was argued that the observed decrease of invasion success with increasing diversity could be caused by an increasing occurrence of a highly suppressive species (Wardle 2001).

Species can be suppressive due to their competitive dominance, but less common species can also significantly influence invasion (Lyons & Schwartz 2001). Specific interactions such as allelopathy (Preston *et al.* 2002) and associations with mycorrhizal fungi (Marler *et al.* 1999; Stampe & Daehler 2003) or pathogens (Mills & Bever 1998; Holah & Alexander 1999) may then be important.

To our knowledge, most experiments performed to date were not designed to adequately separate sampling from diversity effects. As a consequence, it is yet unknown if a decrease of diversity can actually promote invasibility. We report results from an experiment in which grassland communities ranging in plant diversity from one to eight species were created experimentally. Importantly, monocultures of all species from the species pool are present in the

experiment. This enabled us to identify suppressive species and test for their effects. In the experiment species from the local seed rain germinated and established. These plants were weeded out and their numbers were used to address the following questions: 1) does increasing diversity result in decreasing invasibility? And 2), should this phenomenon be attributed to diversity, to particular species, or both?

## **MATERIAL & METHODS**

Spring 2000 102 plots of one m<sup>2</sup> were established in a randomised block design on a nutrient-poor sandy soil. Each of the six blocks contained all diversity treatments: monocultures of all species and mixtures of two, four and eight species. The species pool consisted of four grass species (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., and *Holcus lanatus* L.) and four forbs (*Centaurea jacea* L., *Leucanthemum vulgare* Lamk., *Plantago lanceolata* L., and *Rumex acetosa* L.). Mixtures were created by constraint random selection from the pool: replication of a particular composition was not allowed and all species should be present in an equal number of plots. For detailed information on the experiment see Van Ruijven & Berendse (2003).

The original composition in the plots was maintained by weeding: all other species were removed by hand on approximately monthly intervals. It may be argued that the patterns we show are a result of weeding: if low diversity plots are weeded more than high diversity plots, this could explain the observed patterns in invasibility. However, we weeded each plot once at each interval. Due to intervals of three to six weeks, invading species were still small seedlings at the time of weeding. As a consequence, disturbance due to weeding is limited. Moreover, experimental plots of a study that showed similar patterns were not weeded prior to measurements of invasibility (Kennedy *et al.* 2002).

The plants collected from the inner 60 by 60cm of each plot were used for this analysis. Weeding was done in the last week of August 2002, immediately after harvest of aboveground biomass. The last weeding round had been in the last week of June. These results thus describe germination and establishment from the summer seed rain. Seedlings were counted per species. Species from the species pool ('internal invaders' following Knops *et al.* 1999) were omitted from the analysis, because they are not equally distributed across diversity levels. In monocultures, up to seven species of the species pool would be regarded as invaders, whereas in eight-species mixtures no species would (see also Hector *et al.* 2001).

The number of 'external' species and of external individuals, as well as the number of individuals of particular abundant external species were analysed using a General Linear Model (GLM) with block as

random factor and species richness as fixed factor. External species and external individuals will be further referred to as invading species and invaders, respectively. The relationship with diversity was determined by including species number as a factor in a regression analysis combined with a curve estimation module (SPSS 10.0). This test included two models: linear and loglinear.

Increasing diversity has several consequences, like an increase of biomass (Hector *et al.* 1999; Tilman *et al.* 2001; Van Ruijven & Berendse 2003) a reduction of light intensity at the soil and reduced nutrient availability (Knops *et al.* 1999), which may also affect invasibility. To test for these effects, we included aboveground biomass, light intensity at the soil surface and soil nutrient concentrations as covariates in an extended model. All measurements were done in the last week of August 2002, just before weeding. The biomass of the vegetation was determined by clipping the same 60 by 60cm area at two cm above the soil surface. Light intensities at the soil surface and soil nutrient concentrations were measured prior to clipping. Light intensity was measured as the relative amount of light (PAR) corrected for the PAR above the vegetation. It was measured using a light probe (Sun Scan Canopy Analyses system, Delta T Devices Ltd, UK). The light probe had a light sensitive area of 2 cm x 1 m, but only data from the cells placed inside the centre of plots were used. Soil nutrient concentrations were determined by pooling 12 samples per plot. A sample consisted of a soil core (one cm diameter) taken to a depth of 10 cm. Soil cores had to be taken in the edges of plots because inside the centre of plots the impact of 12 soil cores each year would probably have too great an impact on plant productivity. Nutrient concentrations were determined on 30 gram sub-samples after extraction with 0.01 M CaCl<sub>2</sub> (Houba *et al.* 1994).

Effects of particular species were determined in two steps. First, differences in invasibility between monocultures were determined using a GLM with block as random factor and species identity as factor. Significant differences between species were determined using a Tukey HSD post hoc test. Second, we selected species that differed markedly from other species according to these tests and included the presence of these species as covariates in the extended model. In this model we had to omit the eight-species plots, because in these plots each species from the species pool is always present. We included the interaction term between richness and presence of species in the model, because preliminary data showed differential effects of species across diversity levels. The effect of presence was tested separately at each diversity level using a GLM with block as a random factor and presence of species as covariates. In all tests, the number of invading species and the number of invaders were arcsinh transformed prior to analysis.

Including a covariate that is correlated with treatment factors in an ANOVA results in an unbalanced design. Correlated explanatory variables are typical of biodiversity experiments because different components of diversity tend to have co-linear effects (Allison 1999; Hector *et al.* 2001; Schmid *et al.* 2002). As a consequence, the variation explained by different terms cannot be uniquely separated into additive components. Because the imbalance in biodiversity experiments is often large, the best approach probably is to use sequential (type I) sums of squares (SS). When fitting general linear models in this way, the sequence in which the terms are entered into the analysis may change the statistical significance attached to these terms. Because there is no answer to which sequence is best, it is useful to use different sequences to explore the data (Schmid *et al.* 2002). We decided to use this approach for our extended model and to combine it with an approach of backward exclusion that was recently used in another biodiversity experiment (Craine *et al.* 2003). In this approach, all explanatory variables are included initially in the model. Then, non-significant ( $P \geq 0.01$ ) terms are removed serially in order of increasing  $F$ -value, and new values are calculated each time a term is removed. A more stringent than usual test of significance was used because of the potential for false effects.

We started the first sequence with block followed by species richness and the different covariates (plot biomass, captured PAR, soil nutrient concentrations and presence of particular species). The second sequence started with the terms that were removed from the first sequence (in order of increasing  $F$ -value) and was completed with the sequence of terms that remained after exclusions from the first sequence. Those terms that showed significance in both sequences were included in a third sequence, in which their initial order was reversed. This procedure was repeated for four variables: number of invading species, total number of invaders and the number of *Taraxacum* and *Erigeron* invaders.

Because interactions with the soil community may be important we sampled root-feeding nematodes, being an abundant and diverse group of soil organisms. It is known that nematodes can affect plant competition and succession in grasslands (Verschoor *et al.* 2002; De Deyn *et al.* 2003). Nematodes were extracted from a 100 ml subsample of the same 12 soil cores that were used for soil analysis. They were extracted with Oostenbrink elutriators (Oostenbrink 1960) and incubated for 48 hours on a double cotton wool filter (Hygia milac filter). The plant roots remaining on the funnel top sieve were extracted following the mistifier method ('s Jacob & van Bezooijen 1987). Nematodes from soil and roots were compiled per plot, concentrated in 2 ml and heat-killed and fixed in 8 ml of 4% formalin. All nematodes in 1 ml sub-samples were counted under an inverted microscope (x100-400) and identified to

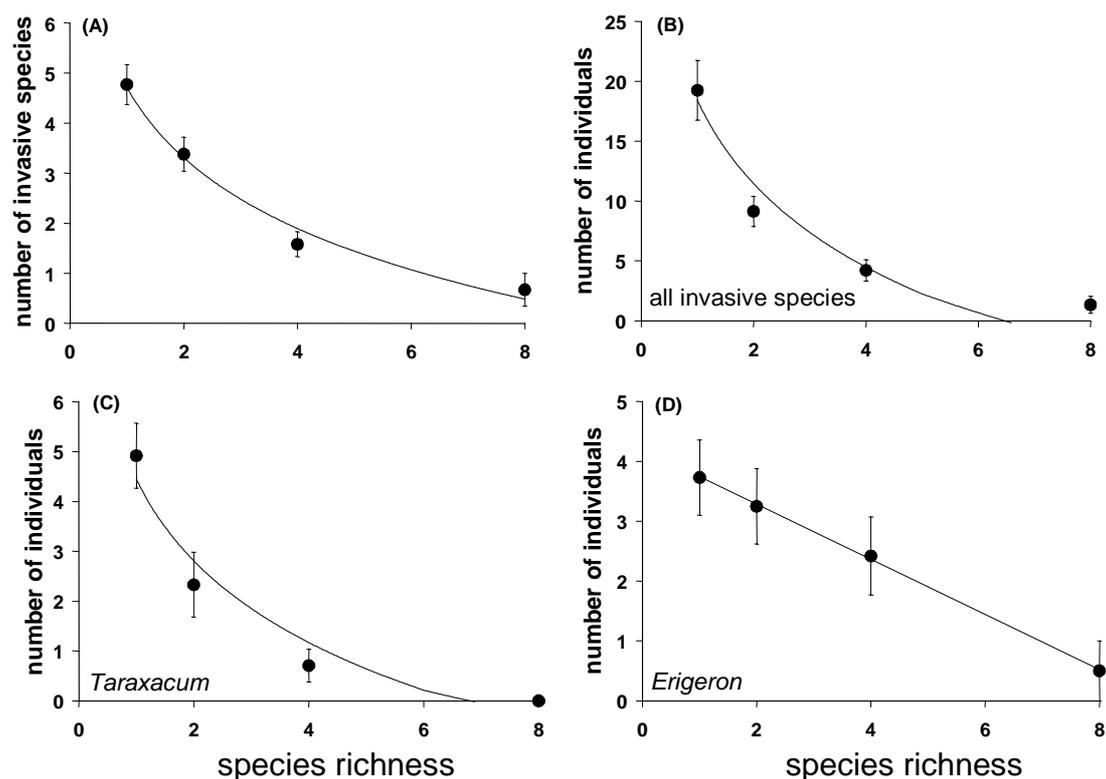
genus level. Numbers were expressed per 100 g of soil fresh weight. These numbers were square-root transformed prior to statistical analysis. Effect of plant species on the abundance of nematodes was tested by using a GLM at each diversity level with block as random factor and presence of plant species as a covariate.

**Table 4.1** External invader species and their number of individuals found in the experimental plots

Invasive species	Total number of invaders
<i>Achillea millefolium</i>	4
<i>Betula pendula</i>	2
<i>Carex ovalis</i>	2
<i>Crataegus monogyna</i>	1
<i>Epilobium montanum</i>	142
<i>Erigeron canadensis</i>	319
<i>Geranium molle</i>	1
<i>Heracleum sphondylium</i>	1
<i>Hieracium pilosella</i>	1
<i>Juncus bufonius</i>	2
<i>Juncus effusus</i>	5
<i>Lamium purpureum</i>	2
<i>Lolium perenne</i>	6
<i>Lotus corniculatus</i>	2
<i>Luzula campestris</i>	2
<i>Oenothera biennis</i>	1
<i>Ornithopus perpusillus</i>	1
<i>Plantago major</i>	1
<i>Poa annua</i>	43
<i>Poa pratensis</i>	138
<i>Poa trivialis</i>	1
<i>Ranunculus repens</i>	3
<i>Rumex acetosella</i>	18
<i>Sagina procumbens</i>	59
<i>Salix</i> sp.	1
<i>Senecio jacobaea</i>	92
<i>Senecio vulgaris</i>	20
<i>Sonchus asper</i>	33
<i>Sorbus aucuparia</i>	2
<i>Stellaria media</i>	1
<i>Taraxacum officinale</i>	309
<i>Trifolium repens</i>	24
<i>Veronica serpyllifolia</i>	17

**Table 4.2** Results of GLM (type I SS) on four separate variables (number of invading species, total number of invaders, and number of invaders of two invasive species) analysing the effects of block and species richness on invasibility. Block is included as a random factor, species richness as a factor. <sup>ns</sup>  $P \geq 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

Source of variation	Number of species <i>F</i>	All invaders <i>F</i>	Taraxacum <i>F</i>	Erigeron <i>F</i>
Block	.46 <sup>ns</sup>	0.59 <sup>ns</sup>	1.17 <sup>ns</sup>	1.15 <sup>ns</sup>
Species richness	14.87***	13.05***	11.43***	3.39*



**Figure 4.1.** Invasibility at each diversity level. Note that graph A shows the number of invading species, whereas the other three show the number of invaders. A) number of invading species. Fitted curve  $y = 4.7718 - 2.1596 * \ln(\text{richness})$ .  $r^2 = .31$ ;  $F = 45.7$   $P < 0.001$ . B) total number of invaders. Fitted curve  $y = 18.4285 - 10.062 * \ln(\text{richness})$ .  $r^2 = .23$ ;  $F = 29.3$   $P < 0.001$ . C) number of *Taraxacum* invaders. Fitted curve  $y = 4.7332 - 2.7858 * \ln(\text{richness})$ .  $r^2 = .22$ ;  $F = 28.2$   $P < 0.001$ . D) number of *Erigeron* invaders. Fitted curve  $4.1892 - 0.4527 * \text{richness}$   $r^2 = .05$ ;  $F = 5.3$   $P < 0.05$ . Data show means  $\pm$  se.

## RESULTS

Apart from the internal species a total of 1256 seedlings belonging to 33 different species were found (table 4.1). Only one species, *Erigeron canadensis* L., is not native but it is questionable if this species can be regarded as an exotic, since it has occurred in the Netherlands for more than 200 years (Van der Meijden 1990). The number of invading species and the total number of invaders were strongly affected by plant diversity (table 4.2).

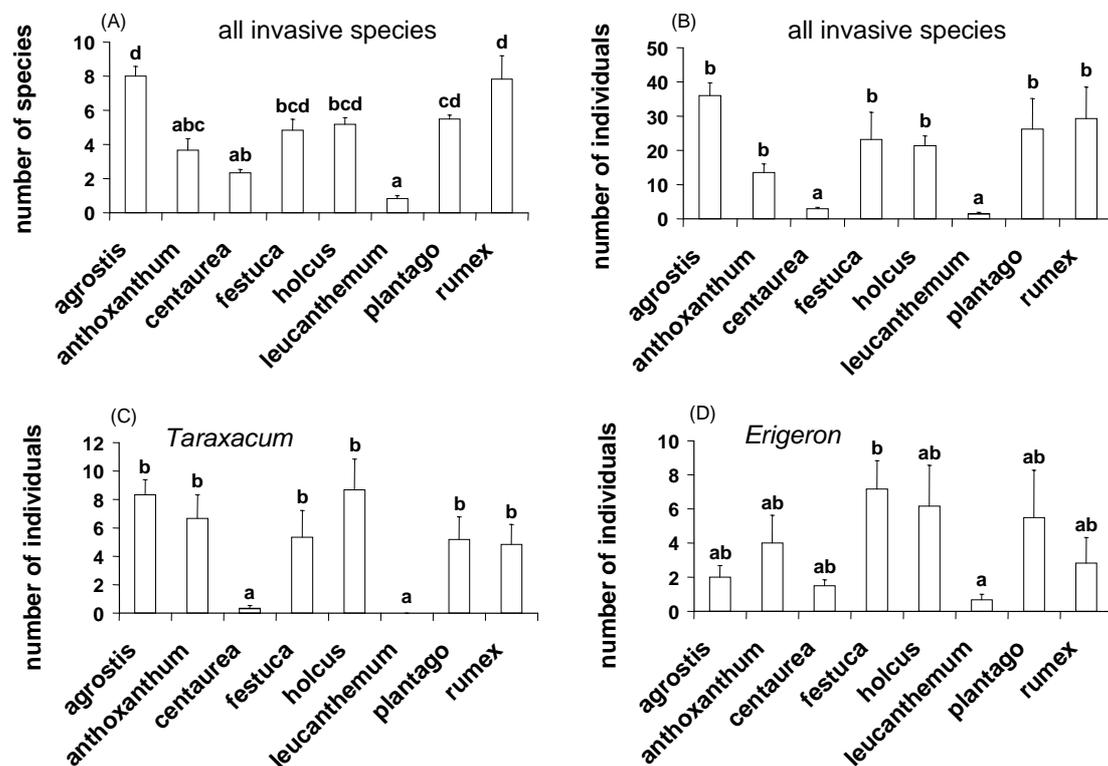
Two abundant invading species (*Taraxacum officinale* Wiggers, s.s. and *Erigeron*), of which we collected sufficient numbers to analyse them separately, showed similar results. In most cases, the relationship with diversity could best be described by a loglinear decrease, but *Erigeron* showed a linear decrease (figure 4.1). Aboveground biomass increased

log-linearly with diversity ( $F = 23.77$ ;  $P < 0.001$ ), whereas light intensity at the soil surface decreased ( $F = 6.73$ ;  $P < 0.05$ ). Soil

nutrient concentrations were not related to diversity (phosphate) or even showed a slight increase ( $P < 0.05$ ) with diversity (potassium and nitrate). Invasibility differed greatly between monocultures. In most cases, monocultures of two species (*Centaurea* and *Leucanthemum*) had significantly lower invasion than most other species (figure 4.2).

However, the number of *Erigeron* invaders was significantly lower than at least one other species only in *Leucanthemum* plots.

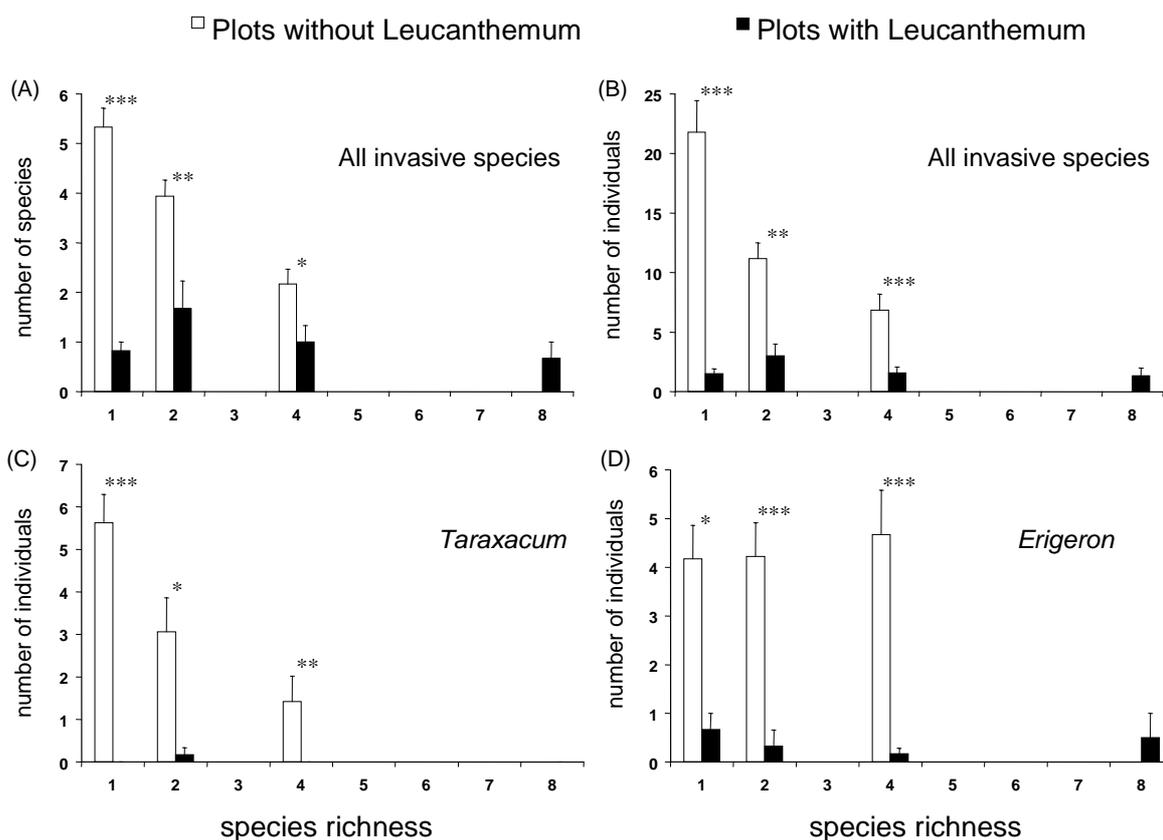
Including the covariates in the model and applying backward exclusion to different sequences greatly affected the effect of diversity (table 4.3). Only the terms that remained significant after the third exclusion procedure are shown. For clarity, we show significances from a GLM with type III SS instead of those from particular sequences in a GLM with type I SS. Terms showed roughly the same significance in both types, with the presence of the two species explaining most of the variance.



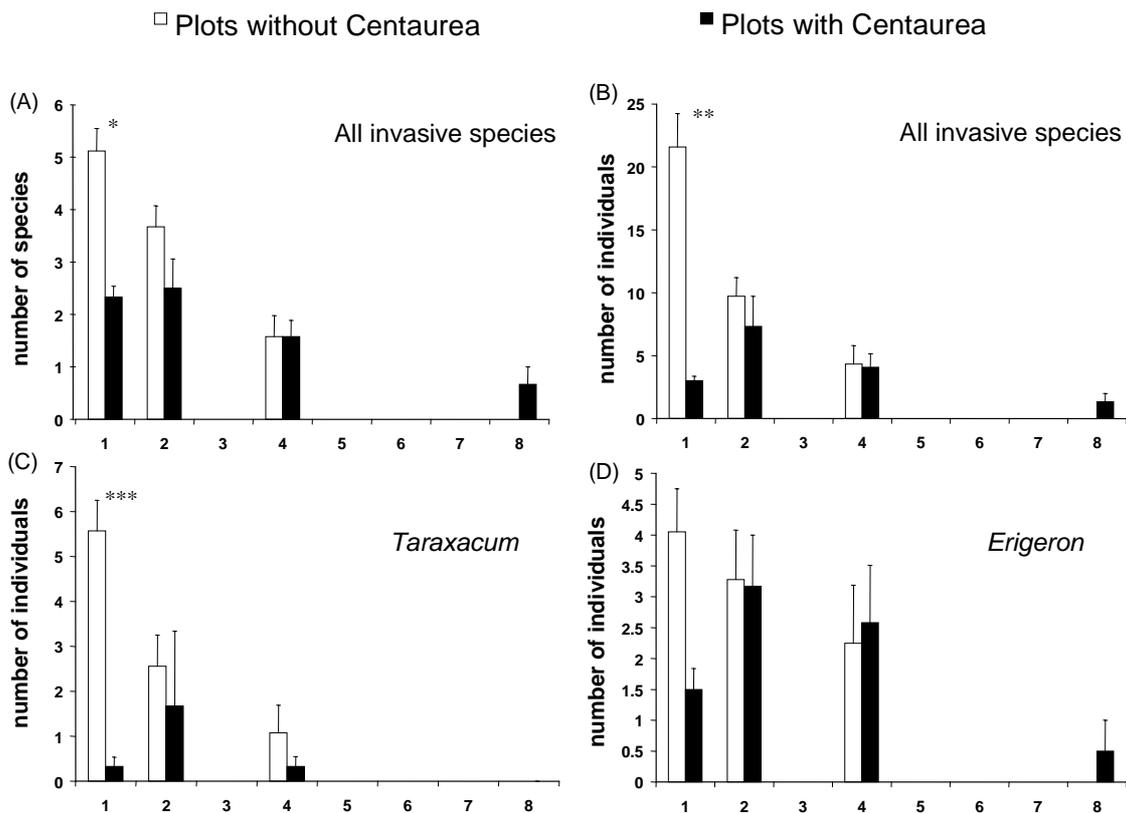
**Figure 4.2.** Invasibility in monocultures. Note that graph A shows the number of invading species, whereas the other three show number of invaders. A) number of invading species. B) total number of invaders. C) number of *Taraxacum* invaders. D) number of *Erigeron* invaders. Different letters indicate significant differences between species ( $P < 0.05$ ). Data show means  $\pm$  se.

**Table 4.3** Results of GLM (type III SS) on four separate variables (number of invading species, total number of invaders, and number of invaders of two invasive species) analysing the effects of the explanatory variables remaining after three exercises with backward exclusion. Species richness was included as a factor, light intensity at the soil surface (captured PAR) as a covariate. For the presence of suppressive species dummy variables were created. Values could either be zero (absent) or one (present). Dummy variables were also included as covariates. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Source of variation	Number of species $F$	Number of invaders $F$	Taraxacum $F$	Erigeron $F$
Species richness	7.19**	Excluded	10.76***	excluded
Captured PAR	11.51***	Excluded	22.41***	excluded
<i>Centaurea</i>	18.68***	20.66***	44.93***	excluded
<i>Leucanthemum</i>	12.24***	99.72***	20.09***	58.60***
<i>Cent. x richness</i>	Excluded	excluded	5.84**	excluded



**Figure 4.3.** Invasibility at each diversity level split into plots with or without *Leucanthemum* being present. Note that at the eight-species level *Leucanthemum* is always present. Also note that graph A shows the number of invading species, whereas the other three show number of invaders. A) number of invading species. B) total number of invaders. C) number of *Taraxacum* invaders. D) number of *Erigeron* invaders. Asterisks indicate significant differences between plots with and without *Leucanthemum* at that diversity level (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ). Data show means  $\pm$  se.



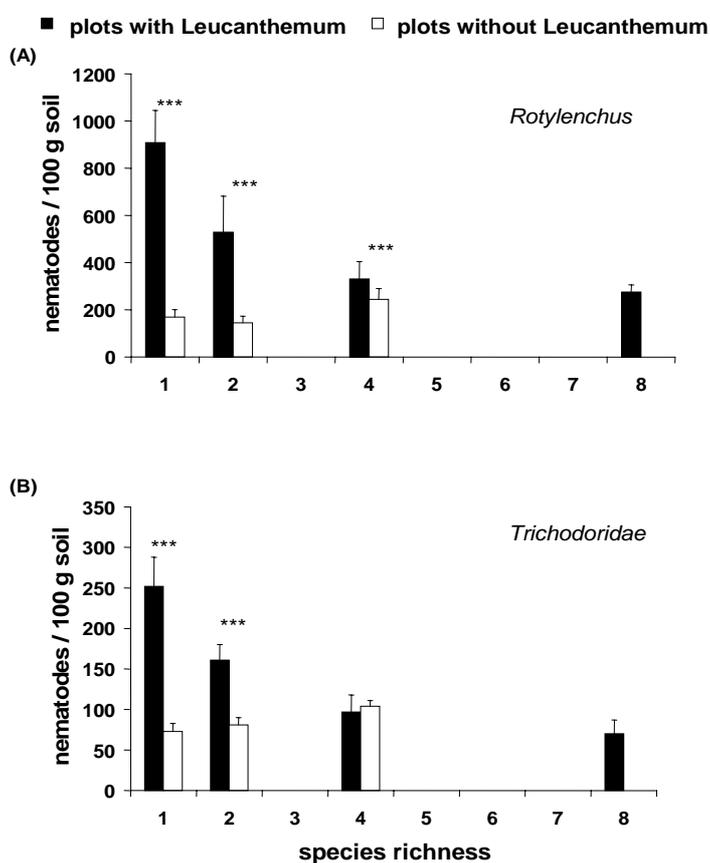
**Figure 4.4.** Invasibility at each diversity level split into plots with or without *Centaurea* being present. Note that at the eight-species level *Centaurea* is always present. Also note that graph A shows the number of invading species, whereas the other three show number of invaders. A) number of invading species. B) total number of invaders. C) number of *Taraxacum* invaders. D) number of *Erigeron* invaders. Asterisks indicate significant differences between plots with and without *Centaurea* at that diversity level (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < .001$ ). Data show means  $\pm$  se.

Block effects were always excluded in our procedure. Soil nutrient concentrations did not show significant effects unless they were entered first in a sequence. Plot biomass showed significant effects, but was also excluded when included later in the sequence. Light intensity at the soil surface only remained in two of the four variables measuring invasibility (table 4.3). Surprisingly, its effect was negative: invasion success increased with decreasing light intensity.

The number of invading species was negatively affected by the presence of *Leucanthemum* and *Centaurea*, but a significant negative effect of species richness remained. The total number of invaders turned out to be affected only by the presence of *Leucanthemum* and *Centaurea*. The number of *Taraxacum* invaders showed similar results as the number of species, but more pronounced. This species hardly occurred in plots with *Leucanthemum*. The invasion success of *Erigeron* was strongly

limited by the presence of *Leucanthemum*. All other explanatory variables (including diversity) were excluded. The effects of *Leucanthemum* and *Centaurea* on invasibility across diversity levels are shown in figure 4.3 and 4.4, respectively. The interaction term between diversity and the presence of *Centaurea* was often excluded in the procedure, but the significant effect of *Centaurea* only occurred in monocultures. In contrast, the effect of *Leucanthemum* was significant at each diversity level.

The presence of two groups of root-feeding nematodes was closely related to the presence of *Leucanthemum*. We found high numbers of nematodes belonging to the family *Trichodoridae* and to the genus *Rotylenchus* in plots with *Leucanthemum* (figure 4.5). Abundance of these nematodes showed no overall correlation with plant diversity.



**Figure 4.5** Number of *Rotylenchus* (A) and *Trichodoridae* (B) nematodes across diversity levels in plots with or without *Leucanthemum*. Note that at the eight-species level *Leucanthemum* is always present. Asterisks indicate significant differences between plots with and without *Leucanthemum* at that diversity level (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < .001$ ). Data show mean  $\pm$  se.

## DISCUSSION

Both the number of invading species and the number of invaders strongly decreased with increasing diversity. The initial models showed strong effects of species richness on all four variables measuring invasibility. These patterns are similar to results reported elsewhere (Knops *et al.* 1999; Naeem *et al.* 2000; Levine 2000; Prieur-Richard *et al.* 2000). The extended models showed highly significant effects of diversity for two of four invasibility variables, indicating that after taking into account the presence of suppressive species, other mechanisms related to diversity still decreased invasibility. It might be possible that the remaining diversity effect is caused by yet another species, but we could not detect such an effect. We included other species in our extended models, but they were often non-significant and always removed in the backward exclusion procedures. The negative effect of diversity is often explained by increased resource use: complementarity of resource use between species results in lower levels of available resources at high diversity, thus inhibiting invasion (Knops *et al.* 1999; Naeem *et al.* 2000). It has been shown that including light and nutrient levels in the analysis eliminated plant diversity as an explanatory variable (Knops *et al.* 1999). We also included light intensity and soil nutrient levels in the analysis, but they hardly had significant effects. Moreover, soil nutrient availability did not decrease with increasing diversity. It should be taken into account, however, that these data are from the top 10cm only. Especially at higher diversity, deep-rooting species may be forced to forage at greater depths in the soil, resulting in lower nutrient availability in the entire soil column, but not necessarily in the top layer. The negative impact of light intensity is hard to explain but may be correlated to the presence of *Leucanthemum*: its monocultures, for example, showed relatively high light intensities at the soil surface due to its low aboveground biomass. Another explanation may be that shading during summer is necessary for seedlings to avoid desiccation.

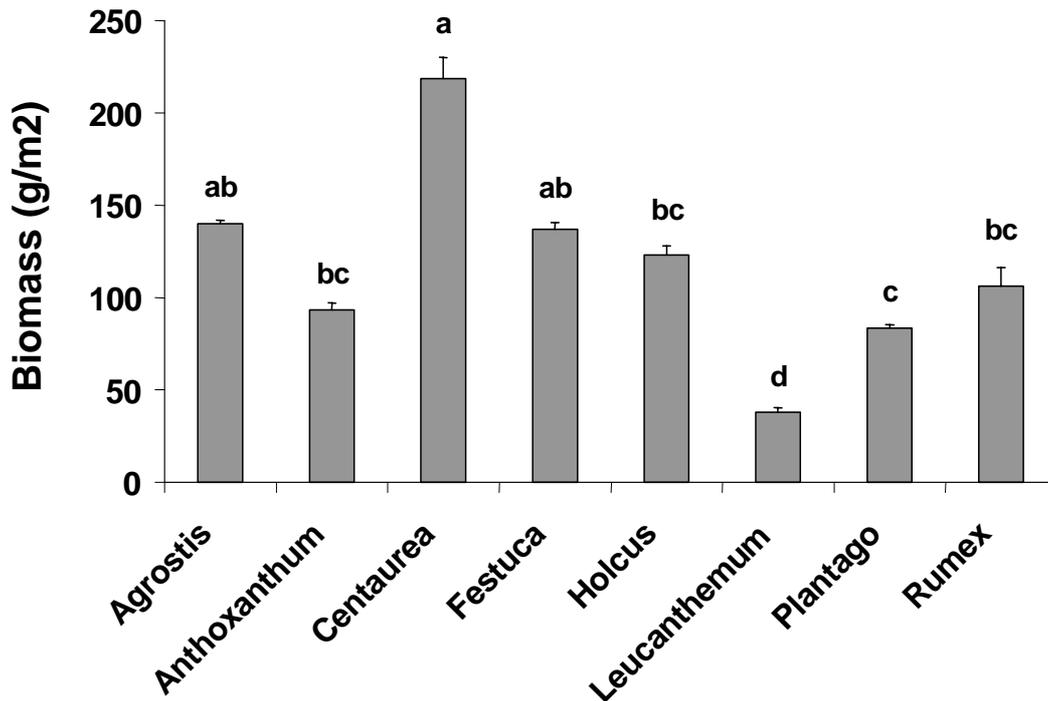
Diversity may also enhance invasion resistance by increased crowding. Plant neighbourhoods become increasingly crowded and competitive with increasing diversity (Kennedy *et al.* 2002). We did not collect data about crowding, but seedling competition for example, may have increased with diversity. Because plant performance increased with diversity, seed input from the initial plant species may have increased as well, resulting in increased seed and seedling densities in more diverse mixtures. Seed density of the initial species pool was recently shown to be an important determinant of invasibility (Brown & Fridley 2003).

Our extended model revealed that plant identity is the most important determinant of invasibility, as was argued earlier

(Crawley *et al.* 1999; Van der Putten *et al.* 2000; Wardle 2001). For two variables measuring invasibility, the effects could only be attributed to the effects of plant species. The most striking example of a sampling effect is the effect on *Erigeron*, which initially showed a linear decrease with diversity (fig 2D). From our extended model it became clear that this species is affected only by the presence of *Leucanthemum*. The overall decrease with diversity is the result of an increasing frequency of *Leucanthemum* with diversity (from 1/8 in monocultures, via 1/4 and 1/2 to 1 in eight-species mixtures).

The composite invaders *Taraxacum* and *Erigeron* were affected by the presence of other composites, especially *Leucanthemum*. In contrast to results from another study, in which the survival of *Conyza* species was favoured by the presence of other members of the same plant family (Prieur-Richard *et al.* 2000), the effect in our study was strongly negative. It is interesting to mention that a grass species (*Poa pratensis*) was not affected by the presence of *Centaurea* and *Leucanthemum*. This species showed a marginal decrease with diversity, but this was caused by a very high invasion success in *Agrostis* monocultures (data not shown). Perhaps the detrimental effects of *Leucanthemum* are limited to closely related species, for example to species within the family of the *Asteraceae* (62% of the external invaders belonged to this family). Unfortunately, data of other species, both composite and non-composite, were too few to test this hypothesis.

The effect of *Centaurea* may be due to its high resource use: its monoculture biomass was significantly greater than that of most other species (figure 6) and it was the dominant species in most mixtures (data not shown). *Leucanthemum*, however, had low biomass in monoculture (figure 6) and although individuals slightly increased in size with diversity, it certainly did not dominate mixtures. More likely, its effects arose from allelopathic or pathogenic interactions. *Artemisia tridentata* (sagebrush), for example, is known to inhibit germination of neighbouring plants by releasing methyl jasmonate (Preston *et al.* 2002). To our knowledge, such a mechanism has not been identified for *Leucanthemum*. Recent research suggests that interactions between plants and pathogens are important in regulating the dynamics of plant species abundance in the field (Olff *et al.* 2000; Klironomos 2002). Many studies show a negative feedback between plant species and their soil community, but there is evidence that the soil community of a plant can suppress the growth of neighbouring species as well (Mills & Bever 1998; Holah & Alexander 1999; Klironomos 2002). Our results suggest that *Leucanthemum* can also control plant community composition through a negative feedback with the soil. It is known that this species can act as a host for root-knot nematodes (Belair & Benoit 1996). We found high numbers of two other groups of root-feeding nematodes associated with



**Figure 4.6.** Aboveground biomass (g/m<sup>2</sup>) of monocultures. Different letters indicate significant differences between species ( $P < 0.05$ ). Differences were determined using a Tukey *post hoc* test in a GLM with block as random factor and plant species as factor. Data show mean  $\pm$  se.

*Leucanthemum*. Especially trichodorid nematodes may play an important role, because they are frequently associated with virus transmission (Ploeg & Decraemer 1997). Unfortunately, we could not reliably disentangle the effect of nematodes from that of the plant species because their occurrence is so closely related to the presence of *Leucanthemum*. It should also be noted that we did not sample other organisms, like pathogenic fungi, which could be equally important in determining the suppressive effect of this plant species. Future research should identify the mechanism by which this species reduces invasibility and also, if the effects are indeed limited to closely related species.

In this experiment, we use the invasion by native species to illustrate the effect of diversity on invasibility. We think that the establishment of native species and the invasion of exotic species are to a large extent affected by the same mechanisms. As a consequence, we would expect strong effects of diversity also when exotic species invade the grassland plots (see Kennedy et al. 2002). An important difference between native and exotic species may be that invaders experience a release from their natural pathogens and parasites (Klironomos 2002; Van der Putten 2002; Mitchell & Power

2003; Torchin *et al.* 2003). Assuming that the effects of *Leucanthemum* are indeed caused by interactions with parasites and pathogens, the presence of *Leucanthemum* would perhaps not reduce the invasion success of an exotic species that is susceptible only to a species-specific pathogen occurring in its native habitat. The question remains if diversity is an important mechanism in determining ecosystem invasibility. At larger scales, other mechanisms (e.g. disturbance, resource heterogeneity) that determine the susceptibility to invasion may be more important (Levine 2000; Wardle 2001). We have shown, however, that invasibility decreases with increasing diversity at the local scale of experimental grassland plots. Our results confirm that this local invasion resistance is to a large extent determined by the presence of particular species. It should be emphasized, however, that if these species are lost from plant communities (which happened in most agricultural grasslands in the Netherlands), the ecosystem loses a very significant barrier to invasion. Moreover, diversity showed additional negative effects. Both effects imply that the current loss of biodiversity may have serious implications for the invasibility of ecosystems.

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Diversity reduces invasibility

**Plant species identity and diversity effects on  
different trophic levels of nematodes in the soil food  
web**

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## ABSTRACT

Previous studies on biodiversity and soil food web composition have mentioned plant species identity, as well as plant species diversity as the main factors affecting the abundance and diversity of soil organisms. However, most studies have been carried out under limitations of time, space, or appropriate controls. In order to further examine the relation between plant species diversity and the soil food web, we conducted a three-year semi-field experiment in which eight plant species (4 forb and 4 grass species) were grown in monocultures and mixtures of two, four and eight plant species. In addition there were communities with 16 plant species. We analyzed the abundance and identity of the nematodes in soil and roots, including feeding groups from various trophic levels (primary and secondary consumers, carnivores, and omnivores) in the soil food web.

Plant species diversity and plant identity affected the diversity of nematodes. The effect of plant diversity was attributed to the complementarity in resource quality of the component plant species rather than to an increase in total resource quantity. The nematode diversity varied more between the different plant species than between different levels of plant species diversity, so that plant identity is more important than plant diversity. Nevertheless the nematode diversity in plant mixtures was higher than in any of the plant monocultures, due to the reduced dominance of the most abundant nematode taxa in the mixed plant communities. Plant species identity affected the abundances of the lower trophic consumer levels more than the higher trophic levels of nematodes. Plant species diversity and plant biomass did not affect nematode abundance. Our results, therefore, support the hypothesis that resource quality is more important than resource quantity for the diversity of soil food web components and that plant species identity is more important than plant diversity *per se*.

**Key words:** *Plant diversity, ecosystem processes, nematode diversity, nematode community, soil food web, plant species-specific effect, above-below ground interactions, trophic interactions*

## INTRODUCTION

There has been considerable interest in how plant species diversity and identity affect plant biomass production (Naeem et al. 1995, Tilman et al. 1996, Tilman et al. 1997, Hector 1998, Hector et al. 1999, Leps et al. 2001) and invasion resistance (Knops et al. 1999, Van der Putten et al. 2000, Wardle 2001, Van Ruijven et al. 2003), but effects on higher trophic level organisms have received less attention. Most studies on the influence of plant species diversity on higher trophic level organisms have focussed on aboveground invertebrates (Southwood et al. 1979, Siemann et al. 1998, Koricheva et al. 2000). Nevertheless, the interest in the effects of plant species diversity on the abundance and diversity of soil organisms and soil food web functioning is rapidly increasing (Wardle et al. 1999, Stephan et al. 2000, Spehn et al. 2000, Hooper et al. 2000, Korthals et al. 2001, Hedlund et al. 2003, Wardle et al. 2003). However, it is still unclear how plant species diversity and plant species identity interact with belowground diversity (Wardle 2002).

Above ground, higher plant species diversity may lead to greater herbivore, predator and parasite diversity of arthropods without a change in their abundance, but increased plant species richness can also reduce total arthropod diversity via a decrease in parasite and predator diversity (Siemann 1998). These contrasting results were explained by differences in the degree of specialisation and mobility between the arthropod species under study. Other studies pointed at the importance of plant functional group traits for aboveground arthropod communities (Koricheva et al. 2000), the role of plant nutritional quality (Symstad et al. 2000) and plant structural diversity (Southwood et al. 1979).

Below ground the effect of plant species diversity on the diversity and abundance of soil organisms is even more unpredictable (Hooper et al. 2000, Wardle 2002). Experimental studies with living plants, as well as with dead plant parts usually showed no or inconsistent effects of plant diversity on the abundance, biomass, activity or diversity of primary consumers and higher trophic levels of soil organisms (Blair et al. 1990, Wardle and Nicholson 1996, Malý et al. 2000, Wardle et al. 2000, Korthals et al. 2001, Hedlund et al. 2003). Other studies, however, report weak to strong positive relations (Wasilewska 1995, Bardgett and Shine 1999, Kaneko and Salamanca 1999, Stephan et al. 2000, Spehn et al. 2000, Porazinska et al. 2003). In cases where no or inconsistent results were found it was suggested that plant specific traits were overruling the effects of plant species diversity or plant functional diversity (Wardle et al. 1999, Wardle et al. 2000, Scheu et al. 2003) or that the response could be hampered by the poor dispersal and colonization abilities of soil organisms (Korthals et al. 2001).

Positive relations between plant species diversity and the abundance or diversity of soil organisms were explained by increased substrate diversity, increased substrate quantity or increased diversity of soil microhabitats (Stephan et al. 2000, Spehn et al. 2000).

The impact of plant species diversity on soil biota has mainly been tested for primary consumers in the decomposer pathway (often neglecting plant feeders), while the effect on plant feeders and higher trophic level consumers in the soil have hardly been studied simultaneously (Wardle 2002). Recent studies, however, suggest important effects of plant species diversity or plant species identity on multiple trophic levels in the soil food web and ecosystem processes (Wardle et al. 2003, Porazinska et al. 2003). Primary consumers (microbes, plant feeders) often appeared to be more responsive than secondary (microbe-feeders) and tertiary consumers (predators) to plant diversity and plant identity (Korthals et al. 2001, Wardle et al. 2003, Porazinska et al. 2003). Spehn et al. (2000) however, found stronger effects of plant diversity on higher than on lower trophic levels of consumers.

Most diversity studies on interactions between plants and soil organisms did not include monocultures of all plant species (Wasilewska 1995, Spehn et al. 2000, Stephan et al. 2000), plant diversity was not maintained by, for example, hand weeding (Malý et al. 2000, Korthals et al. 2001, Hedlund et al. 2003), or the diversity was restricted to combinations of maximally 2 species (Wardle and Nicholson 1996, Scheu et al. 2003, Porazinska et al. 2003). In many field studies (Wardle et al. 1999, Verschoor 2001, Porazinska et al. 2003) the history of changes in plant diversity due to natural colonization or (a)biotic heterogeneity is unknown, which can obscure the interpretation of the results. Pot-experiments can avoid these problems (Wardle et al. 2003), but they are less suitable for long-term experiments. Moreover, many experiments that examined effects of plant diversity on the abundance and species diversity of soil organisms have focused on single trophic levels of soil organisms.

In order to overcome these constraints we carried out a three-year semi-field experiment with controlled plant diversities ranging from 1 to 16 plant species and studied the response of a range of trophic levels simultaneously. Up to 8 plant species diversity treatments all monocultures were present, while legumes (often dominating observed effects, Stephan et al. 2000, Spehn et al. 2000) were not included in our experiment (chapter 2). In this way we were able to discriminate between the effects of plant species diversity and plant species identity on the abundance and diversity of different trophic groups of soil biota.

We analysed the community structure of nematodes in relation to plant species diversity and plant species identity, because

nematodes appear in a wide array of feeding groups reflecting the current or recent availability of their food (herbivores, bacterivores, fungivores, omnivores and carnivores), while they can be isolated simultaneously from single soil samples by floatation techniques. Their abundance is also presumed to mirror that of other important consumers (Ferris and Matute 2003). Nematodes are important components of the soil community with substantial effects on soil ecosystem processes and plant community structure (Bardgett and Cook 1998, Van der Putten and Van der Stoel 1998, Ritz and Trudgill 1999, De Deyn et al. 2003). We investigated the effect of plant taxonomic diversity, plant productivity and plant species identity on the abundance, as well as on the taxonomic and functional diversity of the different feeding groups of nematodes present in the plant roots and in the soil.

We tested the hypothesis that the abundance and diversity of the nematode community is enhanced by plant species diversity. We also tested if the effects of plant species diversity and plant species identity are more pronounced in the nematode trophic levels most closely associated with plants than in higher trophic levels. We expected total nematode abundance and diversity to increase when more plant species are present. Furthermore, we expect that plant feeders are affected most by the composition of the plant community, that bacteri- and fungivores show an intermediate response and that omni- and carnivorous nematodes are the least responsive.

To determine the effect of plant species identity and plant species diversity on the soil food web, the nematode community diversity was evaluated in several ways. We compared the nematode abundance in the different consumer level feeding groups (plant feeders, fungal feeders, bacterial feeders, omnivores and carnivores) and in feeding types within the primary consumer level (ectoparasites, semi-endoparasites, migratory endoparasites and sedentary endoparasites). We also calculated diversity indices over all taxa, as well as functional diversity indices developed for nematode community studies (Ferris et al. 2001).

## **MATERIAL & METHODS**

### **Plant diversity treatments**

We established outdoor semi-field plots, 1 m<sup>2</sup> each, with plant communities of 1, 2, 4, 8 or 16 plant species, distributed over six replicated blocks (chapter 2). The plant communities were monocultures of four grass species [*Holcus lanatus* (L.), *Festuca rubra* (L.), *Anthoxanthum odoratum* (L.) and *Agrostis capillaris* (L.)] and four forb species [*Plantago lanceolata* (L.), *Leucanthemum vulgare* (Lamk.), *Rumex acetosa* (L.) and *Centaurea jacea* (L.)] and mixed cultures of two, four and all eight plant species. The plant

species in the two and four plant species plots were combinations of only grasses, only forbs or of both, randomly drawn from the species pool of grasses, forbs or both and omitting identical combinations. Plant communities with sixteen plant species were established from the previously mentioned eight plant species with in addition *Achillea ptarmica* (L.), *Prunella vulgaris* (L.), *Hypochaeris radicata* (L.), *Cardamine pratensis* (L.), *Filipendula ulmaria* (L.), *Stellaria graminea* (L.), *Sanguisorba officinalis* (L.) and *Hypericum dubium* (L.). In total there were 108 plots: 48 monoculture plots, 24 plots with 2 plant species, 24 plots with 4 plant species, 6 plots with 8 and 6 plots with 16 plant species. Each plot was isolated from the surrounding field soil by 40 cm deep wooden frames (down to the mineral soil layer) and contained a 3:1 mixture of white sand and black soil, originating from an old field. Per plot 144 seedling plants were planted in a grid of 12 x 12. The seedlings had been germinated and pre-grown in a greenhouse for 4 weeks.

### Measurements

After 16 (2001: year 1) and 28 months (2002: year 2) plant shoots were clipped at 2.5 cm above the soil surface, sorted into species, dried and weighed (the data on biomass development from 2000 to 2003 have been discussed in chapter 3). Plants from the inner zone (60 x 60 cm) and from the 20 cm wide outer zone of the plots were collected separately, and the nematodes were collected from this outer zone in order to minimise disturbance of the inner plot. The plant biomass from the outer zone, expressed as dry weight per m<sup>2</sup>, was used in the analyses in order to be able to combine nematode and plant biomass data from the same plot area. The soil samples were collected just before clipping. In each plot, 12 pooled cores of 1 cm diameter and 10 cm depth were collected adjacent to the inner square and the holes were filled with dry sand. Nematodes were extracted from 100 cm<sup>3</sup> of soil by Oostenbrink elutriators (Oostenbrink 1960) and from the roots using a mistifier. After extraction for 48 hours the roots were dried and weighted. Nematodes were counted and identified to family/genus level and divided into feeding groups according to Bongers (1988) and Yeates et al. (1993a). The most dominant plant-feeding genera were identified to species level. The effect of plant species identity on the abundance of the plant-feeding nematodes was studied in more detail by subdividing this group into four plant feeder feeding types: ectoparasites, semi-endoparasites, migratory endoparasites and sedentary endoparasites (Yeates et al. 1993a). Epidermis or root hair feeders were represented as plant-associated nematodes (Yeates et al. 1993b).

### **Calculations and data analyses**

Simpson's evenness index was calculated to determine effects of plant species diversity and plant identity on nematode taxonomic diversity (Magurran 1988). Nematode taxa consisted of genera (mainly) and of families. Plant effects on the nematode community structure were assessed via the Structure index (SI), Enrichment index (EI) and Channel index (CI). These indices are indicators for the soil food web structure and condition (indicating functional diversity). They are calculated via weighted abundances of nematodes in different feeding guilds, taking their position on the coloniser-persister (cp) scale into account (Ferris et al. 2001). The coloniser-persister scale groups nematode taxa with similar response to changes in their soil environment; ranging from enrichment opportunists (cp-1) to taxa which do not tolerate disturbance (cp-5) (Bongers 1990, Bongers and Ferris 1999).

#### *Nematode abundance*

The effects of plant species richness and of plant species identity on nematode abundance in the different feeding groups and feeding types were tested per year by Wilks Lambda multivariate test with block and number of plant species or block and plant species identity as factors. The plant species identity effects were tested using the monoculture data. The effects of plant species identity and time within each nematode feeding group were tested by repeated measures Analysis of Variances, with plant species identity, block and their interaction with time as factors. Differences between plant species were tested with Tukey HSD test ( $P < 0.05$ ) using the data of both years. When the effects of plant species identity interacted with plant development time the differences between plant species were tested per year (plant feeder abundance). The abundance of the bacterial feeders was  $\log_{10}(1+x)$  transformed and the abundances of the fungal feeders, omnivores, plant-associated, carnivores and plant feeders as well as the ratio of fungal to fungal plus bacterial feeders were square root transformed prior to analysis. The plant species identity effects on the different feeding types of the plant feeders were tested per year, since the assumptions for repeated measures Analysis of Variances were not met. The abundance of the sedentary endoparasites in year 1 and the semi- and migratory endoparasites in year 2 were analysed by Kruskal-Wallis (KW), and the difference between the plant species with Mann-Whitney U tests ( $P < 0.05$ ). The effect of plant species identity on the abundance of all other feeding types of plant-feeding nematodes were analyzed by Analysis of Variances, after  $\log_{10}(1+x)$  transformation of the abundances. Block was not included as a factor since it was not significant in the multivariate tests over all feeding types. Differences in the

abundances of the feeding types between the plant species were tested using Tukey HSD test ( $P < 0.05$ ) while omitting one extreme outlier. The abundances of the feeding types in year 2 were used to test how the specific effects of *P. lanceolata*, *H. lanatus*, *L. vulgare* and *C. jacea*, as observed in the monocultures, may have affected the different plant-feeding nematode feeding types in the plant communities of two and four plant species. Per plant species diversity level Mann-Whitney U test was used to compare the nematode abundance in the plant communities that included the plant species under consideration with the nematode abundance in the plant communities where that specific plant species was not present. The relation between plant shoot biomass and nematode abundance or nematode diversity was tested per year by Pearson correlation both over all plots as well as in the monocultures separately. The effect of year on nematode abundances in the feeding groups was tested by Wilks Lambda multivariate test with block and year as factors; per feeding group differences in nematode abundance between years were derived from the repeated measures ANOVA for the monocultures and over all plant communities tested by KW.

#### *Nematode taxonomic and functional diversity*

The effect of plant species richness and plant species identity on the nematode taxonomic (taxa, Simpson's evenness) and functional (SI, EI, CI) diversity over time was tested by repeated measures Analysis of Variances (ANOVA), with plant species richness or plant species identity, block and their interaction with time as factors. Plant species-specific effects on nematode diversity were tested in the monocultures. Effects of plant species diversity treatments and of plant species identity on nematode diversity were tested with Tukey HSD test for unequal numbers of replicates ( $P < 0.05$ ). CI was square root and SI arcsines transformed prior to analysis. The relation between plant shoot biomass and the nematode diversity indices was tested over all plots and in the monocultures using Pearson correlation of plant shoot biomass and the diversity indices. The effect of year on nematode diversity indices was derived from the repeated measures Analysis of Variances for the monocultures and was tested over all plant communities by Analysis of Variances with block as random and year as fixed factor.

#### *Shoot biomass*

Effect of plant species diversity on shoot biomass and plant development time was tested by repeated measures Analysis of Variances, with block, the number of plant species and their interaction with time as factors. Treatment effects on shoot biomass were tested with Tukey HSD test for unequal numbers of replicates ( $P < 0.05$ ).

## RESULTS

### Plant diversity and identity effects on nematode abundance

Plant species richness *per se* did not affect the abundance of nematodes of any feeding group (Wilks  $\Lambda$   $P > 0.05$ ; data not shown). Plant species identity, however, affected the nematode abundance of the different feeding groups in both years (Wilks  $\Lambda$  year 1:  $F_{42,144} = 3.45$ ,  $P < 0.001$  and year 2:  $F_{42,144} = 2.77$ ,  $P < 0.001$ ). Plant species identity affected the numbers of bacterivorous and plant-feeding nematodes and the ratio of fungal to bacterial plus fungal feeders. Plant species identity marginally affected the abundance of the plant-associated and fungal feeding nematodes and did not affect the higher trophic level feeding groups (omni- and carnivorous nematodes; Table 5.1).

The plant-feeding nematodes were the most abundant nematode feeding group and they also showed the strongest response to specific plant species (Table 5.1). Plant-feeding nematodes were present in very small numbers in monocultures of *P. lanceolata*. In year 1 the plant-feeding nematode abundance in *R. acetosa* monocultures resembled those in *P. lanceolata*, while in year 2 their abundance had increased to a level found for the other plant species. The highest total abundance of plant-feeding nematodes was observed in the root zones of *H. lanatus*, *A. capillaris*, *A. odoratum*, *C. jacea* and *L. vulgare*.

Although the total numbers of plant-feeding nematodes did not differ between these plant species, the response of various feeding types of plant-feeding nematodes differed between the plant species (Wilks  $\Lambda$  year 1:  $F_{28,117} = 9.18$ ,  $P < 0.001$  and year 2:  $F_{28,117} = 8.30$ ,  $P < 0.001$ ). Ecto- and semi-endoparasites dominated in the root zone of the grass species, while in the forb species sedentary endo- and ectoparasites were most abundant, especially in *C. jacea* and *L. vulgare* (Table 5.2).

The plant species-specific effects on the plant-feeding nematodes observed in plant monocultures persisted in the mixed plant communities.

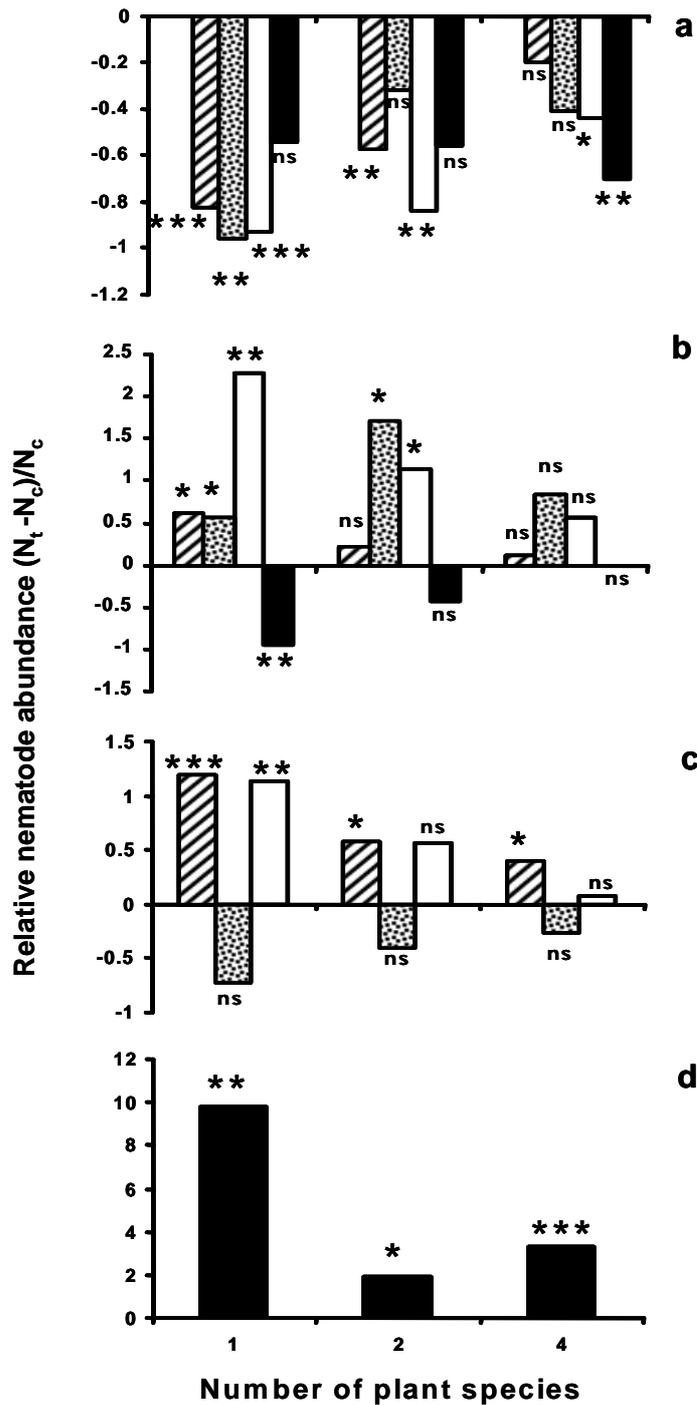
The presence of *P. lanceolata* in the two and four plant species mixtures reduced the abundance of the ecto-, migratory and sedentary endoparasitic nematodes (Fig. 5.1 a). The presence of *H. lanatus* in the two-plant species mixtures enhanced the semi-endo and migratory endoparasitic nematodes (Fig. 5.1b). The presence of *L. vulgare* stimulated ectoparasitic nematodes (Fig. 5.1 c), while *C. jacea* specifically promoted sedentary endoparasitic nematodes (Fig. 5.1 d). The effect of *C. jacea* was due to promotion of the nematode species *Meloidogyne hapla* and the effect was even stronger in the plots with four than with two plant species.

**Table 5.1.** Abundance of nematode feeding groups in single plant species plots (numbers per 100 g soil; back-transformed values of mean over year 1 and year 2); with Pf= plant feeders, Pa= epidermis/root hair feeders, Om= onivores, Ca= carnivores, Bf= bacterial feeders, Ff= fungal feeders. Different letters denote differences within columns at  $P < 0.05$  level.

Monocultures	Bf	Ff	Ff/ (Bf+Ff)	Pa	Ca	Om	Pf year 1	Pf year 2	Time effect on Pf (K-W)
<b>Forbs</b>									
<i>Plantago</i>	224 b	34 a	0.13 ab	22 a	60 a	177 a	52 c	241 c	ns
<i>Rumex</i>	257 ab	14 a	0.04 b	55 a	70 a	230 a	281 bc	1198 ab	< 0.001
<i>Centaurea</i>	251 ab	36 a	0.11 ab	24 a	59 a	210 a	1058 a	1596 a	ns
<i>Leucanthemum</i>	435 ab	58 a	0.12 ab	73 a	85 a	250 a	929 a	1669 a	ns
<b>Grasses</b>									
<i>Holcus</i>	305 ab	50 a	0.12 ab	27 a	62 a	204 a	1469 a	1404 a	ns
<i>Anthoxanthum</i>	482 a	53 a	0.09 ab	39 a	57 a	227 a	1223 a	877 ab	ns
<i>Festuca</i>	320 ab	83 a	0.18 a	24 a	31 a	155 a	793 ab	619 bc	ns
<i>Agrostis</i>	516 a	57 a	0.09 ab	38 a	50 a	240 a	1160 a	1471 a	ns
<b>Repeated measures ANOVA</b>									
	<i>P</i> -values								
Block (B)	0.12	0.08	0.19	0.37	0.01	0.08	0.34		
Plant species (P)	< 0.01	0.06	< 0.05	< 0.05	0.19	0.43	< 0.001		
Time (T)	< 0.001	0.09	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001		
B x T	0.92	0.37	0.22	0.61	0.09	0.32	0.45		
P x T	0.40	0.84	0.90	0.17	0.13	0.25	< 0.001		

**Table 5.2.** Abundance of feeding types of plant-feeding nematodes in single plant species plots (numbers per 100g soil). Ecto= ectoparasite, Semi-endo= semi-endoparasite, Migratory endo= migratory endoparasite, Sedentary endo= sedentary endoparasite. Different letters denote differences at  $P < 0.05$  level. Underlined is a mean with 1 outlier omitted.

Monocultures (N=12)	Year 1				Year 2			
	Ecto	Semi- endo	Migra- tory Endo	Seden- tary endo	Ecto	Semi- endo	Migra- tory endo	Seden- tary Endo
<b>Forbs</b>								
<i>Plantago</i>	27 c	3 c	0 b	14 bc	110 d	9 f	6 d	31 abcd
<i>Rumex</i>	148 b	18 b	26 a	43 b	539 abc	<u>18 ef</u>	57 b	41 abcd
<i>Centaurea</i>	129 b	24 b	27 a	895 a	489 bc	36 e	84 ab	515 a
<i>Leucanthemum</i>	335 ab	51 b	67 a	495 a	1166 a	59 de	137 a	246 ab
<b>Grasses</b>								
<i>Holcus</i>	752 a	567 a	28 a	2 c	906 ab	277 b	188 a	3 d
<i>Anthoxanthum</i>	816 a	291 a	40 a	12 bc	624 abc	163 c	44 bc	14 bcd
<i>Festuca</i>	343 ab	358 a	29 a	4 c	387c	122 cd	48 bc	10 cd
<i>Agrostis</i>	386 ab	609 a	12 a	27 bc	526 abc	606 a	24 c	84 abc
ANOVA or Kruskal-Wallis*	<i>P-values</i>							
Plant species	<0.001	<0.001	<0.001	<0.001 *	<0.001	< <u>0.01</u> *	<0.05	<0.001



**Figure 5.1.** Plant-feeding nematode feeding type abundances in presence relative to absence of specific plant species in monocultures and mixed plant communities, with  $N_t$  the abundance in presence of the plant species and  $N_c$  the abundance when the plant species is not present. Asterisks indicate significant differences after Mann-Whitney U pairwise comparison of the abundance in plant communities with versus without a) *Plantago lanceolata*, b) *Holcus lanatus*, c) *Leucanthemum vulgare* and d) *Centaurea jacea* at diversity levels of 1, 2 and 4 plant species. (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , ns = non significant; hatched = ecto-, dotted = semi-endo-, white = migratory endo- and black = sedentary endoparasitic nematodes).

Over all plant communities the abundance of the nematodes within feeding groups did not correlate with shoot biomass. In the monocultures the abundance of the bacterial feeding nematodes was negatively correlated with the plant shoot biomass in year 2 (Pearson correlation coefficient  $r = -0.38$ ,  $P < 0.05$ ; Table 5.3). Time significantly affected the nematode abundance over all plant communities as well as in the monocultures (Wilks *Lambda* year effect  $F_{6,204} = 63.21$  and  $F_{6,84} = 27.92$ , respectively over all plant communities and in the monocultures,  $P < 0.001$ ). Over all plant communities the nematode abundance increased due to the response of plant-, bacterial and fungal feeders (Table 5.3). In the monocultures the increase in numbers of plant feeders was only significant for *R. acetosa*, while the numbers of bacterial feeders increased independent of plant species identity (Tables 5.1 and 5.3).

The abundance of the higher trophic level feeding groups (carnivores and omnivores), as well as the abundance of the plant-associated nematodes and the proportion of the fungal feeders in the primary decomposers decreased over time, independent of plant species number or plant species identity (Tables 5.1 and 5.3).

Total rainfall and average day temperature did not differ greatly among years: 1039 mm and 10.4 °C in 2001 and 924 mm and 10.8 °C in 2002. The rainfall pattern was slightly different: March and April were drier in 2002 than in 2001, while May, June and July were wetter in 2002 than in 2001; in August rainfall was similar in both years (Koninklijk Nederlands Meteorologisch Instituut 2003).

**Table 5.3.** Mean abundance (m) of nematode feeding groups in all plant communities and in monocultures in relation to plant development time and plant shoot biomass.  $r$  = Pearson correlation coefficient. Different letters and asterisk denote differences at  $P < 0.05$  level; a, b different group than x, y. Legend see Table 1.

		Pf		Pa		Om		Ca	
		M	r	M	r	M	r	M	r
Monocultures	year 1	912 <sup>b</sup>	-0.07	61 <sup>a</sup>	0.10	288 <sup>a</sup>	-0.09	87 <sup>a</sup>	0.12
	year 2	1169 <sup>a</sup>	0.12	37 <sup>b</sup>	0.16	169 <sup>b</sup>	-0.26	54 <sup>b</sup>	-0.26
All plant communities	year 1	869 <sup>y</sup>	-0.02	48 <sup>x</sup>	0.06	286 <sup>x</sup>	-0.11	99 <sup>x</sup>	0.11
	year 2	1341 <sup>x</sup>	0.18	35 <sup>y</sup>	0.14	190 <sup>y</sup>	-0.17	68 <sup>y</sup>	-0.07
		Bf		Ff		Ff/(Ff+Bf)			
		M	r	M	r	M	r		
Monocultures	year 1	246 <sup>b</sup>	-0.11	51 <sup>a</sup>	0.03	0.18 <sup>a</sup>	0.14		
	year 2	624 <sup>a</sup>	-0.38*	71 <sup>a</sup>	-0.20	0.10 <sup>b</sup>	-0.12		
All plant communities	year 1	288 <sup>y</sup>	0.06	54 <sup>y</sup>	0.13	0.16 <sup>x</sup>	0.18		
	year 2	781 <sup>x</sup>	-0.09	72 <sup>x</sup>	-0.16	0.08 <sup>y</sup>	-0.11		

**Table 5.4.** Functional and taxonomic nematode diversity and shoot biomass in relation to the plant species diversity of experimental plant communities. Different letters denote differences at  $P < 0.05$  level.

Number of plant species	Taxonomic diversity		Functional diversity			Shoot biomass (g/m <sup>2</sup> )
	Taxa	Simpson evenness	Structure index	Enrichment index	Channel index	
1	17.3 b	0.38 b	80.2 a	20.1 a	56.3 a	182.0 c
2	18.2 ab	0.43 ab	79.0 a	21.4 a	46.5 a	201.3 bc
4	18.8 a	0.45 a	77.4 a	20.1 a	36.0 a	233.4 ab
8	17.7 ab	0.50 a	78.5 a	23.3 a	35.7 a	286.0 a
16	20.1 a	0.40 ab	75.6 a	22.1 a	54.5 a	244.8 abc
Repeated measures ANOVA		<i>P-values</i>				
Block (B)	0.17	0.23	0.07	< 0.001	0.95	0.32
Nr of plant species (S)	< 0.001	< 0.001	0.40	0.92	0.10	< 0.001
Plant development time (T)	< 0.001	0.89	< 0.001	< 0.001	< 0.05	< 0.001
B x T	0.38	0.45	0.02	< 0.01	< 0.05	0.49
S x T	0.09	0.22	0.93	0.90	0.16	0.21

**Table 5.5.** Functional and taxonomic nematode diversity in single plant species plots (\* back-transformed values). Different letters denote differences at  $P < 0.05$  level.

Monocultures (N=12)	Taxonomic diversity		Functional diversity		
	Taxa	Simpson Evenness	Structure index	Enrichment index	Channel index
<b>Forbs</b>					
<i>Plantago</i>	14.8 b	0.41 a	85.9 a	11.4 a	40.9 a
<i>Rumex</i>	15.1 b	0.34 ab	74.5 a	9.1 a	38.6 a
<i>Centaurea</i>	17.0 ab	0.25 b	77.9 a	15.1 a	65.7 a
<i>Leucanthemum</i>	17.7 a	0.38 a	78.5 a	8.2 a	44.4 a
<b>Grasses</b>					
<i>Holcus</i>	18.0 a	0.43 a	84.9 a	6.9 a	46.7 a
<i>Anthoxanthum</i>	18.6 a	0.44 a	82.0 a	6.9 a	55.6 a
<i>Festuca</i>	18.2 a	0.44 a	76.3 a	16.2 a	68.3 a
<i>Agrostis</i>	18.9 a	0.35 ab	81.7 a	14.0 a	44.8 a
<b>Repeated measures ANOVA</b>					
	<b><i>P</i>-values</b>				
Block (B)	< 0.05	0.14	0.24	< 0.001	0.20
Plant species (P)	< 0.001	< 0.001	< 0.05	0.50	0.63
Plant development time (T)	< 0.001	0.13	< 0.001	< 0.001	< 0.001
B x P	0.16	0.76	0.06	0.20	0.22
P x T	0.53	0.23	< 0.01	0.86	0.67

### **Plant diversity and identity effects on nematode taxonomic diversity**

Plant species diversity clearly had a positive effect on nematode taxonomic diversity (Table 5.4). The Simpson's evenness index of the nematode community increased with increasing plant species diversity and significantly more nematode taxa were found in the four and sixteen plant species communities than in the monocultures. Over time the number of nematode taxa increased and this increase was not significantly affected by the diversity level of the plant community or plant species identity.

Plant species identity affected the nematode taxonomic diversity in the monocultures (Table 5.5). There were less nematode taxa in *P. lanceolata* and *R. acetosa* than in the monocultures of the other plant species, apart from *C. jacea*. The number of nematode taxa in *C. jacea* did not significantly differ from that in any other monoculture, while the nematode community was less diverse in terms of evenness.

In the first year, the taxonomic diversity indices did not correlate with shoot biomass. In the second year in the monocultures and over all plant communities together the Simpson's evenness index was negatively correlated with the shoot biomass (Pearson correlation coefficient  $r = -0.48$  and  $r = -0.23$ , respectively in the monocultures and over all plant communities,  $P < 0.05$ ). In both years, we found a significant positive correlation between the total nematode abundance and the number of nematode taxa (Pearson correlation coefficient year1:  $r = 0.50$  and year2:  $r = 0.43$ ,  $P < 0.001$ ), while in year 2 the Simpson's evenness index was negatively correlated with the total nematode abundance (Pearson correlation coefficient  $r = -0.29$ ,  $P < 0.01$ ).

### **Plant diversity and identity effects on nematode functional diversity**

The nematode functional diversity indices (structure, enrichment and channel index) were not affected by plant species diversity (Table 4). Likewise, plant species identity did not affect most nematode functional indices. The structure index seemed to be affected by plant species identity, but no differences between specific plant species were found (Table 5.5).

## DISCUSSION

### Plant diversity and identity effects on the abundance of nematode feeding groups

In contrast to our hypothesis plant species diversity did not enhance the abundance of individuals in any of the nematode feeding groups, but we observed strong influences of the presence and relative abundance of specific plant species. Plant species identity affected the abundance of both the primary consumers (plant feeders and plant-associated nematodes) and secondary decomposers (bacterial and fungal feeding nematodes), but did not affect the higher trophic levels (carnivores and omnivorous nematodes). Our results, therefore, support the hypothesis that the effects of plant species identity are most pronounced in trophic levels most intimately interacting with the plants. We found that some plant species reduced the abundance of the plant-feeding nematodes, and that some plant species were suitable hosts for specific plant-feeding types, which resulted in increased dominance of the total amounts of plant feeders in these monocultures. Host complementarity, however, did not result in enhanced abundance of plant-feeding nematodes in the mixed cultures. This might be due to reduced search efficiency for suitable hosts and, consequently, increased mortality risk.

Since the nematode abundances did not correlate with shoot biomass, different nematode communities under different plant species may have been due to low plant quality (Bardgett and Wardle 2003), or plant defence compounds in the roots (Van der Putten 2003). The low nematode abundances in the root zone of *P. lanceolata* could be due to plant defensive iridoid-glycosides (Bowers and Puttick 1988, Marak et al. 2002), which might also act against nematodes (Yeates et al. 1993b). Moreover, indirect toxic effects on plant-feeding nematodes via antibiotics produced by root-associated bacteria have been reported for the related *P. major* (Insunza et al. 2002).

### Plant diversity and identity effects on nematode diversity

In agreement with our hypothesis nematode taxonomic diversity was enhanced by plant species diversity. However, the differences between individual plant species were larger than between plant diversity treatments, suggesting that plant species identity is more important for nematode diversity than plant species richness. Observations on host-specialization and selectivity of plant-feeding nematodes are known to occur in agricultural systems (Yeates 1987, Yeates and Bongers 1999) and in natural systems with monospecific plant communities (Van der Putten and Van der Stoel 1998), but

have been rarely described for other non-cropping systems (Yeates 1999, Verschoor 2001). Positive effects of the number of plant species on the diversity of soil biota have been reported (Wasilewska 1995, Kaneko and Salamanca 1999, Porazinska et al. 2003) as well as cases where only plant quality mattered (Wardle et al. 2003, Scheu et al. 2003). Some preference of nematode plant-feeding types was observed in *H. lanatus*, *A. capillaris*, *A. odoratum*, *C. jacea* and *L. vulgare*, suggesting that nematode diversity will be higher in more diverse plant communities when composed by plant species with complementary host suitability. The Simpson's evenness was higher in the 4 and 8 plant species mixtures than in the monocultures of the component species. The more specific plant-feeding sedentary endoparasites are dependent on host plant compatibility at the plant genetic level, while physical barriers of the root tissue mostly affect ectoparasites (Hussey and Grundler 1998). The impact of the number of plant species on the diversity of nematodes may, therefore, be directly linked to the degree of difference between individual plant species. Plant functional groups, based on the communality of the response of soil biota to individual plant species might yield more straightforward results of plant diversity on soil communities than when individual plant species or forbs, legumes, C3- and C4-grasses are considered.

In the monocultures the nematode taxonomic diversity of three of the four forb species was lower than in the other plant species. The low taxonomic diversity in *P. lanceolata* and *R. acetosa* correlated with an overall low nematode abundance, especially of the plant feeders. The low Simpson's evenness in *C. jacea* was due to the disproportionally high abundance of *M. hapla*. This nematode species is specialised on forbs but has a wide host range (Goodey et al. 1965). When nematode abundance was higher, the chance of encountering more nematode taxa also increased, but this often resulted in a decreased evenness. The negative correlation between the Simpson evenness and the plant shoot biomass was due to increased abundance of the already abundant taxa (plant- and bacterial-feeding taxa) and reduced abundance of the already less abundant omnivorous and carnivorous taxa. *C. jacea* was more productive than most other plant species, while it had the lowest nematode diversity due to the dominance of one plant-feeding nematode species. The presence of *C. jacea* could explain the negative relation between plant shoot biomass and nematode diversity to a large extent, since the correlation when only plant communities without *C. jacea* were considered remained only marginally significant ( $r = -0.20$ ,  $P < 0.10$ ).

Despite the enhancement of shoot biomass by plant species diversity (see also Van Ruijven and Berendse 2003), shoot biomass did not positively correlate with nematode abundances, number of nematode taxa or nematode functional diversity indices in the

mixed plant communities and in the monocultures. This suggests that resource quality is more important than resource quantity for nematode abundance and diversity. We can however not exclude that the root biomass showed a different response than the shoot biomass to increasing plant diversity or that shoot biomass did not mirror root biomass in the monocultures. Although the plant community affected nematode abundance and diversity, we did not observe significant effects of plant species diversity or plant species identity on the nematode functional diversity indices. The resolution of the latter indices might thus be too coarse to pick up differences between plant communities (Yeates 2003).

Nematode diversity on a field scale might result from a mosaic of local low diversity spots with high densities of different nematode species (Blomqvist et al. 2000, Ettema and Wardle 2002). Our results empirically support this idea by showing that mainly plant identity affects the diversity and abundance of nematodes in the soil food web. The effect of plant species diversity was attributed to the complementarity in resource quality of the component plant species rather than to an increase in total resource quantity. The abundances of the lower trophic consumer levels were more affected than the higher trophic levels and only by plant species identity, but not by plant species diversity.

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**Effects of AM fungi on plant diversity in grasslands  
on sandy soil**

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## ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are known to strongly enhance plant diversity in European grasslands on calcareous, phosphate-limited soils. The role of AMF on non-calcareous soils, however, remains largely unknown. We set up three experiments to investigate the importance of AMF for plant diversity on sandy soils. The first experiment assessed their role in plant community composition in the greenhouse. AMF increased aboveground biomass of most species, resulting in an overall increase in aboveground biomass and significantly larger aboveground N and P pools of the vegetation.

A two-year outdoor mesocosm experiment with experimental plant communities was done to verify the results of the first greenhouse experiment under natural conditions. In the second year the presence of AMF had resulted in significant shifts in plant community composition. Molecular identification of AMF in roots of dominant plants revealed that plants in the control treatment of this experiment were also colonized, but the AMF community differed from those in the AMF-treatment.

The second experiment in the greenhouse revealed that AMF affected the establishment of dicots in grass vegetation. Both survival and aboveground biomass of dicot seedlings increased in grass vegetation with AMF. Thus, AMF promote seedling establishment and may reduce recruitment limitation in grasslands. These results highlight the important role of AMF in plant community composition on sandy soil and emphasize that especially dicot species depend on AMF for their persistence in these grassland communities.

**Keywords:** *mycorrhiza, grassland, dicots, seedling establishment, plant community composition, phosphorus, germination, seed weight*

## INTRODUCTION

Mycorrhizal associations are common in most ecosystems. Arbuscular mycorrhizal fungi (AMF) form symbioses with 80% of all plant genera (Smith & Read 1997). The hyphal network of the fungus serves as an extension of the plant root and often increases nutrient uptake and consequently, plant growth. Especially the uptake of phosphorus (P) is increased due to root colonisation. The uptake of soluble P is the major nutrient capture mechanism of AMF (Olsson *et al.* 2002). Their widely ramified hyphae allow them to acquire P outside the depletion zone around plant roots. They may also reach into smaller soil pores and compete effectively with other microorganisms (Joner & Jakobsen 1995). In return for P, the fungus receives photosynthates from the plant. The interaction between plants and AMF has traditionally been regarded as a mutualism, in that both partners benefit from the association. Recent evidence, however, indicates that the costs and benefits of the symbiosis can differ significantly for plants (Streitwolf-Engel *et al.* 1997; van der Heijden *et al.* 1998a; Klironomos 2003). Plant growth responses may range from positive to negative and the symbiosis is more accurately defined as a continuum from parasitism to mutualism (Johnson *et al.* 1997; Klironomos 2003). Whether plants benefit from the symbiosis with AMF depends on a number of factors, including nutrient availability (Koide 1991), plant species (Harley & Harley 1987), and AMF taxa (Sanders & Fitter 1992; van der Heijden *et al.* 1998a). AMF may affect competition among plants in two ways: by benefiting some species more than others, and by mediating net transfer of resources between plants (Grime *et al.* 1987). Using isotopic tracers, transfer between plants via AMF has been shown for elements like carbon (Francis & Read 1984), phosphorus (Chiariello *et al.* 1982; Ritz & Newman 1984) and nitrogen (Haystead *et al.* 1988). Evidence for net transfer of ecologically important amounts, however, is under debate (Fitter *et al.* 1998).

As a result, AMF can have a strong influence on competition between plants (Fitter 1977; Hartnett *et al.* 1993; West 1996; Moora & Zobel 1996), which may also affect plant community diversity (Grime *et al.* 1987; Gange *et al.* 1990; Sanders & Koide 1994; van der Heijden *et al.* 1998a,b; Hartnett & Wilson 1999). Effects of AMF on plant diversity are positive when the abundance of subordinate plants is enhanced (Grime *et al.* 1987; van der Heijden *et al.* 1998a,b), but may also be negative. A study on tall-grass prairie showed that especially the dominant C4 grasses profited from AMF (Hartnett & Wilson 1999). Thus, the mycorrhizal dependency of the dominant plant species is important. Other factors, however, may also play a role. Those studies that reported strong positive effects of AMF on plant diversity were performed on calcareous soil, which

often has a very low P-availability. Some plants adapted to low-phosphorus soils excrete acidifying and chelating compounds to increase the availability of phosphate, but many other species lack this capacity and are dependent upon mycorrhizal associations. Among these mycorrhizal species, grasses are generally less dependent upon AMF for their nutrient acquisition (Lambers *et al.* 1998). Thus, especially dicot seedlings are likely to remain exceedingly small on low-phosphate calcareous soil in the absence of AMF. Indeed, dicot species showed strong positive responses to the presence of AMF in the experiments on calcareous soils (Grime *et al.* 1987). On non-calcareous soils, these strong responses to AMF may not occur, but to our knowledge very few studies on the effects of AMF on plant community composition have been performed on other soil types.

Apart from interactions among adult plants, AMF may affect plants in the establishment phase. Communities like perennial grasslands are often recruitment limited (Turnbull *et al.* 2000). Many factors have been proposed that are important for seedling establishment (Grubb 1977; Bakker & Berendse 1999), but the role of AMF in the seedling stage is poorly known. Under recruitment limitation, integration of seedlings into a common mycelial network may be essential for successful establishment (Newman 1988). This network may supply nutrients to the seedlings (Read & Birch 1988; van der Heijden 2004). If net transfer of elements occurs within this network, seedlings may act as sinks, meaning adult plants supply them with carbon and nutrients (Kytöviita *et al.* 2003; van der Heijden 2004). Infection by AMF may also protect seedlings from pathogenic soil fungi (Newsham *et al.* 1995). It remains unclear, however, if seedlings actually benefit from associations with AMF. Two recent studies showed opposite results: one reported no change in growth of seedlings close to an adult plant (Kytöviita *et al.* 2003), whereas the other reported enhanced growth and phosphorus uptake of seedlings in 1-year old grassland microcosms (van der Heijden 2004).

We aimed to test if communities of AMF can change plant community composition by favouring particular species, either by altering competition among adult plants or by enhancing the establishment of dicots in grass vegetation. We performed three experiments to determine the role of AMF in grasslands on non-calcareous sandy soil. In experiment 1, we assessed the influence of an AMF community on plant community development and composition in microcosms with or without AMF under controlled conditions in the greenhouse. The results obtained from this experiment were verified in an experiment under natural conditions. During two years, we determined the role of AMF in outdoor mesocosms measuring 1 m<sup>2</sup>, which were planted with seedlings of 16 different plant species. Molecular identification of AMF in roots

allowed us to determine possible differences in colonization by local AM-fungi between control and AMF treatments. In the third experiment we determined the importance of AMF for the establishment of dicot species. Seeds of dicots were sown in mesocosms consisting of monocultures of different grass species with or without AMF. Because we were interested in belowground interactions between seedlings and established vegetation (i.e. seedling establishment in grazed vegetation) we eliminated competition for light by regular clipping of the vegetation. Importantly, we measured seed weight of each individual seed. Large-seeded species often show higher survival rates (Moles & Westoby 2004) and within species, seed size is often positively correlated to seedling growth and survival (Andersson 1996; Strykstra *et al.* 1998). Thus, it may be an important source of variation in germination experiments. Measuring individual seed weights allowed us to correct for this 'noise' in the analysis.

## MATERIAL & METHODS

In each experiment, soil consisted of a mixture of soil from an old-field, and pure sand (1:3 v/v). We used the diluted soil because the black soil was too nutrient-rich in comparison to the sandy soils on which the studied plant communities normally occur. Soil of all treatments was autoclaved prior to each experiment. AMF-inoculum was added as a thin layer (< 0.5 cm) approximately five cm below the soil surface. Inoculum was obtained from Plantworks Ltd (Sittingbourne, UK) and consisted of the AMF species *Glomus claroideum* (BEG 23), *Glomus intraradices* (BEG 75), *Glomus microaggregatum* (BEG 56) and *Glomus mosseae* (BEG 91). Spores and mycelium of these AMF species as well as root fragments containing them were interspersed in carrier material that consisted of clay particles. Only this carrier material was added as a thin layer in the control treatments.

### Experiment 1

The response of a diverse plant community to the presence of AMF was first assessed in the greenhouse. Experimental plant communities consisting of 13 species were grown in 4.7L pots (20 cm diameter, height 15 cm) from August to November 2001. Two treatments, with and without AMF, were randomly allocated to 20 pots. Pots were placed following a randomised block design in which each block contained two pots of each treatment. Each pot contained 16 plant individuals: two of each grass species (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., and *Festuca rubra* L.) and one each of the dicot species *Achillea millefolium* L., *Centaurea jacea* L., *Cerastium fontanum* Baumg., *Galium mollugo* L.,

*Hypochaeris radicata* L., *Leucanthemum vulgare* (Lamk.), *Plantago lanceolata* L., *Prunella vulgaris* L., *Ranunculus acris* L. and *Rumex acetosa* L. Seeds were germinated in sterilized trays in the greenhouse. After three weeks seedlings were transplanted into the pots. Plants were allowed to grow for 10 weeks. Plants were clipped at the soil surface and sorted to species before drying them at 70° C for at least 48 h, and weighing. Roots of all plants together were washed to remove soil. Random samples were taken from the root material, weighed and stored in 50% alcohol. Roots of four species that could be distinguished were also removed from the root mass, weighed and stored in 50% alcohol. The remaining roots were weighed before drying them at 70° C and reweighing. For each species, roots from five pots were randomly selected and cut in 1 cm fragments. Per sample, 10 root fragments were randomly collected and used to assess colonisation. Root fragments were cleared in 10% KOH at 90° C for 30 minutes or longer if necessary, depending on species. After clearing, root fragments were captured on a sieve and rinsed with water (Brundrett *et al.* 1984). Roots were stained in 0.05% trypan blue in lactoglycerol, by heating at 90° C for several days. Roots were destained in 50% glycerol and stored in lactoglycerol (Brundrett *et al.* 1996). To get a measure of colonization, we used a simplified version of the magnified intersection approach (McGonigle *et al.* 1990). Root fragments were mounted on slides in lactoglycerol and covered with 40 x 22 mm coverslips. The 1 cm fragments were aligned parallel to the long axis of the slide and observed at x 200. Each fragment was aligned along the horizontal crosshair. At each minor tick (1 mm) of the crosshair, the absence/presence of mycorrhizal structures (hyphae, vesicles or arbuscules) was noted. Colonization was calculated per root fragment as the number of ticks where mycorrhizal structures were observed divided by the total number of ticks examined.

## Experiment 2

To check the results of experiment I under natural conditions, we performed a second experiment with a diverse plant community and AMF. Plots were established at an experimental field in Wageningen in early spring 2002. The topsoil was removed to a depth of 45 cm. At this depth, soil consisted of mineral sand. The organic layer below the surrounding vegetation had an average depth of approximately 10 cm. Wooden frames measuring 1 by 1 by 0.5 m (l x w x h) were placed into each hole and filled with sterilised soil. The area outside the frames was filled with the field soil. 144 plants per plot were planted as seedlings following a substitutive design (i.e. total seedling density was equal in all plots). In total, the experiment comprised 12 plots of 1 m<sup>2</sup> distributed over 6 replicated blocks. Each plot contained a mixture of sixteen species: 4 grass

species (*A. capillaris*, *A. odoratum*, *F. rubra*, *Holcus lanatus* L.) and 12 dicot species (*A. millefolium*, *C. jacea*, *C. fontanum*, *G. mollugo*, *H. radicata*, *Leontodon autumnalis* L., *L. vulgare*, *P. lanceolata*, *P. vulgaris*, *R. acris*, *R. acetosa*, and *Senecio jacobaea* L.). These species commonly coexist in European grasslands. The composition was maintained by removing seedlings of all other species at monthly intervals during the growing season. The analysis is mainly based on data on aboveground plant biomass in August 2002 and 2003, four and 16 months after the start of the experiment, respectively. All plants were cut to 2.5 cm above ground level. Plants growing in the centre (60 by 60 cm) were separated from plants growing in the edges of the plot. To avoid any edge effects, only data from the centres were used for this analysis. Plant material was sorted to species before determining dry weight. Next to the biomass measurements, the number of flowering stems of abundant plant species was counted in May and July 2003.

In autumn 2003, five dominant species (*Achillea millefolium*, *Anthoxanthum odoratum*, *Centaurea jacea*, *Plantago lanceolata*, and *Rumex acetosa*) were selected to determine the identity of the AMF taxa that colonised plant roots. Root cores were taken underneath a randomly chosen individual of each species in each plot. Roots were carefully washed from soil and stored at 5° C. Sixty root pieces of approx. 4 cm length (one from each sample) were used for the DNA extraction. DNA was extracted by the Quiagen Dneasy Plant Mini Kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions. A nested PCR reaction was performed with AMF-specific primers (GLOM1310, ACAU1660, ARCH1311 and LETC1670) in the second reaction as described by Redecker et al. (2000).

From each sample, one amplified PCR product was chosen and ligated into the pGEM-T vector (Promega, Madison, WI, USA). Ligation mixtures were transformed into competent *Escherichia coli* DH5 $\alpha$  (Invitrogen, San Diego, CA, USA). Two white colonies were taken from each plate and subcultured. Plasmids were extracted by the Hotprep Plasmid Mini Preparation Kit (Zymo Research, Orange, CA, USA). To check for the presence of the inserts, PCR was done with the original primer combination. Restriction fragment length polymorphism (RFLP) analysis was done on the successful PCR products with Hinf1, Mbo1 and Alu1 restriction endonucleases (Fermentas, Vilnius, Lithuania) to select for the different products. Clones with inserts differing in the RFLP pattern were further cleaned by the Hotprep Plasmid Mini Preparation Kit and were sequenced. All sequences were compared to sequences present in GenBank using the Blast software at the NCBI homepage (<http://www.ncbi.nlm.nih.gov>) to obtain a putative identification.

### Experiment 3

The effect of AMF on dicot establishment was assessed in a second greenhouse experiment. 36 mesocosms measuring 45 by 30 by 15 cm (l x w x d) were randomly assigned to three grass species, *A. capillaris*, *A. odoratum* and *F. rubra*. For each species, six mesocosms received AMF inoculum, whereas the other six mesocosms only received the control inoculum. The experiment was set up following a randomised block design. Seeds of the grass species were sown in September 2001 to create grass monocultures. The number of grass seeds per mesocosms amounted to 2500 m<sup>-2</sup>. Mesocosms were divided into six subplots and each of six dicot species was randomly assigned to a subplot. For each dicot species, we collected seeds from a natural population in the Netherlands. Seeds of *Cirsium dissectum* were collected from three different populations. Per species, 378 seeds were selected at random and weighed individually. The six dicot species used were (mean seed weight (mg) ± sem): *Achillea ptarmica* L. (0.25 ± 0.003), *C. jacea* (2.06 ± 0.034), *Cirsium dissectum* (L.) Hill (1.99 ± 0.038), *H. radicata* (0.78 ± 0.01), *L. vulgare* (0.38 ± 0.005) and *Succisa pratensis* Moench (1.71 ± 0.025). After five weeks, aboveground grass biomass was clipped to reduce light competition for the seedlings. This procedure was repeated every five weeks. Grass dry weight was determined after at least 48 h at 70° C. Seeds were sown in the mesocosms after five weeks. Each subplot received nine seeds. They were randomly selected and placed in a 3 x 3 matrix with a distance of 3 cm between individual seeds. Germination was monitored daily and day of germination was recorded for each seed. Time of death of seedlings was also recorded. Fifteen weeks after the start of the experiment, the seedlings were carefully removed from the soil and divided into shoots and roots. Fresh weight of roots was determined prior to storing them in 50% alcohol. Fresh and dry weights of shoots were also determined. Grass roots were collected from each subplot and washed to remove soil before drying and weighing.

### Statistics

Effects of AMF on individual species were analysed using multivariate General Linear Model (GLM) with block and treatment (+ / - AMF) as factors. Effects on total aboveground biomass were tested using a univariate GLM with the same two factors. Data on germination and survival of seedlings per species (experiment 3) were analysed using univariate GLM with block, grass, dicot species and AMF-treatment as factors and seed weight as a covariate. Germination and survival of individual seeds were analysed per dicot species using binary logistic regression models with AMF-treatment and seed mass as covariates and

grass species as categorical covariate. Differences between grass species were determined using simple contrasts in this procedure. Aboveground biomass of individual seedlings was analysed using a univariate GLM with block, grass species and AMF-treatments as factors. In each experiment, data were ln or square root transformed if assumptions of normality and/or equal variance across groups were violated. If necessary, data were analysed non-parametrically using Mann-Whitney U-tests.

## RESULTS

### Experiment 1

In experiment 1, we determined the effectiveness of the AMF-inoculum. Roots were generally highly colonized in AMF-treatments, whereas virtually no infection was observed in control treatments (Table 6.1). Roots of *Rumex acetosa* hardly showed any colonization, but this species is often reported to be nonmycorrhizal (Harley & Harley 1987).

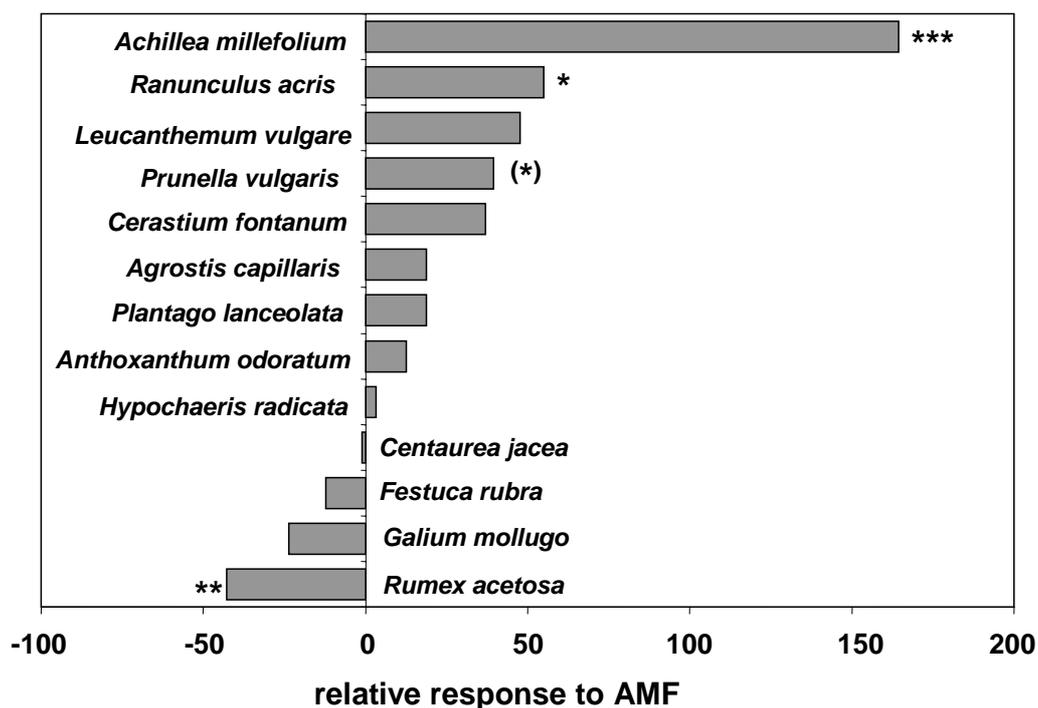
**Table 6.2** Degree of colonization of roots of four plants species in control and AMF treatments (experiment 1). Values in bold are significantly ( $P < 0.05$ ) different from the control values. Data show mean  $\pm$  se

plant species	control	AMF
<i>Achillea millefolium</i>	0	<b>0.85 <math>\pm</math> 0.12</b>
<i>Hypochaeris radicata</i>	0.02 $\pm$ 0.02	<b>0.77 <math>\pm</math> 0.12</b>
<i>Plantago lanceolata</i>	0	<b>0.43 <math>\pm</math> 0.14</b>
<i>Rumex acetosa</i>	0	0.04 $\pm$ 0.03

Most plant species appeared to respond positively to AMF (Figure 6.1). Three dicot species showed a significant ( $P < 0.05$ ) increase in aboveground biomass when inoculated with AMF (*Achillea millefolium*, *Prunella vulgaris* ( $P = 0.051$ ) and *Ranunculus acris*). As a result, total aboveground biomass was significantly higher in pots with AMF. One dicot species (*R. acetosa*) significantly decreased in the presence of AMF. Aboveground N and P-concentrations of seven dicot species (*A. millefolium*, *Centaurea jacea*, *Galium mollugo*, *Hypochaeris radicata*, *Leucanthemum vulgare*, *Plantago lanceolata* and *P. vulgaris*) were significantly enhanced by AMF. Mean N:P ratio of aboveground biomass was 1.64.

### Experiment 2

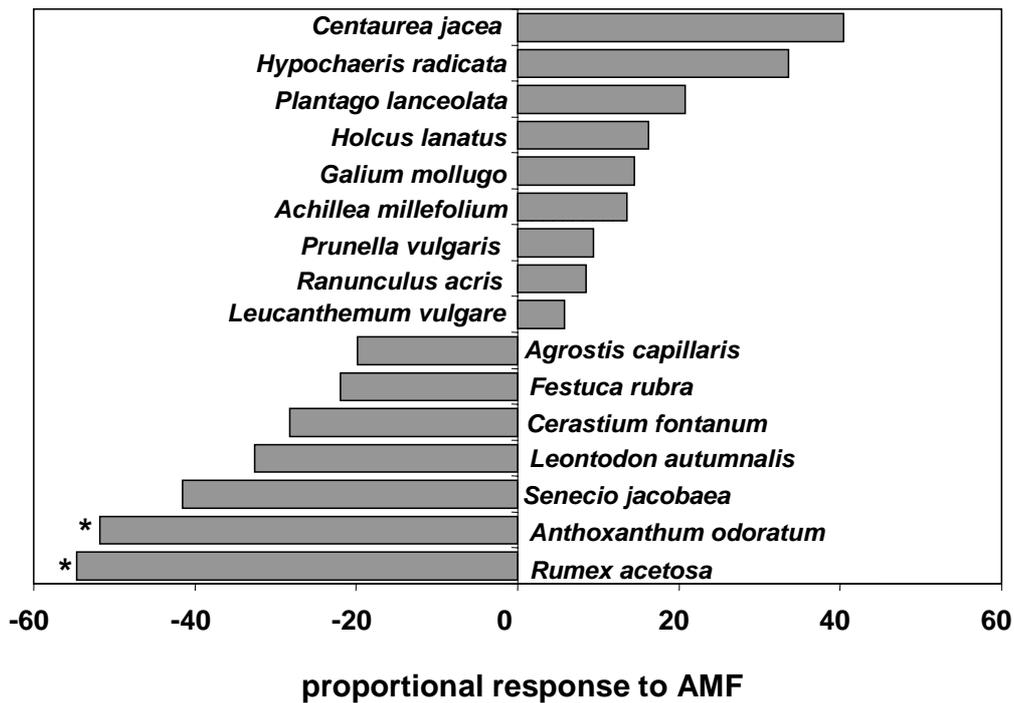
At the first cut, four months after the start of the experiment, aboveground biomass had amounted to an average of  $986.1 \pm 22.9$



**Figure 6.1** The effect of AMF on aboveground biomass of ten species in experimental communities (experiment 1). Bars represent change of aboveground biomass (%) of plants inoculated with AMF relative to the control plants. Asterisks indicate significant differences in biomass between AMF and control treatments. \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; (\*)  $P = 0.051$ .

g/m<sup>2</sup>. Significant differences between treatments could not be detected. Mean N:P ratio of aboveground biomass was 6.67. In July the next year, however, the first effects of AMF became apparent. The number of flowering stems of *R. acetosa* was significantly lower in plots with AMF (9.7 vs. 21.7), whereas *Ranunculus acris* had produced significantly more flowering stems in plots with AMF (3.8 vs. 1.0). Aboveground biomass in 2003 was far less than in the previous year:  $197.8 \pm 9.2$  g/m<sup>2</sup>, similar to values found in another experiment with a similar set up (see (Trannin *et al.* 2000). At this time, species showed shifts in biomass between AMF treatments (Figure 6.2), but in contrast to experiment 1, total aboveground biomass did not differ between treatments. Two species (*Anthoxanthum* and *Rumex*) showed a strong significant ( $P < 0.05$ ) reduction in biomass.

DNA-analysis revealed that plants were colonised by AMF in both treatments. 31 out of 60 root samples contained AMF, 21 of which belonged to species present in the inoculum. AMF were found in roots of each plant species sampled (Table 6.2). In the AMF-treatment, 17 fungal samples were identified, 16 of which were similar to AMF present in the inoculum (*Glomus intraradices* 11;



**Figure 6.2** The effect of AMF on aboveground biomass of sixteen species in experimental communities after two years (experiment 3). Bars represent change of aboveground biomass (%) of plants inoculated with AMF relative to the control plants. Asterisks indicate significant differences in biomass between AMF and control treatments. \*  $P < 0.05$ .

**Table 6.3** AMF found in roots of five plant species in both treatments.

Plant species	control	AMF
<i>Achillea millefolium</i>	No AMF (5)	No AMF (3)
	<i>Glomus intraradices</i> (1)	<i>Glomus intraradices</i> (3)
<i>Anthoxanthum odoratum</i>	No AMF (2)	<i>Glomus intraradices</i> (4)
	<i>Glomus intraradices</i> (2)	<i>Glomus claroideum</i> (2)
	<i>Paraglomus brasilianum</i> (2)	
<i>Centaurea jacea</i>	No AMF (4)	No AMF (3)
	<i>Glomus intraradices</i> (1)	<i>Glomus intraradices</i> (2)
	<i>Paraglomus occultum</i> (1)	<i>Glomus claroideum</i> (1)
<i>Plantago lanceolata</i>	No AMF (3)	No AMF (3)
	<i>Glomus intraradices</i> (1)	<i>Glomus intraradices</i> (1)
	<i>Paraglomus brasilianum</i> (2)	<i>Glomus claroideum</i> (2)
<i>Rumex acetosa</i>	No AMF (2)	No AMF (4)
	<i>Paraglomus brasilianum</i> (3)	<i>Glomus intraradices</i> (1)
	<i>Glomus</i> sp (1)	<i>Acaulaspora trappei</i> (1)

*Glomus claroideum* 5). The remaining sample, found in roots under *Rumex* was identified as *Acaulospora trappei*. In the control treatment, 14 fungal samples were identified. In contrast to the AMF-treatment, more than half of these samples belonged to AMF that were not present in the inoculum and were not found in the AMF-plots (*Paraglomus brasilianum* 7; *Paraglomus occultum* 1). A Mann-Whitney test revealed that the number of recorded AMF taxa not present in the inoculum was significantly higher in the control treatment ( $P < 0.05$ ). One AMF species present in the inoculum (*G. intraradices*) was found in five samples from the control plots. The remaining sample could only be identified to genus level (*Glomus*).

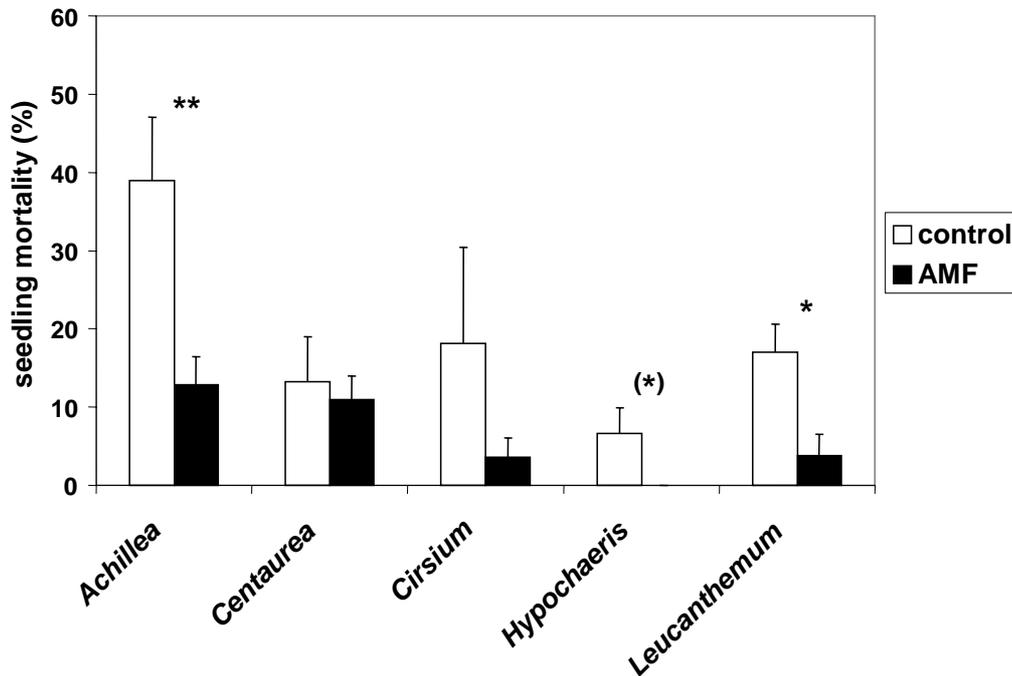
### Experiment 3

The grass species showed differential responses to AMF. At the start of the experiment, aboveground biomass of *Agrostis capillaris* was significantly higher in the presence of AMF, whereas that of *Anthoxanthum odoratum* was significantly lower. *Festuca rubra* showed no response to AMF. Regrowth after clipping, however, was significantly lower for *A. odoratum* and *F. rubra* in the presence of AMF after the first two cuts. This difference disappeared towards the end of the experiment; the total amount of aboveground biomass did not differ between species or AMF treatments. Root biomass of *A. capillaris* and *A. odoratum* was significantly ( $P < 0.05$ ) reduced in the presence of AMF.

One of the dicot species (*Succisa pratensis*) did not germinate after sowing, probably because the seeds were too old and no longer viable. This species is left out of the analysis. Considering the remaining five species, *Cirsium dissectum* showed significantly lower germination (16%) than the other species (48 to 59%). Germination was also affected by grass species. Germination of dicots was significantly higher in mesocosms with *F. rubra* than in those with *A. capillaris*.

**Table 6.3** Impacts of seed mass, grass species and AMF treatment on germination of dicot species in experiment 3. No significant interactions occurred. Data show Wald values from binary logistic regression. Asterisks indicate significance: \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ , \*  $P < 0.05$ .

	seed mass	grass species	AMF
<i>Achillea ptarmica</i>	ns	ns	ns
<i>Centaurea jacea</i>	45.60 ***	ns	ns
<i>Cirsium dissectum</i>	ns	ns	4.39 *
<i>Hypochaeris radicata</i>	ns	11.39 **	ns
<i>Leucanthemum vulgare</i>	16.21 ***	8.90 *	ns



**Figure 6.3** The effect of AMF on the survival of seedlings (experiment 3). Bars show means  $\pm$  se of the percentage of germinated seeds that did not survive the experiment. Asterisks indicate significant differences between treatments. \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; (\*)  $P = 0.057$ .

Germination in containers with *A. odoratum* showed intermediate values. Analysed per seed, separately for each dicot species, germination was affected by several factors. AMF stimulated germination of *C. dissectum*. Germination of *Centaurea jacea* was only affected by seed weight: germinating seeds were, on average, heavier than those that did not germinate (table 6.3). Germination of *Leucanthemum vulgare* was similarly affected by seed mass, but was also dependent upon grass species: germination was highest in *F. rubra*, intermediate in *A. odoratum* and lowest in *A. capillaris*. Grass species also affected germination of *Hypochaeris radicata*: germination was higher in *F. rubra*. Germination of *Achillea ptarmica* was not affected by any factor (Table 6.3).

Survival of seedlings generally was higher in the presence of AMF (Figure 6.3). The survival of *L. vulgare* seedlings was also positively affected by seed mass ( $P < 0.05$ ). Grass species had no effect on survival of seedlings.

AMF significantly enhanced biomass of *C. jacea* and *H. radicata*. Growth of *A. ptarmica* and *H. radicata* was also determined by grass species. For both species, biomass was significantly higher in mesocosms with *A. odoratum*. Growth of seedlings was highly dependent upon seed mass: aboveground biomass at the end of the

experiment was positively related to seed weight, except for *C. dissectum* (Table 6.4).

**Table 6.4** Impacts of seed mass, grass species and AMF treatment on dry weight of seedlings at the end of experiment 3. No significant interactions occurred. Data show F values from a GLM. Asterisks indicate significance: \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ , \*  $P < 0.05$  (\*)  $P = 0.086$ .

	seed mass	grass species	AMF
<i>Achillea ptarmica</i>	15.19 ***	7.96 **	ns
<i>Centaurea jacea</i>	20.52 ***	ns	4.48 *
<i>Cirsium dissectum</i>	ns	ns	ns
<i>Hypochaeris radicata</i>	12.88 ***	4.56 *	12.48 ***
<i>Leucanthemum vulgare</i>	7.68 **	ns	3.0 (*)

## DISCUSSION

Our results show that AMF are important for plant community composition. They affect both competition among adult plants and seedling establishment. Abundance of plant species changed and recruitment success was enhanced when inoculated with AMF. The N:P ratios reported show that the plants were mainly limited by nitrogen. Ratios  $< 14$  indicate that plants are limited by nitrogen (Koerselman & Meuleman 1996; Olde Venterink *et al.* 2003). These results emphasize that AMF also contribute to plant community dynamics (i.e. plant competition, recruitment) in grasslands on non-calcareous soils, when phosphate is not limiting plant-growth.

The results from experiments 1 and 2 show that AMF alter plant community composition by affecting the competition between adult plants. Inoculation with AMF resulted in significant shifts in the abundance of plant species. In the short-term pot experiment, AMF also enhanced aboveground concentrations of N and P, and total aboveground biomass, but this effect disappeared when the experimental period was extended to two growing seasons in the outdoor mesocosms. In these outdoor mesocosms, however, control plots became colonised by AMF, illustrating the difficulties of performing such experiments under more natural conditions. We assume that plants are colonised by AMF to a similar degree in both treatments in the second year, but the results we report may be caused by initial differences in the presence of AMF. It is interesting to note that control plots were mainly colonised by species that were not found in the AMF plots. Apparently, the presence of AMF-inoculum reduces the invasion success of other AMF.

In our experiments, especially dicots were stimulated by the presence of AMF. This has also been found in earlier experiments (Grime *et al.* 1987; van der Heijden *et al.* 1998a; van der Heijden *et al.* 1998b). The proportional increase, however, was less than reported in those studies. This is related to the performance in control treatments, which appeared to be greater in our experiments, and is most likely caused by differences in soil type. Availability of soluble P may be relatively high in the non-calcareous soil we used, but in the calcareous soil used in other experiments (van der Heijden *et al.* 1998a; van der Heijden *et al.* 1998b), phosphorus will readily react with calcium to form highly insoluble salts (Rendig & Taylor 1989). Especially dicots are then dependent on AMF for the acquisition of P. Our results show that this mycorrhizal dependency of dicots also exists on non-calcareous soil. The response of the plants in the experimental communities as shown in figure 1 and 2 are very similar to those of another experiment, in which 64 plant species grown in monoculture with and without a single AMF species (Klironomos 2003). AMF can directly reduce growth of mycorrhizal plants when the costs of the

association outweigh the benefits (Lambers *et al.* 1998). The shifts in abundance observed in the experimental plant communities, however, are most likely caused by indirect effects of AMF on competitive interactions between plants (Smith *et al.* 1999). Three lines of evidence support this. First, the plant species that performed worse in the presence of AMF in experiments 1 and 2 (*Rumex acetosa*) was barely colonized by AMF. AMF taxa were identified in roots collected under *R. acetosa*, but this does not imply that the roots investigated belonged to this species. Other studies also reported *R. acetosa* to be nonmycorrhizal (Grime *et al.* 1987; Harley & Harley 1987) and makes direct reduction by AMF unlikely. Second, when grown in monoculture, *R. acetosa* did not show a difference in growth between AMF-inoculated and control plants (Govaert 2003). Thus, the reduced performance of *R. acetosa* is probably due to improved competitive abilities of the mycorrhizal plants in the community relative to that of the nonmycorrhizal *R. acetosa*. Third, *Anthoxanthum odoratum* showed reduced biomass in the diverse plant community with AMF in experiment 2, but showed no effect of AMF when grown alone in experiment 3. This may be the result of altered competitive interactions, although we cannot reject the alternative hypothesis that this is caused by a positive effect of the AMF colonising the control treatments of experiment 2.

*A. odoratum* was one of the dominant species in the experimental communities and is often reported to be mycorrhizal (Harley & Harley 1987). An earlier mesocosm study also reported that AMF raised biomass of subordinate species at the expense of the dominant mycorrhizal grass species (Grime *et al.* 1987). In that study, exposure of the dominant plants to  $^{14}\text{CO}_2$  suggested that this pattern was caused by export of assimilates from dominant to subordinate mycorrhizal species, but the importance of interplant transfer of carbon remains controversial (Fitter *et al.* 1999; Simard *et al.* 2002). However, interplant transfer is not a prerequisite of net benefit. Two plants connected by a mycelial network could differ in their supply of carbon to the fungus in such a way that one gives more carbon than the other. If the amount of carbon is sufficient for fungal metabolism and nutrient acquisition, both plants could benefit (Kytöviita *et al.* 2003). This may be especially important for seedlings among adult plants.

Results from experiment 3 show that AMF can affect plant communities by enhancing seedling establishment. Similar to another mesocosm study (van der Heijden 2004), the survival and growth of seedlings was enhanced by AMF in established vegetation. Grasslands often are recruitment limited and the establishment of seedlings in existing vegetation contributes to plant diversity (Tilman 1997; Symstad & Tilman 2001; Foster & Dickson 2004). Enhancement of growth and survival of seedlings may be an

important underlying mechanism of the positive effects of AMF on plant diversity reported earlier (Grime *et al.* 1987; van der Heijden *et al.* 1998b). Other experiments reported negative or neutral effects of AMF on seedling establishment (Eissenstat & Newman 1990; Kytöviita *et al.* 2003), but each dicot species in our experiment responded positively to AMF. Competitive interactions are often found to overwhelm any positive effects of AMF. Because competition for light was excluded by clipping the vegetation, the positive effects reported here might be less in high vegetation (i.e. hay meadows).

The nature of the benefit differed between species. Small-seeded species mainly profited from increased survival, but showed no response (*Achillea ptarmica*) or only a minor positive one (*Leucanthemum vulgare*) in terms of growth. In contrast, only growth of the large-seeded species (*Centaurea jacea*) was affected by AMF. Increased survival may become important in the long term. *Hypochaeris radicata* (intermediate in seed weight) benefited both from increased survival and from enhanced growth. *Cirsium dissectum* was the only species to show increased germination due to AMF. This effect is difficult to explain, but AMF are known to alter soil structure (e.g.. increase of water stability of soil aggregates (Rillig *et al.* 1999; Rillig *et al.* 2002), which perhaps affected germination. Growth and survival of *Cirsium* were not affected by AMF. Considering the low number of seedlings due to low germination rates and subsequent mortality, however, it is impossible to draw any conclusions about mycorrhizal dependency of this species.

The positive effects of AMF on seedling establishment may also have contributed to the effects observed in the outdoor experiment on plant community composition. We did not monitor seedlings in that experiment, but the low number of establishing seedlings observed during the two years of that experiment suggests that this was not the case. In the longer term, however, AMF may significantly contribute to plant community composition by increasing the establishment success of dicots.

Experiment 3 also highlights the important role of seed size for establishment. One of the trade-offs in life-history theory is that between seed size and number of offspring (Harper 1977). Small-seeded species can produce more seeds and hence reach more germination sites, but large-seeded species may show higher survivorship during establishment. Across species, recruitment success is often positively related to seed size (Jakobsson & Eriksson 2000). This relationship has also been shown for variation in seed size within some individual species (Andersson 1996; Strykstra *et al.* 1998). Our results also show that germination, seedling survival, and seedling growth of individual species are positively related to seed weight.

It has been argued that the ecological relevance of experiments with an easily culturable AM fungus is questionable (Read 2002). Because we lacked the facilities to isolate and culture a native AMF community, we used an inoculum containing four different AMF species from five collections, all of which are present in the European database of Glomalean fungi (BEG). Moreover, a recent experiment suggested that both native and foreign AMF induce a range of responses in separately grown plant species, although the range of responses and the frequency of positive responses were reduced when using foreign AMF (Klironomos 2003). This suggests that the effects on plant community composition observed in our experiments would be more pronounced when using local plants and AMF. Considering the observed differences in AMF communities between plant species and between plant communities (Helgason *et al.* 1998; Helgason *et al.* 2002; Vandenkoornhuyse *et al.* 2002b), the high variability in plant growth responses to different AMF may itself contribute to plant species coexistence and community composition (van der Heijden *et al.* 1998b; Klironomos & Hart 2002; Gastine *et al.* 2003).

The role of AMF is generally investigated in the greenhouse using pot experiments. Experiments like ours highlight the potential role of AMF in the persistence of dicot species in grasslands. In natural communities, complexity is far greater due to spatial and temporal heterogeneity, disturbance and the presence of other organisms that affect plants and AMF. Some studies used fungicides in the field to determine the role of AMF in natural communities (Gange *et al.* 1990; Hartnett *et al.* 1994). These studies are important because they provide valuable information about the net effect of fungi in plant communities, but they may be less suitable to determine the specific role of AMF, because fungicides also affect other fungi, which may also affect plants. Recent studies indicate significant effects of pathogenic fungi on plant community composition (Clay & Holah 1999; Klironomos 2002). Moreover, a single plant root was found to contain an unexpectedly high diversity of fungi, of which the roles are largely unknown (Vandenkoornhuyse *et al.* 2002a). This raises important questions about how these various groups of fungi interact to affect plant communities.

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**Interactions between above- and below-ground  
herbivores indirectly alter plant diversity**

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## ABSTRACT

Above- and belowground herbivores promote plant diversity when selectively feeding on dominant plant species, but little is known about their combined effects. Using a model system, we show that neutral effects of an aboveground herbivore and positive effects of a belowground herbivore on plant diversity became profoundly negative when adding these herbivores in combination. The non-additive effects were explained by differences in plant preference between the aboveground- and the belowground herbivores and their consequences for indirect interactions among plant species. Simultaneous exposure to aboveground- and belowground herbivores led to plant communities being dominated by a few highly abundant species. As above- and belowground invertebrate herbivores generally differ in their mobility and local distribution patterns, our results strongly suggest that aboveground-belowground interactions contribute to local spatial heterogeneity of diversity patterns within plant communities.

**Keywords:** *above-belowground interactions, plant diversity, insect herbivores, nematodes, indirect effects*

## INTRODUCTION

Terrestrial ecosystems consist of explicit above- and belowground subsystems. Spatially separated inhabitants of these subsystems influence each other, ecosystem properties and processes (Wardle 2002). Most changes in ecosystem processes occur through impacts of above- and belowground organisms on plant community structure and composition (Wardle *et al.* 2004). Above- and belowground herbivores, pathogens, and mutualists have been identified as drivers of plant diversity, and consequently, ecosystem functioning (Crawley 1997; Olff & Ritchie 1998; van der Heijden *et al.* 1998; Clay & Holah 1999; Hartnett & Wilson 1999; Mulder *et al.* 1999; De Deyn *et al.* 2003).

The interdependence of above- and belowground herbivores has been acknowledged (van der Putten *et al.* 2001), but their joint effects on plant communities rarely have been studied (Bardgett & Wardle 2003). On a shared host plant, above- and belowground herbivores affect each other through changes in the production and allocation of primary (Seastedt *et al.* 1988; Masters *et al.* 2001; Poveda *et al.* 2003) and secondary (Bezemer *et al.* 2003) plant metabolites. Experimental studies on these kinds of interactions, however, are often limited to one plant species (van Dam *et al.* 2003). In reality, above- and belowground interactions take place in diverse plant communities. Under these complex conditions, above- and belowground herbivores may not only affect each other through changes in the host plant, but also through changes in plant community structure by affecting interactions among plant species.

The established view on interactions between above- and belowground herbivores and plant community dynamics is mainly based on the application of soil and foliar insecticides in the field. These pioneering studies suggested that aboveground invertebrate herbivores mainly affect grasses, while belowground invertebrates influence dicots (Brown & Gange 1989; Brown & Gange 1992). Most aboveground insect herbivores, however, feed on only one or a few genera or one family of plants (Bernays & Graham 1998). Moreover, recent studies have shown considerable selectivity of belowground herbivores (De Deyn *et al.* 2003; De Deyn *et al.* 2004). If above- and belowground organisms selectively affect individual plant species instead of functional groups of plant species, they may alter the outcome of plant competition at a much finer scale than previously assumed. In that case, interactive effects of above- and belowground herbivores cannot easily be predicted (Wardle 2002). We carried out an outdoor mesocosm experiment to determine if interactions between above- and belowground herbivores, and their impact on plant diversity, can be predicted based on their separate responses. Instead of omitting all herbivores from existing communities, we added common above- and belowground

herbivores to experimental plant communities in a full factorial design. This design enabled us to determine the impacts of three classes of above- and belowground invertebrates, as well as the impact of their interactions, on plant community properties.

## MATERIAL AND METHODS

### Mesocosm design

We established 48 mesocosms in six replicate blocks on an experimental field in Wageningen, the Netherlands. Mesocosms consisted of wooden frames (1 m wide, 1 m long and 45 cm deep) dug into the soil to a depth of 40 cm. The field soil was removed and replaced by a 3:1 mixture of pure sand and black soil from an old field that was treated with nematicide (Nemacur, Phenamiphos) to clear the soil from its nematode community. On top of the frames, cages measuring 1.0 x 1.0 x 1.0 m were placed, consisting of an aluminum frame and synthetic gauze with a mesh width of 300  $\mu\text{m}$  (*Nitex*, Sefar AG, Switzerland), preventing migration of invertebrates into or out of the experimental units. Inside these cages, light intensity was reduced by 25%.

The model system consisted of an experimental plant community, containing 16 plant species, and three different invertebrate herbivores: grasshoppers, wireworms and nematodes. Seeds of plant species and their herbivores were collected from natural communities, characteristic for species-rich grasslands on sandy soil. In September 2001, each mesocosm was planted with 144 seedlings: nine seedlings of *Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., *Holcus lanatus* L. (grasses), *Achillea ptarmica* L., *Centaurea jacea* L., *Cerastium fontanum* Baumg., *Galium mollugo* L., *Hypochaeris radicata* L., *Leontodon autumnalis* L., *Leucanthemum vulgare* Lamk., *Plantago lanceolata* L., *Prunella vulgaris* L., *Ranunculus acris* L., *Rumex acetosa* L. and *Senecio jacobaea* L. (dicots). These species co occur in semi natural grasslands in Western Europe. Seeds were germinated in trays in the greenhouse. Three weeks after germination seedlings were planted in a 12 x 12 matrix. The resulting experimental plant communities are similar to those used to study the relationship between plant diversity and ecosystem functioning (see Van Ruijven & Berendse 2003).

Spring 2002, grasshoppers and wireworms were added in densities representative of those in natural grassland communities. To establish the grasshopper treatment, ten individuals (five males and females) of a common grassland species (*Chortippus parallelus*) were released into each mesocosm. Densities of grasshoppers in grasslands differ considerably, but 10 individuals  $\text{m}^{-2}$  seem to be a natural density for low-productive grasslands (Ingrisch & Köhler 1998). The species we used are annual grasshoppers, which means

that they spend winter as eggs in the soil, emerge in spring, reproduce during summer and die in autumn. In the second year of the experiment the mean number of grasshoppers per mesocosm at harvest time was  $15.7 \pm 2.4$  (mean  $\pm$  se). Natural densities of wireworms in grasslands are also highly variable. One study reported densities of 0 to 185 individuals  $\text{m}^{-2}$  in dairy pastures, with mean values per pasture of 5 to 10  $\text{m}^{-2}$  (Byers & Barker 2000). We added ten wireworms (*Elateridae* larvae) per mesocosm, each in a separate small hole, according to a stratified random pattern. In the second year of the experiment, we occasionally found an adult click beetle in mesocosms. Unfortunately, we cannot provide the number of wireworms in the second year, as sampling them would have destroyed the mesocosms. Nematodes were added as a complete assemblage of root-feeding herbivorous, bacterivorous, fungivorous, omnivorous and carnivorous nematodes (Yeates *et al.* 1993) by extracting all nematodes from soil and root samples taken from the species-rich grasslands. It is important to note that this method of collecting nematodes is based upon active movement of the animals instead of centrifugation, thus avoiding the inclusion of other organisms in the inoculum. The herbivorous and bacterivorous nematodes were the most abundant feeding types (Table 1). Nematodes were introduced at 25 inoculation points per mesocosm according to a stratified random pattern. Eight different treatments were randomly assigned to the mesocosms. The three groups of herbivores were added following a complete factorial design: 1) addition of nematodes, 2) grasshoppers 3) wireworms 4) nematodes and grasshoppers 5) nematodes and wireworms 6) grasshoppers and wireworms 7) all groups 8) none.

## Data collection

### Plants

After 11 and 23 months, shoots were clipped to 2.5 cm above the soil surface and dry weight was determined per species after 48 hours at 70°C. All analyses and data presented are based on the clipping after 23 months. Plant diversity was calculated as the Shannon-Wiener diversity index:  $H = - \sum p_i \ln p_i$ , where  $p_i$  is the proportional contribution of the  $i$ th species to aboveground biomass. Because each mesocosm contained the same number of plant species, this index is a measure of plant evenness. To illustrate the effects of herbivores on plant diversity and shoot biomass, we show relative changes in Fig. 1 and Table 2. These changes were calculated as  $(N_h - N_c)/N_c$ , where  $N_h$  and  $N_c$  are the mean values of the Shannon-Wiener diversity index or shoot biomass in treatments with and without herbivores, respectively.

Nematodes

In order to investigate the nematode abundances per mesocosm 12 soil cores, 1 cm diameter and 10 cm deep, were collected immediately after clipping the plant shoots. Nematodes were extracted from 100cm<sup>3</sup> soil of the pooled 12 cores by Oostenbrink elutriators and from the roots therein in a mist chamber (Oostenbrink 1960). Nematodes were counted and identified according to Bongers (1988) and divided into feeding groups according to Yeates et al. (1993). Although nematodes were able to colonise the mesocosms from the surrounding, nematode inoculation treatment mesocosms maintained significantly higher nematode abundances (Table 7.1).

**Table 7.1** Nematodes per feeding group in the nematode inoculum and 23 months after the start of the experiment (T2) with (+ N) or without (- N) nematode inoculum (mean  $\pm$  1 s.e. per mesocosm, per 100g soil). Asterisks indicate significance between last two columns: \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; ns, not significant.

	Inoculum	T2		
		- N	+ N	
Plant-feeders	6317 $\pm$ 490	66 $\pm$ 32	552 $\pm$ 74	**
Bacterivores	1998 $\pm$ 178	41 $\pm$ 7	96 $\pm$ 13	**
Plant-associated	514 $\pm$ 76	6 $\pm$ 2	12 $\pm$ 2	*
Fungivores	325 $\pm$ 61	3 $\pm$ 1	22 $\pm$ 5	**
Omnivores	98 $\pm$ 17	161 $\pm$ 13	223 $\pm$ 27	ns <sup>1</sup>
Carnivores	18 $\pm$ 7	2 $\pm$ 1	4 $\pm$ 1	ns

<sup>1</sup> significant at  $P < 0.10$

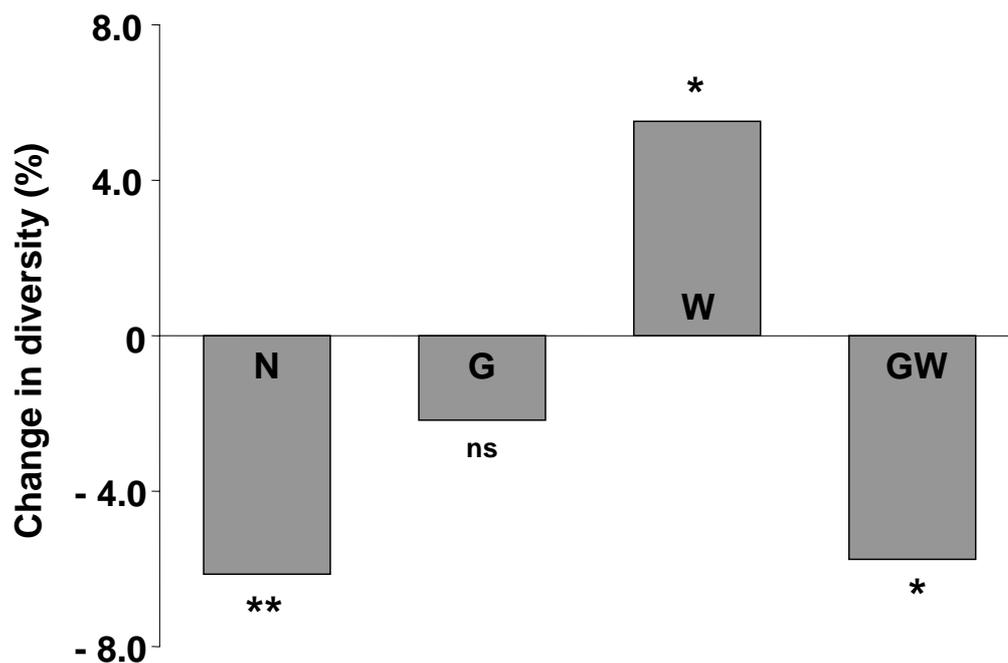
**Statistics**

Data were analysed using SPSS 10.0. Plant diversity data and total biomass were analysed using univariate General Linear Models (GLM). Data of aboveground biomass per species was analysed in a similar way using a multivariate GLM. Presence/absence of the three groups was included as fixed factors and all two-way and three-way interactions between them were included. Block was included as a random factor. Separate impacts of herbivores were derived from this GLM analysis, unless significant ( $P < 0.05$ ) interactions between herbivores occurred. In that case, the separate impact of any herbivore was determined in a similar way, but only in those treatments in which the other, interacting, herbivore was not present. Because we then only use subsets of the entire dataset, we also show changes significant at  $P < 0.10$  in Table 2. In the case of interactions between herbivores  $x$  and  $y$ , differences between

treatments were analysed further using multiple pair-wise comparisons (LSD) in a GLM with a treatment factor consisting of four levels: none, x, y and xy. The presence/absence of the third herbivore was used as a covariate and block was used as a random factor. Nematode feeding group abundances in non- and nematode-inoculated mesocosms were analysed by Mann-Whitney U-tests. Normality was checked for by the Kolmogorov-Smirnov (Liliefors) test and homogeneity of variance with Levene's test. Biomass data were ln-transformed.

## RESULTS

Grasshoppers and wireworms showed a significant interaction in their effects on plant diversity ( $P < 0.05$ ). The effect of grasshoppers was neutral in the absence of wireworms, whereas wireworms had a positive effect on plant diversity in the absence of grasshoppers. In combination, however, their impact was strongly negative (Fig. 7.1).



**Figure 7.1** Change in plant diversity in the presence of above- and belowground herbivores. Nematodes (N) reduced diversity, grasshoppers (G) and wireworms (W) interacted: separately, grasshoppers had no effect and wireworms increased diversity, but together (GW) they reduced plant diversity. Asterisks indicate significance \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; ns, non significant.

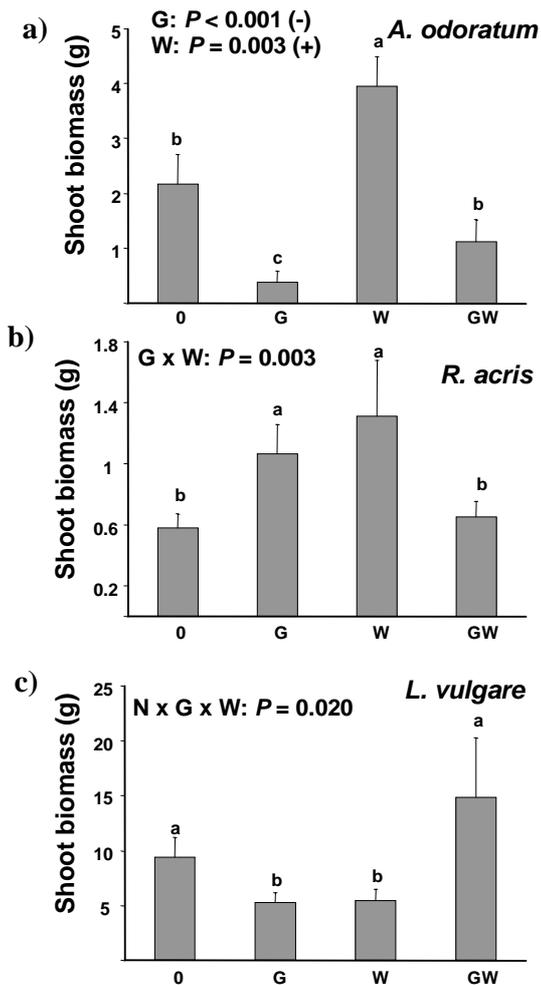
Nematodes showed an overall negative impact on diversity, similar to the combined effect of grasshoppers and wireworms in our experiment, but the nematodes did not interact with the separate and joint effects of grasshoppers and wireworms to affect plant diversity.

We examined changes in shoot biomass of individual plant species to elucidate how the above- and belowground herbivores affected plant diversity. Grasshoppers reduced shoot biomass of all (both dominant and subordinate) grass species, as well as total shoot biomass (Table 7.2).

**Table 7.2** Separate impact of different herbivores on plant shoot biomass. Data show relative change in biomass due to the presence of the herbivores, when significant. Abbr.: abbreviated species names. Species are ranked on abundance in the controls in descending order. Dominant species in the control mesocosms are in bold (see also Fig. 7.3a). Groups: D = dicot, G = grass. Herbivores: N = nematodes, G = grasshoppers, and W = wireworms. Significant interactions between herbivores are shown in the last column. Asterisks indicate significant effects of herbivores: \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; (\*)  $P < 0.10$ ; ns, not significant.

Plant species	Abbr.	Gr.	N	G	W	Inter.
<b><i>Holcus lanatus</i></b>	Hi	G	0.24*	-0.32**	ns	
<b><i>Plantago lanceolata</i></b>	Pl	D	Ns	ns	ns	
<b><i>Hypochaeris radicata</i></b>	Hr	D	0.38*	ns	ns	
<b><i>Leontodon autumnalis</i></b>	La	D	-0.85***	ns	-0.40*	NxW
<b><i>Leucanthemum vulgare</i></b>	Lv	D	-0.55*	-0.43*	-0.42(*)	NxGxW
<b><i>Festuca rubra</i></b>	Fr	G	ns	-0.88***	ns	
<i>Rumex acetosa</i>	Ra	D	ns	ns	-0.65(*)	NxW
<i>Agrostis capillaris</i>	Ac	G	ns	-0.75***	ns	
<i>Anthoxanthum odoratum</i>	Ao	G	ns	-0.77***	0.91**	
<i>Centaurea jacea</i>	Cj	D	-0.30**	ns	ns	
<i>Cerastium fontanum</i>	Cf	D	-0.39(*)	ns	ns	NxGxW
<i>Prunella vulgaris</i>	Pv	D	-0.40**	ns	ns	
<i>Achillea ptarmica</i>	Ap	D	ns	ns	ns	
<i>Ranunculus acris</i>	Ran	D	ns	0.93*	1.34(*)	GxW
<i>Senecio jacobaea</i>	Sj	D	ns	ns	ns	NxG
<i>Galium mollugo</i>	Gm	D	ns	Ns	1.38*	NxW
Total shoot biomass			ns	-0.17**	ns	
Total root biomass			ns	ns	ns	

The shoot biomass of a dominant dicot (*Leucanthemum vulgare*) was also significantly reduced, whereas one subordinate dicot species (*Ranunculus acris*) was significantly enhanced by the presence of grasshoppers. Wireworms reduced two dominant (*Leontodon autumnalis* and *Leucanthemum vulgare*) and one subdominant dicot (*Rumex acetosa*) and strongly enhanced the abundance of three subordinate species (*Anthoxanthum odoratum*, *Galium mollugo* and *Ranunculus acris*; Table 7.2).

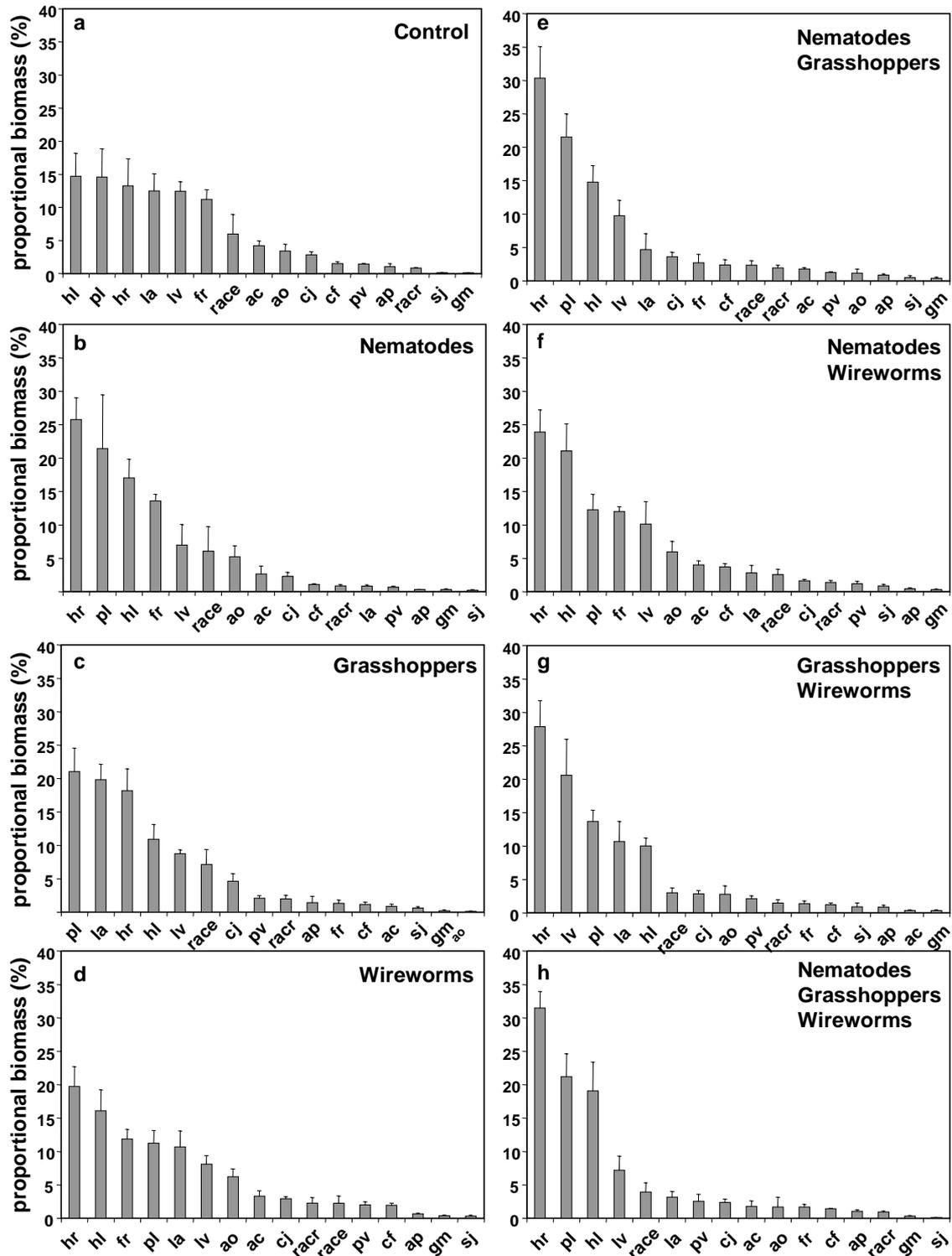


**Figure 7.2** Impacts of separate and simultaneous additions of grasshoppers (G) and wireworms (W) on shoot biomass. 0: shoot biomass in control treatments. a) *Anthoxanthum odoratum*: opposite effects of grasshoppers and wireworms resulted in no impact when added together. b) *Ranunculus acris*: positive effects of grasshoppers and wireworms disappeared when added together. c) *Leucanthemum vulgare*: in the absence of nematodes, both grasshoppers and wireworms reduced plant performance, but their combined effect was not different from the control. In the presence of nematodes, shoot biomass decreased and grasshoppers and wireworms had no effects (not shown). Different letters notate significant ( $P < 0.05$ ) differences between treatments. Data show means  $\pm$  sem.

In mesocosms with nematodes, several dominant (*Leontodon autumnalis*, *Leucanthemum vulgare*) and subordinate (*Centaurea jacea*, *Cerastium fontanum*, *Prunella vulgaris*) dicot species were strongly reduced, whereas one dominant dicot species (*Hypochaeris radicata*) and the dominant grass species *Holcus lanatus* were stimulated (Table 7.2).

Interactions between the above and belowground herbivores occurred at the level of individual plant species (Table 2). Because of their strong interactive effects on plant diversity, we focus on the interactions between grasshoppers and wireworms. Shoot biomass of the subordinate grass *Anthoxanthum odoratum* was enhanced by wireworms and reduced by grasshoppers, but there was no difference from the control when both grasshoppers and wireworms were added (Fig. 7.2a). In this case the effects of above- and belowground herbivores were additive. Biomass of the subordinate dicot *Ranunculus acris* was significantly enhanced in the presence of either wireworms or grasshoppers. These positive effects, however, did not occur when both herbivores were present at the same time (Fig. 7.2b). The third example concerns interactions between all three herbivores. In the absence of nematodes, shoot biomass of the dominant dicot *Leucanthemum vulgare* was significantly reduced in mesocosms with grasshoppers or wireworms, but there was no difference from the controls in plots exposed to both grasshoppers and wireworms (Fig. 7.2c).

The relative abundance of plant species in different treatments clearly illustrates the impact of the decrease of plant diversity caused by simultaneous exposure to above- and belowground herbivores; the addition of herbivores caused a shift from a community co-dominated by six different plant species (Fig. 7.3a) to a community that is dominated by only three species (Fig. 7.3h).



**Figure 7.3** Proportional biomass of each species in the different treatments. a) control, b) nematodes, c) grasshoppers, d) wireworms, e) nematodes and grasshoppers, f) nematodes and wireworms, g) grasshoppers and wireworms, h) nematodes, grasshoppers and wireworms. Abbreviations of species names are given in Table 1. Data show means  $\pm$  sem.

## DISCUSSION

Each of the three groups of invertebrates (grasshoppers, wireworms and nematodes) affected plant diversity. Grasshoppers reduced all grass species and the dicot *Leucanthemum vulgare*. While there was obvious feeding damage found on the grasses, there were no signs of feeding damage on the dicot species. This suggests that grasshoppers reduced performance of *L. vulgare* indirectly by disrupting a facilitative interaction with grasses. In spite of the enhanced performance of a single subordinate dicot, the overall effect of grasshoppers on plant diversity was neutral. Wireworms enhanced plant diversity by feeding preferentially on dominant plant species. Several dominant plant species were reduced while subordinate species were stimulated. The positive effects on subordinate plants were most likely due to a release from interspecific competition, but confirmation of this mechanism would require the inclusion of monocultures and two-species mixtures of all component species in the experimental design. Nematodes reduced diversity by stimulating two dominant plant species and by reducing a number of dicot species. These effects were probably caused by root-feeding nematodes, which were most abundant in the mesocosms (Table 7.1). Bacterial and fungal feeders may affect plant growth by enhancing nutrient mineralization (Bardgett *et al.* 1999; Wardle 2002), but it is unlikely that these effects have led to the plant-species specific shifts in biomass production as has been reported here.

The effects of the belowground herbivores on plant diversity may depend on the species identity of the root feeders. In our study, nematodes reduced plant diversity, whereas wireworms enhanced diversity. In an earlier study, where effects of nematodes and wireworms were not investigated separately, the combined effects of nematodes and wireworms caused an increase of plant diversity (De Deyn *et al.* 2003). However, the nematode community of that study mainly consisted of grass feeders, while in our study, the nematode community was dominated by *Meloidogyne hapla* (79% of the plant-feeders; 13 times more individuals than in the non-inoculated mesocosms; Table 7.1), which is known to prefer dicots (Goodey *et al.* 1965). Such information on host preference of root feeders in natural systems is crucial for predicting their effects on spatio-temporal processes in natural plant communities.

The effects of combined exposure to the above- and belowground herbivores were very different from the sum of the separate impacts of these herbivores. While the impact of simultaneous addition was expected to be positive, the joint effect of grasshoppers and wireworms reduced diversity. Thus, the interactions between the effects of the above- and belowground herbivores on individual plant species indicate that the separate

impacts of wireworms changed in the presence of grasshoppers. This change in impact on plant diversity was the result of differences (both positive and negative changes) in the abundance of component species. Positive effects of wireworms on subordinate plant species and a negative effect on a dominant dicot disappeared in the presence of grasshoppers. Moreover, the abundance of the dominant dicot *H. radicata*, which was not significantly ( $P < 0.05$ ) affected by wireworms or grasshoppers, significantly increased when wireworms and grasshoppers were added simultaneously.

Adding aboveground and belowground invertebrate herbivores to field mesocosms, as we did in our study, shows that feeding preferences of these herbivores are far more selective than can be demonstrated in biocide experiments carried out in the field (Brown & Gange 1989; Brown & Gange 1992). The results also emphasize that nematode effects can be more selective than generally assumed (Mortimer *et al.* 1999). Moreover, our results show that the effects of combined exposure to these herbivores cannot be deduced from their separate impacts due to changes in indirect interactions within the plant community.

Natural plant communities are exposed to a more diverse assemblage of above- and belowground herbivore species than added in our study. Diversity effects may be additive, as suggested for arbuscular mycorrhizal fungi (van der Heijden *et al.* 1998), or counteracting, as suggested for soil invertebrates of different size classes (Bradford *et al.* 2002). Moreover, over the longer term – exceeding our experimental period of two years – interaction patterns may change due to altered plant community dynamics. Nevertheless, our results strongly suggest that interactions between spatially separated herbivores alter the composition and diversity of natural plant communities in a way that is very different from the sum of their separate effects.

It has been acknowledged that above- and belowground organisms are involved in small-scale spatial heterogeneity in grasslands (Ritchie & Olf 1999; Blomquist *et al.* 2000; Olf *et al.* 2000; Ettema & Wardle 2002). Due to the clustered occurrence of belowground invertebrate herbivores and the better mobility of aboveground invertebrates (Mortimer *et al.* 1999) natural plant communities will be exposed to a spatial variety of above-belowground interactions. Our results show that these interactions may enhance the spatial heterogeneity of plant diversity in natural vegetation.

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## Interactions between herbivores alter plant diversity

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**Chapter**  
**8**

**General discussion**

Jasper van Ruijven



## Plant diversity – ecosystem functioning

The results presented in chapters 2 and 3 clearly show that primary productivity, as a measure of ecosystem functioning, increases with plant species richness. This pattern has been shown before (Hector *et al.* 1999; Tilman *et al.* 2001), but in these earlier experiments the nitrogen-fertilising effects of legumes played an important role (Huston *et al.* 2000; Mulder *et al.* 2002; Hille Ris Lambers *et al.* 2004). Our results provide the first evidence that this relationship emerges in the absence of legumes. Effects of legumes are important in grassland communities, but we found that other plant species are involved in interactions that result in a similar relationship between plant species richness and productivity.

Moreover, the results presented in chapter 3 emphasize that a positive relationship between diversity and productivity appears to be a long-term property of these perennial communities (Tilman *et al.* 2001; Mulder *et al.* 2002). The relationship emerged in the second growing season and became increasingly strong with time. This pattern strongly argues against alternative hypotheses that the observed results were caused by high initial growth rates of just a few plant species or that it was determined by differences in initial plant densities (Huston 1997; Huston *et al.* 2000).

In most experimental studies, including ours, biodiversity is reduced to species number and the abundances of the individual species are similar at the start of the experiment. Natural communities, however, are dominated by a few species whereas most other species are rare (Preston 1948; May 1975). As a consequence, biodiversity-ecosystem functioning studies have higher species evenness than normally encountered in natural communities (Schwartz *et al.* 2000; Wardle 2002). Recent studies show important effects of plant evenness (Nijs & Roy 2000; Wilsey & Polley 2002; Polley *et al.* 2003). Similar to the Cedar Creek experiment (Tilman *et al.* 2001), however, our experimental communities shifted to less even ones (resembling natural communities) over time due to competitive interactions.

### Complementarity vs. sampling effects

Much of the debate about the interpretation of the biodiversity experiments centred on the distinction between niche complementarity and sampling effects. The additive partitioning method (Loreau & Hector 2001) allowed a distinction to be made between the two effects. The results in chapters 2 & 3 show that complementarity effects prevailed in our experiment. Sampling effects generally were small and often not significant. This method, however, only identifies the effect caused by the increased probability of including a highly productive species (in monoculture),

which also performs best in mixtures. This is the 'original' sampling effect (Aarssen 1997; Huston 1997; Tilman *et al.* 1997). It is possible that the species that is very productive in mixtures does not perform best in monoculture: a species that is a superior interspecific competitor may experience increased intraspecific competition relative to other species. In particular, this may be true for legumes, which profit from fixing nitrogen instead of competing with other species for soil N. Clearly, this advantage is absent in monocultures. Other plant species, however, can show the same pattern (Hille Ris Lambers *et al.* 2004). Indeed, some of the species identified as contributors to increased mixture performance in chapter 3 were not among the species with high monoculture yields. This is identified as a negative sampling effect (see chapter 2). If different species give rise to positive and negative sampling effects, the overall sampling effect may be close to zero. An alternative method to separate the sampling effect from complementarity effects is to exclude all plots containing a particular species and consequently re-determine the relationship between diversity and productivity (Špaěková & Lepš 2001). Following this approach, however, we have to omit all plots at the highest level of diversity (because all eight species of the species pool are always present at that level) plus a number of two and four species plots<sup>1</sup>.

Nevertheless, we used this method to determine relationships between diversity and productivity across plots containing 1, 2 and 4 species. As an example, we omitted plots with *Plantago lanceolata*. In 2002 and 2003, it was very productive in mixtures, but showed low biomass in monoculture. Omitting all plots with *Plantago*, we still found highly significant positive relationships between diversity and productivity ( $F = 18.2$  and  $18.5$ , respectively,  $P < 0.001$ ). Similar results were obtained when omitting plots with *Centaurea jacea* and *Rumex acetosa* (these three species showed the highest contributions to mixture biomass). This shows that our results cannot be explained by the positive effects of a single species. This is confirmed by the results discussed in chapter 3, which show that more than half of the plant species contributed to increased productivity at high diversity in the last year. Even rare species can affect the functioning of ecosystems (Lyons & Schwartz 2001).

<sup>1</sup> To optimally use this method, an experiment with eight plant species should include - besides replicated monocultures of all species and eight-species mixtures - eight series of replicated plots containing 2, 4 and 7 species, each series lacking a different species. Although some plots could be used in multiple series (a plot containing species A and B can be used in all series except those that should lack A or B), this setup would require at least a doubling of the number of experimental plots!

**Box 8.1 An additional controversy?**

In recent discussions about the interpretation of biodiversity experiments, the role of productivity in regulating diversity also attracted attention. The 'hump-backed' relationship observed in field surveys (Al-Mufti *et al.* 1977; Grime 1979), showing highest diversity at intermediate levels of productivity, seems to be contradictory to the positive relationship between diversity and productivity (Fig. 8.1.1).

First of all, it is important to note that cause and effect are reversed in these two relationships. In the hump-backed curve, productivity is a measure of site fertility, which is assumed to determine the number of species. In contrast, the biodiversity experiments are carried out at constant site fertility. At a given level of fertility, productivity (yield) is treated as an effect of species diversity.

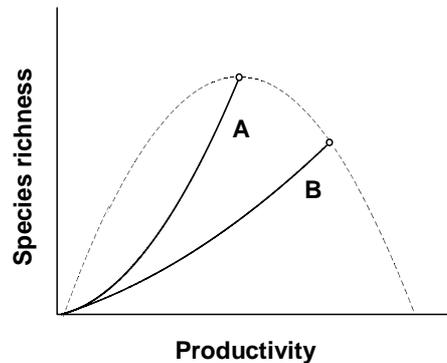
In a recent paper, Schmid (2002) provided a graphical resolution to the apparent contradiction. In a 3D graph, site fertility is introduced as the third axis. The hump-backed curve can be drawn above the diagonal between fertility and productivity (Fig. 8.1.1). This diagonal reflects maximum productivity at a given level of fertility when the species pool is intact. As fertility does not change in experimental studies, their diversity-productivity curves will be constrained to rectangular planes (A and B in Fig. 8.1.2). The two curves thus represent the effects of species loss at two site fertilities, with maximum site productivities following the hump-backed curve.

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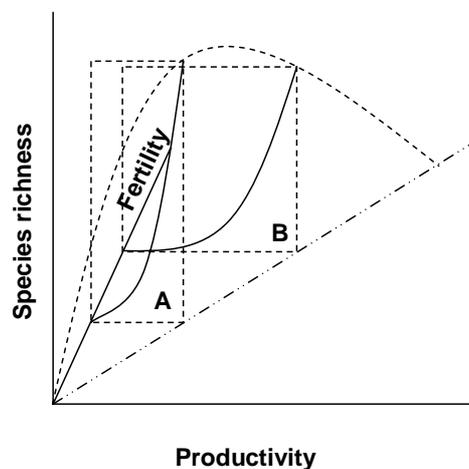
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**Figure 8.1.1** The relationship between plant species richness and productivity in grasslands. The dashed line represents the relationship that has been observed in field surveys; the solid lines (A and B) indicate curves that are typically found in experiments that simulate species loss (Schmid 2002).



**Figure 8.1.2** 3D representation of figure 8.1.1 (Schmid 2002).

**Underlying mechanisms**

Greater productivity is caused more by complementarity than by sampling effects (Chapter 3; Loreau & Hector 2001; Tilman *et al.* 2001), yet the underlying mechanisms of complementarity are poorly known. We found, however, that the dominant dicots

(*Centaurea jacea*, *Plantago lanceolata* and *Rumex acetosa*) acquired greater amounts of nutrients at high diversity. This indicates that they are superior competitors for nutrients (mainly nitrogen), similar to the C4 grasses in Minnesota grasslands (Hille Ris Lambers *et al.* 2004). In contrast to these C4 grasses, however, the dicot species did not reduce the performance of most other species in our experiment. The positive values of RYT-N (chapter 3) strongly suggest that niche differentiation in nutrient uptake is important. Both spatial and temporal differences in nutrient uptake may be important (McKane *et al.* 1990; Craine *et al.* 2003) and plant species may also differ in the chemical form of nitrogen they take up (McKane *et al.* 2002).

This is not the only mechanism that contributed to increased biomass at high diversity. We also found that total aboveground biomass at high diversity was enhanced by plants increasing the amount of aboveground biomass per unit nitrogen. How this works is not clear. Perhaps these plant species invested relatively more biomass into flowering stems in mixtures. Because stems generally show lower nutrient concentrations than leaves, this might explain the observed pattern.

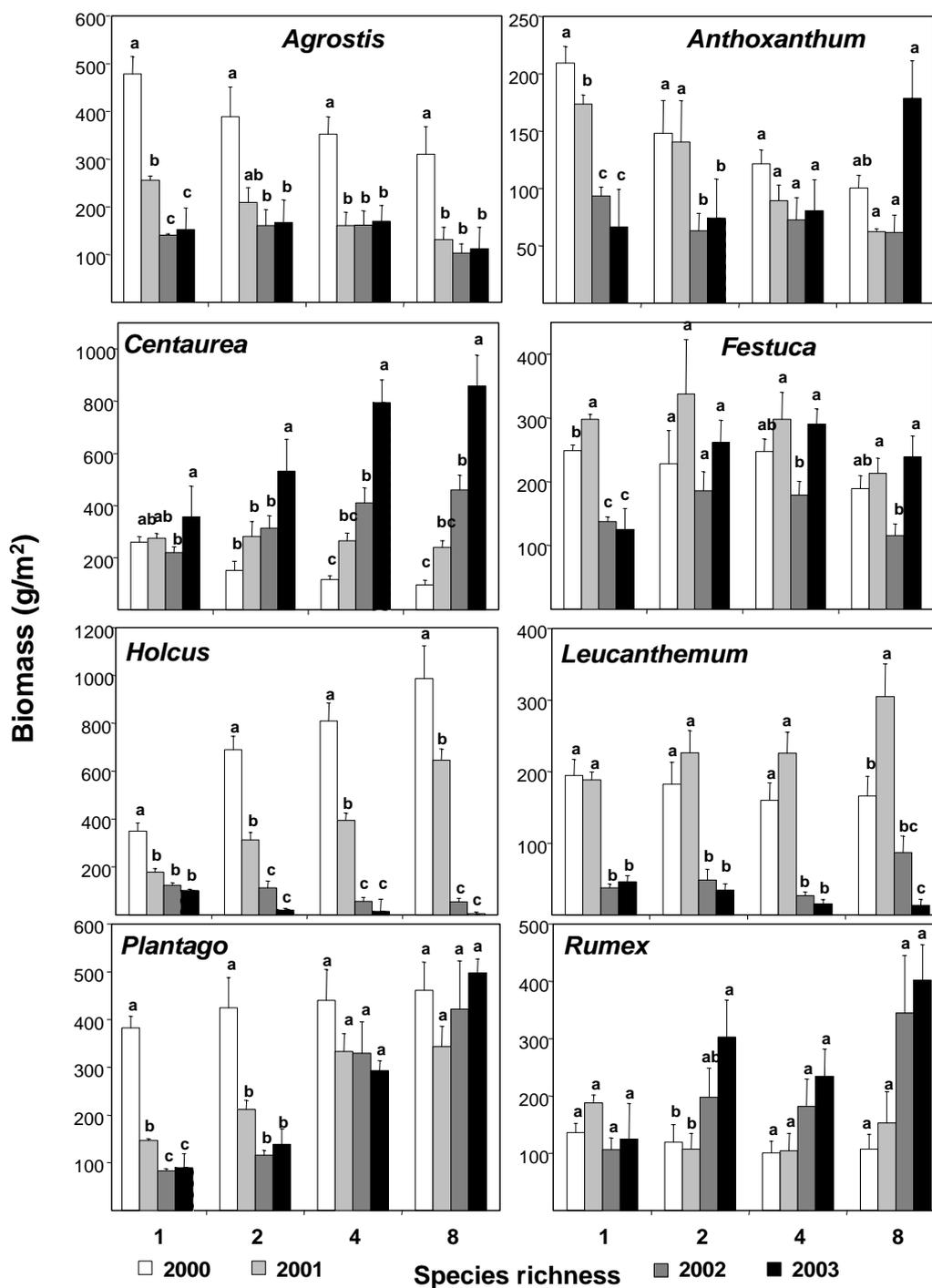
Facilitation may be an important additional mechanism (Cardinale *et al.* 2002; Bruno *et al.* 2003; Callaway *et al.* 2003), but we found no indication of significant contributions of facilitative interactions. On larger temporal scales, species may respond differently to climatic fluctuations (Tilman & Downing 1994; Craine *et al.* 2003) and may be offset in their successional status. Niklaus *et al.* (2001) found that communities containing fast- and slow-growing species maintained higher productivity over time. Early in the experiment, fast-growing species had high productivity due to high nitrogen availability. As nitrogen availability declined, they were replaced by slow-growing species. This is very similar to what we observed: the fast-growing *Holcus lanatus* initially showed high productivity and dominated the mixtures, but was steadily replaced by the slow-growing dicot *Centaurea jacea*.

### **Biotic control of the relationship between diversity and productivity?**

So far we focussed on abiotic circumstances and properties of the plant species. Several studies, however, have shown that higher trophic-level organisms may have strong effects on the relationship between diversity and productivity (van der Heijden *et al.* 1998; Mulder *et al.* 1999; Klironomos *et al.* 2000; Rudgers *et al.* 2003; Thebault & Loreau 2003). Host-specific natural enemies such as herbivores and pathogens may contribute to overyielding if their effects are diversity-dependant (Carson & Root 2000; Klironomos 2002). In Chapter 5, we have shown that the diversity of

nematodes depends on plant diversity. The plant-species specific accumulation of plant-parasitic nematodes, however, was not diversity-dependant. Patterns of plant-specific accumulation of nematodes could be detected both in monocultures and in mixtures. Other natural enemies, however, might show different patterns. The spread of plant fungal diseases, for example, was found to be higher at low plant diversity (Knops *et al.* 1999).

Is there any evidence supporting the hypothesis that diversity-dependant effects of natural enemies contributed to complementarity in our experiment? If true, we would expect the change in monoculture biomass over time to be different from that in mixtures. Figure 8.1 shows the biomass per species at each level of diversity in the four experimental years. For each plot, species biomass was standardized by multiplying it to the number of species. Many species showed reductions of aboveground biomass with time, but often the patterns are quite similar at all levels of diversity. This pattern is probably caused by a reduction of nutrient availability. Initially, nutrient availability was relatively high in the unoccupied soil. Because of plant roots exploiting the soil volume and the annual removal of aboveground plant material, nutrient availability decreased. In addition, plants may have responded to the decreased availability by a shift in the shoot-root ratio. Over time, plants probably invested relatively more biomass into roots and less into aboveground biomass. *Centaurea* and *Rumex* show stable monoculture biomass, and moderate to large increases of biomass at higher levels of diversity. One could argue that the lack of increase in monoculture is caused by the accumulation of natural enemies, but it is more likely that the increase in diverse plots is caused by complementary interactions (e.g. differences in rooting depth) that cannot occur in monocultures. *Anthoxanthum odoratum* and *Festuca rubra* show increased biomass at high diversity, but not in monoculture, in the last year. This pattern, however, is probably the result of the strong decline of *Holcus* at higher diversity (chapter 3). *Plantago* is the only species to show a marked decline of biomass in monocultures, whereas that pattern is less or absent at higher levels of diversity. It is unlikely that this pattern is caused by plant parasitic nematodes as this plant species showed very slow accumulation of nematodes (chapter 5). We cannot rule out, however, that the decline in monocultures is caused by accumulation of other natural enemies, such as pathogenic fungi (Marak *et al.* 2002; Dudycha & Roach 2003). Thus, it is possible that complementarity has increased due to poor monoculture performance caused by the accumulation of natural enemies, but only for one of the five species that showed overyielding in the final year of the experiment.



**Figure 8.1** Aboveground biomass of each species at the different levels of diversity. Biomass data have been multiplied by the number of species in the plot to allow comparison. Different letters denote significant ( $P < 0.05$ ) differences between years at a given level of diversity

## Diversity – invasion resistance

In natural communities, fluctuation in resource availability (coinciding with propagule availability) has been identified as the key factor controlling the susceptibility of a community to the invasion of non-resident species (Davis *et al.* 2000). When variation in extrinsic factors is removed, however, plant diversity is a strong determinant of plant invasion (chapter 4, see (Levine *et al.* 2002) for a review). The increase of invasion resistance, however, is not caused by diversity *per se*. Our results strongly support earlier work that showed that species identity, rather than species diversity, determines the reduction of invasion (Crawley *et al.* 1999; Wardle 2001). One of the suppressive species was the canopy dominant (*Centaurea jacea*). Dominant species often are superior competitors and have strong impacts on ecosystem properties (Smith & Knapp 2003; Smith *et al.* 2004; Dangles & Malmqvist 2004). The strong impact of the other species (*Leucanthemum vulgare*) on invasibility was unexpected, because it was a poor competitor in our experiment and it constituted only a minor fraction of the total biomass of mixtures. After a sudden decline in 2002, it almost disappeared from high-diversity plots (chapter 3). Still, it had a strong negative impact on plant establishment. We hypothesized that this may have been caused by the accumulation of plant-parasitic nematodes (chapter 4). Several studies have shown that feedbacks with soil communities limit the abundance of plant species (van der Putten & Peters 1993; Bever 1994; Mills & Bever 1998; Klironomos 2002). Our results indicate that these negative feedbacks may be less selective. As the majority of the invading species in our experiment belong to the same family of plants as *Leucanthemum* (Asteraceae), accumulation of plant-parasitic nematodes under individuals of *Leucanthemum* might result in patches that cannot be colonized by *Leucanthemum* and related plant species. Similar to plant-species specific ones, these feedbacks may give rise to shifting mosaics in natural vegetation (Blomquist *et al.* 2000; Olf *et al.* 2000).

## Biotic controls of plant diversity

A large number of studies have demonstrated significant impacts of a range of organisms on plant diversity. Vertebrate herbivores (Pacala & Crawley 1992; Bever 1994; Olf & Ritchie 1998), insect herbivores (Brown & Gange 1989a; Brown & Gange 1989b; Carson & Root 2000; Fine *et al.* 2004), soil pathogens (van der Putten & Peters 1993; Bever 1994; Holah & Alexander 1999; Olf *et al.* 2000; Klironomos 2002), soil fauna (De Deyn *et al.* 2003), AMF (chapter 6; van der Heijden *et al.* 1998) and fungal endophytes (Clay & Holah 1999) are important determinants of plant diversity. The

most prominent mechanism by which these organisms promote plant diversity is by altering the competitive balance between plants. Subordinate (Grime 1977) species can be stimulated directly, for example through enhanced nutrient acquisition (AMF). Indirectly, subordinates may profit from suppression of dominant plant species by above- and belowground herbivores and pathogens. In addition, these organisms may play important roles by promoting the colonization of plant species and altering soil nutrient dynamics.

Considering the fact that many of the organisms mentioned above exert their effects simultaneously in natural communities, it is perhaps surprising that the joint effects of these organisms received relatively little attention. Several studies examined interactions between different species within a single group of organisms, like vertebrate herbivores (Huisman & Olff 1998; Arsenault & Owen-Smith 2002; Bakker 2003) but interactions among different groups are rarely studied. Experimental studies on interactions between above- and belowground herbivores are limited to two insect herbivores on a single host plant (Masters & Brown 1992; Masters *et al.* 1993; Maron 2002; Bezemer *et al.* 2003; Poveda *et al.* 2003; van Dam *et al.* 2003). Field studies are mainly limited to experiments comparing undisturbed and insecticide-treated plots (Brown & Gange 1989a; Brown & Gange 1989b), but biocides are unlikely to selectively eliminate one trophic group. Thus, the effects of above- and belowground herbivores in diverse plant communities remained unknown (Wardle 2002). The results presented in chapter 7 provide the first experimental evidence of significant interactions between above- and belowground herbivores affecting plant diversity. Our study clearly shows that the joint effects of above- and belowground herbivores are very different from the sum of their separate impacts. Thus, it is necessary to include multiple 'drivers of plant diversity' in experiments to fully understand the dynamics in plant communities.

### **Future challenges**

Progress has been made in describing the relationship between diversity and ecosystem processes, in identifying important species, and in revealing underlying mechanisms. There is, however, uncertainty how to scale up the results of recent experiments and how to generalize across ecosystem types and processes (Loreau *et al.* 2001).

Functional effects of biodiversity changes at landscape and regional scales are largely unknown, yet may be particularly important (Bengtsson *et al.* 2002). Experiments at small temporal scales are also difficult to extrapolate to long-term patterns. The current experiments may underestimate the importance of diversity

because species typically replace each other along gradients. The insurance hypothesis and related hypotheses propose that diversity provides a buffer against environmental fluctuations because different species respond differently to these fluctuations, leading to more predictable ecosystem processes (Yachi & Loreau 1999).

So far, experiments mainly assessed the relationship between plant diversity and productivity in grasslands. Different interactions between species, and between species and abiotic factors, may lead to differences in biodiversity effects on ecosystem processes (Loreau *et al.* 2001). It is questionable if the results obtained from plant diversity-productivity experiments can be generalized to other ecosystem processes and other trophic levels. For example, few consistent trends in the relationship between diversity and the soil community and soil processes have emerged (Wardle 2002; Wardle & van der Putten 2002).

Evidently, interactions between plants and other organisms play key roles. Plant-organism relationships that are based on host specificity or involve relatively few key species which carry out a unique process (e.g. symbiotic nitrogen fixation) are likely to show positive effects of diversity (Wardle & van der Putten 2002). Many interactions, however, are based on differential susceptibility of plant species to certain mutualists, pathogens or herbivores. These interactions may be particularly important for the regulation of plant community composition (chapter 7; Wardle & van der Putten 2002). Moreover, we have shown that the effects of these interactions depend on the effects of other interactions occurring simultaneously (chapter 7). Our study was limited to above- and belowground invertebrate herbivores, but many other organisms are likely to show similar interactions.

In conclusion, future research should move beyond approaches in which diversity is either cause or effect, and address interactions among changes in biodiversity, ecosystem functioning, and environmental factors to understand and predict changes in biodiversity and ecosystem processes at large spatial and temporal scales (Loreau *et al.* 2001). Furthermore, it is necessary to extend current knowledge to other ecosystem types and processes. Finally, a combined aboveground-belowground approach to community and ecosystem ecology is required to enhance our understanding of the regulation and functional significance of biodiversity (van der Putten *et al.* 2001; Bardgett & Wardle 2003; Wardle *et al.* 2004).

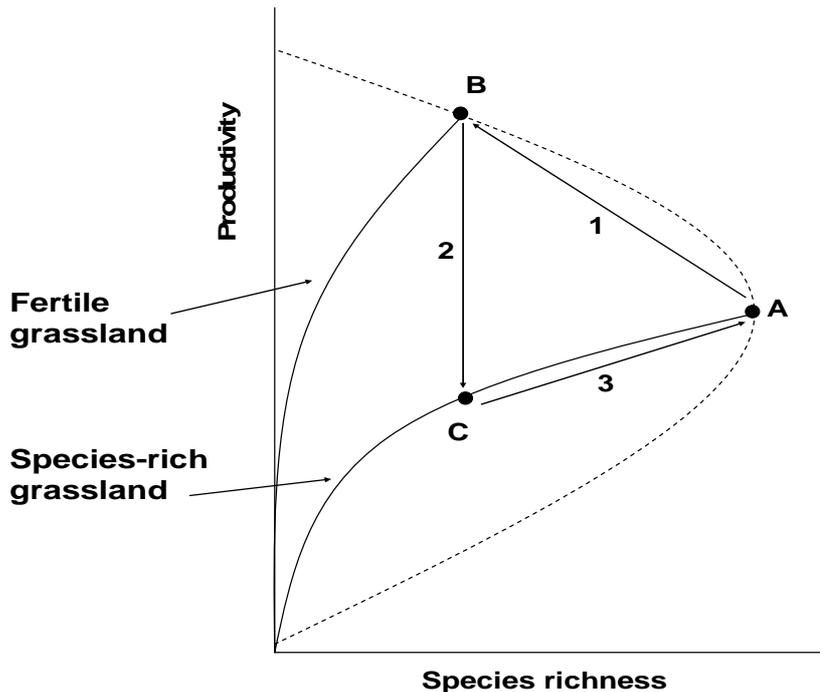
## **Prospects for the restoration of grasslands in the Netherlands**

The previous chapters have shed some light on the consequences and causes of plant diversity in nutrient-poor, relatively species-rich grasslands. Intensification of agricultural practices in such species-rich grasslands has led to an increase of productivity and a loss of species. As a consequence, this type of grassland has disappeared from the agricultural landscape and is nowadays restricted to nature reserves and road verges. Can the knowledge about important interactions in grasslands, as described in the previous chapters, be used to overcome bottlenecks during the restoration process?

Restoration of these grasslands usually starts with reducing soil fertility (Marrs 1993). This can be achieved by mowing and hay-removal, extensive grazing or top soil removal (Bakker 1989; Olff & Bakker 1991; Bakker *et al.* 2002; Moog *et al.* 2002). The theory behind these restoration practices is based on the hump-backed relationship between fertility (productivity) and diversity Al-Mufti *et al.* 1977; Grime 1979; see also Box 8.1). When fertility is very low, species richness is low because only a few stress-tolerant species can survive. At the other end of the range, at high levels of fertility (current agricultural grasslands), species richness is also low. Plants compete for light and most plant species disappear because of competitive exclusion by a few fast-growing grass species. The highest levels of species richness are generally found at intermediate levels of fertility.

Although the approach of reducing fertility has been successful, there are also examples in which these restoration efforts were not (Berendse *et al.* 1992). This may depend on abiotic factors other than soil fertility. Years of agricultural practices may have resulted in an altered nutrient balance, which is not necessarily restored by reducing fertility (Olde Venterink *et al.* 2003). Furthermore, it may be necessary to restore the former hydrological conditions (Roelofs *et al.* 1996). Biotic constraints, however, may also play a role. The low availability of seeds often poses a serious problem, because many species are no longer present in the seedbank and seed dispersal is severely limited due to habitat fragmentation (Bakker *et al.* 1996; Bakker & Berendse 1999; Jones & Hayes 1999; Bekker *et al.* 2000; Lindberg & Eriksson 2004). Figure 8.2 provides an integration of biodiversity-ecosystem functioning research, the hump-backed curve and restoration management.

Even when these problems have been solved, however, restoration of plant diversity may be difficult. We hypothesized that soil-biotic constraints were responsible for the lack of success of these restoration measures. Soil communities also respond to years of intensive agricultural practices. AMF, which play an important role in maintaining plant diversity (van der Heijden *et al.* 1998), are known



**Figure 8.2.** Relationships between species richness and productivity. The dashed curve is the hump-backed curve, the dashed lines are the relationships observed in biodiversity experiments. Fertilization in species rich grasslands (point A) leads to an increase of productivity and a loss of species (arrow 1). Extensification reduces productivity (arrow 2), but may not increase species richness if the species lost in step 1 are also lost from the surrounding region. Note that point C can also be reached if species loss is simulated in species-rich grassland. Adding species increases both productivity and species richness (arrow 3). After Schmid (2002).

to severely decrease in intensively-used agricultural fields (Eason *et al.* 1999; Eriksson 2001). Many other soil organisms are also known to respond strongly to intensive agricultural practices (Korthals *et al.* 2001; Berg & Hemerik 2004). Because of the limited dispersal capacities of many soil organisms (Korthals *et al.* 2001), it is reasonable to assume that even after years of reducing fertility the soil community will be very different from the original one. It has been suggested that the introduction of soil biota from species-rich grasslands may accelerate vegetation development on former agricultural fields (van der Putten *et al.* 2001). As nutrient availability declines, the impact of interactions with soil organisms on plant competition generally increases (Bardgett & Wardle 2003; Reynolds *et al.* 2003).

We tested the hypothesis that introducing soil biota from species-rich grasslands stimulates the restoration of these grasslands on former agricultural fields. Next to the experiments described in the previous chapters, we performed a field experiment in two grasslands in the vicinity of Wageningen, the Netherlands (51° 59' N, 05° 40' E). Both sites were intensively used agricultural pastures before restoration management (mowing and hay removal) started.

At site one ("Mansholt"), this management started in 2000, whereas at the other (Born) it had already begun in 1973. Details of the two field sites are given in Table 1.1. The reference site ("Smalbroeken"), a species-rich grassland site managed as a hay meadow for more than 150 years, was located in a nature reserve in the vicinity of Boxtel, The Netherlands (51° 34' N, 05° 17').

We introduced soil biota in two different ways in May 2000: 1) by placing entire soil columns (25 x 25 x 15 cm) into the soil of two field sites. Per 3 x 3m plot nine columns were placed following a regular 3 x 3 matrix. 2) We added the soil as a thin-layer of soil suspension on top of the vegetation. The three treatments (control, soil blocks and soil suspension) were duplicated per block. One of these plots received a seed mixture (2500 seeds/m<sup>2</sup>), whereas the other received none. Seeds were added twice, both in 2000 and in 2001. Seed addition was done to overcome the dispersal bottleneck, because the seed bank did not contain any seeds of plant species that were expected to occur in the former species-rich grasslands.

**Table 8.1** Characteristics of the field sites. Numbers of species refer to all species found in 40 0.25 m<sup>2</sup> plots (12 plots for Smalbroeken). Numbers for individual species denote their mean proportional biomass in these plots. Only the most abundant species are shown.

	Mansholt	Born	Smalbroeken
<b>Plant productivity (tonnes/ha/y)</b>	10.2	6.3	3.7
<b>pH</b>	5.03	4.67	4.36
<b>Organic matter (%)</b>	4.84	6.62	8.36
<b>Available P (P(H<sub>2</sub>O) mg/100g)</b>	0.92	0.36	0.21
<b>Number of grass species</b>	<b>6</b>	<b>5</b>	<b>6</b>
<i>Agrostis canina</i>	--	--	22
<i>Agrostis capillaris</i>	--	41	--
<i>Anthoxanthum odoratum</i>	--	20	< 1
<i>Festuca rubra</i>	--	15	< 1
<i>Holcus lanatus</i>	20	8	< 1
<i>Lolium perenne</i>	8	--	--
<i>Molinia caerulea</i>	--	--	41
<i>Phleum pratense</i>	69	--	--
<b>Number of dicot species</b>	<b>5</b>	<b>8</b>	<b>14</b>
<i>Achillea ptarmica</i>	--	--	1
<i>Cirsium dissectum</i>	--	--	2
<i>Lotus pedunculatus</i>	--	--	1
<i>Lysimachia vulgaris</i>	--	--	4
<i>Plantago lanceolata</i>	--	1	--
<i>Ranunculus acris</i>	--	1	--
<i>Rumex acetosa</i>	1	10	--
<i>Succisa pratensis</i>	--	2	2
<b>Number of sedges / rushes</b>	<b>0</b>	<b>1</b>	<b>5</b>
<i>Carex hirta</i>	--	< 1	--
<i>Carex nigra</i>	--	--	3
<i>Carex panicea</i>	--	--	16
<i>Juncus acutiflorus</i>	--	--	3
<i>Juncus conglomeratus</i>	--	--	3

Moreover, nearby populations of these species were at least 200m to more than 5km away and separated from the experimental fields by forest, hedgerows and buildings.

Each year, we monitored vegetation development by harvesting aboveground biomass, sorting it to species and determining dry weight. In addition seedling establishment of the sown species was monitored. During three years of observations, however, we could not detect any effects of the introduced soil organisms. No changes in vegetation composition were observed and establishment of the sown species generally was very poor (1-5 individuals per 0.25 m<sup>2</sup> subplot, which did not survive beyond the seedling stage). In fact, only at the fertile site two of the sown species (*Centaurea jacea* and *Prunella vulgaris*) actually established, but this was restricted to plots of an additional treatment, which were mown every month during the growing season (to mimic cattle grazing).

It is perhaps tempting to conclude that soil organisms are not important for the restoration of species-rich grasslands. This conclusion requires that the introduction of these organisms has been successful. We found no changes, however, in nematode communities or colonization by AMF. Most likely, the treatments had no effect because the introduction failed. This may have several reasons. First, the differences in abiotic conditions (soil characteristics, hydrology) between the target site and the two field sites may have prevented establishment of the introduced soil community. Second, the resident soil communities in the two field sites may have made establishment of the 'new' community impossible. It has been hypothesized that the residential species are a barrier against introduced soil organisms (De Deyn 2004). Degraded communities can sometimes be resilient to restoration efforts (Suding *et al.* 2004). Third, the establishment of a new soil community may require more time than the three experimental years.

The previous chapters provide multiple examples of strong biotic control of plant diversity, yet application of this knowledge under field conditions remains difficult. To overcome the bottlenecks reported here, restoration management should pay attention to the following points: 1) Important abiotic characteristics (pH, nutrient-ratios, soil texture, hydrology) of the fields under restoration should be similar to those of the target community 2) The ecological constraints or bottlenecks should be identified. If there are multiple constraints, it is crucial to prioritize these and to determine if they should be addressed separately or together to restore the community (Suding *et al.* 2004). 3) If local extinction of plants and other organisms is widespread, recolonization will require deliberate reintroduction (Schmid 2002). 4) To overcome the barrier formed by the resident community, it is perhaps better to remove that community. Indeed, in recent experiments the introduction of soil

organisms and plant seeds is combined with top soil removal. The preliminary results are promising: several target plant species established on former arable fields (P. Kardol, pers. comm.).

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## SUMMARY

There are many reasons (cultural, aesthetic, ethical, economic) reasons why we should halt the current wave of species extinctions. It was the notion that the functioning of ecosystems might be affected by the current loss of biodiversity, however, that has led to the emergence of biodiversity-ecosystem functioning research (BEF). The issue was formally postulated on a conference in Germany in 1991. The idea of a relationship between diversity and ecosystem functioning inspired ecologists to formulate a range of hypotheses. Obviously, these hypotheses had to be tested experimentally. Small-scale experimental grassland plots proved an ideal experimental setting for this purpose. Grasslands constitute an important ecosystem type that occurs worldwide. They are often grazed by one to many species of vertebrate herbivores, but especially in Europe there is a long history of haymaking in grasslands. Instead of being grazed, these hay meadows are mown regularly, often when vegetation reaches peak standing crop. When they are mown every year, the hay is a reasonable estimate of annual plant productivity. Primary productivity is one of the most important processes in many terrestrial and aquatic ecosystems and is considered a good predictor of ecosystem functioning.

Many studies with experimental grasslands communities have been performed, the most extensive of which are the pan-European BIODEPTH programme and the experiments at Cedar Creek (Minnesota, USA). These studies often showed a positive relationship between diversity and plant productivity. It was proposed that this was caused by niche complementarity: species have different strategies to acquire and use nutrients, which results in more complete use of the available resources in diverse communities.

This interpretation, however, sparked an intense debate. Although there were additional reasons for this unusual intensity, the scientific debate basically centred around two issues. The first one was the fact that the results could also be explained by a so-called sampling effect. This means that high-diversity plots have a higher probability of containing species with major impacts on whatever ecosystem process measured. Initially, it proved difficult to distinguish complementarity from sampling effects, but a new partitioning method enabled a distinction between the two effects. The second point involved the role of legumes. Legumes can strongly increase both productivity and complementarity because they do not compete with other plants for soil nitrogen, but fix atmospheric  $N_2$ . In addition, the fixed N can be transferred to co-occurring plants by root exudation and decomposition of dead roots. As a consequence, the greater probability of including legume species at greater species numbers will increase both the

productivity and the degree of complementarity in species-rich mixtures. Several studies indeed showed an important role of legumes in the positive relationship between diversity and productivity. These effects of legumes are difficult to detect using the additive partitioning method. Moreover, the two major sets of biodiversity experiments were not designed to adequately disentangle the effects of legumes from other diversity effects.

We performed an experiment similar to those in earlier studies, but we simply omitted legumes. This approach yielded several important results. In the first year, a sampling effect occurred: the fast-growing and high-productive grass *Holcus lanatus* strongly dominated the mixtures. This dominance, however, did not result in a positive relationship between diversity and productivity because the increase of *H. lanatus* was nullified by a decrease of several other species. In the second year of the experiment, a positive relationship between productivity and diversity emerged. This relationship became stronger in later years. We found that this pattern was mainly caused by niche complementarity. Nutrient analysis revealed that spatial and/or temporal complementarity in nutrient uptake was one of the underlying mechanisms. In addition, more than half of the plant species used nutrients more efficiently for aboveground biomass production at high diversity. The number of species contributing to the positive effect of diversity increased with time. In 2003, five of the eight species in the species pool had significant positive effects on productivity. Two species, however, showed a negative contribution. The fast-growth strategy of the initial dominant, *H. lanatus*, became unfavourable when nutrient availability declined, whereas *Leucanthemum vulgare* probably suffered from the accumulation of large numbers of parasitic nematodes. Both species were gradually outcompeted by the other species and virtually disappeared from high-diverse plots in the last year of the experiment.

A long-standing issue in ecology is that of the relationship between diversity and invasion success. Already in 1958, Elton proposed that diverse communities would be more resistant to the invasion by exotic species. Invasion by exotic (not native) species poses a serious problem worldwide, and it has been proposed that the loss of biodiversity might facilitate the expansion of exotic species. Experiments with artificial communities provide an ideal setup to test if there is a relationship between diversity and invasion success, because other factors (disturbance, nutrient availability) that frequently co-vary with diversity are kept constant. Our analysis of weeding data in 2002 revealed that the invasion by species that were not present in our species pool was indeed reduced at high diversity. Careful analysis revealed, however, that

this was mainly caused by the increased presence of two suppressive plant species at higher diversity, although diversity *per se* had significant additional effects. Using the terms of the debate about diversity-ecosystem functioning, this would be a sampling effect. Those two species that had a strong negative impact on invading species were very different. One of them, *Centaurea jacea*, was the dominant species in the mixtures. It is not surprising that a species, which effectively competes with the other species in the mixture, also reduces invading plants. The other species, however, was *Leucanthemum vulgare*, which was not abundant. We hypothesized that the accumulation of parasitic nematodes, which seemed to have reduced the growth of this plant species in the experiment, has also limited the success of invading species.

Changes in plant diversity may also affect organisms at higher trophic levels, like herbivores. Studies investigating the effects of plant diversity on invertebrates, however, showed conflicting results, which may be explained by differences in specialisation and mobility of the herbivores, plant nutritional quality and specific plant traits. We investigated the role of plant diversity in regulating the nematode community and found that nematode diversity increased with increasing plant diversity. The abundance of nematodes was not affected by plant diversity. Detailed analysis showed that especially plant identity had a strong impact on the nematode community. Each plant species accumulated a slightly different community of nematodes in its rooting zone. As a result, diverse plant communities harboured different nematode communities, leading to higher overall nematode diversity.

Herbivores and other organisms respond to plant diversity, but they can also control plant diversity. Studies have shown impacts of vertebrate herbivores, plant-feeding insects, mutualistic fungi and soil fauna on plant diversity. This indicates that changes in the diversity and abundance of these organisms may affect plant diversity and, consequently, ecosystem functioning. Our work with AMF, a group of fungi that enhance the acquisition of phosphorus by plants, also shows that these organisms are important for plant diversity. In contrast to earlier studies, we performed our experiments on non-calcareous soil, in which phosphorus is not limiting plant growth. We also found that AMF affect plant diversity. They altered competition among adult plants and enhancing seedling establishment of dicot species in vegetation completely dominated by grasses.

Many studies (including our study on AMF), however, have considered these organisms in isolation from other organisms which may simultaneously affect plant diversity. Studies with different

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groups of organisms include either different species of the same group (e.g. vertebrate herbivores) or are carried out on a single host plant. Our experiment was done to test if interactions between different organisms that have the potential to affect plant diversity result in changes in the impact on plant diversity. We exposed experimental communities to aboveground insect herbivores, belowground insect herbivores, and nematodes, separate and in combination. The results show that interactions indeed lead to different impacts on plant diversity. Especially interactions between aboveground and belowground insect herbivores led to a shift in the effect on plant diversity, which cannot be predicted based on the separate impacts of these herbivores. A neutral effect of grasshoppers on plant diversity and a positive effect of wireworms turned into a strong negative impact on plant diversity when both grasshoppers and wireworms were present in the plant community. This shift is caused by differences in feeding preferences among the herbivores and indirect changes in interactions among plants.

In conclusion, plant diversity enhances primary productivity through complementarity interactions among plant species. Invasion success is largely determined by the presence of certain species instead of diversity. Nevertheless, the loss of these species might accelerate the expansion of exotic species. Plant diversity is also important for other organisms, like nematodes. Biodiversity, however, is more than plant diversity. We have shown that many other organisms can affect plant diversity, which may feedback on ecosystem functioning. In this respect, especially the role of interactions among different groups of organisms in regulating plant diversity and ecosystem functioning deserves further attention.

Are the data presented in this thesis sufficient to conclude that the loss of biodiversity reduces the functioning of ecosystems? The answer is an obvious no, but together with well-known examples of the importance of keystone-species they do provide indications that the loss of biodiversity has important consequences.

Ultimately, biodiversity is the result of a range of mutual interactions among abiotic factors, primary producers, higher-trophic level organisms and ecosystem processes. Integration of these interactions into a unified picture, across multiple ecosystem types and processes, is crucial if we want to predict the world-wide consequences of the current wave of species extinctions.

## Samenvatting

Er zijn verscheidende redenen waarom het uitsterven van soorten beëindigd dient te worden – ethische, economische, culturele en esthetische. Het was echter de veronderstelling dat de huidige achteruitgang van de biodiversiteit op aarde invloed zou kunnen hebben op het functioneren van ecosystemen die leidde tot de opkomst van een nieuw onderzoeksveld. Dit biodiversiteitsonderzoek (biodiversity-ecosystem functioning research) heeft zijn oorsprong op een congres in Duitsland in 1991, waar ecologen werd gevraagd hoe de relatie tussen biodiversiteit en het functioneren van ecosystemen eruit zou zien. De vraag leidde tot een heel scala aan hypothetische lijnen (zie figuur 1.3). Uiteraard diende deze hypothesen middels experimenteel onderzoek getoetst te worden. Graslanden bleken een ideaal onderzoekssysteem. Dit type ecosysteem komt over de hele wereld voor. Vaak worden ze gevormd en in stand gehouden door groepen grote grazers, maar vooral in Europa is er ook een eeuwenlange traditie van zogenaamde hooilanden. Deze worden niet continu begraasd, maar regelmatig gemaaid. Zeker als dat maaien één keer per jaar plaatsvindt, op het moment dat de vegetatie haar hoogste punt bereikt, vormt het maaisel een goede schatting van de jaarlijkse productie van de planten in het grasland. Deze productie door planten is één van de belangrijkste processen in een ecosysteem en is daardoor een geschikte indicator voor het functioneren van het ecosysteem.

Na 1991 zijn er een aantal experimenten met experimentele graslanden uitgevoerd. De meest bekende zijn het Europese BIODDEPTH programma en de experimenten in Cedar Creek (Minnesota, V.S.). Deze studies lieten zien dat er een positief verband is tussen diversiteit en produktiviteit: de biomassa van de vegetatie neemt toe met een toenemend aantal plantensoorten. De onderzoekers stelden dat dit fenomeen werd veroorzaakt door het zogenaamde niche-complementariteit effect. Niche complementariteit betekent dat soorten verschillende strategieën hebben om de aanwezige resources (nutrienten, water, licht, etc.) te benutten, die elkaar aan vullen. Als een gevolg daarvan benutten soorten de resources samen (in mengsels) beter dan elk apart (in zogenaamde monoculturen).

Deze interpretatie leidde echter tot verhitte discussies. Die draaiden eigenlijk om twee punten. Ten eerste werd gesteld dat de resultaten ook verklaard konden worden door een zogenaamd selectie-effect. Deze verklaring hield in dat gemeenschappen met meer soorten een hogere kans hebben om een soort te bevatten die zeer productief is en daardoor de biomassa van het mengsel sterk vergroot. In eerste instantie bleek het moeilijk deze twee verklaringen (complemen-

tariteit en selectie) uit elkaar te halen, maar na de publicatie van een nieuwe methode in 2001 leek dit probleem opgelost.

Het tweede punt van discussie was de rol van vlinderbloemige planten, zoals klavers. Deze soorten hebben een groot effect op de primaire productie omdat ze niet met andere soorten concurreren om stikstof, maar hun stikstof uit de lucht halen met behulp van stikstofbindende bacteriën. Deze stikstof kan ook in andere planten terchtkomen via de afbraak van de dode wortels van vlinderbloemigen. Op die manier kunnen ze zowel de totale biomassa als de mate van complementariteit sterk verhogen. Dit werd ook gevonden in verschillende experimenten. In die experimenten bleek het bovendien erg lastig om de effecten van deze vlinderbloemigen te onderscheiden van die van plantendiversiteit.

Wij hebben een vierjarig diversiteitsexperiment uitgevoerd dat weliswaar vergelijkbaar is met eerdere experimenten, maar waarin de rol van vlinderbloemigen werd uitgesloten, simpelweg door ze niet in het experiment op te nemen. Dit experiment, met slechts grassen en kruiden, leverde interessante resultaten op.

In het eerste jaar trad er een selectie-effect op: de snelgroeiende soort *Holcus lanatus* haalde een hoge productie in z'n monoculturen en domineerde ook de biomassa in de mengsels. Dit leidde echter niet tot een positief effect van diversiteit op de totale biomassa van de planten. Dit werd veroorzaakt door de sterke competitie tussen *H. lanatus* en de andere soorten: de toename van *H. lanatus* ging ten koste van de groei van de andere soorten.

In het tweede jaar echter, vonden we een positieve relatie tussen plantendiversiteit en -biomassa. Deze relatie bleef ook in de volgende jaren bestaan en werd zelfs sterker (zie fig. 3.1). Dit patroon bleek niet veroorzaakt te worden door selectie-effecten, maar door complementariteit. Uit de analyse van de nutriënten in de planten bleek dat ze samen meer nutriënten opnamen dan apart. Waarschijnlijk komt dat doordat de verschillende soorten op verschillende diepten gaan wortelen om aan hun nutriënten te komen. Daarnaast kan ook belangrijk zijn dat ze op verschillende momenten tijdens het groeiseizoen hun nutriënten opnemen. Zo begint de grassoort *Anthoxanthum odoratum* vroeger te groeien dan de meeste andere soorten, terwijl *Centaurea jacea* als laatste gaat bloeien.

Niet alleen namen de soorten samen meer nutriënten op, ze gebruikten die nutriënten ook efficiënter om biomassa te produceren. Dit betekent dat ze met dezelfde hoeveelheid nutriënten meer biomassa produceren in mengsels dan in monoculturen. Op deze twee manieren droeg in het laatste jaar van het experiment meer dan de helft van de plantensoorten bij aan de positieve relatie tussen diversiteit en productiviteit. Het lijkt er sterk op dat niet de aanwezigheid van een enkele soort, maar juist de

diversiteit aan soorten belangrijk is. Bovendien blijkt uit ons onderzoek duidelijk dat er ook zonder vlinderbloemigen een positieve relatie tussen soortenaantal en het functioneren van het ecosysteem is.

Binnen de ecologie bestaat allang het idee dat er relatie is tussen diversiteit en de invasie door uitheemse of exotische soorten. In 1958 veronderstelde Charles Elton dat soortenrijke gemeenschappen resistenter tegen invasies zouden zijn dan soortenarme. Op het moment zijn invasies een wereldwijd probleem, omdat de oorspronkelijke soorten verdrongen worden. De huidige achteruitgang van biodiversiteit zou kunnen leiden tot meer invasies, wat vervolgens weer kan leiden tot het verlies van nog meer soorten.

In experimenten zoals de onze kan een mogelijke relatie tussen diversiteit en invasies goed onderzocht worden, omdat andere factoren die ook een rol kunnen spelen, zoals bodemeigenschappen, constant zijn. In het experiment komen het hele jaar door onkruiden op en die kunnen goed als een model voor invasies gebruikt worden. In de zomer van 2002 telden we alle onkruiden die we verwijderden. Uit de analyses bleek dat er inderdaad meer onkruiden waren bij een lage diversiteit en veel minder in de gemeenschappen met alle acht soorten. Een uitgebreide statistische analyse liet echter zien dat het niet zozeer diversiteit, als wel vooral de aanwezigheid van twee soorten was die de invasies beïnvloedden (een selectie-effect). Deze twee soorten hadden een sterk negatief effect op de onkruiden. Eén van deze twee, *Centaurea jacea*, was de dominante soort op dat moment. Het is niet verrassend dat er weinig onkruiden kunnen groeien onder grote planten. De andere soort, *Leucanthemum vulgare*, had echter helemaal geen hoge biomassa. Het lijkt er sterk op dat deze soort grote hoeveelheden plant-parasieten ophoopte in de wortelzone, die de groei van onkruiden belemmerden.

Veranderingen in plantendiversiteit kunnen ook gevolgen hebben voor andere organismen in een grasland, maar uit de studies die tot nu toe zijn uitgevoerd volgt nog geen eenduidig patroon. Wij onderzochten de effecten op nematoden (aaltjes) in de bodem en vonden dat de diversiteit van nematoden toenam met de diversiteit aan planten. Nadere analyse liet zien dat het vooral de identiteit van de plantensoort was die de nematodensamenstelling bepaalde. In de wortelzone van bijna elke plantensoort vormde zich een aparte gemeenschap van nematoden. Daardoor waren er in mengsels van meerdere plantensoorten meer verschillende nematoden aanwezig.

Organismen zoals nematoden reageren op plantendiversiteit, maar het is ook bekend dat ze zelf die diversiteit kunnen beïnvloeden.

Eerder onderzoek liet zien dat grote grazers, plantenetende insecten, mycorrhiza-schimmels, pathogenen en nematoden een groot effect op de vegetatie kunnen hebben. Dat betekent dat als deze organismen achteruitgaan, dit effecten kan hebben op plantendiversiteit die doorwerken naar het ecosysteem als geheel. Wij hebben onderzoek gedaan aan arbusculaire mycorrhiza-schimmels (AMF). Deze schimmels vormen associaties met veel planten en leveren fosfaat in ruil voor suikers. Ons onderzoek laat zien dat ze een belangrijke rol spelen bij de competitie tussen planten. Bovendien verhogen ze de diversiteit van planten door de vestiging van jonge planten in de vegetatie bevorderen.

Uit ander onderzoek is bekend dat AMF in intensief gebruikt agrarisch grasland maar weinig voorkomen. Herstel van de soortenrijkdom van planten op deze percelen verloopt vaak moeizaam. Gezien de belangrijke rol van AMF is het goed mogelijk dat dat wordt veroorzaakt door de afwezigheid van deze schimmels.

In veel onderzoek, inclusief ons onderzoek aan AMF, wordt het effect van een bepaalde groep organismen op plantendiversiteit onderzocht zonder rekening te houden met andere groepen. Onderzoeken met meerdere organismen zijn zeldzaam en bovendien beperkt tot meerdere soorten binnen één groep (bijvoorbeeld grote grazers) of verschillende groepen organismen op slechts één plant. In een ecosysteem is er echter een grote diversiteit aan planten en andere organismen. Wij wilden weten wat het effect van interacties tussen verschillende organismen op de plantengemeenschap zou kunnen zijn en voerden een experiment uit met 16 plantensoorten en drie groepen planteneters: sprinkhanen, ritnaalden (ondergronds levende larven van kniptorren) en nematoden. Deze planteneters werden zowel apart als samen toegevoegd aan plots met 16 plantensoorten die waren overdekt met kooien. Na twee jaar bleek uit de resultaten dat de afzonderlijke effecten van de planteneters heel anders waren als ze samen werden toegevoegd. Sprinkhanen hadden geen effect op de plantendiversiteit en ritnaalden hadden een positief effect, maar samen hadden ze een sterk negatief effect. Door het eten van een aantal verschillende plantensoorten, traden er verschuivingen op in de abundantie van planten. Maar als ze samen aanwezig waren, werd één bepaalde plantensoort ineens sterk bevoordeeld. Deze soort kon daardoor de vegetatie gaan domineren, wat leidde tot een afname van de plantendiversiteit.

Uit dit onderzoek kunnen een aantal belangrijke conclusies getrokken worden. Ten eerste dat plantendiversiteit belangrijk is voor het functioneren van het ecosysteem. Dit komt omdat er bij interacties tussen meerdere soorten complementariteit optreedt, die leidt tot een efficiëntere opname en gebruik van nutriënten en

daarmee tot een hogere biomassa. Anders gezegd: een achteruitgang van het soortenaantal leidt tot een afname in de productie.

Ten tweede is een hoge soortenrijkdom belangrijk om invasies van uitheemse soorten te onderdrukken, maar het is vooral de aanwezigheid van slechts twee bepaalde soorten die dit patroon veroorzaakt. Achteruitgang van deze soorten betekent echter wel dat invasies kunnen toenemen.

Ten derde: een hoge plantendiversiteit is belangrijk voor andere organismen. Nematodendiversiteit neemt toe met soortenrijkdom van planten door specifieke associaties tussen plantensoorten en nematoden.

Ten vierde kunnen organismen zoals nematoden en AMF een belangrijk effect op de soortenrijkdom van planten hebben. Veranderingen in de diversiteit van deze organismen kan leiden tot allerlei veranderingen in de plantengemeenschap.

Tot slot is het heel belangrijk om deze organismen niet apart te onderzoeken. Juist hun onderlinge interacties leiden tot onverwachte effecten op plantendiversiteit.

Zijn de resultaten in dit proefschrift voldoende om te concluderen dat de achteruitgang van biodiversiteit wereldwijd leidt tot een reductie van allerlei ecosystem processen? Natuurlijk niet. Maar samen met een aantal beroemde gevallen van zogenaamde 'keystone' soorten (waarbij het verdwijnen van die ene soort leidt tot een ineenstorting van een complete voedselketen) geven ze wel aanwijzingen dat de achteruitgang van biodiversiteit grote gevolgen kan hebben.

Uiteindelijk is biodiversiteit het resultaten van een reeks van wederzijdse interacties tussen organismen, omgevingsfactoren en ecosystem processen. Integratie van deze interacties over verscheidende ecosystem typen in de tijd is noodzakelijk om daadwerkelijk te kunnen voorspellen wat wereldwijd de consequenties van de achteruitgang van biodiversiteit zullen zijn.



## Dankwoord

"All things move towards their end..."<sup>1</sup> Deze schijnbaar fatalistische uitspraak is soms geruststellend. Na 4,5 jaar is het in ieder geval mooi dat het boekje is afgerond. Dat het gelukt is (en dat het nog een leuke tijd was ook) dank ik aan aardig wat mensen. Ik zal hieronder tenminste proberen alle bijdragen te noemen. Niets is te gek voor woorden, maar vind maar eens de juiste...

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jasper

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