Invertebrate herbivory, plant diversity and ecosystem processes in experimental grassland

Dissertation
zur Erlangung des akademischen Grades doctor rerum naturalium
(Dr. rer. nat.)

vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät
der Friedrich-Schiller- Universität Jena

von Dipl.-Biol. Christoph Scherber

geboren am 12. Oktober 1976 in München

Gutachter: 1. Prof. Dr. Wolfgang W. Weisser, Jena
2. Prof. Dr. Stefan Halle, Jena
3. Prof. Dr. Teja Tscharntke, Göttingen

Datum der Promotionsprüfung: 14.12.2006

Contents

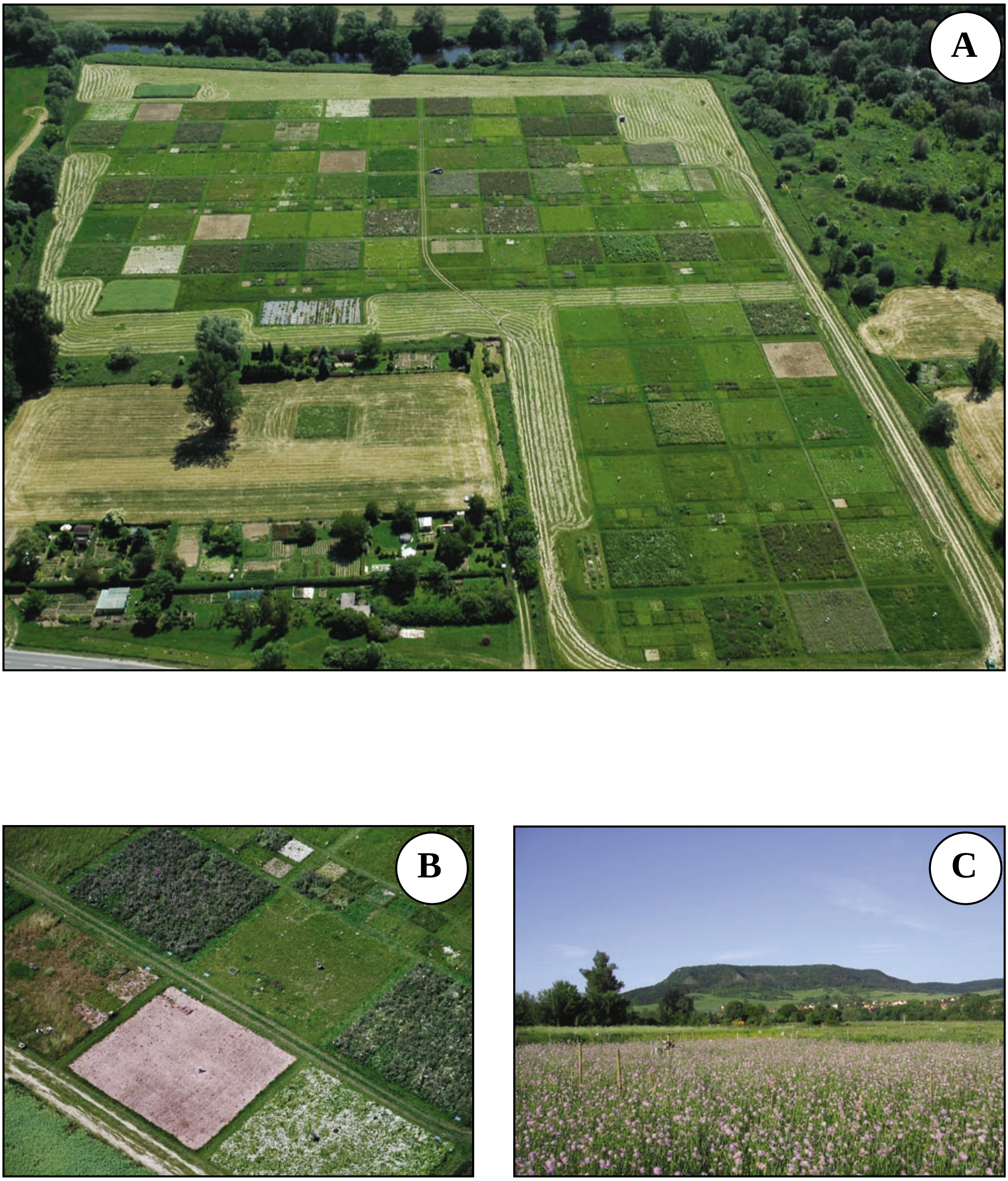


Plate 1: General experimental design. (A) Aerial view of the field site taken from west to east on 14 June 2006 using a Bell Jet Ranger 206B Helicopter. Note the four blocks of the experiment, starting with each two rows of plots at the top, with each block separated by small 3.5 x 3.5 m plots (except for block 4) (B) Aerial view of a bare ground control plot and several multi-species mixtures; note the small replicates at the top right. (C) A single 20 x 20 m plot dominated by *Knautia arvensis* L. (A)&(B) © Forschergruppe Biodiversität (photograph taken by Winfried Voigt); photograph (C) © Christoph Scherber, 27 May 2005.

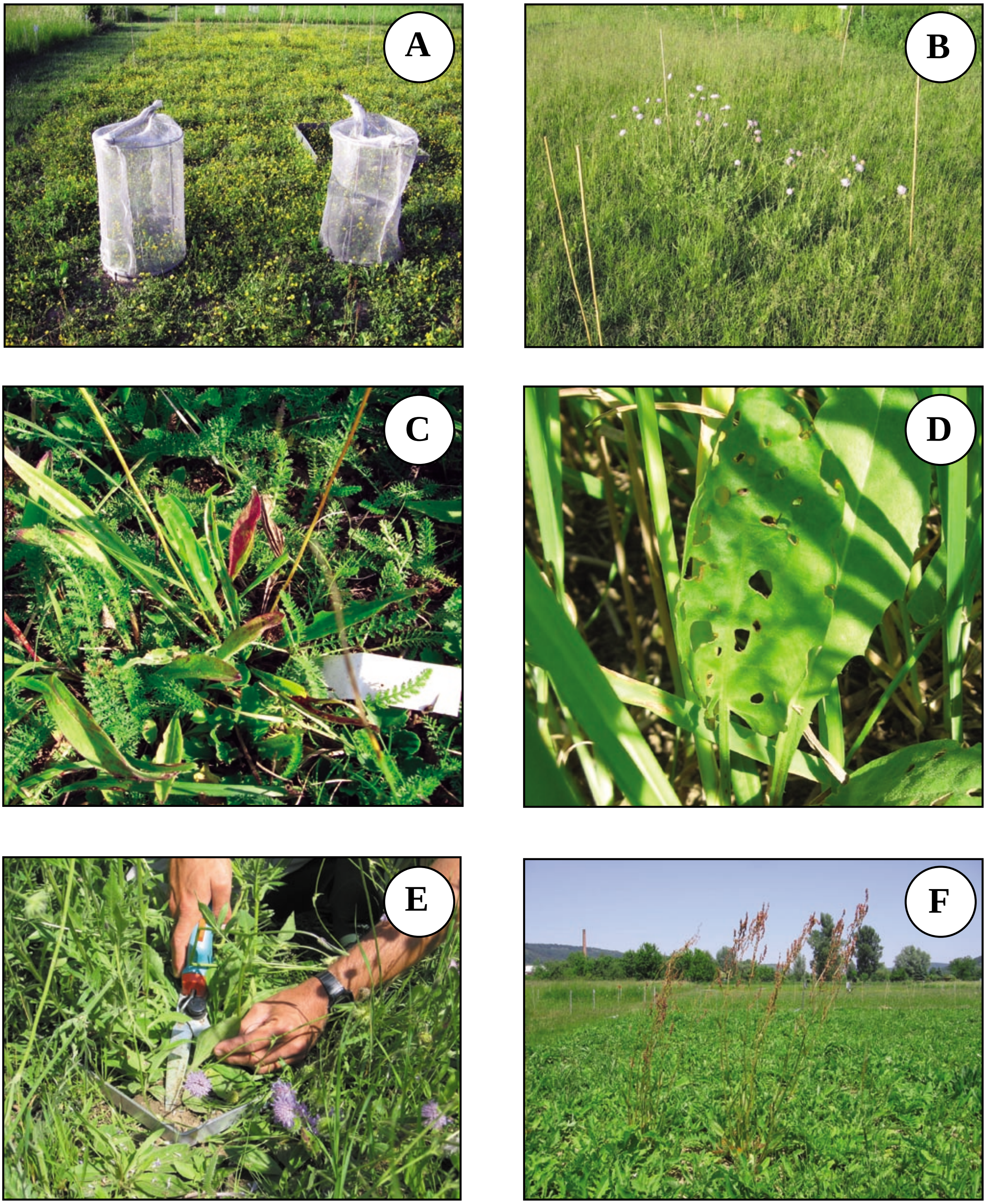


Plate 2: Details of some of the experiments presented in this thesis. (A) Two experimental cages used in the grasshopper caging experiment (chapter 6), 01 June 2005; (B) Flowering *Knautia arvensis* L. phytometers in a plot dominated by grasses (chapter 5); photograph taken on 1 June 2005; (C) *Plantago lanceolata* L. phytometers, marked with a white stick label (chapters 3 and 4); photograph taken on 01 September 2005; (D) Hole feeding damage in *Rumex acetosa* phytometers (chapter 4); photograph taken on 13 June 2006; (E) Biomass harvest using a metal of 20 x 50 cm size (chapters 3,4,5,6); photograph taken on 06 September 2005; (F) Flowering *R. acetosa* phytometers (chapter 4); photograph taken on 14 June 2006. All photographs © Christoph Scherber.

Theory is the fishing net that scientists cast to catch the world, to explain it and to control it.

Sir Karl Raimund Popper, "The Logic of Scientific Discovery" (1959)

1. Introduction

1.1 Plant-herbivore interactions in the history of life on earth

Plants and their herbivores have a long history of co-existence, with the first multicellular herbivorous organisms emerging in marine ecosystems mostly in or after the Cambrian Period, 490-543 Million years ago (Ma) (Vermeij and Lindberg 2000).

The **colonization of land** by plants at the Ordovician-Silurian boundary (400-500 Ma; Kenrick and Crane 1997) marked the beginning of plant-arthropod[1] interactions in the broad sense, with some of the earliest confirmed land plants belonging to the genus Cooksonia (Chaloner *et al*. 1991), and first assemblages of terrestrial arthropods dating back to the late Silurian (Chaloner *et al*. 1991); these arthropods, however, are believed to have been detritivorous and zoophageous rather than herbivorous, and herbivory in those early times was mainly restricted to spore feeding and, sap-sucking (Labandeira 1998; Scott *et al*. 1992).

Terrestrial life expanded in the Carboniferous, and it is in that period that first signs of leaf feeding (**Figure 1**) can be found in fossils[2].

Whether or not the appearance of angiosperms had an influence on insect familial or species diversity is still debated (Labandeira and Sepkoski 1993), but undoubtedly the diversity and number of feeding strategies increased as the angiosperms came to dominate the terrestrial flora towards the close of the Cretaceous (Chaloner *et al*. 1991).

Figure 2 shows that major insect herbivore feeding damage types were already present long before the appearance of angiosperms in the geologic record.

The parallel evolution of winged insects (Pterygota s.str.) then formed the foundations of a major radiation, leading to the emergence of most extant insect orders.

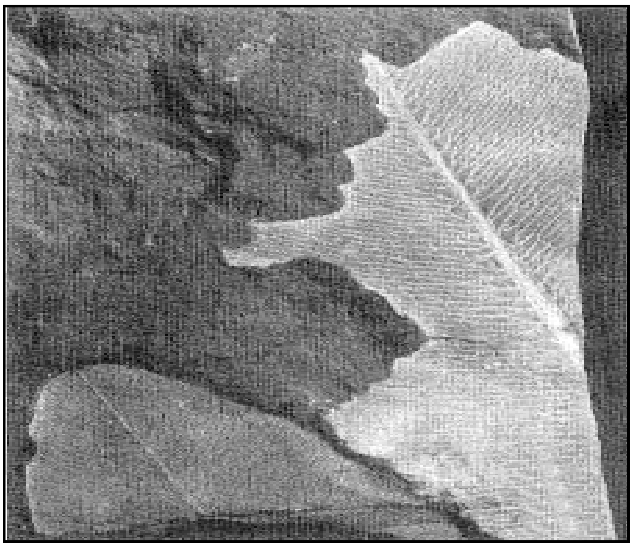


Figure 1 Leaf damage on a Permian seed fern of the genus *Glossopteris* (Cycadophytina, Pteridospermales). Reprinted from Chaloner *et al*. 1991, with permission from The Royal Society, London, UK.

---

[1] Other herbivorous metazoans with triploblastic organization, such as gastropods, have colonized the land in the early Cambrian; see, e.g., Barker (2001); Rosenberg (1996).

[2] Notably, this was well before the appearance of angiosperms (Kenrick & Crane 1997).

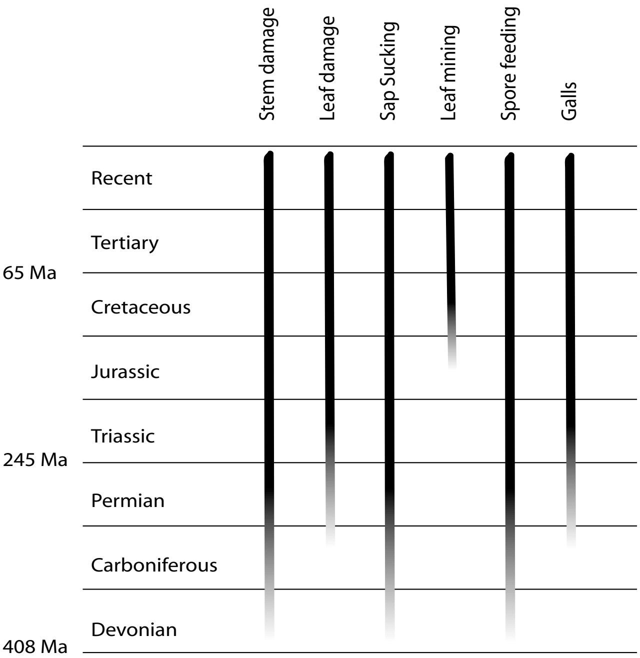


Figure 2 Types of invertebrate herbivore feeding damage in the geologic record. Note the early onset of stem damage, spore feeding and sap sucking (after Scott *et al.* 1992).

Then, after the so-called Permian-Triassic bottleneck (Labandeira and Sepkoski 1993), insect diversity[3] in the fossil record increased almost steadily to present-day levels (**Figure 3**).

The most convincing evidence for an angiosperm-driven **coevolution** between (largely herbivorous) insects[4] and plants has been presented by Farrell (1998), who states that "the success of the order Coleoptera (…) seems to have been enabled by the rise of flowering plants."

Present-day levels of angiosperm species richness, and the species richness of other groups of invertebrates, cannot be interpreted or experimentally studied without a look back on their geological past. Only then will we be able to investigate patterns and processes in present-day biodiversity.

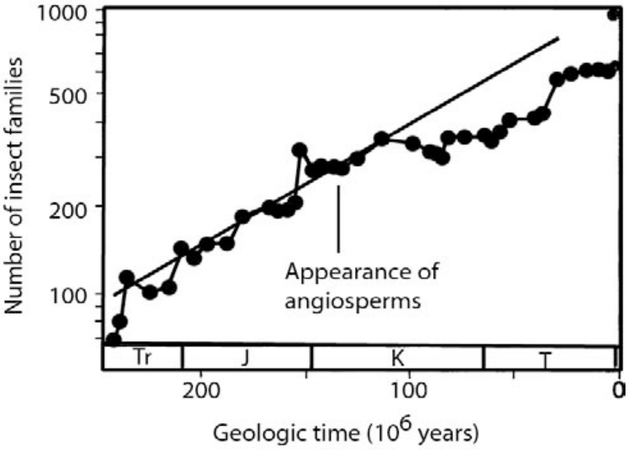


Figure 3 The number of insect families in the geological record. Note that the number of insect families increased steadily and ± independently from the appearance of angiosperms. Tr, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary. (redrawn after Labandeira and Sepkoski 1993)

1.2 Rise and fall of present-day biodiversity

The history of life on earth has seen both phases of rapid **speciation** and phases of **mass extinctions**. These mass extinctions are comparatively short intervals in geologic time in which substantial amounts of global biodiversity are lost due to physical or biological causes (Willis and McElwain 2002). Yet, as Rosenzweig (2001) points out, "in ordinary times, life has replaced such losses by speciation".

Background **rates of extinction** have been estimated to amount to 1 to 0.1 extinctions per 10^6 species-years (Pimm *et al.* 1995). Recent extinction rates are up to several hundred-fold[5] (Pimm *et al*, *ibd.*); in a study on species losses in butterflies, birds and plants in the UK, Thomas *et al.* (2004) have estimated that within the last 40 years 28% of native plants, 54 % of native birds, and 71% of native butterflies had decreased in abundance, with extinction rates of up to 3.4% in 40 years[6].

---

[3] as measured at the family level

[4] Focusing on the Phytophaga clade within the Coleoptera

[5] In fact, for some taxonomic groups the estimated extinction rates are between 10^3 and 10^4 extinctions per 10^6 species-years (Pimm *et al* 1995)

[6] This rate was found for butterflies, but "because insect populations typically respond more rapidly to adverse environmental change than longer-lived organisms" extinction rates in other groups of organisms may rise accordingly (Thomas *et al.* 2004)

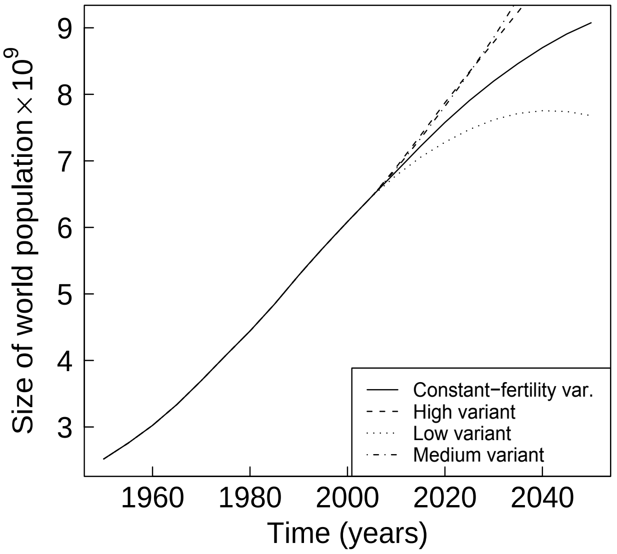


Figure 4 Predicted growth of the world´s population for four different scenarios: Constant fertility, as well as predictions for high-, medium- and low-fertility countries. Source: Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat, World Population Prospects: The 2004 Revision and World Urbanization Prospects: The 2003 Revision, http://esa.un.org/unpp, 02 July 2006; 11:30:12 AM. Graph created by C. Scherber.

This means, **human-caused** rates of extinction are many orders of magnitude higher than background levels, and "the process of re-achieving a steady state after this biotic crisis will not resemble any previous recovery from a mass extinction" (Rosenzweig 2001).

The major drivers of these high extinction rates have been summarized by Sala *et al.* (2000) as land use changes, climatic changes, nitrogen deposition, biotic introductions, and rise in global levels of atmospheric CO_2.

While it is not known whether future extinctions will be directional or stochastic, studies on the effects of different types of species loss on ecosystem properties are required, because

- Global human population size is predicted to continuously grow exponentially at least until 2040 (see **Figure 4**);
- Growing population sizes may be coupled with a non-linear rise in resource demands, e.g. for food production (Daily *et al.* 1998)
- Increased resource demands will lead to further agricultural intensification
- This, in turn, will negatively affect biodiversity at a global scale (Tscharntke *et al.* 2005)

It is currently not known how global declines in species numbers will affect components of the earth system, such as temperature, precipitation, or productivity (Schmid and Hector 2004).

Thus, there is a clear need for manipulative experiments relating biodiversity changes to changes in ecosystem processes.

1.3 Biodiversity, and ecosystem processes, services and functions

Understanding the effects of biodiversity changes on ecosystems requires a clear understanding of the terms that are used to describe these changes. In the context of experimental biodiversity research (such as the experiments presented in this thesis), it is useful to apply terms from systems biology. We define a **system** according to Dale (1970) in the following way:

A system is a collection of interacting entities, together with statements on the relationships between these entities.

Importantly, the entities studied do not need to be in one-to-one correspondence with existing things; rather, they can be seen as *classes* of things, or classes of *processes*. All those entities that are used to describe the state of the system at a given point in time are called **endogeneous**, while variables affecting the state of the system are called **exogeneous** (e.g. changes in local climatic conditions, such as drought).

We define an **ecosystem** here according to Dale (1970) as "a system open[7] for at least one property, in which at least one part of the entities is classed as living". This is not fundamentally different from Tansley´s classical definition (Tansley 1935), "*the whole system (in the sense of physics), including not only the organism-complex, but also*

---

[7] An open system exchanges entities with other systems; in thermodynamic systems, these entities are restricted to be either matter, energy or heat.

the whole complex of physical factors forming what we call the environment of the biome[8] – the habitat factors in the widest sense". Extent, compartimentation, and ´emergent properties´ of ecosystems are further noteworthy issues (Schulze *et al*. 2005), but these shall not be dealt with in detail here.

Ecosystems may be described using **intensive variables** (whose values are not size-dependent; e.g. relative abundances of organisms) or **extensive variables** (proportional to the size of the system; e.g. energy, biomass, volume). Extensive variables may be expressed relative to a common time base (e.g. rate of biomass formation per unit time and area), and they always need to be expressed relative to a unit of (usually two-dimensional) space.

The terms "**ecosystem functions**" and "**ecosystem functioning**" are misleading concepts (Jax 2005) that, albeit frequently used in the ecological literature, shall be replaced by more specific terms throughout this thesis. The same is true for "**ecosystem services**" and related terms that are useful from a management perspective, but will not be further treated in this thesis; a concise review on this topic can be found in Christensen *et al*. 1996.

Specifically, we focus here on **ecosystem processes** (e.g. herbivory, i.e. consumption of living plant tissue by animals; biomass production; hydrologic flux; decomposition; biogeochemic cycling, etc.) and related extensive variables, and their relationship with the number and identity of components within ecosystems ("biodiversity" s.l.)

Throughout this thesis, the term "**biodiversity**" is used to describe the numbers of entities in communities and ecosystems[9], be it the number of biological species present (**species richness**) or the number of functionally similar species (**number of functional groups**), or the functional identity of species (**functional group identity**). Further, the evenness of the distribution of species within a sample, and interactions between species richness and components of functional richness are used to describe diversity. Thus, species richness and functional group composition are both subcomponents of diversity, and in all quantitative descriptions and interpretations of data, these subcomponents of diversity are named explicitly. The term "**diversity**" is only used to describe more general broad-scale patterns.

In the special case of the experiments conducted in this study, species richness is manipulated in discrete steps, making it possible to use species richness rather than diversity indices as a descriptor for diversity. However, diversity indices are calculated for every dataset collected.

Spehn *et al* 2005 have noted that biodiversity is "not a one-dimensional ecological variable" and may simultaneously affect several interrelated ecosystem processes. This is why throughout this thesis, several ecosystem processes are measured simultaneously in order to allow generalizations about how diversity changes these processes.

1.4 Biodiversity and herbivory

1.4.1 Present-day plant biodiversity

There are about **250,000-260,000 species** of **flowering plants**[10] worldwide (Heywood 1993; Kubitzky 1993), with other groups such as Bryophyta, Psilophyta, Lycopodiophyta, Equisetophyta, and Filicophyta only contributing a small proportion to overall plant biodiversity in terms of the total number of described species (see **Figure 5**).

Flowering plants comprise only about 20% of all named organisms on earth, by far outreached by about **one million species** of **insects** (Herrera and Pellmyr 2002). Nevertheless, as a starting point in experimental biodiversity research, it is reasonable to start with primary producers in studying **plant-herbivore interactions** in terrestrial herbaceous ecosystems – and, hence, to experimentally modify plant species richness in order to study the resulting changes in ecosystem processes. Throughout this thesis, therefore, plant species richness will be experimentally varied to

---

[8] In Tansley´s sense, a biome is "the whole complex of organisms inhabiting a given region".

[9] The term "community" will be used for collections of species that co-occur in space and time, e.g. experimental grassland communities; while the term "ecosystem" will be used whenever generalizations across different organismic groups and communities are made.

[10] i.e. higher plants of the group Angiospermae.

study ecosystem effects of biodiversity changes. Specifically, the focus will lie on herbivory as one exemplary ecosystem process.

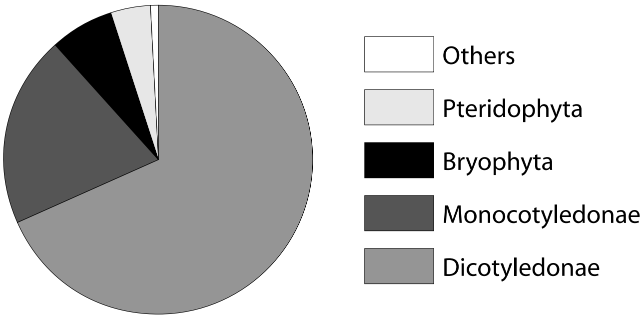


Figure 5 Present-day species richness of the major terrestrial plant groups. Angiospermae (with Dicotyledonae and Monocotyledonae) have the highest numbers of species. The calculations are based on an assumed total number of 250,000 species (after Crawley 1997). Graph © C. Scherber.

1.4.2 Herbivorous groups of organisms

By definition, **herbivores** are organisms that consume living plant tissues. Strictly speaking, this definition would also include plant parasitic[11] fungi, nematodes and viruses (Dobson and Crawley 1994). Throughout this thesis, only certain groups of organisms will be treated and others excluded.

Unicellular organisms, marine or aquatic organisms, as well as Nematoda, Tardigrada, Myriapoda, Arachnida and Vertebrata will not be considered here, although some of these groups are very important herbivores in ecosystems.

The **focus of this thesis** will be on herbivorous insects and their interactions with plants; they represent a "dominant component of biodiversity in most terrestrial ecosystems" (Weisser and Siemann 2004), yet there is only limited knowledge about their effects on ecosystem processes, and their interactions with plant species richness.

This lack of knowledge is in sharp contrast to the almost ubiquitous presence of herbivorous insects in most terrestrial ecosystems, making up at least 26% of all living organisms (Schoonhoven *et al.* 1998). Hence, it seems at least surprising that so little is known about their effects on processes at the ecosystem level.

The study system we use is a Central European *Arrhenaterum* grassland community, and throughout this thesis we will consider mainly herbivory by sap-feeding and chewing above-ground insect herbivores (Orthoptera, Heteroptera, Homoptera, Hymenoptera, Coleoptera, Lepidoptera, Diptera).

1.4.3 First-order consumers in ecosystem carbon flow

In terrestrial ecosystems, total **consumption of biomass** by herbivores has been estimated at about 36%[12] (Cebrian 2004), while older sources suggest a median value of 18% (Cyr and Pace 1993). **Insect herbivores** remove on average about 7% of leaf biomass in deciduous forests (Coley 1996), 10% of leaf biomass in tropical forests (Coley, ibd.), and between 0.5 and 15% in temperate grasslands (Crawley 1983).

Such estimates of percentage herbivory, however, may by far underestimate actual losses due to herbivory, with corrected levels amounting to more than 2.6-fold the baseline estimates (Sandjensen *et al.* 1994, cited in Zangerl *et al.* 2002).

The impact of herbivores on **biogeochemical cycles** of carbon and other elements is complicated by feed-back loops with the decomposer subsystem; these issues, however, shall not be treated in any more detail here.

The impact of insect herbivory at the ecosystem level, and especially its relationships with primary productivity and plant species richness, are still only poorly known. Cebrian and Lartigue (2004) state that "[…] **ecosystem-level experimental manipulations** [of herbivory] would certainly improve our understanding of carbon and nutrient

---

[11] Parasitism has been defined by Anderson & May (1078) as "an ecological association between species in which one, the parasite, lives on or in the body of the other, the host. […] To classify an animal species as parasitic we therefore require that three conditions be satisfied: utilization of the host as a habitat; nutritional dependence; and causing ´harm´to its host".

[12] This value has been calculated for an assumed net primary production of 1000 g C $m^{-2}a^{-1}$, based on the regression equation given in Fig. 3a of Cebrian (2004)

cycling in ecosystems". The thesis presented here tries to fill this gap.

1.4.4 Theories relating insect herbivory to plant diversity

More than 90 percent of all extant herbivorous insect species can be classified as **specialists** (Bernays and Graham 1988). But it is not at all clear whether this high degree of host specificity will mean that herbivory impact will generally be highest in plant monocultures or not.

If one assumes for simplicity that all insect herbivores were strictly monophageous, then there would be a perfect linear correlation between plant species richness and insect species richness – but this would not tell anything about the amounts of damage inflicted by each herbivore per plant or unit area.

Root (1973) was the first to describe a relationship between insect herbivore abundance and plant species richness, the **resource concentration hypothesis**: "*Many herbivores, especially those with a narrow host range, are more likely to find hosts that are concentrated (i.e., occur in dense or nearly pure stands)*".

However, the resource concentration hypothesis is neither quantitative in its predictions, nor does it allow predictions other than herbivore load per plant. Further, if herbivores are strongly controlled by natural enemies, a negative correlation between patch size and herbivore load might be expected (Fahrig and Jonsen 1998). The enemies hypothesis (Root 1973) on the other hand predicts that rates of predation and parasitism should be higher in polycultures, again resulting in higher herbivore load in monocultures.The way in which herbivore load translates into herbivore damage in plants depends on the aggregative functional responses[13] of the herbivore community (which is, in turn, difficult to measure). For single herbivore species feeding on single plant species or groups of plant species, Crawley (1983) stated that both linear (type I), asymptotic (type II) or sigmoid (type III) functional responses may be expected when herbivore food intake is plotted against available plant biomass.

However, functional response curves for invertebrate herbivores are rare (e.g. Islam and Crawley 1983), and under experimental conditions it is often sufficient to assume a simple type I functional response, especially if most plant species are consumed and feeding occurs until complete resource depletion (as is the case, for example, in caging experiments using insect herbivores).

Further hypotheses have been put forward to explain and predict relationships between plant diversity and host plant selection in insect herbivores in its widest sense. Mechanisms made responsible for higher (specialist) insect herbivore densities in pure stands are, e.g.:

- **plant architecture** and the complexity of physical structures (Lawton 1983);
- **associational resistance**, described by Tahvanainen and Root (1972) as "the biotic, structural and microclimatic complexity of natural vegetation [that] greatly ameliorates the herbivore pressure" on individual plants in diverse mixtures
- the **distance** between plant individuals in monocultures vs. mixtures (Tahvanainen and Root 1972);
- **altered physiology** of host plants (reviewed in Finch and Collier 2000),
- the ´**appropriate/inappropriate landings**´ hypothesis (Finch and Collier 2000),
- **plant apparency** (Feeny 1976): if plants grow in pure stands, "the apparency[14] of each individual [...] plant to some of its adapted en-

---

[13] A functional response is "the change in rate of predation by an individual predator in response to a change in density of the prey"; see. e.g., Fussmann *et al.*, 2005.

[14] Feeny defined apparency as "the vulnerability of an individual plant to discovery by its enemies"; ´apparent´ plants would contain ´quantitative´ defensive substances, such as tannins, and have tough leaves with a low nutritive value for herbivores; ´unapparent´ plants would contain ´qualitative´ defensive substances, such as glucosinolates, that are effective in small concentrations; unapparent plants would be rather ´ephemeral´ and characteristic for early successional stages

emies is […] increased", and

- the **toxin dilution hypothesis** (Freeland and Janzen 1974), which states that generalist herbivores should "consume a variety of plant foods at any one time" to avoid toxification by plant secondary compounds; thus, one could conclude that higher generalist herbivore loads might be expected in species-rich plant communities.

The problem with all these experiments comparing ´monocultures´ with ´mixtures´ of plant species is two-fold: First, if **density** is held constant throughout all plant diversity levels, there will be more host plants per unit area for any specialist herbivore in the monocultures, leading to host plant density rather than diversity effects. Second, if **number of host plants** is held constant across all diversity levels, plant density will increase in high-diversity mixtures, leading to increased interspecific competition and decreased plant size per individual (Crawley 1983). Hence, inferring diversity effects from both kinds of approaches is a non-trivial task.

The situation is further complicated by another distinct set of theoretical approaches, mainly stemming from **chemical ecology**, where predictions are made about the relationship between **resource availability** in the environment, and plant **anti-herbivore defense**:

- the **Carbon-nutrient balance hypothesis** (Bryant *et al.* 1983): the concentration of plant secondary metabolites is controlled by the availability of carbon and nitrogen in the environment; under low-resource conditions, secondary metabolites will be carbon-based rather than nitrogen-based, and this could have effects on the relative abundances of generalist and specialist herbivores, for example in plant communities containing legumes;

- the **Growth/differentiation balance hypothesis** (Herms and Mattson 1992), which relates resource availability to a plant´s investment into growth (biomass, height) or differentiation (enhancement of structure or function) processes; under low resource availability, plants invest more into growth than into defense;

- the **Resource availability hypothesis** (Coley *et al.* 1985), which predicts that "both the nature and quantity of plant defenses are determined by the resources available in the local habitat".

These theories are especially important in interpreting possible effects of **legume** vs. **grass presence** on invertebrate herbivory (Symstad 2000), because legumes tend to contain higher concentrations of leaf nitrogen (see Figure 4 in chapter 7). Yet, as Stamp (2003) has noted, "*the theory of plant defense has the characteristics of immature theory*", and so do many of the other hypotheses stated above. For example, the resource concentration hypothesis has recently been challenged by the formulation of a "**Resource dilution hypothesis**", predicting lower specialist herbivore loads in species-poor mixtures (Otway *et al.* 2005).

> There is a clear need for a rigid theoretical framework relating invertebrate herbivory to plant species richness. It is hoped that the evidence presented in this thesis will help in the formulation of this theoretical framework.

1.5 Biodiversity and other ecosystem processes

Changes in plant diversity will of course influence many ecosystem processes, of which herbivory is only one part. Though it is not the aim of this section to give an in-depth introduction into this subject, some recent findings from other studies shall briefly be summarized, as far as these results are **relevant to the present study**.

In experiments where plant species richness has been experimentally altered using a random extinction scenario and species additions from seed rather than species removal, a positive relationship between plant species richness and **plant community biomass** per unit area and time has been found (reviewed in Hooper *et al.* 2005). Other variables that are correlated with community biomass, such as **vegetation cover**, **canopy height**, and **light interception** also tend to be positively influenced by plant species richness (Spehn *et al.* 2005).

In addition, the diversity of primary producers[15] has been found to be either positively or negatively correlated with the **diversity of higher trophic levels** (Knops *et al.* 1999; Koricheva *et al.* 2000; Siemann *et al.* 1998; Symstad 2000). Some aspects of this relationship will be further discussed in chapter 7.

Further, resident plant species diversity has been shown to have a negative effect on establishment of **invading species**[16] (see chapter 7), and on performance of individual invader species (reviewed in Levine *et al.* 2004). Yet, Crawley *et al.* (1999) have questioned the role of species richness for invasion resistance, and argued that **invasion resistance** was mainly related with **species identity**. Invasion resistance is treated separately in chapter 5, and there is a more in-depth treatment of this subject in chapter 7.

The effects of plant species richness on the performance of **individual plant species** have been investigated by several authors; Diemer and Schmid (2001) have shown that the growth, number of inflorescences and survival of two *Ranunculus* species (Ranunculaceae) were negatively affected by increasing plant species richness. How the performance of individual plant species is altered by plant species richness shall be further explored in chapters 4, 5 and 7.

The last ecosystem property that shall be considered here is "ecosystem stability" and its relationship with plant diversity; we define community stability[17] using the two properties **resistance** and **resilience**. When reporting the results of diversity-stability experiments, it is important to state exactly what the **reference state** is, which **variables** are used to describe stability, and what **temporal and spatial scales** are considered (Grimm and Wissel 1997). In general, both theory and experiments are still inconclusive, but many authors have pointed out that diversity and stability should be positively correlated. These issues are further discussed in chapters 6 and 7.

1.6 Experimental approaches to the study of biodiversity and ecosystem processes

In principle, there are just two basic approaches to studying biodiversity and ecosystem processes: First, existing ("**natural**") ecosystems can be investigated; and, second, **experimental** ecosystems can be designed, e.g. using climate chambers, mesocosms, cages or experimental grassland plots. These two principal approaches should not be seen as controversial, but as complementary; for example, starting with a "natural" grassland ecosystem, natural diversity gradients could be studied, or diversity gradients could be artificially imposed by removal of species, seed addition or fertilization. These experimental procedures, however, could also be used in artificially assembled communities. The task is, then, to **integrate** the results from both "natural" and "artificial" communities to give a **general picture** of biodiversity effects on ecosystem processes.

It is out of the scope of this thesis to give a complete overview of previous works. We refer to two recent reviews published in Ecological Monocgraphs (Hooper *et al.* 2005; Spehn *et al.* 2005) for a state-of-the art introduction.

The first ecological experiment to investigate the relationship between biodiversity and ecosystem properties was the **Park Grass Experiment** in Rothamsted, UK (Silvertown *et al.* 2006), with sharp diversity gradients imposed by long-term addition of different mineral nutrients, organic fertilizers and lime treatments. The data from the Park Grass Experiment "show unequivocally that, over the range of values observed, biomass (productivity) has a negative effect upon species richness" (Silvertown *et al.* 2006); studies on **invertebrate herbivory** in several transplanted **phytometer** species showed that herbivory decreased with increasing plant diversity, but "there was little evidence that one hypothesis satisfactorily accounts for the whole range of variation observed." Other studies using **natural gradients** in species richness are, for example, Thompson *et al.* (2005).

---

[15] either in terms of species richness, or in terms of functional group richness

[16] Invaders were in many cases defined as "all plant species that were observed in a plot, but that were not specifically planted in a plot" (Knops *et al.* 1999), i.e. this term included both native and ´exotic´ invaders. Terminological issues shall not be discussed here, we refer to Richardson *et al.*, 2000 for details.

[17] In contrast to temporal stability (Grimm & Vissel, 1997)

Experiments similar to Park Grass were set up in 1982 by David Tilman (Tilman 1987) to study the effects of long-term nitrogen addition on plant communities. Later, in 1994, Tilman and colleagues set up one of the largest biodiversity experiments in existence (the **Cedar Creek** Biodiversity Experiment), consisting of two sub-experiments (Tilman *et al.* 1997; Tilman *et al.* 1996) with different plot sizes (3 x 3 m, "Biodiversity I" and 9x9 m, "Biodiversity II"). The ecosystem properties studied were, e.g., plant productivity, plant and soil nitrogen, and arthropod abundances.

Naeem *et al.* (1994) used 14 climate-controlled growth chambers (the **ECOTRON** facilities at Silwood Park, UK) with a size of each $8m^3$ to establish model communities with different numbers of species of several trophic levels and combinations of herbaceous annual plants, herbivorous insects and snails, insect parasitoids, Collembola and earthworms. These experiments were the first[18] to indicate that plant species richness may be positively correlated with several ecosystem processes, including productivity.

The aim of the European **BIODEPTH** experiment (Spehn *et al.* 2005) has been to find out about the consistency of these effects in space and time, covering a wide range of local abiotic conditions, and using different local species pools. The experiment lasted three years and had eight study sites[19] along gradients throughout Europe, with a plot size of 2x2 m[20]. Plant communities with 1-12(-32) herbaceous plant species were established from seed, and about ten different parameters were measured (e.g. aboveground biomass, stand height, decomposition).

The most recent experiment, established in 2000 and using very small plots (1 x 1 m), has been performed by van Ruijven and Berendse (2005). This experiment used planted seedlings rather than growing experimental mixtures from seeds, and the authors reported a positive relationship between plant species richness and productivity that strengthened over time.

The experiments performed so far have been **criticized** for several reasons (e.g. Huston 1997; Huston *et al.* 2000; Thompson *et al.* 2005). For example, the ECOTRON experiment used combinations of species where the members of each trophic level were subsets of the higher trophic levels (i.e. **non-random selection** of species), and species richness had zero replication. This means, **species identity** effects can not be separated from **species richness** effects in this experiment. Similary, some of Tilman´s Cedar Creek experiments (e.g. Tilman 1996) have been criticized for using **nutrient additions** to create diversity gradients, making it impossible to separate the effects of nutrient addition from species richness effects. Finally, experiments using random assemblages of plant species, such as BIODEPTH, have been criticized because the probability of including species with specific traits increases with increasing species richness (the "**sampling effect**", Wardle 1999).

This overall very mixed picture of studies on biodiversity and ecosystem processes (Cameron 2002) has lead to the justified claim that a **new generation of biodiversity experiments** is required to resolve some of the most challenging questions:

> "While the first generation of biodiversity experiments has generated a lot of new information about the relationship between diversity and ecosystem processes, […] only a new generation of more mechanistic experiments will reveal more about the underlying biology" (Spehn *et al.* 2005).

---

[18] Actually, Charles Darwin was the first to propose such a relationship as early as 1859 (see Hector and Hooper, 2002).

[19] in Germany, Greece, Ireland, Portugal, Sweden, Switzerland, and two sites in the UK.

[20] except for the Swedish site, which used a size of 2x5 m.

1.7 The Jena Experiment, a new multidisciplinary research project in experimental biodiversity research

1.7.1 General aspects of the experimental design

This section gives a brief description of the overall experimental design used for the experiments conducted in this study, without the intention of being exhaustive[21]. Further details on how the Jena Experiment differs from previous experimental approaches will be presented in chapters 3, 4 and 7.

The **Jena Experiment** (Plate 1) was established on former arable land[22] in spring 2002. It is a field research site located near the city of Jena (Germany, 50°55′N, 11°35′ E, 130 m a.s.l.). The mean annual air temperature at the site is 9.3°C (1961-1990), with a mean annual precipitation of 587 mm (Roscher *et al*. 2004). The soil at the field site can be classified as Eutric Fluvisol (FAO 1998), with organic carbon concentrations of 33 g kg^{-1}, an organic C to total N ratio of 8 to 15, and pH (H_2O) between 7.1 and 8.4 (Oelmann *et al*. 2005).

The whole field site was divided into **four blocks** perpendicular to a gradient in soil sand content to account for differences in abiotic soil conditions. The overall experimental design is a randomized incomplete block design[23], with six levels of plant species richness crossed with four levels of plant functional group richness. Further experimental treatments, e.g. insecticide applications, are applied at **sub-plot** scales, resulting in a two-level nested design.

The experiment comprises 90 plots of 20 x 20 m size, with an additional 206 smaller plots 3.5 x 3.5 m in size (Plate 1 A,B). Only the large plots (Plate 1 C) were used for the experiments reported in this study. There were several reference plots (2 free succession, 2 succession with mowing, and 4 bare ground; Plate 1 B) that were also not used in this study, leaving a total of 82 plots for the measurements reported here.

All plant species used for the establishment of the experimental communities were members of Central European *Arrhenaterum* meadows (Arrhenatherion community, Molinio-Arrhenatheretea, Ellenberg 1996). The **experimental plant communities** consisted of 1, 2, 4, 8, 16, or 60 plant species. The number of replicates for these treatment levels was 16, except for the 16- and 60- species plots that had 14 and 4 replicates, respectively. In addition, the experimental plant communities contained 1-4 **functional groups** (legumes, grasses, small herbs, and tall herbs); these had been defined *a priori* according to a cluster analysis of 17 functional traits. All biologically possible combinations of species richness x number of functional groups were implemented, except for the 60-species mixtures, because of limited seed availability.

The plots are weeded and mown twice a year, according to traditional management of hay meadows in the area. Chapter 2 will describe the specific experiments that were performed using the experimental plots of the Jena Experiment. Further details can be found in the following chapters 3, 4, 5 and 6. Plate 2 shows details of some of the experiments conducted, and Chapter 7 gives a general discussion of the methods used for the experiments presented in this thesis.

1.7.2 Specific experiments conducted in this study

One of the key differences between the Jena Experiment and its precursors is that experiments manipulating **trophic interactions** are explicitly included into the experimental design. In fact, the comparatively large plot size of 20 x 20 m has been selected because it was assumed that specific invertebrate communities could only establish when plot sizes exceeded those of previous experiments. Thus, the experimental setup chosen should allow for the first time to study ecosystem processes other than plant biomass production[24]

---

[21] The reader is referred to Roscher *et al.*, 2004 for more details.

[22] Agricultural use for the growing of vegetables and wheat has been documented for about 40 years.

[23] The design was a randomized *complete* blocks design only with regard to species richness, but not with regard to the combinations of species richness and functional group identity; e.g., block three contained more plots with grasses than the other blocks.

[24] The overall focus of biodiversity experiments conducted so far has been on the effects of plant species richness on aboveground primary productivity, simply because this variable is assumed to be most

in considerable detail. Specifically, it should allow a rigid test of Root´s resource concentration hypothesis and several other aspects of plant-herbivore interactions in ecosystems differing in plant species richness.

If such an approach were successful, the consequences could be of far-reaching importance to our overall understanding of herbivore impact in simple vs. complex terrestrial systems.

How, then, could reasonable experiments across several trophic levels be designed? Clearly, there would be an almost infinite number of combinations of organisms that could be monitored or whose presence could be manipulated. **Monitoring** species richness across several groups of organisms *per se* would, however, not increase our knowledge about ecosystem processes, because it is the **physical interactions** between these groups of organisms that matter (e.g. predator-prey interactions, with plant-herbivore interactions as a specific subset of these). Thus, the experiments to be conducted should allow inferences about these physical interactions to be drawn.

Keeping the experimental design as straightforward as possible, it makes sense to start with a simple system consisting of **two trophic levels** (plants and herbivores; Plate 2A). Further, it makes sense to differentiate between effects of **single species** (Plate 2A-D,F), and effects at the **community level** (Plate 2E). Therefore, there are four basic experimental approaches that are summarized in **Figure 6**:

- First, changes in plant diversity can be related to the **performance of whole plant communities**. This is what is generally done when the effects of plant diversity on community biomass or related variables are studied. As community biomass is an integral variable relevant to all experiments conducted in the study presented here, examples for this approach can be found throughout chapters 3, 4, 5 and 6 of this thesis.

- Second, interactions between plant diversity and **whole invertebrate herbivore communities** can be studied. This approach is followed in chapters 3 and 4 of this thesis, for example by applying insecticide treatments or studying community herbivory.

- Third, changes in plant diversity can be related to the **performance of single plant species**. (Plate 2B-D,F) An example for this can be seen in chapters 4 and 5 of this thesis.

- Fourth, interactions between plant diversity and **single herbivore species** can be studied (Plate 2A). In this thesis, chapter 6 gives an example for this kind of approach.

It is worth noting, however, that neither of these approaches is mutually exclusive. By contrast, **all four approaches** are needed to provide the framework for an in-depth understanding of **plant-invertebrate herbivore interactions** in differentially diverse plant communities.

The way in which the boxes in Figure 6 are arranged already indicates that the classical "bottom-up or top-down" view of trophic interactions[25] has been replaced by a view incorporating species richness and a **hierarchy of embedded systems** (as proposed by Nielsen 2000), in which further hierarchies (e.g. the level of secondary consumers) could be added as additional semi-circles above or below the horizontal centre line[26]. For example, of one would like to add a **third trophic level** to the system, studying plant-herbivore-parasitoid interactions, there would be two new outer sub-layers ("single parasitoid species" and "multiple parasitoids") that would interact with "single herbivore species" or "multiple herbivores", with corresponding experimental treatments (e.g. removal or inclusion experiments). For simplicity, the experiments reported in this study are restricted to just the "simple" two-layer interactions

---

influential from an applied point of view, and to be most important for the overall "functioning" of the systems under study .

[25] In the sense of a reductionist scalar interpretation of hierarchies in ecosystems.

[26] Nielsen´s approach is even more restrictive in that it represents the systems as consisting of concentrical ring-like layers and turns the lowermost trophic level outward, such that top-carnivores would form the inner part of the system; this approach has been modified here to include a diversity component, and is oversimplified for illustratory purposes.

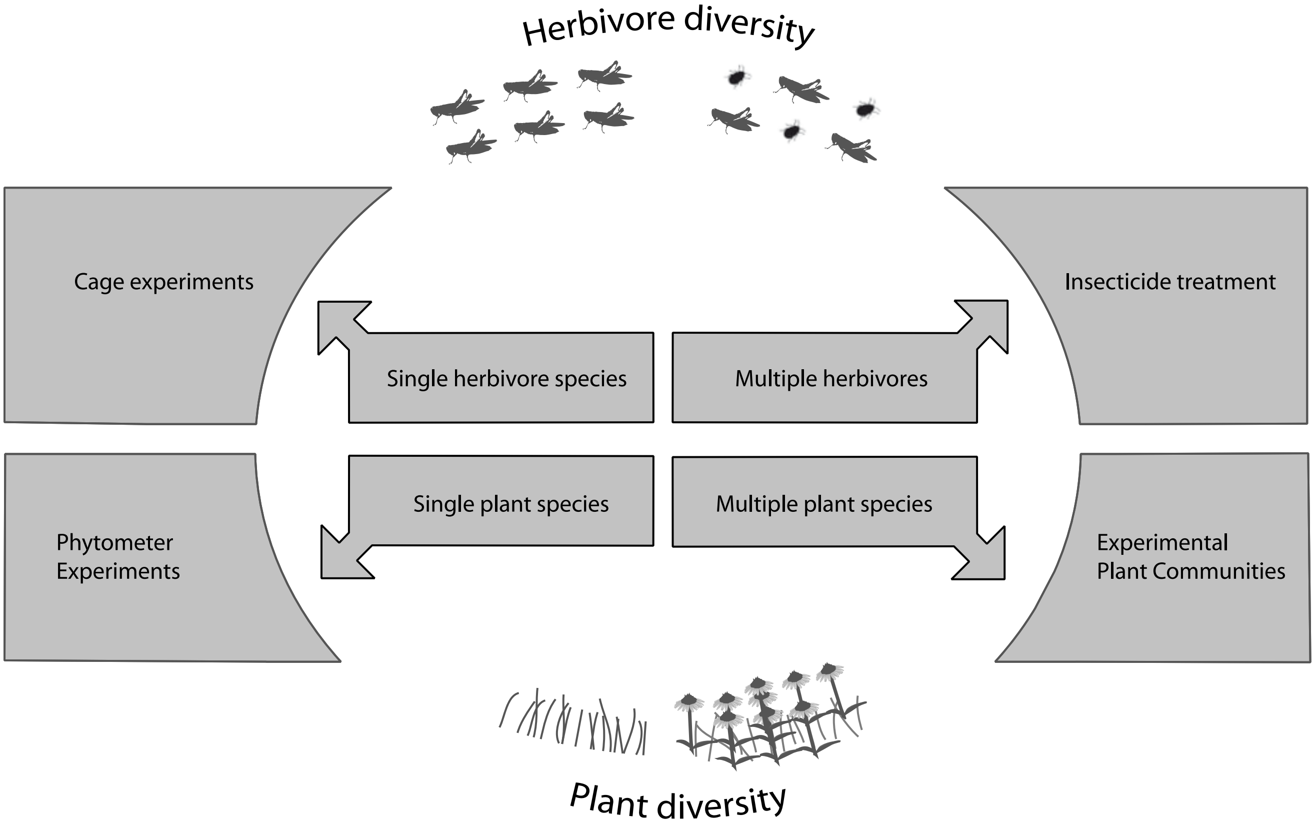


Figure 6: The structure of this thesis. Relationships between single vs. multiple plant species, and single vs. multiple herbivore species, are studied using four principal experimental approaches: (1) Phytometer experiments; (2) Cage experiments; (3) Insecticide treatments; and (4) the use of experimental plant communities. Further explanations can be found in the text. Drawing by C. Scherber.

between **plants** and invertebrate **herbivores** – but additional complexity could be added in further experiments elucidating multitrophic interactions in experimental grassland.

2 Hypotheses and the structure of this thesis

2.1 Questions and hypotheses

This thesis is centred around a sequence of three interrelated questions:

- **Question 1:** Is there a relationship between plant diversity and invertebrate herbivory?
- **Question 2:** What is the shape of that relationship?
- **Question 3:** Why does this relationship occur?

The third question is a so-called evaluative question, i.e. finding an answer to such a question may require complex chains of causation, involving indirect effects of diversity on other ecosystem processes.

Answering these three questions will start from three different perspectives:

- Interactions between whole plant communities and whole invertebrate herbivore communities (Chapter 3)
- Interactions between single plant species and whole invertebrate herbivore communities (Chapter 4)
- Interactions between whole plant communities and single invertebrate herbivores (Chapter 6)

In addition, interactions between single plant species and whole plant communities will be studied (Chapters 4 and 5) because such an approach allows links to other ecosystem properties, such as **invasion resistance**.

The following set of **null hypotheses** shall be tested in the following chapters:

Hypothesis A: There is no effect of plant species richness on invertebrate herbivory at the level of whole plant communities, individual plant species, or individual herbivore species[27].

Hypothesis B: Number of plant functional groups and plant functional group identity are not better predictors of invertebrate herbivory than plant species richness.

Hypothesis C: Plant community biomass and related parameters do not significantly influence invertebrate herbivory.

Hypothesis D: The performance of individual plant species is not significantly influenced by plant species richness, plant functional group richness, identity of plant functional groups, or plant community biomass.

In case of a rejection of each sub-hypothesis, alternative hypotheses will be proposed based on the data. These hypotheses will be stated in the discussion (chapter 7) and the prerequisites necessary for the development of a general theory of plant diversity and invertebrate herbivory will be proposed.

2.2 Overview of manuscripts

This thesis comprises the following manuscripts:

Manuscript 1: Effects of plant diversity on invertebrate herbivory in experimental grassland

by Christoph Scherber, Peter N. Mwangi, Vicky M. Temperton, Christiane Roscher, Jens Schumacher, Bernhard Schmid, and Wolfgang W. Weisser. **Oecologia** (2006) 147: 489-500. DOI 10.1007/s00442-005-0281-3. Accepted: 14 September 2005; **Published online:** 18 December 2005; **Published in print:** March 2006.

---

[27] Note that this hypothesis consists of three sub-hypotheses, one of each relating to whole plant communities, individual plant or individual herbivore species. Similar principles apply to the following hypotheses.

This manuscript forms the basis of this thesis, examining the relationship between invertebrate herbivory and various aspects of plant diversity based on datasets collected over a period of two years. It is shown that

- Invertebrate herbivore damage at the level of whole plant communities increases with plant species richness and number of functional groups, while herbivory in individual plant species is mostly explained by the functional composition of plant communities.

- The presence of particular plant functional groups is the most important factor for invertebrate herbivory in grassland.

Christoph Scherber is the overall author of this manuscript. He has genuinely developed the main ideas and experimental setup. He has personally written the whole manuscript, collected and analyzed the data, created the graphs and tables, corresponded with referees, editors and typesetters, and accomplished the whole publication process from submission in December 2004 until print publication in March 2006.

Peter N. Mwangi has helped in cultivation and transplantation of the phytometer individuals, and in implementing the overall phytometer approach in the Jena Experiment. He has also commented on earlier versions of this manuscript.

Vicky M. Temperton has provided data on community biomass for May and August 2003. She also commented on earlier versions of this manuscript.

Christiane Roscher has been involved with the overall experimental setup and maintenance of the Jena Experiment. During her work as an overall project coordinator, she was – amongst other things – responsible for organizing mowing and weeding. Her contribution to the manuscript was mainly in the form of comments on earlier versions.

Jens Schumacher has been involved with the implementation of the overall experimental design of the Jena Experiment, and worked as a statistical advisor. His contribution to the manuscript was mainly in the form of comments on earlier versions.

Bernhard Schmid has been involved with the implementation of the overall experimental design of the Jena Experiment and the phytometer sub-experiment. His contribution to the manuscript was mainly in the form of comments on earlier versions.

Wolfgang W. Weisser has been involved with the implementation of the overall experimental design of the Jena Experiment and the sub-experiments presented in this manuscript. He has critically reviewed all previous versions of this manuscript, and served as a supervisor for the experiments presented.

Manuscript 2: The effects of plant diversity and insect herbivory on performance of individual plant species in experimental grassland

by Christoph Scherber, Alexandru Milcu, Stephan Partsch, Stefan Scheu, and Wolfgang W. Weisser. **Journal of Ecology** (2006) Volume 94(5), Pages 922-931, doi:10.1111/j.1365-2745.2006.01144.x; **Published online:** June 2006; **Published in print:** September 2006.

This manuscript provides a detailed analysis of the joint influences of insect herbivores, plant species richness and plant functional identity on the performance of an individual plant species over a period of two years, using transplanted phytometer individuals. We show that:

- Plant species richness positively affects survival, but has negative effects on reproductive parameters.

- Insect herbivory and plant functional identity are the most important factors affecting growth, survival and reproduction in a focal plant species.

Christoph Scherber is the overall author of this manuscript. He has genuinely developed the main ideas and experimental setup. He has personally written the whole manuscript, collected and analyzed the data, created the graphs and tables, corresponded with referees, editors and typesetters, and accomplished the whole publication process from submission in January 2006 until print publication in September 2006.

Alexandru Milcu has helped in collecting biomass data used as a covariate in the analyses. He has also commented on earlier versions of this manuscript.

Stephan Partsch has helped in collecting biomass data used as a covariate in the analyses. He has also commented on earlier versions of this manuscript.

Stefan Scheu has been involved in the setup of the insecticide treatments. He has also commented on earlier versions of this manuscript

Wolfgang W. Weisser has been involved with the implementation of the overall experimental design of the Jena Experiment and the sub-experiments presented in this manuscript. He has critically reviewed all previous versions of this manuscript, and served as a supervisor for the experiments presented.

Manuscript 3: Niche pre-emption increases with species richness in experimental plant communities

by Peter N. Mwangi, Martin Schmitz, Christoph Scherber, Christiane Roscher, Jens Schumacher, Michael Scherer-Lorenzen, Wolfgang W.Weisser, and Bernhard Schmid; **Journal of Ecology**; **Accepted with minor revisions:** August 2006.

In this manuscript, we study the effects of plant diversity on growth and reproduction of four individual plant species belonging to four different functional groups; these individual plant species are used as a model system to study invasion resistance of plant communities. It is shown that:

- Plant species richness negatively affects growth and reproduction of individual plant species – and, hence, promotes invasion resistance.
- Plant functional identity is an important driver of invasion resistance, determining invasion resistance through functional similarity (niche overlap) between invaders and plant communities.

Peter N. Mwangi is the overall first author and has written the whole manuscript, collected data, performed statistical analyses, and created the graphs and tables.

Martin Schmitz has helped in biomass harvests and during field work. He has also commented on earlier versions of this manuscript.

Christoph Scherber has helped with cultivation and transplantation of the phytometer species *Trifolium pratense*, *Plantago lanceolata*, and *Knautia arvensis*. He has collected data on number of leaves, number of ramets, plant height, number of inflorescences, and phytometer aboveground biomass. He has commented on earlier versions of this manuscript, and especially helped in a clarification of the statistical analyses performed.

Christiane Roscher has been involved with the overall experimental setup and maintenance of the Jena Experiment. During her work as an overall project coordinator, she was – amongst other things – responsible for organizing mowing and weeding. Her contribution to the manuscript was mainly in the form of comments on earlier versions.

Jens Schumacher has been involved with the implementation of the overall experimental design of the Jena Experiment, and worked as a statistical advisor. His contribution to the manuscript was mainly in the form of comments on earlier versions.

Michael Scherer-Lorenzen has been involved with the implementation of the overall experimental design of the Jena Experiment, and served as a co-supervisor of the experiments presented in this manuscript. His contribution to the manuscript was mainly in the form of comments on earlier versions.

Wolfgang W. Weisser has been involved with the implementation of the overall experimental design of the Jena Experiment and the sub-experiments presented in this manuscript. His contribution to the manuscript was mainly in the form of comments on earlier versions.

Manuscript 4: Selective herbivory affects stability and composition of plant communities, irrespective of plant species richness

Bernhard Schmid has been involved with the implementation of the overall experimental design of the Jena Experiment and the phytometer sub-experiment. He was the main supervisor of the experiments presented in this manuscript, and gave input at various stages, especially in statistical questions. He also commented on earlier versions of this manuscript.

by Christoph Scherber, Juliane Specht, Günter Köhler, Nadine Mitschunas & Wolfgang W. Weisser; **In preparation** for *Ecology*.

We use selective herbivory as a “perturbation” to study changes in plant community stability and composition. It is shown that:

- Selective herbivory affects resistance and resilience of vegetation biomass, irrespective of the number of plant species present.

- Herbivory leads to persistent changes in the functional composition of plant communities, and – if the analysis is restricted to plots containing the preferred food plant – these changes do depend on plant species richness, with monocultures being more severely affected than species-rich mixtures.

Christoph Scherber is the overall author of this manuscript. He has genuinely developed the main ideas and experimental setup. He has personally written the whole manuscript, collected and analyzed the data, created the colour plates, graphs and tables, and developed the simulation models presented in the appendix.

Juliane Specht has helped during experimental setup, collected the test organisms and provided data on grasshopper survival. She has also commented on earlier versions of this manuscript.

Günter Köhler has helped during experimental setup, especially during identification, sexing and sorting of the grasshoppers. He has been an advisor especially in questions relating to the feeding ecology of grasshoppers. He has also commented on earlier versions of this manuscript.

Nadine Mitschunas has helped during data collection and setup. She has also commented on earlier versions of this manuscript.

Wolfgang W. Weisser has been involved with the implementation of the overall experimental design of the Jena Experiment. He was the main supervisor of the experiments presented in this manuscript, and gave input at various stages, especially with regard to the simulation models. He also commented on earlier versions of this manuscript.

2.3 References for chapters 1 and 2

Anderson RM, May RM (1978) Regulation and Stability of Host-Parasite Population Interactions: I. Regulatory Processes. Journal of Animal Ecology 47:219-247

Barker GM (2001) Gastropods on Land: Phylogeny, Diversity, and Adaptive Morphology. In: Barker GM (ed) The Biology of Terrestrial Molluscs. CAB International, Wallingford, Oxfordshire, UK

Bernays E, Graham M (1988) On the Evolution of Host Specificity in Phytophagous Arthropods. Ecology 69:886-892

Bryant JP, Chapin FS, Klein DR (1983) Carbon Nutrient Balance of Boreal Plants in Relation to Vertebrate Herbivory. Oikos 40:357-368

Cameron T (2002) 2002: the year of the "diversity-ecosystem function" debate. TRENDS in Ecology & Evolution 17:495-496

Cebrian J (2004) Role of first-order consumers in ecosystem carbon flow. Ecology Letters 7:232-240

Cebrian J, Lartigue J (2004) Patterns of herbivory and decomposition in aquatic and terrestrial ecosystems. Ecological Monographs 74:237-259

Chaloner WG, Scott AC, Stephenson J (1991) Fossil evidence for plant-arthropod interactions in the Palaeozoic and Mesozoic. Philosophical Transactions of the Royal Society of London Series B 333:177-186

Christensen NL, Bartuska AM, Brown JH, Carpenter S, Dantonio C, Francis R, Franklin JF, MacMahon JA, Noss RF, Parsons DJ, Peterson CH, Turner MG, Woodmansee RG (1996) The report of the ecological society of America committee on the scientific basis for ecosystem management. Ecological Applications 6:665-691

Coley P (1996) Herbivory and plant defenses in tropical forests. Annual review of ecology and systematics 27:305-335

Coley PD, Bryant JP, Chapin FS (1985) Resource Availability and Plant Antiherbivore Defense. Science 230:895-899

Crawley MJ (1983) Herbivory. Blackwell Scientific Publications, Oxford

Crawley MJ (1997) Biodiversity. In: Crawley MJ (ed) Plant Ecology. Blackwell Science, 2nd ed., Oxford

Crawley MJ, Brown SL, Heard MS, Edwards GR (1999) Invasion-resistance in experimental grassland communities: species richness or species identity? Ecology Letters 2:140-148

Cyr H, Pace ML (1993) Magnitude and Patterns of Herbivory in Aquatic and Terrestrial Ecosystems. Nature 361:148-150

Daily G, Bartuska AM, Brown JH, Carpenter S, Dantonio C, Francis R, Franklin JF, MacMahon JA, Noss RF, Parsons DJ, Peterson CH, Turner MG, Woodmansee RG (1998) Food Production, Population Growth, and the Environment. Science 281:1291-1292

Dale BM (1970) Systems analysis and ecology. Ecology 51:2-16

Diemer M, Schmid B (2001) Effects of biodiversity loss and disturbance on the survival and performance of two *Ranunculus* species with differing clonal architectures. Ecography 24:59-67

Dobson A, Crawley M (1994) Pathogens and the structure of plant communities. Trends in Ecology & Evolution 9:393-397

Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen, 5 edn. Ulmer, Stuttgart

Fahrig L, Jonsen I (1998) Effect of habitat patch characteristics on abundance and diversity of insects in an agricultural landscape. Ecosystems 1:197-205

FAO (1998) World reference base for soil resources. Food and agriculture organization of the United Nations (FAO), International Society of Soil Science, Rome

Farrell BD (1998) “Inordinate Fondness” Explained: Why Are There So Many Beetles? Science 281:555-559

Feeny P (1976) Plant apparency and chemical defense. Recent Advances in Phytochemistry 10:1-40

Finch S, Collier RH (2000) Host-plant selection by insects - a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomologia Experimentalis et Applicata 96:91-102

Freeland WJ, Janzen DH (1974) Strategies in Herbivory by Mammals - Role of Plant Secondary Compounds. American Naturalist 108:269-289

Fussmann GE, Weithoff G, Yoshida T (2005) A direct, experimental test of resource vs. consumer dependence. Ecology 86:2924-2930

Grimm V, Wissel C (1997) Babel, or the ecological stability discussions: An inventory and analysis of terminology and a guide for avoiding confusion. Oecologia 109:323-334

Hector A, Hooper R (2002) Darwin and the first ecological experiment. Science 295:639-640

Herms DA, Mattson WJ (1992) The Dilemma of Plants - to Grow or Defend. Quarterly Review of Biology 67:478-478

Herrera CM, Pellmyr O (eds) (2002) Plant-Animal Interactions. An Evolutionary Approach. Blackwell Science Ltd., Oxford

Heywood VH (1993) Flowering plants of the world. BT Batsford Ltd, London

Hooper DU *et al*. (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75:3-35

Huston MA (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110:449-460

Huston MA, Aarsen LW, Austin MP, Cade BS (2000) No consistent effect of plant diversity on productivity. Science 289:1255a

Islam Z, Crawley MJ (1983) Compensation and regrowth in ragwort (*Senecio jacobaea*) attacked by cinnabar moth (*Tyria jacobaeae*). Journal of Ecology 71:829-843

Jax K (2005) Function and "functioning" in ecology: what does it mean? Oikos 111:641-648

Kenrick P, Crane PR (1997) The origin and early evolution of plants on land. Nature 389:33-39

Knops JMH, Tilman D, Haddad NM, Naeem S, Mitchell CE, Haarstad J, Ritchie ME, Howe KM, Reich PB, Siemann E, Groth J (1999) Effects of plant species richness on invasion dynamics, disease outbreaks, and insect abundances and diversity. Ecology Letters 2:286-293

Koricheva J, Mulder CPH, Schmid B, Joshi J, Huss-Danell K (2000) Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grassland. Oecologia 125:271-282

Kubitzky K (ed) (1993) The families and genera of vascular plants. Springer, Berlin

Labandeira CC (1998) Early history of arthropod and vascular plant associations. Annual Review of Earth and Planetary Sciences 26:329-377

Labandeira CC, Sepkoski JJ (1993) Insect diversity in the fossil record. Science 261:310-315

Lawton JH (1983) Plant Architecture and the Diversity of Phytophagous Insects. Annual Review of Entomology 28:23-39

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7:975-989

Naeem S, Thompson LJ, Lawler JH, Lawton JH, Woodfin RM (1994) Declining biodiversity can alter the performance of ecosystems. Nature 368:734-737

Nielsen SN (2000) Thermodynamics of an ecosystem interpreted as a hierarchy of embedded systems. Ecological Modelling 135:279-289

Oelmann Y, Wilcke W, Bol R (2005) Nitrogen-15 in NO_3^- characterises differently reactive soil organic N pools. Rapid Communications in Mass Spectrometry 19:3177-3181

Otway S, Hector A, Lawton JH (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. Journal of Animal Ecology 74:234-240

Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The Future of Biodiversity. Science 269:347-350

Richardson DM, Pysek P, Rejmanek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6:93-107

Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (*Brassica oleracea*). Ecological Monographs 43:95-124

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze ED (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology 5:107-121

Rosenberg G (1996) Independent Evolution of Terrestriality in Atlantic Truncatellid Gastropods. Evolution 50:682-693

Rosenzweig ML (2001) Loss of speciation rate will impoverish future diversity. PNAS 98:5404-5410

Sala OE, Chapin III S, F., Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff LN, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for year 2100. Science 287:1770-1774

Sandjensen K, Jacobsen D, Duarte CM (1994) Herbivory and Resulting Plant-Damage. Oikos 69:545-549

Schmid B, Hector A (2004) The value of biodiversity experiments. Basic & Applied Ecology 5:535-542

Schoonhoven LM, Jermy T, van Loon JJA (1998) Insect-Plant Biology. Chapman&Hall, London

Schulze ED, Beck E, Müller-Hohenstein K (2005) Plant Ecology. Springer, Heidelberg

Scott AC, Stephenson J, Chaloner WG (1992) Interaction and Coevolution of Plants and Arthropods during the Palaeozoic and Mesozoic. Philosophical Transactions of the Royal Society of London: Biological Sciences 335:129-165

Siemann E, Tilman D, Haarstad J, Ritchie M (1998) Experimental tests of the dependence of arthropod diversity on plant diversity. American Naturalist 152:738-750

Silvertown J, Poulton P, Johnston E, Edwards G, Heard M, Biss PM (2006) The Park Grass Experiment 1856–2006: its contribution to ecology. Journal of Ecology doi: 10.1111/j.1365-2745.2006.01145.x

Spehn EM, Hector A, Joshi J, Scherer-Lorenzen M, Schmid B, Bazeley-White E, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Jumpponen A, Koricheva J, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Palmborg C, Pereira JS, Pfisterer AB, Prinz A, Read DJ, Schulze ED, Siamantziouras ASD, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (2005) Ecosystem effects of biodiversity manipulations in European grasslands. Ecological Monographs 75:37-63

Stamp N (2003) Out of the quagmire of plant defense hypotheses. The Quarterly Review of Biology 78:23-55

Symstad AJ (2000) A test of the effects of functional group richness and composition on grassland invasibility. Ecology 81:99-109

Tahvanainen JO, Root RB (1972) Influence of Vegetational Diversity on Population Ecology of a Specialized Herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Oecologia 10:321-346

Tansley A (1935) The use and abuse of vegetational terms. Ecology 16:284-307

Thomas JA, Telfer MG, Roy DB, Preston CD, Greenwood JJD, Asher J, Fox R, Clarke RT, Lawton JH (2004) Comparative losses of British butterflies, birds, and plants and the global extinction crisis. Science 303:1879-1881

Thompson K, Askew AP, Grime JP, Dunnett NP, Willis AJ (2005) Biodiversity, ecosystem function and plant traits in mature and immature plant communities. Functional Ecology 19:355-358

Tilman D (1987) Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecological Monographs 57:**189-214**

Tilman D (1996) Biodiversity: Population versus ecosystem stability. Ecology 77:350-363

Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E (1997) The influence of functional diversity and composition on ecosystem processes. Science 277:1300-1305

Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379:718-720

Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. Ecology Letters 8:857-874

van Ruijven J, Berendse F (2005) Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. Proceedings of the National Academy of Sciences of the United States of America 102:695-700

Vermeij GJ, Lindberg DR (2000) Delayed herbivory and the assembly of marine benthic ecosystems. Paleobiology 26:419-430

Wardle DA (1999) Is "sampling effect" a problem for experiments investigating biodiversity-ecosystem function relationships? Oikos 87:403-407

Weisser WW, Siemann E (2004) The Various Effects of Insects on Ecosystem Functioning. In: Weisser WW, Siemann E (eds) Insects and Ecosystem Function. Springer, Berlin, Heidelberg

Willis KJ, McElwain JC (2002) The Evolution of Plants. Oxford University Press, Oxford

Zangerl AR *et al.* (2002) Impact of folivory on photosynthesis is greater than the sum of its holes. PNAS 99:1088-1091

Copyright notice

The following four chapters (chapter 3, 4, 5 and 6) have been either published, accepted for publication, or are in preparation for publication in international journals.

In accordance with the exclusive licences signed with the publishers, the versions printed in the following sections are completely **newly formatted versions** that do not violate any of the copyright transfer statements.

Copyright for text and graphs is with the authors. **However, the publishers own the exclusive rights to publish or use the material for their purposes.**

Reprint of any of the materials presented in this thesis requires permissions of the publishers and of the author of this thesis.

Oecologia (2006) 147: 489-500; DOI 10.1007/s00442-005-0281-3; © Springer-Verlag 2005

Plant-Animal Interactions

Chapter 3

Effects of plant diversity on invertebrate herbivory in experimental grassland

Christoph Scherber(1),Peter N. Mwangi (3), Vicky M. Temperton (2), Christiane Roscher (1), Jens Schumacher (1), (2), Bernhard Schmid (3) and Wolfgang W. Weisser(1)

(1) Institute of Ecology, University of Jena, Dornburger Str. 159, 07743 Jena, Germany; (2) Max Planck Institut für Biogeochemie, PO Box 10 01 64, 07701 Jena, Germany; (3) Institute of Environmental Sciences, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland; **Corresponding Author:** Christoph Scherber Email: Christoph.Scherber@web.de Phone: +49-3641-949406 Fax: +49-3641-949402;

Received: 23 December 2004 Accepted: 14 September 2005 Published online: 18 October 2005 ; Communicated by Christian Koerner

Abstract The rate at which a plant species is attacked by invertebrate herbivores has been hypothesized to depend on plant species richness, yet empirical evidence is scarce. Current theory predicts higher herbivore damage in monocultures than in species-rich mixtures. We quantified herbivore damage by insects and molluscs to plants in experimental plots established in 2002 from a species pool of 60 species of Central European *Arrhenatherum* grasslands. Plots differed in plant species richness (1, 2, 4, 8, 16, 60 species), number of functional groups (1, 2, 3, 4), functional group and species composition. We estimated herbivore damage by insects and molluscs at the level of transplanted plant individuals (“phytometer” species *Plantago lanceolata*, *Trifolium pratense*, *Rumex acetosa*) and of the entire plant community during 2003 and 2004. In contrast to previous studies, our design allows specific predictions about the relative contributions of functional diversity, plant functional identity, and species richness in relation to herbivory. Additionally, the phytometer approach is new to biodiversity-herbivory studies, allowing estimates of species-specific herbivory rates within the larger biodiversity-ecosystem functioning context. Herbivory in phytometers and experimental communities tended to increase with plant species richness and the number of plant functional groups, but the effects were rarely significant. Herbivory in phytometers was in some cases positively correlated with community biomass or leaf area index. The most important factor influencing invertebrate herbivory was the presence of particular plant functional groups. Legume (grass) presence strongly increased (decreased) herbivory at the community level. The opposite pattern was found for herbivory in *T. pratense* phytometers. We conclude that (1) plant species richness is much less important than previously thought and (2) plant functional identity is a much better predictor of invertebrate herbivory in temperate grassland ecosystems.

Keywords Biodiversity - Ecosystem functioning - Functional diversity - Resource concentration - Trophic interactions

Flowering plants comprise more than 260,000 species worldwide (Kubitzky 1993) and constitute a major component of all biomass in terrestrial ecosystems. About 400,000 known species of phytophagous insects feed on them, with about 90% exhibiting high degrees of host specialization (Bernays and Graham 1988). While there is a wealth of studies on the relationship between plant diversity and diversity of herbivorous insects (e.g. Haddad *et al.* 2001; Koricheva *et al.* 2000; Mulder *et al.* 1999; Siemann *et al.* 1998), surprisingly little is known about the relationship between plant diversity and the damage caused by herbivorous insects, i.e. the amount of herbivory itself, both at the level of the entire plant community and of individual plants. Agricultural experiments suggest that because of larger densities of specialist herbivores, greater herbivore damage at the individual level should occur in plant monocultures than in plant mixtures (Andow 1991; Finch and Collier 2000). The 'resource concentration' and the 'enemies' hypotheses (Root 1973) make predictions about herbivore loads in simple and diverse environments, but there is a shortage of studies actually measuring invertebrate herbivore damage in ecosystems across gradients of plant species richness. Most notably, previous studies were not specifically designed to investigate whether, in addition to plant species richness per se, the presence/absence of particular plant functional groups plays a role for invertebrate herbivory.

Mulder *et al*. (1999) used a limited number of plots to study the effects of plant species richness on herbivore damage in only a selected number of plant species. The authors found that, in contrast to theory, herbivory tended to increase with plant species richness (over the range of 1–12 species), mainly due to low damage in monocultures and higher damage on some dominant legumes in higher diversity mixtures (*Trifolium hybridum*, *T. pratense*), but the role of functional groups was not tested.

In the present study, we manipulate plant species richness, number of functional groups (FG), and presence of particular functional groups using all possible combinations of these (Roscher *et al*. 2004) on experimental plots measuring 20×20 m. In contrast to previous studies, we explicitly chose a comparatively large plot size in order to account for local mobility of invertebrates. We then monitor invertebrate herbivory both at the community level and at the level of individual plant species transplanted into all plots. Thus, in addition to community measures, we introduce several given plant species into all of the experimental mixtures, at sufficiently low densities. We call these plant species 'phytometers' (sensu Gibson 2002), because they serve us to measure the internal properties of our experimental ecosystems almost without a direct effect on the system. The phytometers can be seen as small components systematically added to each of the experimental systems; and by following rates of herbivory in these components, we try to infer what is happening at a rather small scale inside the system.

We restrict our study to invertebrate herbivores of the phyla Mollusca (Gastropoda, e.g. Hulme 1996) and Insecta, explicitly excluding other groups such as Annelida or plant-parasitic Nematoda. Although the original hypotheses were formulated with insect herbivores in mind, they also apply to herbivory by other invertebrates.

We ask the following questions: (1) Do the components of plant diversity, species richness and number of FG, affect herbivore damage by insects and molluscs at the level of the plant community and of phytometer individuals? (2) Does plant functional group composition, i.e. the presence of particular plant functional groups in experimental communities, affect herbivore damage by insects and molluscs at the level of the plant community and of phytometer individuals?

In contrast to previous studies, our study is the first to systematically analyze plant damage by herbivores over a large range of plant species richness (1–60 species) and number of FG (1–4), using replicated and different measures of herbivory at community and plant species level.

Materials and methods

Experimental design

Eighty-two experimental grassland plots 20×20 m in size were established at a field site located on the floodplain of the Saale river (altitude 130 m) at the northern edge of Jena (Jena-Löbstedt, Thuringia, Germany; Roscher *et al*. 2004). The field was previously used for arable crops before establishment of the experimental plots with plant communities representing various plant diversity treatments. The species pool consisted of 60 herbaceous plant species commonly occurring in semi-natural, mesophilic grasslands in the region surrounding the field site (Molinio-Arrhenatheretea meadows, Arrhenatherion community, Ellenberg 1996). The 60 plant species were divided a priori into the four FG grasses, legumes, small herbs and tall herbs using cluster analysis of a trait matrix (see Roscher *et al*. 2004 for a detailed species list and allocation to functional groups). The plots were seeded with 1, 2, 4, 8, 16 or all 60 species containing 1, 2, 3 or 4 FG in May 2002. Species to be used in monocultures and mixtures were selected randomly with replacement from the pool, ensuring that all possible combinations of species richness and number of FG occurred in the experiment. This yielded 16 replicates for monocultures (1 FG), 16 for 2-species mixtures (1–2 FG), 16 for 4-species mixtures (1–4 FG), 16 for 8-species mixtures (1–4 FG), 14 for the 16-species mixtures (1–4 FG, except for the "16 species × 1 FG" combination, because fewer than 16 species of legumes and small herbs were available) and 4 replicates for the 60-species mixture (Roscher *et al*. 2004). The 82 plots were grouped into four blocks to account for gradients in abiotic conditions with increasing distance from the Saale river (mainly soil sand content; Roscher *et al*. 2004). All experimental communities were manually weeded to maintain the given diversity treatments and mown twice a year. Each plot was divided systematically into subplots. Two different subplots within each 20×20 m plot were used for planting of phytometers (see below).

Cultivation and transplantation of phytometers

We selected three species of phytometers, *Plantago lanceolata* L. (Plantaginaceae), *Trifolium pratense* L. (Fabaceae) and *Rumex acetosa* L. (Polygonaceae). These species were expected to support species-rich specialist invertebrate herbivore communities. In addition, the selected phytometer species were already present in at least 10% of the experimental plots and had leaf shapes that allowed quick assessment of invertebrate herbivore damage. Seeds of wild-type origin of phytometer species were obtained from a commercial supplier (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). On 14 March 2003, seeds of *P. lanceolata* and *T. pratense* were pre-germinated on moist filter paper for 6 days, and individually transferred to random positions of 40-cell plug trays filled with a 1:1:1 mixture of standard compost (TKS 1, OBI Merchandise Center GmbH, Wermelskirchen, Germany), homogenized top soil collected from the field site, and perlite (OTAVI Perlit GmbH, Eschborn, Germany). The trays were transferred to a glasshouse (night:day temperature 18°C:22°C, supplementary lighting –1 h/+2 h before/after sunrise/sunset, average day-light intensity 210 W/m^2). About 20 days after sowing, trays were transferred to the field site where plants were hardened for 1 week prior to transplanting. Five individuals were selected using size class-specific randomization for each phytometer species and transplanted into one 2×2 m subplot of each main plot at an interplant distance of 28 cm. On April 2003, *R. acetosa* phytometers were pre-germinated on standard compost and treated in a similar way as the other two phytometer species except that they were grown on a mixture of standard compost and perlite (4:1) in a different glasshouse (night:day cycle 10 h:14 h and night:day temperature 15°C:22°C). On 25 June, after 7 days of hardening, five *R. acetosa* individuals were selected using size class-specific randomization and transplanted into a different 2×5 m subplot within each main plot at an interplant distance of 50 cm. In total, 1,230 phytometer individuals of the three species were transplanted into field plots. Immediately after transplanting, plant size (number of fully unfolded leaves) was recorded for each individual and included as a covariate into all subsequent statistical analyses. It is important to note that phytometer herbivory differs from community herbivory in a sense that "monoculture" means the phytometer individuals were surrounded by monocultures of a different plant species.

Assessment of herbivory at the level of phytometer individuals

Invertebrate herbivory was assessed in a cumulative way as the sum of all events of herbivore damage over a given period of time. Thus, we did not account for age-specific differences in the susceptibility of leaves to herbivory; this holds also for our transect estimates of community herbivory.

May 2003 *P. lanceolata* phytometers were cut at 3 cm above ground between 30 May and 10 June 2003, just before the first mowing of the plots, and stored in sealed clear plastic bags at 4°C before processing. In order to assess invertebrate herbivore damage to leaves, all leaves of all individuals were scanned (O'Neal *et al*. 2002) using a flatbed scanner (HP ScanJet 4570 c, Hewlett-Packard, Palo Alto, USA) at 100×100 dpi resolution

(16.7 million colours). Total leaf area (TL) was determined in three steps: (1) we measured total remaining leaf area (TLR) using the magic wand tool in Adobe Photoshop 5.5 (1989–1999 Adobe Systems, Inc.) with tolerance set to 100 and summing over all leaves; (2) due to low damage levels, we visually estimated total leaf area damaged (TLD) on the screen, using a system of damage classes; (3) TL was then calculated as the sum of TLR and TLD. Square pixels were transformed to centimetre square using calibration quadrates. To estimate TLD in step (2), we determined NH_i, the number of holes of size class i (i=1–3) per plant, where midpoints of hole-size classes AH i were 0.005 cm² (i=1), 0.065 cm² (i=2) and 0.125 cm² (i=3), and, for feeding damage on the leaf margins, NM, the number of leaf sites that were damaged with an area of $AM \leq 0.5$ cm². The total leaf area eaten per plant (TLD) in cm² was then calculated as

$$TLD = NM \times 0.5 + \sum_i (NH_i \times AH_i)$$

For every plant, the proportion p of tissue removed by invertebrate herbivores was calculated as

$$p = \frac{TLD}{TL}$$

August 2003 In contrast to the May data, herbivore damage in *P. lanceolata*, *T. pratense* and *R. acetosa* phytometers was visually and non-destructively estimated between 4–18 August 2003. To obtain an estimate of total undamaged leaf area per leaf, we assessed the sizes of the smallest and the largest leaf laminas per plant using ellipsoid templates made for each plant species, calculating mean leaf size as the mean of these two leaf sizes. We then estimated total damage per plant by holding millimetre-grid graph paper next to each leaf and summing up the damaged area (in mm²). The proportion of leaf area eaten was then calculated by dividing total damage by mean leaf size.

Assessment of herbivory at the level of the plant community

Herbivory at the community level was assessed with a line-transect method, i.e. individuals were sampled according to their relative abundance.

May 2003 Between 22–29 May 2003, we estimated community herbivory at fixed 20 cm intervals along two parallel line transects in a randomly allocated 2×5 m subplot of each main plot. Transects measured 2.8 m each, starting 1 m from the edge of the main plot, with 15 observation points per transect. The distance between the two transects on a main plot was 1 m. We measured the plant that was rooted at or closest to the desired point, in the direction towards the centre of the subplot. In case of clonally growing plants (e.g. grasses), a set of five randomly chosen identifiable ramets or tillers was assessed. Herbivory per plant was visually estimated as the total percentage of leaf area affected by invertebrates, including molluscs and chewing, sucking (if visible) or mining insects using a class system (e.g. Mitchell 2003), where upper class limits for four herbivory classes were 1, 5, 10, and 30% of leaf area. No damage higher than 30% occurred. Means of the percentage of leaf area damaged for a plant community were obtained by averaging damage class midpoints across plants using arcsine-square root transformed values and back-transforming.

August 2003 Community herbivory was estimated between 21 August and 10 September 2003 by harvesting 30 individuals every 10 cm along a 3 m line transect located in the middle and parallel to the 5 m side of the 2×5 m subplot. We selected individuals as described above. For every plant (group of ramets or tillers), we measured both leaf size and leaf area damaged of a randomly chosen medium-sized leaf using templates. The proportion of leaf area damaged was calculated by dividing herbivore damage by leaf size. Thus, in contrast to the May transect, we used proportions of leaf area damaged per leaf, instead of proportional damage of whole plants, for further analysis.

May and August 2004 In order to find out whether herbivory patterns would change or stabilize over time, we collected two more sets of data on community herbivory between 18 and 25 May 2004, and between 11 and 16 August 2004. The methods were the same as in May 2003, except that in May 2004 we directly estimated proportions of damaged leaf area per plant. In August 2004, we used the same system of damage classes as in May 2003 to estimate damaged leaf area per plant.

Assessment of community performance and invertebrate herbivore communities

We harvested community biomass in four randomly placed 20×50 cm quadrats in each 20×20 m main plot at 3 cm above mean soil surface at the end of May and the end of August 2003. The plant material was sorted into species, oven-dried at 70°C for 48 h and weighed. Community leaf area index (LAI) was measured on 12–13 August 2004 using a LAI-2000 Plant Canopy Analyzer (Li-Cor BioSciences Lincoln, USA). As we were working in mixed communities, we did not adjust the LAI-2000 for leaf angles. Both community productivity and LAI were used as covariates in the analyses of invertebrate herbivore damage. The effects of plant diversity on productivity have been reported elsewhere (Roscher *et al.* 2005).

Relative population densities of invertebrate herbivores were estimated in all 1, 4, 16 and 60-species mixtures at five regular intervals during May and October 2003 using suction samplers coupled with biocenometers at six randomly allocated locations per plot. Molluscs were sampled repeatedly by another project group at eight weekly intervals using two 50×50 cm2 cardboard sheets placed systematically on each plot (Bahr 2005). These results will be published elsewhere, yet we will provide a short comment on composition of the herbivore communities.

Statistical analysis

Estimations of herbivory were conducted blockwise, ensuring that effects of time of measurement could be statistically accounted for as block effects. Data were analyzed using the statistical software package S-Plus 6.1 Professional (Release 1, (c) 1988, 2002 Insightful Corp., Crawley 2002). Data were square-root or log transformed if Q–Q plots and Shapiro-Wilk tests showed deviations from normality. Missing data were excluded from the analyses. Proportions were arcsine-square-root transformed. Data were analyzed using analysis of covariance with type-I sums of squares (Crawley 2002; Schmid *et al.* 2002). First, a maximal model was fitted with the following sequence: block, initial leaf number, plant species richness, number of FG, leaf area index, community biomass, presence of grasses, legumes, small herbs and tall herbs. Species richness was always fitted before number of FG. The only interaction terms tested in statistical models were species richness and number of FG, and grass × legume presence.

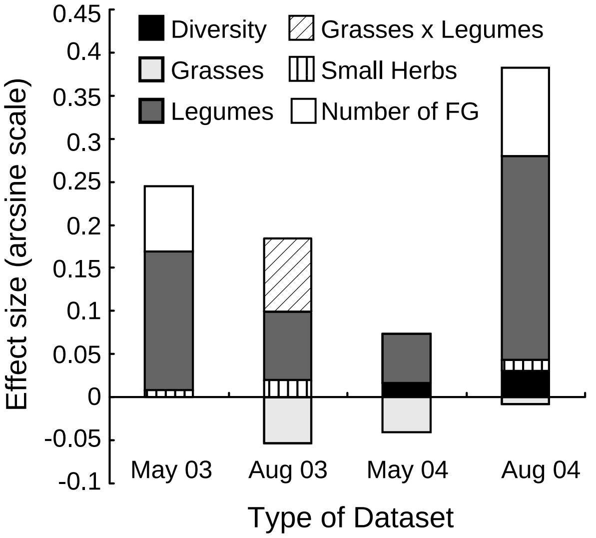


Fig. 1 Community herbivory. Bars show factorial effect sizes of explanatory variables retained in the minimal adequate statistical models. Effect sizes are untransformed proportions, p (i.e. arcsin $\sqrt{p}$). Block effects not included. Note the overall minor contribution of plant species richness or number of FG in comparison with legume and grass effect sizes

After fitting the full model, we simplified it by (1) factor level reduction and (2) sequential deletion of terms from the full model (Crawley 2002). For instance, if the explanatory variable was plant species richness, we started with a six-level factor to test for overall differences between diversity levels; during model simplification, we then constructed an alternative model where species richness was treated as a numerical variable to test for (log-) linear trends. The two alternative models were compared using F-tests (Crawley 2002), and work was continued with the simpler (log-linear) model if changes in deviance were non significant. We then generated a series of simplified models using single-term deletions from the maximal model, and compared the resulting model with its predecessor using F-tests. During automated software-based model simplification, the Akaike Information Criterion (AIC, Burnham and Anderson 1998) was used. Smaller values of AIC indicate higher predictive power of the respective statistical model. Model simplification always started with highest-order interaction terms and was continued until the minimal adequate model was obtained. We defined the minimal adequate model at the point where no further deletions of terms were possible, i.e. until further deletion would have lead to significant changes in deviance (Crawley 2002, p. 449 f.). All main effects that were retained in minimal adequate models are listed in Table 1, with their respective F- and P-values. Figure 1 shows estimated factorial effect sizes (differences between overall and treatment means) for some of the models.

A power analysis conducted with community herbivory data from August 2004 and all levels of plant species richness revealed that in order to detect a maximum difference in herbivory of about 5% with a power of 0.9 at a significance level of 0.05, about 10 replicates would be needed per diversity level. Thus, the design we applied is capable of detecting even small differences in mean levels of herbivory.

Results

Invertebrate herbivore communities

A detailed study on the invertebrate communities across all trophic levels, in particular the insect complex, will be published elsewhere (W. Voigt *et al.*, in preparation). In 2003, 1 year after establishment, the fauna consisted partly of species that can be considered to be present as a legacy of the previous agricultural use of the field (e.g. the slug *Deroceras reticulatum* Müll., Mollusca: Gastropoda). In total, more than 18,000 individuals of cicadas, 2,000 individuals of herbivorous heteropteran bugs, and over 20,000 individuals of leaf beetles were collected (Kowalski 2005). Typical grassland insect herbivores were, for example, *Longitarsus pratensis* Panz. (Chrysomelidae) on *P. lanceolata*, three *Chaetocnema* species on grasses, and *Hypera meles* F. (Curculionidae) on *T. pratense*. Typical grassland leafhopper (Homoptera: Auchenorrhyncha) species included *Philaenus spumarius* L., *Arthaldeus pascuellus* Fall. and *Javesella pellucida* F.

Herbivory on phytometers

Initial number of leaves did not significantly influence damage in any of the species and was always dropped during model simplification (*P. lanceolata*: May 2003: $F_{1,49}$=0.14, P=0.70, August 2003 $F_{1,42}$=0.64, P=0.81; *T. pratense*: $F_{1,61}$=0.11, P=0.74, *R. acetosa*: $F_{1,63}$=0.009, P=0.92). Table 1 summarizes the results of all statistical analyses with respect to the main explanatory variables analyzed.

Plantago lanceolata

In May 2003, invertebrate herbivores damaged on average 0.83±0.11% of available leaf area per leaf. Damage was not dependent on plant species diversity or the number of FG in the plant community (Table 1). Damage tended to increase in the presence of grasses but the effect was not significant. The presence of the other functional groups also had no significant influence on damage (Table 1). Damage was not correlated with community leaf area index (LAI) or community biomass (Table 1). The minimal adequate model included number of FG and presence of grasses (AIC=−223.3540 and df=4, R^2=0.06).

In August 2003, 2 months after mowing, invertebrate herbivory on the newly produced leaves averaged 1.15±0.08%. Herbivore damage increased slightly but not significantly with species richness (Fig. 2) and was not dependent on the number of FG in the community (Table 1). Damage was slightly in but not significantly larger in plots with grasses than in those without grasses (1.3±0.12% vs. 0.99±0.12%, Fig. 2). The presence of other functional groups in the community had no influence on herbivory. Damage was significantly positively correlated with community LAI (Fig. 2) but not with plant community biomass (Table 1). The minimal adequate model included block, presence of grasses, and community LAI (AIC=−310.630, df=7, R^2=0.23).

Trifolium pratense

In August 2003, mean herbivore damage on leaves was 2.61±0.44%. Damage was independent of plant species richness and the number of FG in the community (Fig. 2, Table 1). Damage was significantly higher in presence than in absence of grasses (Table 1). Presence of legumes significantly decreased herbivory (Fig. 2, Table 1), while the presence of other functional groups had no influence on herbivory. Community biomass and LAI

Table 1 Summary of main results

	Response variable	Block	Plant species richness	FG	Community biomass	Leaf area index	Grasses	Legumes	Small Herbs
Phytometers									
P.lanceolata,	Herbivory [a]	NA	NA	$F_{1.70}$=1.01,	NA	NA	$F_{1,70}$=3.42,	NA	NA
May 2003	per plant	NS	NS	P=0.318 (0)	NS .	NS	P=0.068 (0)	NS	NS
P.lanceolata,	Herbivory	$F_{3,74}$=3.91,	NA	NA	NA	$F_{1,74}$=7.47,	$F_{1,74}$=3.36,	NA	NA
Aug 2003	per leaf	P=0.012	NS	NS	NS	P=0.008 (+)	P=0.071 (0)	NS	NS
T.pratense,	Herbivory	NA	NA	NA	NA	NA	$F_{1,76}$=6.52,	$F_{1,76}$=7.37,	NA
Aug 2003	per leaf	NS	NS	NS	NS	NS	P=0.012 (+)	P=0.008 (-)	NS
R. acetosa,	Herbivory	NA	$F_{1,77}$=3.08,	NA	$F_{1,77}$=5.49,	NA	NA	NA	NA
Aug 2003	per leaf	NS	P=0.08 (0)	NS	P=0.022 (+)	NS	NS	NS	NS
Transects									
May 2003	Herbivory	$F_{3,74}$=5.57,	NA	$F_{1,74}$=0.62,	NA	NA	NA	$F_{1,74}$=36.76,	$F_{1,74}$=5.92,
	per plant	P=0.002	NS	P=0.43 (+)	NS	NS	NS	P<0.001 (+)	P=0.017 (+)
	Leaf size[b]	$F_{3,57}$=4.47,	$F_{1,57}$=8.45,	NA	$F_{1,57}$=16.74,	NA	NA	NA	NA
	(mm^2)	P=0.006	P=0.005 (+)	NS	P<0.001 (+)	NS	NS	NS	NS
Aug 2003	Leaf dam-	NA	NA	NA	$F_{1,60}$=6.26,	NA	NA	$F_{1,60}$=10.10,	NA
	age[c] (mm^2)	NS	NS	NS	P=0.015 (+)	NS	NS	P=0.002 (+)	NS
	Herbivory	NA	NA	$F_{1, 59}$=6.22,	NA	NA	F1,59=4.68,	F1,59=7.49,	NA
	per leaf	NS	NS	P=0.015 (+)	NS	NS	P=0.034 (-)	P=0.008 (+)	NS
May 2004	Herbivory	NA	$F_{1,73}$=5.94,	NA	NA	NA	F1,73=4.53,	F1,73=9.18,	NA
	per plant	NS	P=0.017 (+)	NS	NS	NS	P=0.036 (-)	P=0.003 (+)	NS
Aug 2004	Herbivory	NA	$F_{1,74}$=5.22,	$F_{1,74}$=0.43,	NA	NA	$F_{1,74}$=0.04,	$F_{1,74}$=19.72,	$F_{1,74}$=4.44,
	per plant	NS	P=0.025 (+)[d]	P=0.512 (0)	NS	NS	P=0.848 (0)	P<0.001 (+)	P=0.038 (+)

Rows show F- and P-values, and direction of effects, for the response variables listed in the second column. Columns 3-12 show the main factors and covariates tested in the models, in the sequence in which they were fitted. “NS” indicates non-significant terms removed from the maximal model; the corresponding F-values are indicated by “NA”. (0), (+) and (-) indicate no, positive or negative effects of the factors on the response variable. Interaction effects and effects of tall herbs not shown. [a] Percent leaf area damaged; [b] Total available leaf area per leaf; [c] Total damage per leaf; [d] Terms only significant when fitted first in the model.

had no significant influence on damage. The minimal adequate model contained presence of grasses, and presence of legumes (AIC=−179.9297, df=4, R^2=0.15).

Rumex acetosa

In August 2003, invertebrate herbivore damage was on average 3.03±0.3% of leaf area. Damage was independent of plant species richness (Fig. 2) and the number of FG in the community (Table 1). The presence of particular functional groups in the community had no influence on herbivory. Damage increased significantly with community biomass but was independent of LAI (Table 1). The minimal adequate model contained sown species number and community biomass (AIC=−135.2514, df=4, R^2=0.10).

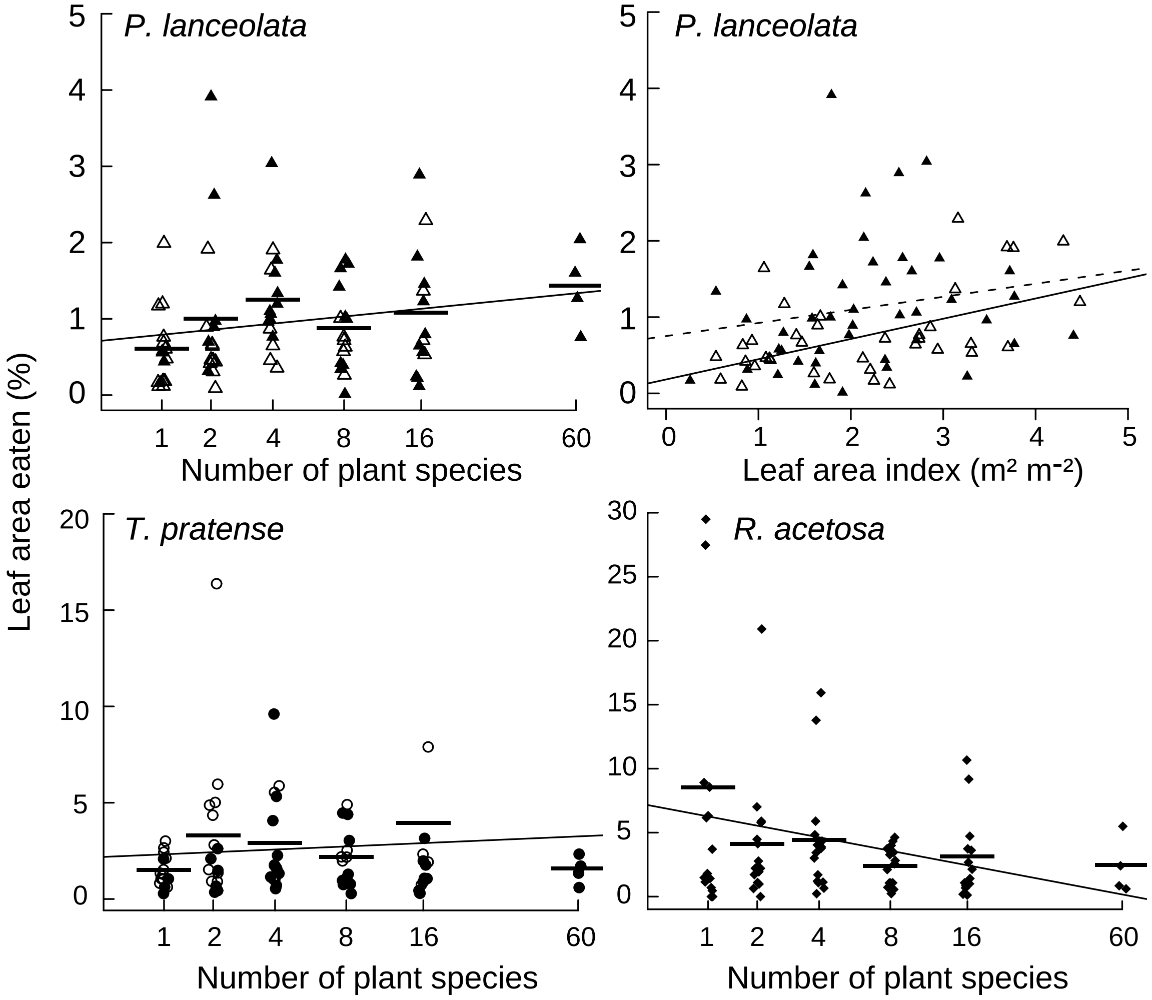


Fig. 2 Phytometer herbivory. Effects of plant species richness and community leaf area index on invertebrate herbivory in phytometers. Random noise added along the x-coordinate of each point to improve readability. Open (closed) triangles represent plots where grasses were absent (present). Similarly, circles refer to the absence (presence) of legumes. Filled diamonds show effects regardless of functional group identity. Solid lines in top left and bottom panels represent mean squares fit lines. Solid vs. dashed lines in top right panel show mean squares fits for subgroups (with vs. without grasses). For *T. pratense*, one outlier (16 species, herbivory=30%) has been omitted from plotting to improve plot appearance

Community herbivory

Relative effect sizes for all main factors tested in the statistical models are shown in Fig. 1. Note especially the overall minor contribution of plant species richness or number of FG to invertebrate herbivory.

May 2003 In May 2003, herbivore damage at the level of the plant community was 1.33±0.16% of leaf area. Estimated damage differed significantly between blocks (Table 1). Herbivory was independent of plant species richness and the number of FG in the community (Table 1). While the presence of grasses in the community had no influence on herbivory, damage was significantly higher in plots with legumes than without legumes and in plots with small herbs than without small herbs (Table 1; Fig. 1). The minimal adequate model included block, number of FG, presence of legumes, and presence of small herbs (AIC=−259.5689, df=8, R^2=0.45).

August 2003 In the August 2003 transect, we were able to compare absolute levels of herbivore damage with proportional damage. Total available leaf area per leaf differed significantly between blocks (Table 1). With increasing plant species richness, total available leaf area increased significantly, while number of FG had no significant influence. Community biomass was significantly positively correlated with available leaf area. The minimal adequate model contained block, community biomass, and sown species number (AIC=164.4347, df=7, R^2=0.40).

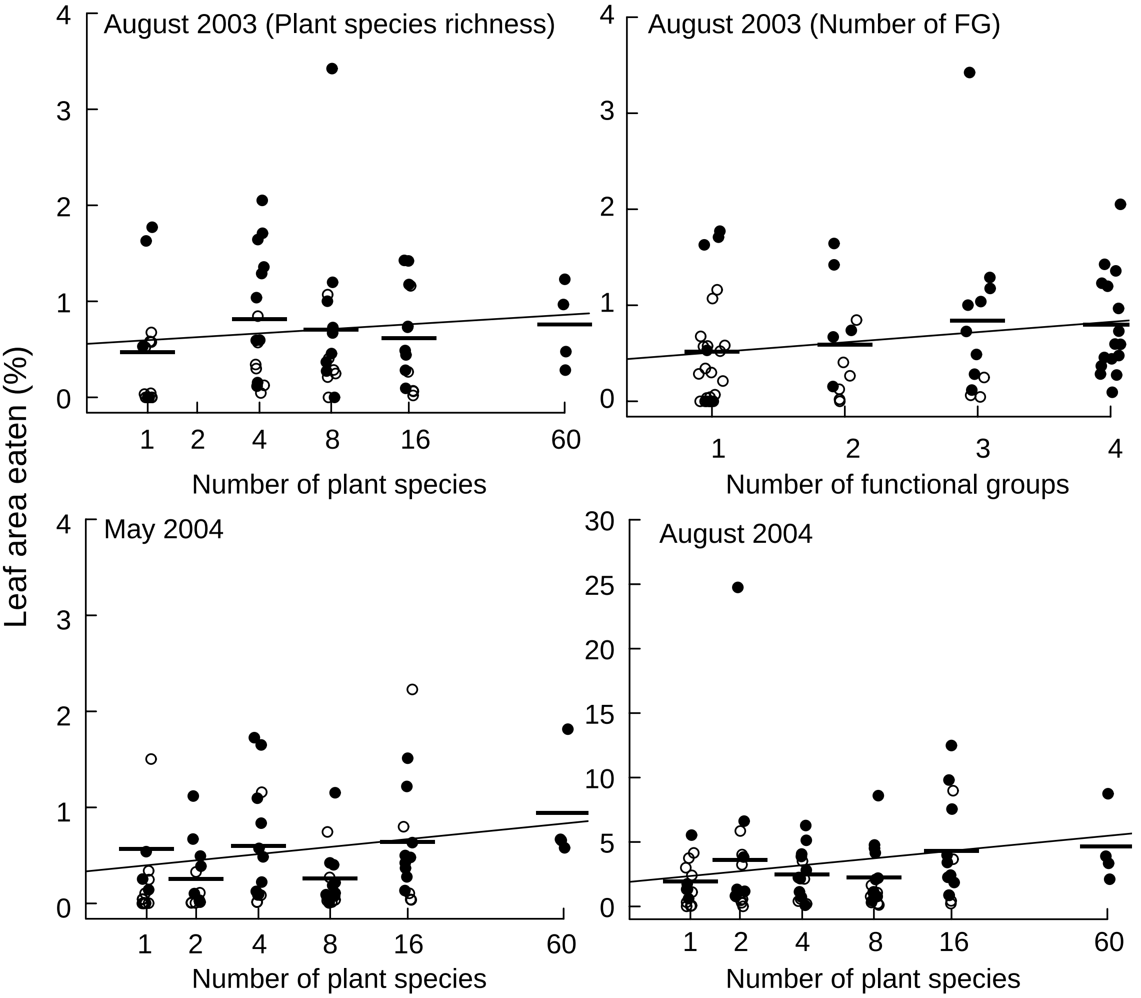


Fig. 3 Effects of plant species richness and number of FG on invertebrate herbivory at the community level between August 2003 and August 2004. Open (filled) circles show absence (presence) of legumes. Random noise added along the x-coordinate of each point to improve readability. Solid lines show mean squares fits

Absolute leaf damage per leaf was not affected by species richness and number of FG (Table 1). However, absolute leaf damage increased significantly with community biomass. Absolute damage was significantly higher in plots with than without legumes (Table 1). The minimal adequate model contained community biomass and presence of legumes (AIC=167.3327, df=4, R^2=0.21).

Because both absolute damaged and available leaf area increased with plant species richness or community biomass, the proportion of damaged leaf area remained constant across the richness gradient (Fig. 3), at a level of 0.65±0.08%, and the effect of species richness on proportional damage wasnot significant (Table 1). However, the proportion of damaged leaf area increased significantly with increasing number of FG in the community (Table 1; Figs. 1, 3). There was a significant interaction between grass and legume presence (Figs. 1, 4). Damage was larger in plots with legumes than in those without legumes and smaller in plots with than without grasses (Figs. 1, 3, 4). The presence of other functional groups had no influence on damage (Table 1). Community biomass and LAI had no effect on damage. The minimal adequate model contained number of FG, an interaction term between grasses and legume presence, plus the corresponding main effects (AIC=–230.0477, df=6, R^2=0.29).

May 2004 In May 2004, herbivore damage by invertebrates measured as proportion of damaged leaf area per plant was on an average 0.46±0.08%. Herbivory increased linearly with log plant species richness (Fig. 3), while number of FG was not significant (Table 1). The orthogonal contrasts between (1) the monocultures and the 60-species mixtures and (2) the monocultures and all mixtures were not significant. When number of FG was fitted first in the model, sown species number was not significant any more. Herbivore damage was significantly higher in plots with legumes than without legumes but lower in plots with grasses than without grasses (Figs. 1, 4). The presence of other functional groups and community biomass or LAI did not significantly influence damage (Table 1). The minimal adequate model contained sown species number, presence of grasses, and presence of legumes (AIC=−276.6479, df=5, R^2 =0.21).

August 2004 In August 2004, invertebrate herbivore damage measured as proportion of damaged leaf area per plant was on an average 2.87±0.39%. Log-linear species richness had a significant positive effect on herbivore damage (Fig. 3, Table 1), but the effect size was small compared with the other model terms (Fig. 1). The orthogonal contrasts between (1) monocultures and 60-species mixtures and (2) monocultures and all mixtures were not significant. When number of FG was fitted first, neither number of FG (F 1,74=3.76, P=0.056) nor sown species number (F 1,74=1.89, P=0.172) were significant. Presence of grasses had no effect on herbivore damage, while legume presence and presence of small herbs strongly increased herbivory (Figs. 1, 4; Table 1). The minimal adequate model contained sown species number, number of FG, presence of grasses, legumes, and small herbs (AIC=−173.6320, df=7, R^2=0.29).

Discussion

Comparison with previous studies

While there have been extensive studies on invertebrate herbivore diversity in relation to plant diversity (e.g. Haddad *et al.* 2001; Knops *et al.* 1999; Koricheva *et al.* 2000; Siemann 1998; Siemann *et al.* 1998), much less is known about actual levels of herbivory across plant diversity. Haddad *et al.* (2001, p. 32) used plots from the large biodiversity experiment in Cedar Creek, Minnesota, USA, and found that "the abundance of chewing insects [...] was most strongly and positively related to plant biomass"; Koricheva *et al.* (2000) used plots created within the BIODEPTH pan-European study and found no significant plant diversity effects on abundance of chewing insects and molluscs. Both studies indicate that species richness might not be the main determinant of herbivore damage as measured in our study.

Pfisterer *et al.* (2003), also in the framework of the BIODEPTH experiment, used a model system with caged grasshoppers and found that "the presence of grasshoppers did not significantly change the slope of the relationship between plant biomass and plant diversity" (p. 237); in addition, they found that "proportional cover change" due to herbivory was "independent of species richness".

Finally, the BIODEPTH experiment also includes two studies that are comparable to ours. Giller and O´Donovan (2002, p. 135) found that herbivore damage in *T. pratense*, which was present in a number of mixtures differing in plant diversity, was lower in eight-species mixtures than in monocultures, with a general trend of herbivory decreasing with plant species richness. In contrast, the study by Mulder *et al.* (1999) reports a significant positive effect of plant diversity on herbivory. However, there are aspects of the experimental design that make it difficult to disentangle the relative contributions of plant functional identity and plant species richness: Plots with 100% legumes were restricted to the 1- and 2-species mixtures at the Swedish site (Mulder *et al.* 2002); thus, in contrast to our design, legume abundance was negatively correlated with species number. All other functional groups were only present as combinations in plots with 4, 8 or 12 plant species; this means, the contribution of single functional groups could not be tested. All Swiss plots except monocultures always contained grasses (Pfisterer *et al.* 2003). In addition, herbivory measurements were not made on all species (Mulder *et al.* 1999, p. 240) and in only one vegetation period (July and August 1997) across a richness gradient of 1–12 species.

Thus, while important in being the first experimental study showing effects of insects on ecosystem processes in a biodiversity-ecosystem functioning context, we think that studies like the ones listed above should be seen as a springboard for further research.

Invertebrate herbivore communities

While the data collected so far clearly show that typical grassland specialist and generalist invertebrate herbivores have already established at our site, it is well possible that after 2 years they may not yet have reached equilibrium densities. Some of the patterns observed in this study may therefore become stronger over time.

Unfortunately, it is difficult to determine the exact links between given plant species and their specific herbivore communities (Unsicker *et al.* 2005). For this reason, in line with other published studies, we do not differentiate between specialist and generalist herbivores in our analysis even though Root´s initial hypotheses (1973) assumed that most damage was attributable to specialists with high monoculture abundance.

Levels of herbivory

We measured herbivory at single-species and community level using a broad range of methods, from fine-scale digital imaging to coarse-scale assignment of herbivory classes, as part of a fine-tuning process to find most suitable measures of herbivory. All measures we employed lead to similar mean values and ranges of the response variables. However, our methods did not account for non-visible damage caused by sucking insects (e.g. Voigt *et al.* 2003), and indirect effects of tissue damage – e.g. due to changed rates of photosynthesis (Zangerl *et al.* 2002).

The levels of leaf damage reported in our study were generally low, but within the range found by other authors. Brown and Gange (1989) suggested that *P. lanceolata* may escape aboveground herbivory in early successional plant communities, and this is possibly attributable to iridoid glycoside defences (Stamp and Bowers 2000). *T. pratense* and R. acetosa showed higher levels of invertebrate herbivory than *P. lanceolata*, indicating species-specificity of herbivore damage (Mulder *et al.* 1999).

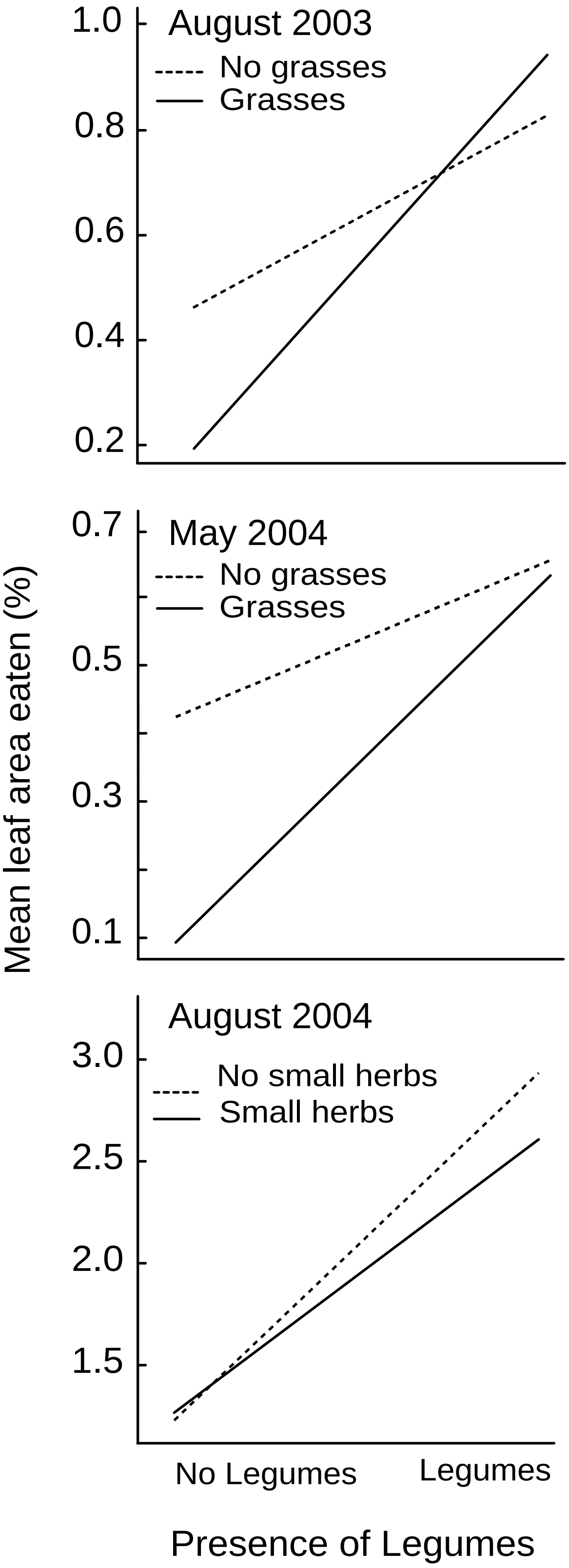


Fig. 4 Effects of particular combinations of functional groups on community herbivory in August 2003, May and August 2004

Community herbivory was relatively low at the onset of our experiment, but tended to increase with time. Crawley (1989, p. 15) reports annual insect herbivory damage of 0.5–15% in grasslands. Carson and Root (1999), however, found that leaf area damage in early successional plant communities almost never exceeded three percent. Mulder *et al.* (1999) found herbivory levels below 2% in a similar grassland biodiversity experiment. As our experimental plots were mown twice a year, annual herbivory levels would sum up to 2–6% of total leaf area. Thus, we think that the low values of invertebrate herbivory reported by us are not the exception, but the rule.

Effects of plant species richness and plant functional diversity

Herbivory in phytometers and at the community level measure different aspects of the same phenomenon. While community transects are suitable for measuring monoculture herbivory, phytometers surrounded by monocultures of different plant species should have a comparatively low probability of being found by specialist invertebrate herbivores. In our study, however, they were found and damaged in a similar way across all levels of plant species richness.

All datasets analyzed in our study show a clear pattern: Plant species richness and plant functional diversity are weak predictors of plant damage by invertebrate herbivores. This result is in contrast to most studies published so far; in our opinion, the overall importance of plant species richness for invertebrate herbivory has been overrated, especially in comparison to other factors, such as plant functional identity, that seem much more important.

Mulder *et al.* (1999, p. 244) found that “herbivory increased as a function of plant species richness”, yet they did not provide a measure of relative importance of the terms in their models. Diversity was only significant “when only [...] species on which herbivore damage was measured were included” (p. 241), but not across all plant species.

Several hypotheses have been proposed to relate plant diversity to the densities of invertebrate herbivores and to damage to plants (Root 1973, Tahvanainen and Root 1972). While being very important in generating research in the area, these hypotheses are qualitative and do not predict a particular shape for the relationship between plant diversity and herbivore densities; hence, they are difficult to reject using experiments.

Notably, Root (1973, p. 104) himself writes that “to measure the impact of an entire consumer fauna on a plant would be a difficult task”. Thus, his resource concentration hypothesis only applies to the densities of insect herbivores “with a narrow host range”, and not to the impact of the entire fauna on plant communities. It additionally only applies to the linear contrast between “pure stand” and “mixture”, which in our analyses always proved non-significant. In case we did find a significant diversity effect, it was positive instead of negative. This is in line with some results of Mulder *et al.* (1999), and observations of Siemann (1998) of higher insect herbivore abundance in more diverse plots, but it remains to be seen if this trend continues over time. If so, the pattern cannot be explained using current theory and needs to be investigated in more detail.

As is well-known from predator-prey models, mutual interference or other changes in the functional response of individual consumers may complicate the relationship between resource density, consumer density, and hence the damage level inflicted on the resource (Siemann 1998; Hassell 2000). Complicated rather than simple dependencies of herbivore diversities and abundances have also been found in other studies (Tonhasca and Byrne 1994; Siemann 1998; Koricheva *et al.* 2000; Perner *et al.* 2005, Andow 1990, 1991).

Experiments within an agricultural context (reviewed in Andow 1991) often found the predicted negative effect of plant diversity on herbivory, but in our opinion these effects are plant-species specific or site-specific and not true diversity effects, as only few combinations of plant species were tested.

A similar conclusion can be drawn for the number of FG (FG), which can be seen as a measure of species redundancy in a community. In general, even when fitted first in statistical models, number of FG was less important for herbivory than species richness per se. Note, however, that a full separation of the effects of plant species richness and plant functional diversity is not possible (e.g.

Tilman *et al*. 1997). At least for the datasets we analyzed, neither species nor functional diversity showed consistent effects on herbivory in either direction.

Effects of particular plant functional groups

In contrast to the weak effects of plant species and functional diversity on herbivory, there were pronounced effects of the presence of particular plant functional groups on herbivore damage. For example, the presence of grasses decreased herbivory levels in transects. Grasses are known to have lower rates of herbivore attack than other plant functional groups (Tscharntke and Greiler 1995), and therefore such a community-level pattern directly translates into overall herbivory rates whenever grasses are present. For the phytometer *T. pratense*, however, the presence of grasses in the plant community increased the levels of herbivory. If surrounded by grasses, a higher percentage of leaf area was consumed by herbivores. However, this effect was only significant for *T. pratense*. We found a similar positive effect on herbivory with increasing community productivity (*R. acetosa*) or increasing leaf area index (*P. lanceolata*). Thus, herbivory in all phytometer species was to some extent significantly influenced by diffuse interspecific competition between phytometers and the surrounding community. We hypothesize that competition for light or water increased phytometers´ susceptibility to invertebrate herbivory.

In addition, community biomass will also directly affect the number of insect herbivores present in a community (Sedlacek *et al*. 1988; Kyto *et al*. 1996; Siemann 1998), possibly also leading to increased herbivore damage with increasing community productivity.

In contrast to grasses, the presence of legumes increased herbivory in all community transects. We hypothesize that this positive effect on community herbivory can be explained by (1) higher levels of herbivory in legumes, which are included in the transect estimates, and (2) a fertilizing effect of legume presence on other plant species via the transfer of fixed nitrogen (Spehn *et al*. 2002), making the plant community more attractive to invertebrate herbivores. In the case of phytometers, however, there was no consistent effect of legume presence on herbivory. Herbivory in *T. pratense*, which is itself a legume, was negatively affected by presence of other legumes in the surrounding community. This might, again, be interpreted as an effect of interspecific plant competition or a special dynamic related to nitrogen-assimilation in legume-rich systems.

Regardless of their direction, we are confident that the effects of legumes, grasses or small herbs on herbivory are at least five to ten times greater than any plant species richness effects.

General conclusions

We have shown that invertebrate herbivore damage in experimental plant communities is independent of the number of plant species present in the communities. All ten datasets collected in two growing seasons support this assertion. This finding sheds new light on many aspects of herbivory-diversity studies, highlighting that species richness may be much less important for patterns of invertebrate herbivory than previously thought.

In addition, almost all datasets we analyzed show that herbivore damage depends on the identity of plant functional groups present in the communities. Processes that involve specific interactions between trophic levels therefore seem to be more dependent on species composition than on species richness alone. We conclude that plant species identity and not species diversity is one of the main factors influencing invertebrate herbivory in temperate grassland ecosystems.

Acknowledgements We thank Mick J. Crawley for his generous encouragement and advice in statistical analysis. Winfried Voigt kindly provided data on invertebrate abundances, and the technical assistance of Sylvia Creutzburg and Nadine Mitschunas is greatly acknowledged. We thank Ernst-Detlef Schulze, Christian Körner, and two anonymous referees for many helpful comments on a previous version of this manuscript. This study was supported by the Deutsche Forschungsgemeinschaft through grants WE2618/4-1 and FOR 456 to WWW. CS was supported by the Studienstiftung des Deutschen Volkes. All experiments

conducted comply with current German laws.

References

Andow DA (1990) Population dynamics of an insect herbivore in simple and diverse habitats. Ecology 71:1006–1017

Andow DA (1991) Vegetational diversity and arthropod population response. Ann Rev Entomol 36:561–586

Bahr C (2005) Effects of plant species diversity, identity and architectural density on the density of slugs in an experimental grassland. Diploma Thesis, University of Jena (in German)

Bernays E, Graham M (1988) On the evolution of host specificity in Phytophagous Arthropods. Ecology 69:886–892

Brown VK, Gange AC (1989) Differential effects of above- and below-ground insect herbivory during early plant succession. Oikos 54:67–76

Burnham KP, Anderson DA (1998) Model selection and inference—a practical information—theoretic approach. Springer, Berlin Heidelberg New York

Carson WP, Root RB (1999) Top-down effects of insect herbivores during early sucession: influence on biomass and plant dominance. Oecologia 121:260–272

Crawley MJ (1989) Insect herbivores and plant population dynamics. Ann Rev Entomol 34:531–564

Crawley MJ (2002) Statistical computing. An introduction to data analysis using S-Plus. Wiley, Chichester

Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen, 5 edn. Ulmer, Stuttgart

Finch S, Collier RH (2000) Host-plant selection by insects—a theory based on ‘appropriate/inappropriate landings’ by pest insects of cruciferous plants. Entomol Exp Appl 96:91–102

Gibson DJ (2002) Methods in comparative plant population ecology. Oxford University Press, Oxford

Giller PS, O´Donovan G (2002) Biodiversity and ecosystem function: do species matter? Biology and environment Proc R Ir Acad 102B:129–139

Haddad NM, Tilman D, Haarstad J, Ritchie ME, Knops JMH (2001) Contrasting effects of plant richness and composition on insect communities: a field experiment. Am Nat 158:17–35

Hassell MP (2000) The spatial and temporal dynamics of host-parasitoid interactions. Oxford University Press, Oxford

Hulme PE (1996) Herbivores and the performance of grassland plants: a comparison of arthropod, mollusc and rodent herbivory. J Ecol 84:43–51

Knops JMH, Tilman D, Haddad NM, Naeem S, Mitchell CE, Haarstad J, Ritchie ME, Howe KM, Reich PB, Siemann E, Groth J (1999) Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecol Lett 2:286–293

Koricheva J, Mulder CPH, Schmid B, Joshi J, Huss-Danell K (2000) Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grassland. Oecologia 125:271–282

Kowalski E (2005) Density patterns of invertebrate herbivores (Auchenorrhyncha and Heteroptera) along a diversity gradient in an experimental grassland. Diploma Thesis, University of Jena (in German)

Kubitzky K (ed) (1993) The families and genera of vascular plants. Springer, Berlin Heidelberg New York

Kyto M, Niemela P, Larsson S (1996) Insects on trees: population and individual response to fertilization. Oikos 75:148–159

Mitchell CE (2003) Trophic control of grassland production and biomass by pathogens. Ecol Lett 6:147–155

Mulder CPH, Jumpponen A, Hogberg P, Huss-Danell K (2002) How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. Oecologia 133:412–421

Mulder CPH, Koricheva J, Huss-Danell K, Högberg P, Joshi J (1999) Insects affect relationships between plant species richness and ecosystem processes. Ecol Lett 2:237–246

O'Neal ME, Landis DA, Isaacs R (2002) An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. J Econ Entomol 95:1190–1194

Perner J, Wytrykush C, Kahmen A, Buchmann N, Egerer I, Creutzburg S, Odat N, Audorff V, Weisser WW (2005) Effects of plant diversity, plant productivity and habitat parameters on arthropod abundance in montane European grasslands. Ecography 28:429–442

Pfisterer AB, Diemer M, Schmid B (2003) Dietary shift and lowered biomass gain of a generalist herbivore in species-poor experimental plant communities. Oecologia 135:234–241

Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (Brassica oleracea). Ecol Monogr 43:95–124

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze ED (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic Appl Ecol 5:107–121

Roscher C, Temperton VM, Scherer-Lorenzen M, Schmitz M, Schumacher J, Schmid B, Buchmann N, Weisser WW, Schulze ED (2005) Overyielding in experimental grassland communities—irrespective of species pool or spatial scale. (vol 8, pg 419, 2005). Ecol Lett 8:576–577

Schmid B, Hector A, Huston MA, Inchausti P, Nijs I, Leadley PW, Tilman D (2002) The design and analysis of biodiversity experiments. In: Inchausti P (ed) Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxford University Press, Oxford, pp 61–75

Sedlacek JD, Barrett GW, Shaw DR (1988) Effects of nutrient enrichment on the Auchenorrhyncha (Homoptera) in contrasting grassland communities. J Appl Ecol 25:537–550

Siemann E (1998) Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. Ecology 79:2057–2070

Siemann E, Tilman D, Haarstad J, Ritchie M (1998) Experimental tests of the dependence of arthropod diversity on plant diversity. Am Nat 152:738–750

Spehn EM, Scherer-Lorenzen M, Schmid B, Hector A, Caldeira MC, Dimitrakopoulos PG, Finn JA, Jumpponen A, O'Donnovan G, Pereira JS, Schulze ED, Troumbis AY, Korner C (2002) The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos 98:205–218

Stamp NE, Bowers MD (2000) Foraging behaviour of caterpillars given a choice of plant genotypes in the presence of insect predators. Ecol Entomol 25:486–492

Tahvanainen JO, Root RB (1972) Influence of vegetational diversity on the population ecology of a specialized herbivore, Phyllotreta cruciferae (Coloeoptera: Chrysomelidae). Oecologia 10:321–346

Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E (1997) The influence of functional diversity and composition on ecosystem processes. Science 277:1300–1305

Tonhasca A, Byrne DN (1994) The effects of crop diversification on herbivorous insects: A meta-analysis approach. Ecol Entomol 19:239–244

Tscharntke T, Greiler H-J (1995) Insect communities, grasses, and grasslands. Ann Rev Entomol 40:535–558

Unsicker S, Renker C, Kahmen A, Spindler S, Buchmann N, Weisser WW (2005) Testing the efficiency of three 15N-labeled nitrogen compounds for indirect labeling of grasshoppers via plants in the field. Entomol Exp Appl 116 (in press)

Voigt W, Perner J, Davis AJ, Eggers T, Schumacher J, Bahrmann R, Fabian B, Heinrich W, Kohler G, Lichter D, Marstaller R, Sander FW (2003) Trophic levels are differentially sensitive to climate. Ecology 84:2444–2453

Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, de Lucia EH (2002) Impact of folivory on photosynthesis is greater than the sum of its holes. PNAS 99:1088–1091

Journal of Ecology, Volume 94, Issue 5, Page 922-931 September 2006

Chapter 4

The effects of plant diversity and insect herbivory on performance of individual plant species in experimental grassland

Christoph Scherber, Alexandru Milcu*†, Stephan Partsch*, Stefan Scheu* and Wolfgang W. Weisser

*Institute of Ecology, University of Jena, Dornburger Str. 159, D-07743 Jena, Germany, * Institute of Zoology, Technische Universität Darmstadt, Schnittspahnstr. 3, D-64287 Darmstadt, Germany, and † NERC Centre for Population Biology, Division of Biology, Imperial College, Silwood Park, Ascot SL5 7PY, UK*

Summary

1 There is increasing evidence that components of biodiversity affect processes at the ecosystem level; yet, the effects of biodiversity on the performance of individual organisms or particular trophic interactions are largely unexplored.

2 We transplanted 10 individuals of *Rumex acetosa* into 82 experimental grassland plots differing in plant species and functional group richness. Half of the plants received an insecticide treatment to manipulate insect herbivory.

3 We measured the amount of herbivory, plant size, survival and reproductive parameters in 2003 and 2004.

4 Insect herbivores removed on average 4.3% (2003) and 5.1% (2004) of leaf area in unsprayed plants. Spraying significantly reduced damage levels on average by approximately 50%. Herbivory significantly decreased plant weight, leaf size and number, and inflorescence length and size.

5 Plant height and inflorescence size of *R. acetosa* significantly decreased with an increase in species diversity. Mortality was slightly higher in the species-poor mixtures. Plant functional group diversity had little effect on plant performance. The presence of legumes generally increased, while the presence of grasses generally decreased, morphological parameters and fitness in *R. acetosa*.

6 Overall, the presence of particular plant functional groups was more important than functional group or species richness *per se*, and insect herbivores had additive effects of the same magnitude as the presence of particular plant functional groups.

7 Insect herbivory and plant functional identity, rather than species richness, determine the performance of individual plant species in temperate grasslands.

Key-words: biodiversity, ecosystem functioning, mixed effects models, multitrophic interactions

Introduction

Changes in the biodiversity of an ecosystem can affect ecosystem properties. If components of biodiversity are lost, the overall state and functioning of the system can be impaired (Loreau *et al.* 2001; Tilman *et al.* 2001). For a number of experimental ecosystems, for example artificially assembled plant communities, there is now a wealth of studies showing that decreasing biodiversity can have negative effects on processes measured at the ecosystem level (Hooper *et al.* 2005; Spehn *et al.* 2005). In contrast, the effects of biodiversity on the performance of individual organisms or particular trophic interactions are largely unexplored. The study presented here tries to fill this gap, focusing on the performance of one particular plant species in communities differing in plant species and functional richness, combined with an experimental manipulation of insect herbivory.

While previous studies have mainly concentrated on the relationship between plant diversity and diversity of herbivorous insects (Siemann 1998; Haddad *et al.* 2001), there is only limited knowledge on the extent of herbivory and its feedback on the performance of individual plant species within a diversity gradient. Even those studies that report significant relationships between plant species richness and insect herbivory have come to opposing conclusions: while Root's classic study on herbivore load in *Brassica oleracea* L. (Root 1973) has resulted in the formulation of a 'resource concentration' hypothesis, more recent results obtained by Otway *et al.* (2005) have been interpreted as showing 'resource dilution'. This, in turn, would mean that 'host plants in high diversity mixtures' should experience 'greater herbivore pressure' (Otway *et al.* 2005).

In this study, we use experimental grassland communities (described in detail in Roscher *et al.* 2004) differing in the number of plant species (1–60) and the number and identity of functional groups (one to four functional groups: grasses, legumes, small herbs, tall herbs). We introduce a given plant species into the experimental mixtures, at a density of five individuals per subplot ('phytometer' approach *sensu* Gibson 2002). Phytometer performance measures both direct and indirect effects of the resident plant and insect herbivore communities. Disentangling these interacting processes is the major aim of the experiments reported here.

Rumex acetosa L., a north-temperate member of the Polygonaceae, was selected as a phytometer species, because: (i) its insect herbivore fauna is well-known; (ii) leaf morphology allows quick and easy determinations of foliar herbivory; and (iii) it is present in the species pool of the experimental mixtures. We address three main questions. (i) How do plant size, survival and reproduction of *R. acetosa* change with plant species richness, number of functional groups, and presence of particular functional groups? (ii) What is the effect of insect herbivory on plant size, survival and reproduction of *R. acetosa*? (iii) What is the relationship between insect herbivory and plant diversity in *R. acetosa*? We hypothesize that: (i) plant functional identity will be more important for performance of *R. acetosa* than species richness *per se* (Scherber *et al.* 2006); and (ii) insect herbivory will increase with plant species richness (Otway *et al.* 2005).

Materials and methods

Study site and general experimental design

Plant communities of increasing species richness (1, 2, 4, 8, 16, 60 species) were established on former arable land near Jena (Germany) in 2002 from a pool of 60 grassland plant species. Plant species were divided *a priori* into four functional groups (grasses, legumes, small and tall herbs; Roscher *et al.* 2004). The overall design is a randomized complete blocks design with 82 plots each 20 × 20 m in size, systematically divided into four blocks (see Roscher *et al.* 2004 for details), and containing all possible combinations of (number of plant species) × (number of functional groups). Due to non-orthogonality in the design, the correlation coefficients for log-species richness and number of functional groups, legumes and grasses were 0.63, 0.35 and 0.38, respectively. Plots are continuously weeded to maintain the target communities.

Study species

Rumex acetosa is a dioecious perennial with a rosette growth habit, racemose flowers and hastate leaves that have characteristically extended basal lobes and long petioles (Clapham *et al.* 1987). Several monophagous beetles of the families Apionidae and Curculionidae have been described for *R. acetosa* (Böhme 2001). There is extensive literature on the effects of selective herbivory, plant competition and fungal infection on other (mostly weedy) *Rumex* species (e.g. Hatcher *et al.* 1994; Keary & Hatcher 2004). In contrast, *R. acetosa* has been studied in much less detail so far.

Transplantation of *R. acetosa* phytometers into diversity plots

Rumex acetosa seeds were obtained from Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany. In early April 2003, *R. acetosa* seeds were pre-germinated on standard compost, and grown on a standard compost: perlite mixture (4:1) in a glasshouse with night:day cycles of 10:14 hours (15:22 °C). Prior to final transplantation, all plants were sorted into three size classes and hardened for 7 days. In mid-June 2003, $n = 820$ plants were randomly selected and transplanted into the diversity plots. Each main plot was divided into two subplots (split-plot design), whose positions were randomized along the north-south axis of each main plot. Each of these two subplots received one row of plants, containing one large, two medium and two small individuals, 50 cm apart. While one subplot (2 × 4 m) served as a control, another subplot was used for insect exclusion ('insecticide') and covered a larger area (5 × 5 m) to allow for efficient insect herbivore exclusion. The initial sizes (as determined by leaf number) of all plants were recorded and included as covariates into all analyses (Crawley 2002).

Insecticide application

The 'insecticide' subplots on all 82 large plots were sprayed with an aqueous solution (30 mL m^2) of an above-ground, semi-systemic organothiophosphate insecticide (Dimethoate, $C_5H_{12}NO_3PS_2$, BASF, Ludwigshafen, Germany) at 4-weekly intervals between April and August 2003 and 2004. Dimethoate has been shown to be both effective in reducing insect herbivory and having little direct effects on plants (Hector *et al.* 2004; Schädler *et al.* 2004). Subplots were sprayed using a backpack sprayer (Birchmeier Senior 20 L) at 6×10^5 Pa operating pressure. As every *R. acetosa* individual received only about 2 mL of solution per month, we decided to leave the plants on the 'control' subplots unsprayed (as, for example, in Keary & Hatcher 2004). To assess the effectiveness of insecticide treatments, we repeatedly quantified insect herbivory (as suggested by Siemann *et al.* 2004).

Measurements in 2003

Initial size (number of fully unfolded leaf laminas) of *R. acetosa* plants was determined 2 weeks after transplanting on 9 July 2003. All other measurements were performed between 7 and 18 August 2003. For each *R. acetosa* plant, we measured: (i) L, the absolute leaf area damaged by insect herbivores per plant (in mm^2); (ii) S_S, S_L, the sizes of the smallest and largest leaves (in cm^2); (iii) T, the total number of leaves; and (iv) plant dry weight (in g). The sizes of the smallest and largest leaves were estimated by comparing leaves with a set of standard ellipsoid paper templates of known area. Absolute leaf area damaged by insect herbivores was estimated using a 1-mm^2 grid. Leaves clipped by vertebrate herbivores or severely damaged by molluscs were excluded. Percentage herbivory, p, was calculated, after adjustment of units, as

$$p = \frac{2\mathrm{L}}{\mathrm{T}(\mathrm{S_S} + \mathrm{S_L})} \times 100\ [\%] \qquad \text{eqn 1}$$

Plants were harvested 3 cm above soil surface between 1 and 3 September 2003, oven-dried at 70 °C for 48 hours and weighed. Community leaf area index (LAI) per subplot was measured between 12 and 13 August 2004, using a LAI-2000 Plant Canopy Analyser (Li-Cor BioSciences, Lincoln, USA). Each LAI measurement consisted of a reference value taken above the canopy, and five measurements of light interception 5 cm above soil surface. Measurements were not adjusted for leaf angles.

Measurements in 2004

Between 5 and 7 April 2004, we counted the number of surviving individuals since transplantation. Reproductive parameters were only measured in 2004, as plants did not flower in 2003. We use morphological parameters of the inflorescences as a measure of potential plant fitness (Conn & Blum 1981). Morphological measurements in *R. acetosa* were performed between 17 and 18 May 2004. For each plant individual, we measured: (i) the absolute leaf area damaged by insect herbivores on a randomly chosen leaf; (ii) the size of that leaf; (iii) the maximum length of the main inflorescence axis using a metering rule; (iv) the number of first-order branches on the inflorescence axis; and (v) the number of main inflorescence axes. For herbivory and leaf area measurements, one leaf per plant was selected by hypothetically constructing a cylinder around each plant and dividing it into eight cylinder sections, of which we selected one at random and picked one leaf from it. Total and damaged leaf areas were measured to the nearest mm^2 using clear plastic sheets with a mm^2 grid. Leaves clipped by vertebrate herbivores or severely damaged by molluscs were excluded. Percentage herbivory was calculated as damaged area divided by total area, multiplied by 100. Community biomass was harvested between 27 May and 10 June 2004 in two randomly placed 20 × 50 cm quadrates per subplot at 3 cm above ground, oven-dried at 70 °C for 48 hours and weighed. In addition, we measured relative cover of every plant species for all mixtures, using an integer cover degree scale with two independent observers.

Statistical analysis

We used linear mixed-effects analysis of covariance models (Pinheiro & Bates 2000; Crawley 2002) implemented in S-Plus 6.1.2 Professional for Windows (Copyright 2002, Insightful Corp., Seattle, USA) for all analyses. Proportion and mortality data were arcsine-square root transformed and count data were square-root or log-transformed to account for heteroscedasticity and non-normality of errors. Standard errors for geometric means and for means calculated from square-root transformed data were derived using bootstrap resampling with 1000 replications. Because of intrinsic aliasing in the design (*sensu* McCullagh & Nelder 1989), construction of the full model follows two main principles: (i) we fit covariates first; and (ii) the sequence of terms directly relates to the scientific hypotheses of interest. As insecticide treatments were applied to subplots within plots, models contained random effects at two levels. Blocks were entered as a fixed rather than random effect (a view that is supported, for example, by Piepho *et al.* 2004), because: (i) functional group treatments were unequally represented within blocks; and (ii) block positions were not randomized, and blocks cannot be considered random samples from an infinite population. The fixed-effects structure of the maximal model fit by maximum likelihood was:

[y initial number of leaves + block + community biomass + insecticide + number of functional groups + $\log_2$ (sown number of plant species + 1) + grass presence + legume presence + insecticide:initial number of leaves + insecticide: block + insecticide:log species richness + number of functional groups:log species richness + grass presence:legume presence], where ':' indicates interactions (Chambers & Hastie 1992).

For 2003, we used community leaf area index instead of community biomass. The significance of terms was assessed using conditional *F*-tests. Variance functions were used to model heteroscedasticity in the within-group errors (Pinheiro & Bates 2000). We simplified the maximal model by sequentially deleting non-significant terms (starting with highest-order interactions) and comparing each model with its predecessor using Akaike information criterion (AIC, Burnham & Anderson 1998) and likelihood ratio tests, until minimal adequate models were retrieved. For graphical representation of data, we use trellis displays (Becker & Cleveland 1996) of the Lattice graphics package (version 0.12–9) in R 2.2.0 (R Development Core Team, 2005). Observations are divided into distinct groups according to several grouping factors.

Table 1 Summary of linear mixed-effects models for August 2003 data. For each of the six response variables (columns), the details of the minimal adequate models are listed in the rows, with explanatory variables (1st column) retained in the models, their corresponding F- and P-values, denominator degrees of freedom, plus additional information (bottom rows). For the purpose of clarity, explanatory variables are ordered (i) by their denominator d.f. and (ii) by the sequence in which they were entered into the maximal models. Bold font indicates significant P-values

August 2003			Percentage herbivory[a]		Number of leaves[b]		Plant dry weight (g)[b]	
Source	Numerator d.f.	Denominator d.f.	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Intercept	1	Plot	355.345	**<.0001**	3962.650	**<.0001**	319.9612	**<.0001**
Block	3	Plot	Excluded	Excluded	2.600	0.0586	Excluded	Excluded
Number of plant species (S)	1	Plot	4.8713	**0.0302**	Excluded	Excluded	Excluded	Excluded
Grasses	1	Plot	5.6628	**0.0197**	2.915	0.0920	4.8652	**0.0303**
Legumes	1	Plot	Excluded	Excluded	7.093	**0.0095**	5.8568	**0.0178**
Grasses:Legumes	1	Plot	Excluded	Excluded	3.404	0.0691	Excluded	Excluded
Initial number of leaves	1	Subplot	Excluded	Excluded	31.425	**<.0001**	22.1559	**<.0001**
Insecticide Treatment	1	Subplot	8.572	**0.0044**	21.082	**<.0001**	5.1809	**0.0259**
Community leaf area index	1	Subplot	Excluded	Excluded	1.645	0.2035	Excluded	Excluded
Number of observations			164		160		154	
Number of groups (plots)			82		80		81	
Number of d.f. used up in minimal model			4		10		5	
Denominator d.f. (plot level)			81		77		78	
Denominator d.f. (subplot level)			79		73		71	
AIC of maximal model			-258.5666		121.0696		116.7885	
AIC of minimal model			-290.4181		105.5165		97.03667	

Annotations: [a]arcsine-square root, [b]log transformed; d.f., degrees of freedom; AIC, Akaike information criterion; ´excluded´ indicates terms excluded during model simplification.

Results

The results of the statistical analyses are summarized in Tables 1 (2003) and 2 (2004). These tables also list non-significant terms, which were retained in the minimal adequate models during model simplification.

Insect herbivory

In 2003, natural levels of herbivory in *R. acetosa* averaged 4.3 ± 0.7%. Insecticide treatment significantly reduced herbivory to 2.4 ± 0.3% (Table 1). Herbivory decreased significantly with increasing plant species richness (Table 1). Figure 1(a) shows that insecticide treatment partly decoupled the diversity-herbivory relationship, but this interaction was not significant. In addition, herbivory was significantly higher when grasses were present in the communities, than when grasses were absent (3.8 ± 0.6% vs. 2.9 ± 0.5%; Table 1).

Average herbivory levels in 2004 were slightly higher than in 2003 (5.1 ± 0.5%). With increasing initial number of leaves (2003), herbivory in 2004 increased significantly (Table 2). Insecticide-treated plants showed significantly lower herbivore damage (Table 2), while plant species richness did not have an effect in 2004 (Fig. 1b). Both the block effect, and the interaction between number of functional groups and plant species richness were significant (Table 2). In contrast to 2003, the presence of grasses or legumes did not have a significant effect on herbivory. To test whether *R. acetosa* density in the surrounding community had an effect on herbivory in the transplanted *R. acetosa* individuals, we plotted phytometer herbivory against the relative cover (range: 0–20%) of *R. acetosa*. Herbivory was fully independent of *R. acetosa* cover (linear regression; intercept 4.2 ± 0.36%, slope – 0.05 ± 0.10, overall $P = 0.63$, $r^2 = 0.001$).

Number of leaves

In 2003, plants had on average 5.3 ± 0.2 leaves. Initial number of leaves, fitted as a covariate, had a highly significant effect on leaf number in August 2003 (Table 1). Plants treated with insecticide had significantly more leaves than control plants (5.5 ± 0.3 vs. 5.1 ± 0.3, Table 1). Plants had significantly fewer leaves in the presence rather than in the absence of grass species (6.1 ± 0.4 vs. 4.6 ± 0.2, Table 1), while the presence of legumes always increased leaf number (6 ± 0.4 vs. 4.5 ± 0.2, Table 1).

Plant dry weight

In 2003, plant weight was on average 0.95 ± 0.09 g, and increased significantly with increasing initial number of leaves (Table 1). Insecticide-treated plants had a significantly higher dry weight than control plants (0.98 ± 0.12 g vs. 0.93 ± 0.14 g, Table 1). When legumes were present in the communities, plants weighed significantly more than in the absence of legumes; grasses had the opposite effect (Table 1, Fig. 2a).

Plant mortality

Approximately 10 months after transplantation, there were 190 dead individuals out of the 820 original transplants (= 23.2%). Mortality across plots followed a negative exponential distribution, with in the majority of cases ($n = 52$) 0–1 dead plants per plot. There was a significantly higher survival in plants with a higher initial leaf number in comparison with plants with a small initial size (Table 2). Plant mortality decreased significantly and linearly with increasing plant species richness, while legume presence generally increased mortality (Table 2).

Figure 3 gives an overview of the combined effects of legumes and plant species richness.

Plant height

In 2004, the maximum length of the main inflorescence axes was on average 57.0 ± 2.1 cm. Plants with a higher initial size produced significantly longer main inflorescence axes than plants with a smaller initial size (Table 2). Insecticide treatment also had a significant positive effect on axis length, leading to an increase from 54.9 ± 2.1 cm to 59.0 ± 2.1 cm. Presence of grasses significantly decreased plant height, while the presence of legumes had a significant positive effect (Table 2). Figure 2(b) shows an interaction plot with both legume and grass effects on plant height.

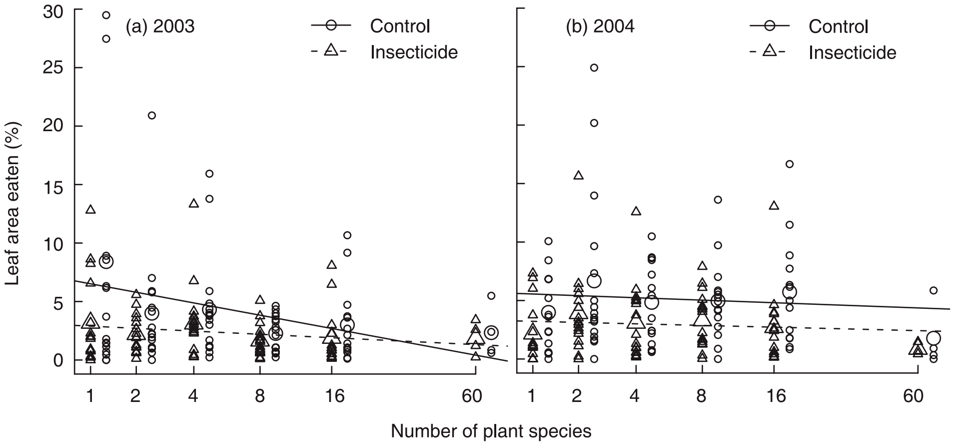


Fig. 1 Relationship between number of plant species and leaf area eaten (%) in *R. acetosa* (A) 2003 and (B) 2004. Open circles and solid lines: control plants; open triangles and dashed lines: insecticide-treated plants. Lines show mean squares fits. r^2 values for 2003 are 0.017 (insecticide) and 0.024 (control); r^2 values for 2004 are 0.001 (insecticide) and 0.008 (control). Larger symbols show means.

Table 2 Summary of linear mixed-effects models for May 2004 data. For each of the six response variables (columns), the details of the minimal adequate models are listed in the rows, with explanatory variables (first column) retained in the models, their corresponding *F*- and *P*-values, denominator degrees of freedom, plus additional information (bottom rows). For the purpose of clarity, explanatory variables are ordered (i) by their denominator d.f. and (ii) by the sequence in which they were entered into the maximal models. Bold font indicates significant *P*-values.

May 2004			Percentage mortality[a]		Percentage herbivory[a]		Plant height (cm)		Number of branches[b]		Number of main axes[c]	
Source	Numerator d.f.	Denominator d.f.	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Intercept	1	Plot	119.2787	**<.0001**	523.2211	**<.0001**	719.8119	**<.0001**	630.9144	**<.0001**	1201.156	**<.0001**
Block	3	Plot	Excluded	Excluded	3.1178	**0.0310**	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Number of FG	1	Plot	Excluded	Excluded	0.8239	0.3670	Excluded	Excluded	0.9978	0.3211	5.420	**0.0226**
Species Richness (S)	1	Plot	6.4580	**0.0130**	1.5800	0.2127	2.7288	0.1027	9.3103	**0.0032**	8.281	**0.0052**
Grasses	1	Plot	Excluded	Excluded	Excluded	Excluded	14.4954	**0.0003**	38.5587	**<.0001**	32.208	**<.0001**
Legumes	1	Plot	4.3943	**0.0393**	Excluded	Excluded	25.2185	**<.0001**	22.6474	**<.0001**	23.341	**<.0001**
Number of FG:S	1	Plot	Excluded	Excluded	4.8256	**0.0311**	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Initial number of leaves	1	Subplot	5.9899	**0.0166**	4.6302	**0.0346**	7.2747	**0.0087**	14.9495	**0.0002**	24.673	**<.0001**
Biomass (gm^{-2})	1	Subplot	Excluded	Excluded	Excluded	n.s.	0.8387	0.3628	0.2253	0.6365	1.915	0.1708
Insecticide treatment	1	Subplot	Excluded	Excluded	9.0667	**0.0035**	17.5489	**0.0001**	21.7716	**<.0001**	19.080	**<.0001**
Number of observations			164		159		155		154		154	
Number of groups (plots)			82		82		80		80		80	
Number of d.f. used up in minimal model			5		9		7		8		8	
Denominator d.f. (plot level)			80		75		76		75		75	
Denominator d.f. (subplot level)			79		75		72		71		71	
AIC of maximal model			-0.0167		-234.6253		1417.33		414.926		148.9746	
AIC of minimal model			-4.9059		-274.8168		1391.193		395.9124		128.9068	

Annotations: [a]arcsine-square root, [b]log, [c]square-root transformed; FG functional groups; d.f., degrees of freedom; AIC, Akaike information criterion; ´excluded´ indicates terms excluded during model simplification.

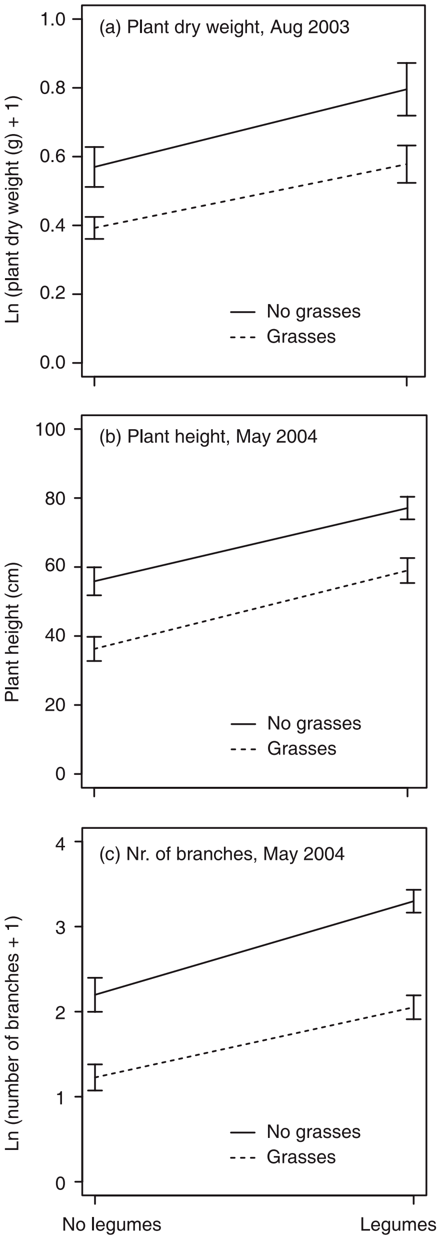


Fig. 2 Effects of particular functional groups on (A) plant dry weight, (B) maximum inflorescence length and (C) number of first-order inflorescence branches in *R. acetosa*. Solid (dashed) lines indicate plots without (with) grasses. Lines connect means ± 1 s.e.

Number of first-order branches on inflorescence axis

Rumex acetosa inflorescences had on average 17.4 ± 2.4 first-order branches. Plants with a greater initial size had more branches than those with a smaller initial size (Table 2). Insecticide-treated plants had significantly more first-order branches (17.5 ± 2.2) than control plants (17.4 ± 4.3); see Table 2. We found a significant effect of plant species richness: with increasing number of plant species, the number of first-order branches significantly decreased (Table 2). When grasses were present, the number of branches significantly decreased, while legume presence generally increased branch number (Table 2). These combined effects of legumes and grasses are presented in Fig. 2(c).

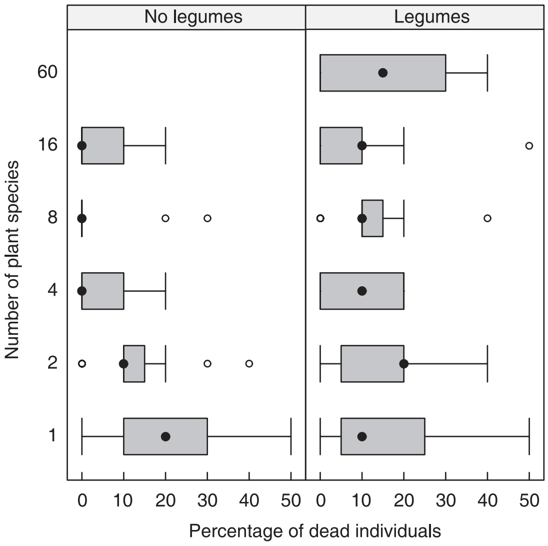


Fig. 3 Effects of plant species richness and legume presence on mortality in *R. acetosa*. Boxes represent lower and upper quartiles; black dots indicate the median. Whiskers indicate observations within 1.5 times the interquartile range from the top (bottom) of the boxes. Open circles show outliers.

Number of main inflorescence axes

Plants had on average 1.8 ± 0.2 main inflorescence axes. The initial number of leaves in 2003 had a significant positive effect on the number of main axes (Table 2). Insecticide-treated plants had significantly more main axes than control plants (Table 2). The number of main axes was significantly reduced in communities that contained more functional groups, or more plant species (Table 2), and there was a significant interaction between these two terms. Plants growing in communities that contained grasses had significantly fewer main axes, while legume presence generally increased the number of main axes (Table 2). Figure 4 shows the combined effects of legumes, grasses, number of plant species, and insecticide treatment, on the number of main axes. It can be clearly seen that there are no significant interactions, and that legume presence and insecticide treatments generally lead to a parallel shift in the regression lines.

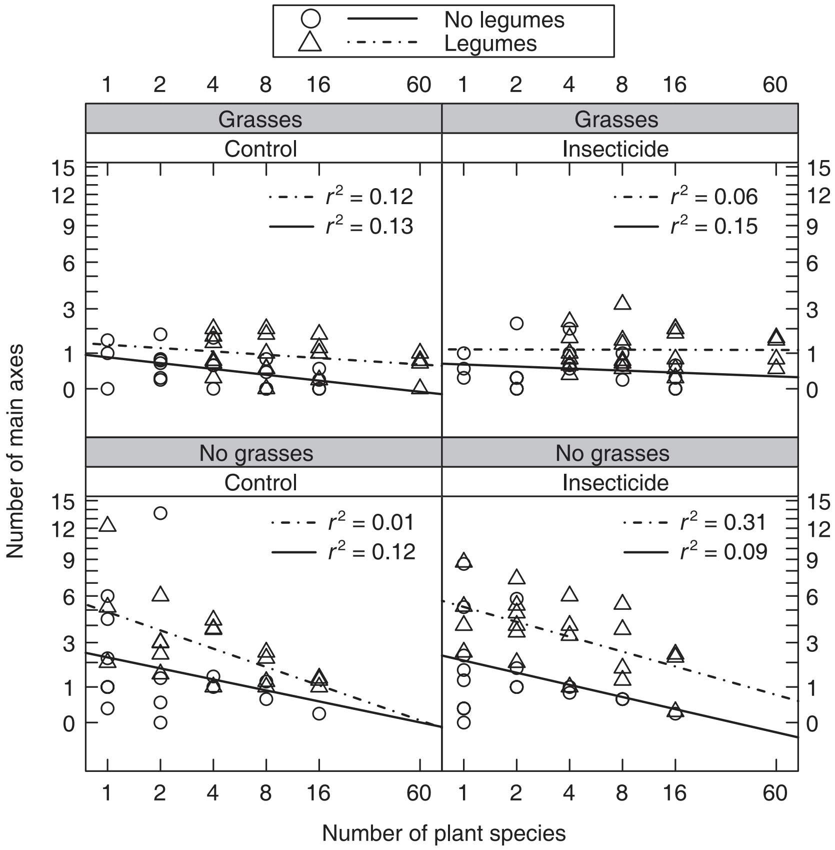


Fig. 4 Relationships between plant species richness (x-axis) and number of main inflorescence axes (y-axis: square root scale) in *R. acetosa.* Open circles and solid lines show plots without legumes; open triangles and dashed lines show plots with legumes. Lines show mean squares fits; r² values are for these mean squares fits only.

Discussion

The results presented in this manuscript clearly demonstrate that different components of biodiversity are of different importance for the overall performance of individual plant species. While species richness *per se* has only weak effects on herbivory, reproductive traits and the survival of *R. acetosa*, plant functional identity affects herbivory, mortality, and all the morphological traits measured in this study. Insect herbivory, in general, acts as an additional factor, independent of diversity effects. Of course, these findings are specific to the system we studied, but we nevertheless believe that generalizations are possible and necessary, not least because we think that these results can be seen as a step towards a more mechanistic understanding of processes acting in grassland ecosystems of differing diversities.

Previous experiments have mainly focused on the community- and ecosystem-level consequences of biodiversity decline (reviewed in Hooper *et al.* 2005). For example, several studies have focused on the relationship between plant and arthropod diversity in experimental grasslands (Knops *et al.* 1999; Koricheva *et al.* 2000; Haddad *et al.* 2001). As part of the BIODEPTH biodiversity experiment, Mulder *et al.* (1999) published one of the first studies on the relationship between insect herbivory and plant species diversity, combined with an insecticide treatment. However, their study focused on community herbivory, and the BIODEPTH experimental design does not allow a full separation of legume and grass effects from other components of biodiversity, because grasses were present in all multispecies mixtures, and plots with 100% legumes were restricted to the one- and two-species mixtures. Giller & O'Donovan (2002) demonstrated single-species herbivory (*Trifolium pratense* L) in relation to plant species richness from a study conducted at the Irish BIODEPTH site. Recently, Otway *et al.* (2005) published a detailed analysis of insect herbivore abundance at the Silwood Park BIODEPTH site, demonstrating that insect herbivore damage (if it translated linearly from insect herbivore load) in several plant species, including *R. acetosa*, might be negatively related to plant species richness.

The study presented here tries to extend the work from previous studies, using a design that enables a separation of the effects of species richness from the effects of plant functional diversity, and from plant functional identity, combined with a manipulation of insect herbivory.

Insect herbivory

The rates of insect herbivory reported are consistent with the literature. Carson & Root (1999) found a rate of 0.9 vs. 0.7% leaf area damage in control vs. insecticide-treated plants in a closely related *Rumex* species. According to Scheidel & Bruelheide (1999), other invertebrate herbivores, in particular molluscs, seem to avoid *R. acetosa* in free-choice feeding trials with other grassland plants. We excluded leaves damaged by molluscs, but these were only encountered very infrequently. It is also important to note that we tested for herbivory effects in established plants, as seedlings may be more severely affected (e.g. Keary & Hatcher 2004). Our insecticide treatment did not have a significant effect on plant mortality; this finding is not surprising, as insect herbivores only rarely kill their hosts (Crawley 1983; Crawley 1997). The visible damage recorded did translate into morphological differences between sprayed and unsprayed plants. The reduction of insect herbivory, using Dimethoate, increased plant dry weight, leaf number, inflorescence length and the number of inflorescence main axes and branches. As plant dry weight and inflorescence parameters are directly correlated with potential fitness (Ainsworth *et al.* 2005), we deduce that potential fitness of a transplant phytometer, *R. acetosa*, is significantly negatively affected by insect herbivory. This view is also supported by a study on the biological control of *R. obtusifolius* L. (Grossrieder & Keary 2004); these authors have found that leaf beetle grazing can lead to fewer and lighter seeds, i.e. the potential fitness of a *Rumex* species can be significantly altered by insect herbivores. Notably, insect herbivore effects were independent of plant diversity. Herbivory acted in an additive way, without an indication of either resource concentration (Root 1973) or resource dilution (Otway *et al.* 2005) effects. The negative trend in the 2003 herbivory data set is mainly caused by two monoculture outliers. Two notes on testing of the above-mentioned hypotheses need to be made. First, neither of the two hypotheses has been formulated in a way that allows a test by experimentation; and secondly, our experimental design does not allow a deliberate manipulation of *R. acetosa* density. Instead, our experiment tests whether diversity *per se* changes insect herbivory at the level of a single plant species, independent of whether taxonomically related individuals co-occur in the communities. Interestingly, even when we incorporated *R. acetosa* cover into our analyses, phytometer herbivory proved fully independent of host plant density. Thus, we conclude that there is no indication of either diversity or host plant density effects on herbivory in our data sets.

Species richness effects

Before testing for species richness and other components of plant diversity, community biomass or leaf area index was fitted as a covariate in our models, because all the additional variation that could then be attributed to plant diversity was corrected for pure biomass (or LAI) effects (sequential fitting of terms, *sensu* Schmid *et al.* 2002). For the same reason, plant species richness was always fitted following the number of functional groups. Our data suggest moderate but significant influences of plant species richness on mortality and reproductive traits in *R. acetosa*. While survival was generally greater in species-rich mixtures, the number and size of inflorescences generally decreased. We emphasize, however, that this result should not be interpreted as 'invasion resistance' (cf. Diemer & Schmid 2001). Rather, we conclude that because *R. acetosa* is a subdominant component of *Arrhenatherum* grasslands, it generally decreases its resource allocation to reproductive tissues when growing in mixtures of increasing plant species richness. This interpretation is in accordance with recent analyses from the Cedar Creek biodiversity experiment, where Lambers *et al.* (2004) found several forb species with yield exponents smaller than −1, indicating an overall negative effect of plant species richness on individual plant species' performance. In addition, a work published by van Ruijven & Berendse (2003) showed similar effects. However, in this study, the overall negative effect of increasing plant diversity was small in terms of its effect and was outperformed by the effects of particular plant functional groups (see below), a finding that has recently been reported at the community level (Scherber *et al.* 2006).

The role of plant functional identity

In our opinion, plant functional identity is one of the most important factors influencing morphological parameters and reproductive traits in *R. acetosa* phytometers. Mortality, inflorescence length, number of first-order branches, number of main axes, herbivory, number of leaves, and plant dry weight, were all highly significantly modulated by the presence or absence of particular functional groups of plants in the communities. While the presence of grasses generally had adverse effects on most morphological parameters, presence of legumes enhanced the plant size of *R. acetosa* in almost every case. Many recently published studies present similar findings, highlighting the overall importance of plant functional identity for the performance of individual species and even whole plant communities (Mulder *et al.* 2002; Spehn *et al.* 2002; Lambers *et al.* 2004; Petchey 2004; Scherber *et al.* 2006), with mostly negative effects as a result of the presence of grass species, and positive effects as a result of legume presence, on the performance of individual plant species.

Conclusions

The results presented here clearly indicate that size, survival and reproduction of an individual plant species are significantly influenced by: (i) the functional identity (rather than diversity) of other plant species in the surrounding plant community; and (ii) the degree of insect herbivory. Increasing plant species and functional diversity leads to enhanced survival, but decreased potential fecundity. Grass presence decreases individual plant performance, while legume presence has the opposite effect. Reduction of insect herbivory results in enhanced growth and potential fecundity of individual plant species. Insect herbivore effects are additive and independent of plant diversity. Extrapolating from species-specific results to whole communities with multi-species interactions is a major challenge for future studies on insect herbivory and plant diversity. We hope that these results can help to further elucidate some of the responsible mechanisms.

Acknowledgements

We thank Mick J. Crawley for his generous encouragement and advice on statistical analysis. We thank Sylvia Creutzburg, Jule Specht, Nadine Mitschunas, Peter N. Mwangi and Sybille B. Unsicker for their enthusiastic support at various stages of the experiments. The comments of two anonymous referees, Lindsay Haddon, Meran Owen, Jens Schumacher, Bernhard Schmid and Christiane Roscher on earlier versions of this manuscript have been very helpful. This study was supported by the Deutsche Forschungsgemeinschaft through grants WE2618/4–1 and FOR 456 to WWW. CS was supported by the Studienstiftung des Deutschen Volkes. All experiments conducted comply with current German laws.

References

Ainsworth, C., Rahman, A., Parker, J. & Edwards, G. (2005) Intersex inflorescences of *Rumex acetosa* demonstrate that sex determination is unique to each flower. *New Phytologist*, **165**, 711–720.

Becker, R.A. & Cleveland, W.S. (1996) The design and control of trellis display. *Journal of Computational and Statistical Graphics*, **5**, 123–155.

Böhme, J. (2001) *Phytophage Käfer und ihre Wirtspflanzen in Mitteleuropa*. Bioform, Heroldsberg, Germany.

Burnham, K.P. & Anderson, D.A. (1998) *Model Selection and Inference: a Practical Information-Theoretic Approach*. Springer, Berlin.

Carson, W.P. & Root, R.B. (1999) Top-down effects of insect herbivores during early sucession: influence on biomass and plant dominance. *Oecologia*, **121**, 260–272.

Chambers, J.M. & Hastie, T.J. (1992) *Statistical Models in S*. AT & T Bell Laboratories, Wadsworth & Brooks/Cole Advanced Books & Software, Pacific Grove, California.

Clapham, A.R., Tutin, T.G. & Moore, D.M. (1987) *Flora of the British Isles*, 3rd edn. Cambridge University Press, Cambridge.

Conn, J.S. & Blum, U. (1981) Differentiation between the sexes of *Rumex hastatulus* in net energy allocation, flowering and height. *Bulletin of the Torrey Botanical Club*, **108**, 446–455.

Crawley, M.J. (1983) *Herbivory*. Blackwell Scientific, Oxford.

Crawley, M.J. (1997) Plant-herbivore dynamics. *Plant Ecology* (ed. M.J. Crawley), pp. 401–474. Blackwell Science, Oxford.

Crawley, M.J. (2002) *Statistical Computing. An Introduction to Data Analysis Using S-Plus*. John Wiley & Sons, Chichester.

Diemer, M. & Schmid, B. (2001) Effects of biodiversity loss and disturbance on the survival and performance of two *Ranunculus* species with differing clonal architectures. *Ecography*, **24**, 59–67.

Gibson, D.J. (2002) *Methods in Comparative Plant Population Ecology*. Oxford University Press, Oxford.

Giller, P.S. & O'Donovan, G. (2002) Biodiversity and ecosystem function: do species matter? *Biology and Environment: Proceedings of the Royal Irish Academy*, **102B**, 129–139.

Grossrieder, M. & Keary, I.P. (2004) The potential for the biological control of *Rumex obtusifolius* and *Rumex crispus* using insects in organic farming, with particular reference to Switzerland. *Biocontrol News and Information*, **25**, 65N–79N.

Haddad, N.M., Tilman, D., Haarstad, J., Ritchie, M.E. & Knops, J.M.H. (2001) Contrasting effects of plant richness and composition on insect communities: a field experiment. *American Naturalist*, **158**, 17–35.

Hatcher, P.E., Paul, N.D., Ayres, P.G. & Whittaker, J.B. (1994) The effect of an insect herbivore and a rust fungus individually, and combined in sequence, on the growth of two *Rumex* species. *New Phytologist*, **128**, 71–78.

Hector, A., Wilby, A., Latsch, O.G. & Brown, V.K. (2004) Phyto-activity of biocides used to manipulate herbivory: tests of three pesticides on fourteen plant species. *Basic and Applied Ecology*, **5**, 313–320.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Keary, I.P. & Hatcher, P.E. (2004) Combining competition from *Lolium perenne* and an insect-fungus combination to control *Rumex obtusifolius* seedlings. *Weed Research*, **44**, 33–41.

Knops, J.M.H., Tilman, D., Haddad, N.M., Naeem, S., Mitchell, C.E., Haarstad, J. *et al.* (1999) Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters*, **2**, 286–293.

Koricheva, J., Mulder, C.P.H., Schmid, B., Joshi, J. & Huss-Danell, K. (2000) Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grassland. *Oecologia*, **125**, 271–282.

Lambers, J.H.R., Harpole, W.S., Tilman, D., Knops, J. & Reich, P.B. (2004) Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. *Ecology Letters*, **7**, 661–668.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A. *et al.* (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, **294**, 804–808.

McCullagh, P. & Nelder, J.A. (1989) *Generalized Linear Models*, 2nd edn. Chapman & Hall, London.

Mulder, C.P.H., Jumpponen, A., Hogberg, P. & Huss-Danell, K. (2002) How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia*, **133**, 412–421.

Mulder, C.P.H., Koricheva, J., Huss-Danell, K., Högberg, P. & Joshi, J. (1999) Insects affect relationships between plant species richness and ecosystem processes. *Ecology Letters*, **2**, 237–246.

Otway, S., Hector, A. & Lawton, J.H. (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *Journal of Animal Ecology*, **74**, 234–240.

Petchey, O.L. (2004) On the statistical significance of functional diversity effects. *Functional Ecology*, **18**, 297–303

Piepho, H.P., Büchse, A. & Richter, C. (2004) A mixed modelling approach for randomized experiments with repeated measures. *Journal of Agronomy and Crop Science*, **190**, 230–247.

Pinheiro, J.C. & Bates, D.M. (2000) *Mixed-Effects Models in S and S-PLUS*. Springer, New York.

R Development Core Team (2005) *R : A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

Root, R.B. (1973) Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95–124.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W.W. *et al.* (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology*, **5**, 107–121.

van Ruijven, J. & Berendse, F. (2003) Positive effects of plant species diversity on productivity in the absence of legumes. *Ecology Letters*, **6**, 170–175.

Schädler, M., Jung, G., Brandl, R. & Auge, H. (2004) Secondary succession is influenced by belowground insect herbivory on a productive site. *Oecologia*, **138**, 242–252.

Scheidel, U. & Bruelheide, H. (1999) Selective slug grazing on montane meadow plants. *Journal of Ecology*, **87**, 828–838.

Scherber, C., Mwangi, P.N., Temperton, V.M., Roscher, C., Schumacher, J., Schmid, B. *et al.* (2006) Effects of plant diversity on invertebrate herbivory in experimental grassland. *Oecologia*, **147**, 489–500.

Schmid, B., Hector, A., Huston, M.A., Inchausti, P., Nijs, I., Leadley, P.W. *et al.* (2002) The design and analysis of biodiversity experiments. *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives* (eds M. Loreau, S. Naeem & P. Inchausti), pp. 61–75. Oxford University Press, Oxford.

Siemann, E. (1998) Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology*, **79**, 2057–2070.

Siemann, E., Carson, W.P., Rogers, W.E. & Weisser, W.W. (2004) Reducing herbivory using insecticides. *Insects and Ecosystem Function* (eds W.W. Weisser & E. Siemann), Ecological Studies 173. Springer, Berlin.

Spehn, E.M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E. *et al.* (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecological Monographs*, **75**, 37–63.

Spehn, E.M., Scherer-Lorenzen, M., Schmid, B., Hector, A., Caldeira, M.C., Dimitrakopoulos, P.G. *et al.* (2002) The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *OIKOS*, **98**, 205–218.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T. & Lehman, C. (2001) Diversity and productivity in a long-term grassland experiment. *Science*, **294**, 843–845.

Journal of Ecology, accepted with minor revisions

Chapter 5

Niche pre-emption increases with species richness in experimental plant communities

Peter N. Mwangi[1,2,3], Martin Schmitz[1,2,3], Christoph Scherber[3], Christiane Roscher[2], Jens Schumacher[2], Michael Scherer-Lorenzen[2,4], Wolfgang W.Weisser[3], Bernhard Schmid[1]

[1]Institute of Environmental Sciences, Zurich University, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland; [2]Max Planck Institute for Biogeochemistry, Hans-Knoll Strasse 10, D-07745 Jena, Germany; [3]Institute of Ecology, Jena University, Dornburger Strasse 159, D-07743 Jena, Germany; [4]Institute of Plant Sciences, ETH Zentrum, Universitätstrasse 2, CH-8092 Zurich, Switzerland
Address for correspondence:Bernhard Schmid, e-mail: bschmid@uwinst.unizh.ch; Tel: +41 (0)44 635 5205; Fax: +41 (0)44 635 5711

Running headline: Niche pre-emption and invasion resistance

Summary

1. In plant communities, invasion resistance is has been hypothesized to increase with diversity because empty niche space decreases simultaneously. However, it is not clear if this only applies to exotic species or also to native species arriving at a site with few other native species during community assembly. We tested this latter and more general ecological question about invasion resistance by transplanting four native species into experimental grassland communities varying in species richness form 1–16 (–60) species. In addition, we tested the hypothesis that invasion is less successful if the invading species belongs to a functional group that is already present in the community.

2. The phytometer species included a grass (*Festuca pratensis*, FP), a small herb (*Plantago lanceolata*, PL), a tall herb (*Knautia arvensis*, KA) and a legume (*Trifolium pratense*, TP). The same four functional groups also occurred alone or in all possible combinations in the different experimental communities.

3. The overall performance of the transplants decreased with increasing resident species richness. Plant biomass declined by 58%, 90%, 84% and 62% in FP, PL, KA and TP, respectively, from monocultures to 16-species mixtures, indicating lower invasiveness of the two herbs than of the grass and the legume.

4. Resident grasses showed a strong negative effect on the performance of all phytometer species, whereas resident small and tall herbs had neutral and resident legumes had positive effects. The case of the legumes indicates that contribution to invasion resistance need not parallel invasiveness. Communities containing resident species of only one functional group were most inhibitive to transplants of the same functional group.

5. These results indicate that taking native species as a null model, invasion resistance of experimental plant communities is related to the degree of niche overlap between resident species and invaders. This niche overlap can be high due to generally low amounts of empty niche space in species-rich resident communities or due to the occurrence of the same functional group as the one of the invader in the resident community.

Key words: functional groups; invasion resistance; niche overlap; phytometers; plant diversity; The Jena Experiment

---

Introduction

Understanding the mechanisms behind the relationship between resident species richness and the establishment of non-resident species (i.e. invaders in the broad sense) in natural communities is a major goal in ecology. This relationship has potential applications in conservation, restoration and prediction of community invasion resistance. Elton (1958) and Levine & D'Antonio (1999) provide evidence for a generally negative relationship between diversity and the likelihood that an intruder will be able to establish itself in a community. Such relationships have been found in a large number of experimental studies using temperate plant communities (Tilman, 1997; Knops *et al.*, 1999; Joshi *et al.*, 2000; Naeem *et al.*, 2000; Prieur-Richard *et al.*, 2000; Diemer & Schmid, 2001; Kennedy *et al.*, 2002; Pfisterer *et al.*, 2004). In contrast, observational studies, which necessarily assess invasion by exotic invaders, often report higher numbers of invading species in species-rich than in species-poor plant communities (Stohlgren *et al.*, 1999; Stadler *et al.*, 2000; Pysek *et al.*, 2002; Stohlgren *et al.*, 2002), though Stohlgren *et al.* (1999) found the opposite at one site in their study. The results of observational studies may be attributed to uncontrolled extrinsic factors, whose effect on native and exotic species is the same (Stohlgren *et al.*, 1999; Levine, 2000; Shea & Chesson, 2002). Additionally, observational studies mostly analyse the number of invading species (e.g. Stohlgren *et al.*, 1999; Meiners *et al.*, 2004) whereas many experimental studies also assess the performance of particular invaders (see e.g. Prieur-Richard *et al.*, 2000; Diemer & Schmid, 2001; Hector *et al.*, 2001).

In most cases, species richness is the only component of diversity manipulated in experimental studies (e.g. Prieur-Richard *et al.*, 2000; Troumbis *et al.*, 2002), though some studies have demonstrated the importance of functional diversity in competitive suppression of invaders (Crawley *et al.*, 1999; Hector *et al.*, 2001; Prieur-Richard *et al.*, 2002; Xu *et al.*, 2004; Fargione & Tilman, 2005). Functional groups are sets of species (not necessarily taxonomic) that show close similarities in traits related to ecosystem functioning, e.g. traits related to resource uptake and biomass production. Increasing evidence suggests that the influence of functional diversity in a community might be more important than pure species richness (Diaz & Cabido, 2001; Garnier *et al.*, 2004; Heemsbergen *et al.*, 2004; Petchey *et al.*, 2004).

It is supposed that empty niche space (Hutchinson, 1957) declines with increasing species richness in a community (MacArthur, 1970). As a consequence, species-rich communities can utilize the total resources available in a biotope more completely than do species-poor communities (e.g. Scherer-Lorenzen *et al.*, 2003; Dimitrakopoulos & Schmid, 2004), thereby pre-empting resources for potential invaders (Tilman, 1999; Hector *et al.*, 2001; Fargione *et al.*, 2003). This effect occurs because generally an increase in species richness should also increase functional richness, suggesting that the number of functional groups in an experimental community may be a good predictor of these diversity effects. Conversely, the effect should be minimal if species richness is increased without increasing the number of functional groups at the same time. In addition, a community should be more resistant to invaders belonging to functional groups already present among the resident species (e.g. Fargione *et al.*, 2003; Turnbull *et al.*, 2005).

In most cases, invasion studies compare a set of species used as test invaders with a separate set of species used as residents of host communities (Tilman, 1997; Knops *et al.*, 1999; Hector *et al.*, 2001; Prieur-Richard *et al.*, 2002; Fargione *et al.*, 2003; Pfisterer *et al.*, 2004, but see Turnbull *et al.* 2005). This approach mimics biological invasions into communities by introduced species, which presumably do not share a common evolutionary history with natives. For example, the average competitive ability of exotic invaders may change with diversity (e.g. Bossdorf *et al.*, 2004; Colautti *et al.*, 2004; e.g. Vila & Weiner, 2004; Hierro *et al.*, 2005). Although exotic species invasions and invasion as a process of community assembly within a pool of native species are similar in principle and there are no a-priori ecological reasons for expecting them to be different, a different approach can be used to test the latter. Because the host communities and invaders belong to the same species pool, it is possible to distinguish between the *invasiveness* of a particular species or functional group as an invader and its contribution to *invasion resistance* of the host community.

Here we present the results of an experiment using this approach. We selected four native species representative of four functional groups and used in a biodiversity experiment to create experimental communities as test invaders or “phytometers”. Specifically, we wanted to find out, (a) if increasing species richness or number of functional groups in plant communities suppresses the performance of invaders; (b) whether the presence of a particular functional group in a host community enhances suppression of the test invaders; and (c) whether the test invaders are most suppressed by host communities containing species belonging to the same functional group.

Materials and methods

Our study was part of a large biodiversity experiment, The Jena Experiment in Germany (50°55’ N, 11°35’ E, 130 m altitude). This experiment was established in May 2002 on a former agricultural field in the flood plain of the Saale river (Roscher *et al.*, 2004). Plant communities were assembled by constrained random selection from a pool of 60 species typical to Central European mesophilic grasslands. The species were categorized into the four functional groups grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species), based on multivariate analyses of their traits (Roscher *et al.*, 2004). Seventy-eight plots, each measuring 20 x 20 m, were sown with 1, 2, 4, 8, or 16 species. A factorial design was formed with all possible species richness x functional group richness mixtures. At each level of species richness, 16 replicate mixtures with different species composition were established, except at the highest level with 14 replicates only. Four additional large plots contained mixtures of all 60 species in the pool. The field was partitioned into four blocks following a gradient in soil characteristics perpendicular to the river (Roscher *et al.*, 2004). The plots were mown twice a year (June, September) to mimic the typical management of meadows in the region and weeded twice a year to maintain the original species composition. Mowing and weeding were done block-wise such that these management effects could be accounted for with the block term in statistical analysis.

Our test invaders were pre-grown phytometer individuals of four species that also occurred in a large number of experimental communities as resident species. Each belonged to one of the four functional groups used in the experiment: *Festuca pratensis* Huds. (grass), *Plantago lanceolata* L. (small herb), *Knautia arvensis* L. (tall herb) and *Trifolium pratense* L. (legume). They are all perennial plant species, form clearly defined compact individuals and are relatively easy to transplant.

In mid March 2003, we germinated the phytometers on moist filter paper in a greenhouse. Individual seedlings were planted in 132-cm^3 cells of potting trays filled with a soil-compost-perlite mixture (3:2:1 in terms of volumes). We used a 14-h light regime with 22°C day temperature and 15°C night temperatures. In mid April 2003, we hardened the plants by placing them outside the greenhouse for one week before transplanting them into the experimental communities at an average size of 4 to 7 leaves. Five phytometer individuals of each test species were randomly allocated to positions at 28-cm intervals in a 2 x 2-m subplot within each large plot and the initial size determined by counting their number of leaves and number of ramets (the latter only for *F. pratense* and *T. pratense*). Transplanted phytometers were marked by fixing numbered plastic labels next to the plants to ease identification during data collection.

In mid August, in addition to counting the number of leaves, we measured the maximum height of the phytometers. For *T. pratense* and *F. pratensis,* we also counted the number of ramets as before. We calculated the relative growth rate of the transplants using the formula

$$RGR = \left(\ln l_{t2} - \ln l_{t1}\right)/\, d,$$

where l_{t2} is the mean number of leaves in August, l_{t1} is the mean number of leaves in April and *d* is the length of time interval in days (Harper, 1977). As a measure of plant fitness, in August, we also counted the number of inflorescences of *P. lanceolata* and *T. pratense* transplants. No individuals of *F. pratensis* or *K. arvensis* were flowering at this time. In the last week of August 2003, shortly before mowing, transplants were cut at 3 cm above the ground and dried at 70°C for at least 48 h to determine the average biomass of each transplant species per plot. In early June 2004, we once again measured the height of the transplants, counted the number of inflorescences in *P. lanceolata* and *T. pratense*, which were flowering at this time, and harvested the transplants per species per plot to determine the average aboveground biomass as described above.

Before each harvest, we determined the leaf area index (LAI) of the resident community in an undisturbed area next to the phytometers using an LAI-2000 Plant Canopy Analyzer (LI-COR Inc., Lincoln, Nebraska, USA).

Statistical analysis

We used general linear models with sequential sum of squares (Type I) for data analysis using Genstat 6th Edition, Release 6.2. (Payne *et al.*, 2002). Since individual plants were pseudo-replicates within plots, we analysed means of the response variables plant biomass, plant height, number of leaves and relative growth rate. The data were transformed if residuals showed deviation from the normal distribution. According to the experimental design, the analysis of variance (ANOVA) model consisted of the terms block, sown species richness (partitioned into linear and deviation from linear), functional group richness, species composition, phytometer species and phytometer species x diversity interactions. Separate contrasts for the presence/absence of each functional group and their interactions with species richness were tested in alternative models. Similarly, separate contrasts were made to compare each phytometer species and its interactions with diversity terms against the other three phytometer species. The diversity terms (species richness, functional group richness, presence of particular functional groups) had to be tested at the between-plot level (Error = composition) whereas phytometer terms and their interactions with diversity terms could be tested at the within-plot level (Schmid *et al.*, 2002). We also analysed the data of each phytometer species separately. To determine if the effect of diversity terms was related to a change in the leaf-area index (LAI) of the community we did post-hoc analyses with LAI as a covariate. In addition, LAI was tested as a dependent variable itself, using the between-plot ANOVA as explained above.

To test if the phytometer species were more affected by their own than by other functional groups, we used a reduced data set of communities with only one functional group (n = 34 plots). To do this, the resident x transplant functional group interaction was decomposed into a “home versus away” contrast and remainder (tacking all “away” treatment combinations together; see Table 3). To illustrate the home versus away contrast we use an equivalent of the relative-neighbour-effect of (Markham & Chanway, 1996), using the formula $(P_h - P_a) / \max(P_h, P_a)$. Here, P_h is the performance (e.g. biomass) of phytometers in communities with their own functional group (home), P_a the performance in communities with other functional groups (away) and $\max(P_h, P_a)$ is the larger of the two.

Results

Effects of species richness and functional richness

Except for plant height in *P. lanceolata* and *K. arvensis*, the measured morphological variables of phytometers were highly correlated with their aboveground biomass (Table 1), indicating that the latter is a good measure of overall phytometer performance.

Table 1 Partial correlation of phytometer vegetative and reproductive traits measured in summer 2003 with aboveground biomass

Plant Trait	*F. pratense* (N=71)	*P. lanceolata* (N=78)	*K. arvensis* (N=76)	*T. pratense* (N=68)
Number of leaves	0.919***	0.887***	0.855***	0.776***
Number of ramets	0.904***	-	-	0.866***
Height	0.467***	0.128^{ns}	0.228*	0.415***
Number of flowers	-	0.974***	-	0.938***
Relative growth rate	0.804***	0.610**	0.720***	0.680***

At the first harvest in summer 2003, i.e. 4 months after transplanting, the performance of phytometer individuals was negatively affected by increasing species richness (reduced number of leaves or number of ramets, reduced biomass and reduced growth rate, Fig. 1a, c, d and Table 2a). The height of the phytometers was, however, not affected and even increased with species richness in one of the phytometer species (*F. pratensis*; $F_{1,70} = 9.30$, $p < 0.01$ in separate analysis), suggesting a typical allometric response to increased competition for light (etiolation); i.e. a faster increase in height, independent of size (Fig. 1b, Table 2a; see also lower correlations of plant height than of other variables with biomass in Table 1). The length of the leaves in *F. pratensis* also increased with increasing species richness ($F_{1,73} = 15.64$, $p < 0.001$). The influence of resident species richness on phytometer performance varied among phytometer species; the herbs (*P. lanceolata* and *K. arvensis*) were more strongly affected than the grass (*F. pratensis*) and the legume (*T. pratense*) (see species richness x phytometer species (PS) interaction in Table 2a). The negative effect of species richness on phytometer aboveground biomass was still significant in spring 2004 ($F_{1,73} = 27.80$, $p < 0.001$, Fig. 2b), but again plant height was not affected by species richness ($F_{1,73} = 1.19$, $p < 0.172$, Fig. 2a).

Functional richness had no effect on the performance of the phytometers after controlling for species richness in both seasons ($p > 0.05$). By contrast, if fitted before species richness, functional richness also had significant negative effects on all phytometer variables except height (aboveground plant biomass: $F_{1,67}=4.74$, $p=0.03$; plant height: $F_{1,67}=1.38$, $p=0.24$; number of leaves: $F_{1,67}= 7.13$, $p = 0.01$; growth rate: $F_{1,67} = 6.12$, $p = 0.01$; Fig. 1e-h); and in addition the species richness effects remained significant ($p < 0.05$) except for plant height, as before. The pattern was the same in spring 2004 (Fig. 2c and d). This highlights the importance of species richness even if functional richness in statistical terms is "held constant", i.e. the species richness effect remains negative within a particular level of functional richness.

Separate analyses showed that increasing species richness led to a significant reduction in number

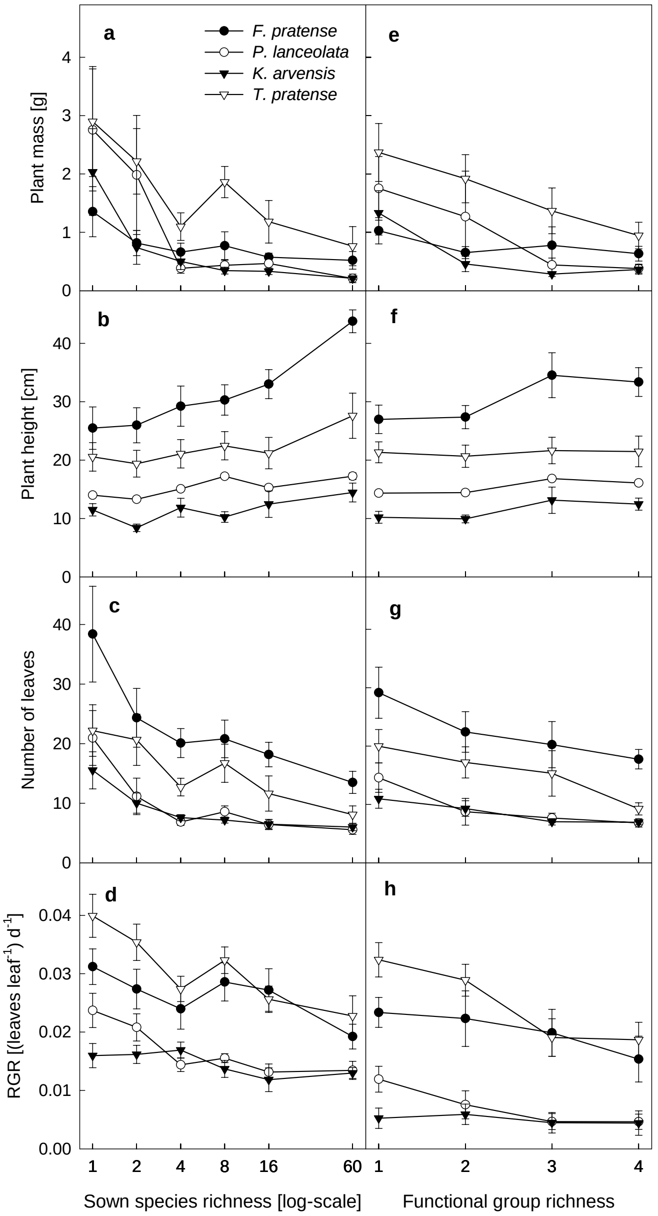


Fig. 1. Effect of plant species richness (a–d) and functional group richness (e–h) on performance of four transplanted phytometer species in the first season (summer 2003). Points and vertical bars represent means ± 1 standard error. All panels use the legend in panel (a).

Table 2a Summary of analyses of variance of the performance of the four phytometer species in summer 2003. Residual d.f. are a=225, b=219, and total c=308 for number of leaves and plant height and a=240, b=234 and total c=327 for growth rate. PS is the phytometer species (also represents the transplant functional group). Due to hierarchical design of our experiment, the terms above composition are tested at plot-level error term, i.e. composition. The terms presence of functional groups and their interaction with SR were added alternatively because they are intrinsically related, same communities contained presence/absence of different groups. PS and SR x PS was tested against within plot error (residual). Significance levels are * P < 0.05; ** P < 0.01; *** P < 0.001.

		Biomass		Leaves		Height		Growth Rate	
Source	d.f.	MS	F	MS	F	MS	F	MS	F
Block	3	6.23	2.63	1.06	1.23	0.79	1.61	<0.01	1.53
Species richness (SR)	1	22.22	9.39**	14.84	17.23***	1.17	2.36	<0.01	13.59***
Deviation	3	5.17	2.18	1.26	1.46	0.26	0.53	<0.01	0.71
Composition	70	2.37	3.28***	0.86	4.28***	0.49	4.30***	<0.01	3.07***
Grasses (GR)	1	19.52	9.25**	3.20	3.89*	4.93	11.66**	<0.01	4.41*
SR x GR	1	2.72	1.29	1.10	1.33	0.93	2.19	<0.01	2.98
Composition	68	2.11	2.89***	0.82	4.21***	0.42	3.82***	<0.01	3.11***
Short-herbs (SH)	1	3.33	1.41	0.15	0.17	1.06	2.19	<0.01	0.46
SR x SH	1	1.60	0.68	0.00	<0.01	0.77	1.59	<0.01	0.99
Composition	68	2.37	3.39***	0.88	4.36***	0.48	4.13***	<0.01	3.07***
Tall-herbs (TH)	1	4.22	1.78	1.27	1.46	0.70	1.41	<0.01	0.04
SR x TH	1	0.06	0.02	0.08	0.09	0.31	0.62	<0.01	<0.01
Composition	68	2.38	3.31***	0.87	4.23***	0.49	4.38***	<0.01	3.12***
Legumes (LG)	1	13.15	5.86*	0.82	0.94	7.18	17.88***	<0.01	<0.01
SR x LG	1	0.03	0.01	0.35	0.41	0.08	0.20	<0.01	0.17
Composition	68	2.24	3.29***	0.87	4.76***	0.40	3.64***	<0.01	3.31***
PS[1]	3	10.44	14.47**	14.82	73.58**	13.31	115.84**	<0.01	82.60***
SR x PS	3	2.07	2.88*	0.25	1.26	0.17	1.45	<0.01	3.08*
Residual	216[a]	0.72		0.20		0.11		<0.01	
Total	299[c]	1.39		0.56		0.34		<0.01	

of inflorescences per plant in *P. lanceolata* ($F_{1,70} = 25.58$, $p < 0.001$) and *T. pratense* ($F_{1,69} = 6.07$, $p = 0.01$), the two phytometer species which flowered before the first harvest in August 2003 (Fig. 3a). The same negative effect of species richness on number of inflorescences per plant was observed in *P. lanceolata* ($F_{1,69} = 18.31$, $p < 0.001$) and *K. arvensis* ($F_{1,68} = 18.88$, $p < 0.001$) in spring 2004 (Fig. 3a). Again, the effect of functional richness on the number of inflorescences was not significant after controlling for species richness, but it was highly significant if fitted first (summer 2003: *P. lanceolata*; $F_{1,70} = 11.15$, $p < 0.001$, *T. pratense*; $F_{1,69} = 6.13$, $p = 0.016$; spring 2004: *P. lanceolata*; $F_{1,69} = 12.04$, $p < 0.001$, *K. arvensis*; $F_{1,68} = 14.71$, $p < 0.001$; Fig. 3b), with the effect of species richness fitted afterwards again remaining significant ($p < 0.01$).

Effects of the presence of particular functional groups

The presence of grasses or legumes in the host communities had significant overall effects on phytometers, but this was not the case for the other two functional groups (Table 2a). Grasses significantly reduced number of modules (number of leaves or number of ramets), aboveground biomass, and growth rate of all the phytometer species (Table 2a) as well as number of inflorescences in *P. lanceolata* in summer 2003 ($F_{1,68} = 5.66$, $p = 0.02$) and spring 2004 ($F_{1,67} = 7.56$, $p = 0.008$). For example, in summer 2003, the average biomass of an individual phytometer (all species together) was 0.8 g in plots with grasses compared to 1.9 g in plots without grasses (Fig. 4). In spring 2004, the figures were 3.5 g and 10.8 g for plots with

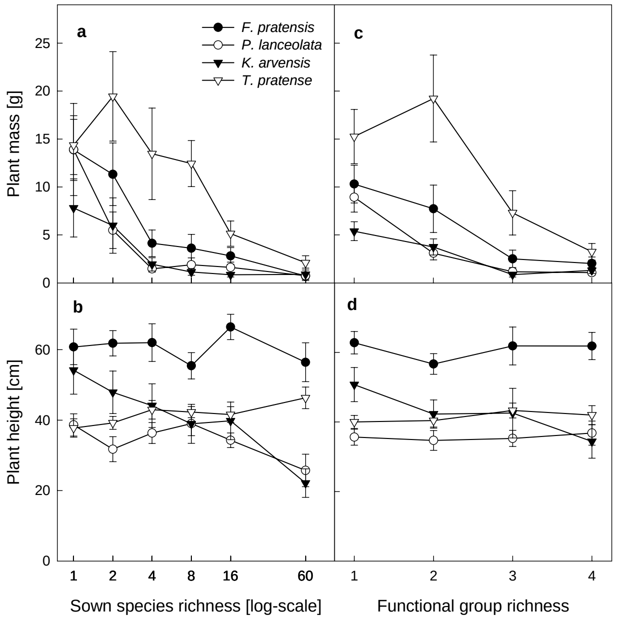


Fig. 2. Effect of plant species richness (a–b) and functional group richness (c–d) on performance of four transplanted phytometer species in the second season (spring 2004). Points and vertical bars represent means ± 1 standard error. All panels use the legend in panel (a).

and without grasses respectively. The presence of legumes had an overall significantly positive effect on the performance of the phytometer species (Table 2a, Fig. 4 and 5). Separate analysis for each phytometer, however, revealed that the presence of legumes actually reduced aboveground biomass of the legume phytometer, *T. pratense*, at least in the spring 2004 ($F_{1,61} = 8.97$, $p = 0.004$). The negative effect of legume presence on the legume phytometer as opposed to a positive effect on the other phytometers is also evident in the significant LG x TP interaction in Table 2b and last rows in figure 4 and figure 5.

There were no significant interactions between species richness and the presence of particular functional groups in the communities on phytometer performance. We mention this explicitly because such interactions might be expected if the sown proportion of a functional group would influence invasion resistance; where present, the proportion of a functional group decreases with increasing species richness.

In both seasons, the leaf area index (LAI) of the resident community increased with increasing species richness (August 2003: $F_{1,68} = 6.42$, $p = 0.014$, May 2004: $F_{1,70} = 6.70$, $p = 0.012$) but was not affected by functional richness. Although there was no effect of the presence of any functional group on LAI in August 2003, in May 2004, LAI was high in mixtures containing legumes ($F_{1,68} = 28.01$, $p < 0.001$) low in mixtures containing small herbs ($F_{1,68} = 6.52$, $p < 0.013$). This suggests that belowground competition may responsible for the observed high suppression of phytometers in communities containing grasses. As a covariate, in August 2003, LAI had significant negative effects on number of leaves and growth rate, positive effects on plant height ($P < 0.05$) but neutral effects on biomass of the phytometers. In May 2004 however, LAI had negative effects on aboveground biomass, plant height, and number of ramets of the phytometers ($P < 0.05$). However, where present, the effects of LAI did not explain the significant effects of species richness; that is, species richness effects remained significant after controlling for the effect of LAI.

Effects of the functional group of the phytometer species

As suggested by hypothesis (c) in the Introduction, comparing the suppression of invaders by communities containing different functional groups is not the same as looking at the performance of invaders belonging to different functional groups. In the first case (hypotheses (a) and (b) in the Introduction), the panels in Fig. 4 and Fig. 5 are compared row-wise, in the second case they are compared column-wise. If the two approaches are combined, the performance of particular phytometer species in assemblages containing only species of its functional group can be compared with its performance in assemblages containing only each of the other functional group (-1 diagonal in Fig. 4 and Fig. 5). We refer to this as a “home-vs.-away”

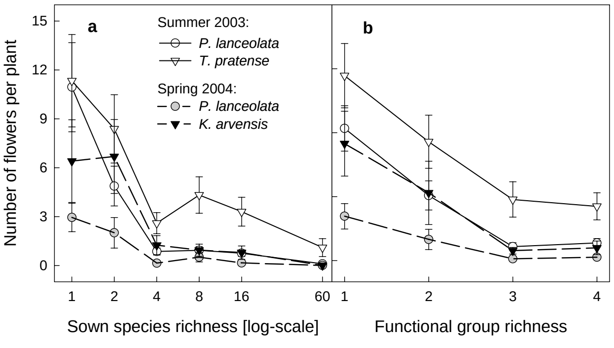


Fig. 3. Effect of plant species richness (a) and functional groups richness (b) on number of inflorescences of four transplanted phytometer species in summer 2003 (solid lines) and spring 2004 (broken lines). Points and vertical bars represent means ± 1 standard error.

Table2b Summary of analyses of variance of contrasts of phytometers species and their interaction with the diversity terms. Contrasts for each phytometer species were added alternatively into model in table 2a and tested against their respective residuals. Abbreviations not included in table 2a are; FP = *Festuca pratensis*, PL = *Plantago lanceolata*, KA = *Knautia arvensis*, TP = *Trifolium pratense*, for the phytometer species.

		BIOMASS		LEAVES		HEIGHT		GROWTH RATE	
Source of variation	d.f.	MS	F	MS	F	MS	F	MS	F
FP	1	<0.01	<0.01	33.52	171.49**	25.57	231.06**	<0.01	36.33***
SR x FP	1	3.55	4.86*	0.42	2.14	0.50	4.49*	<0.01	2.26
GR x FP	1	0.19	0.26	1.46	7.48**	0.01	0.08	<0.01	18.02***
GR x PS	2	0.23	0.32	0.52	2.68	0.34	3.04*	<0.01	3.89*
SR x GR x FP	1	0.38	0.52	0.00	0.02	0.15	1.31	<0.01	2.26
SR x GR x PS	2	0.64	0.88	0.00	0.00	0.40	3.57*	<0.01	0.13
Residual	210^b	0.73		0.20		0.11		<0.01	0.61
PL	1	1.49	2.13	12.46	61.43**	2.15	18.44**	<0.01	50.16***
SR x PL	1	3.68	5.27*	0.53	2.60	0.04	0.34	<0.01	0.77
SH x PL	1	1.67	2.39	0.29	1.43	0.05	0.46	<0.01	0.15
SH x PS	2	2.06	2.96	0.06	0.28	0.10	0.84	<0.01	0.66
SR x SH x PL	1	2.01	2.87	0.00	0.01	0.00	0.01	<0.01	0.86
SR x SH x PS	2	0.66	0.94	0.24	1.20	0.03	0.28	<0.01	0.93
Residual	210^b	0.70		0.20		0.12			
KA	1	14.31	19.96**	12.03	58.72**	23.91	212.05**	<0.01	107.17***
SR x KA	1	0.56	0.78	0.02	0.11	0.08	0.71	<0.01	3.49
TH x KA	1	0.00	0.00	0.01	0.04	0.18	1.56	<0.01	0.17
TH x PS	2	1.69	2.35	0.15	0.74	0.10	0.89	<0.01	0.75
SR x TH x KA	1	0.02	0.02	0.01	0.03	0.37	3.31	<0.01	0.56
SR x TH x PS	2	0.87	1.22	0.07	0.33	0.21	1.84	<0.01	0.45
Residual	210^b	0.72		0.20		0.11			
TP	1	25.83	37.88**	1.38	7.56**	1.56	14.17**	<0.01	144.96***
SR x TP	1	0.56	0.82	0.05	0.28	0.05	0.49	<0.01	6.25*
LG x TP	1	8.17	11.97**	2.56	14.02**	0.44	3.97*	<0.01	7.61**
LG x PS	2	0.76	1.11	0.82	4.50*	0.06	0.51	<0.01	2.35
SR x LG x TP	1	0.55	0.81	0.16	0.89	0.54	4.89*	<0.01	0.77
SR x LG x PS	2	1.16	1.71	0.45	2.45	0.31	2.78	<0.01	2.53
Residual	210^b	0.68		0.18		0.11		<0.01	

contrast (see e.g. Joshi *et al.*, 2001; Turnbull *et al.*, 2005), for which hypothesis (c) predicts a particularly strong negative effect.

The effect of different single-functional-group assemblages on number of modules (leaves or ramets), aboveground biomass and growth rate of the phytometers was similar (Table 3). However, the height of the phytometers significantly differed among these assemblages: it increased from grass < small-herb < tall-herb < legume communities, suggesting that competition for light increased in this order. Overall, the two herbaceous phytometer species were least affected by differences between these one functional group assemblages, whereas the grass (*F. pratensis*) and the legume (*T. pratense*) phytometer were more affected by these differences. This is evident in Fig. 5 by comparing differences between open and filled symbols in monocultures. A contrast between monocultures versus multi-species assemblages containing one functional group showed that the number of modules (leaves or ramets), aboveground biomass and growth rate of the phytometers was significantly lower in the latter (Table 3). This reinforces the statistical observation made above, that competitive suppression increases with species richness of a community even if functional richness is held constant, in this case at the lowest level. The home-disadvantage was similar in mono-specific and multi-species single-functional-group assemblages (interaction home x mono not significant in

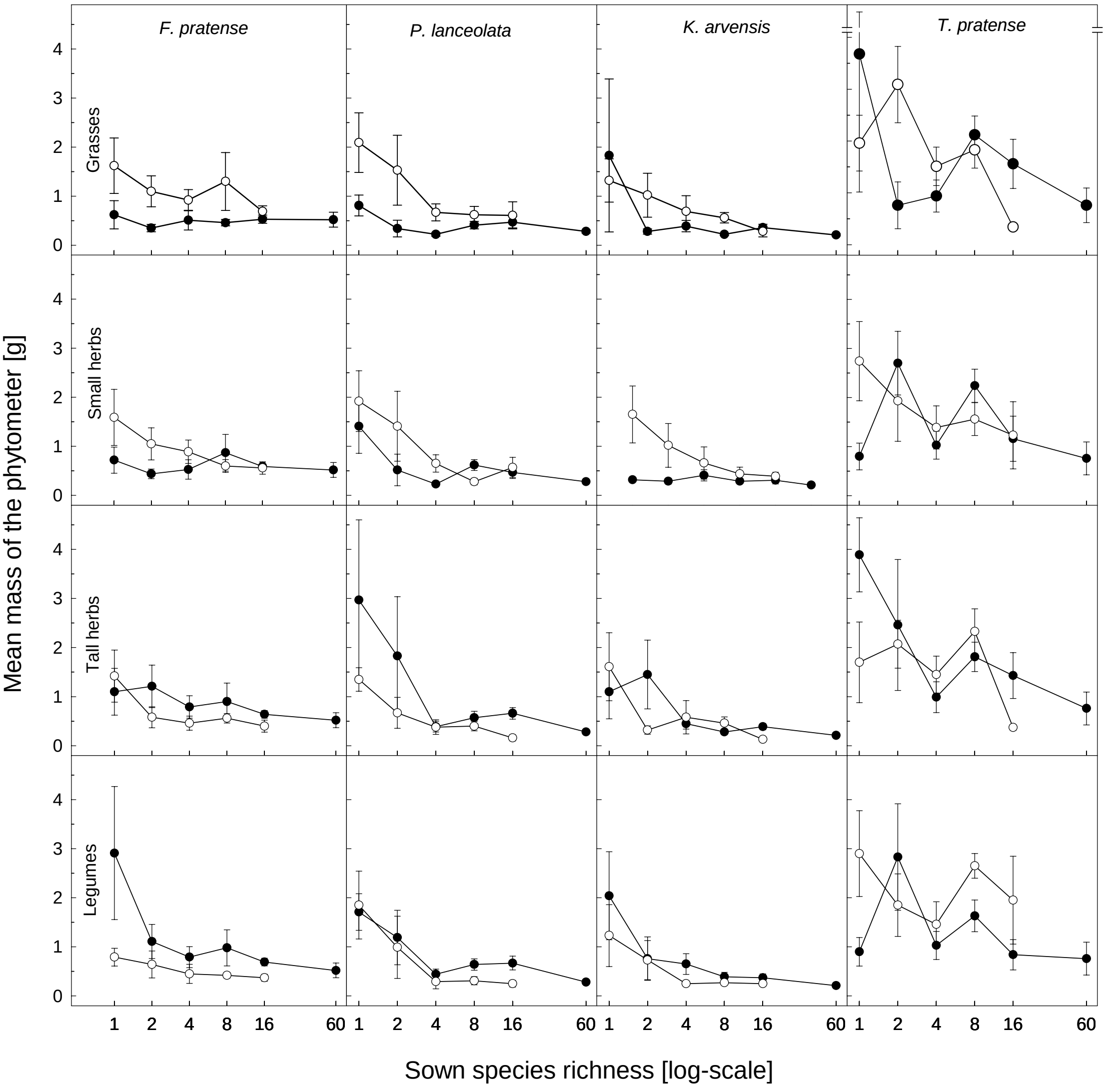


Fig. 4. Effect of plant species richness and presence/absence of different functional groups on biomass of four transplanted phytometer species in the first season (summer 2003). Columns represent phytometers species *F. pratensis, P. lanceolata, K. arvensis* and *T. pratense* from left to right and rows represent presence/absence of grasses, small-herbs, tall-herbs and legumes from top to bottom. Closed and open symbols indicate, respectively, presence and absence of the corresponding functional groups in the resident communities. For example, the second panel in the first row shows the response of P. lanceolata to species richness in the plots containing grasses (closed symbols) and in plots without grasses (open symbols). Points and vertical bars represent means ± 1 standard error.

Table 3).

Except for plant height, the home vs. away contrast almost fully explained the RFG x PS interactions (Table 3). That is, as predicted, the phytometers had significantly lower performance when transplanted into assemblages consisting of the same rather than a different functional group (the effects of home-functional groups were stronger than of away-functional group, i.e. negative bars in Fig. 6). The significant residual RFG x PS interaction for plant height indicates that the home effect on plant height is not as clear-cut; for example, *F. pratense* phytometers were taller in non-grass single function group assemblage whereas *T. pratense* phytometers were shorter in non-legume single functional group assemblages (Fig. 6). The four-phytometer species responded differently to mono-specific versus multi-species single-functional-group assemblages (Table 3): the biomass of *P. lanceolata* declined from 5.3 g in mono-specific to 0.9 g in multi-species single-functional-group assemblages whereas the other three-phytometer species showed little reduction in aboveground biomass. For two phytometer species that also occurred as monocultures, *P. lanceolata* was greatly suppressed by its own monoculture in both seasons. It attained less than 1.5 g in its own monoculture in both seasons compared to an average of 5.6 g and 14.7 g in other monocultures in summer 2003 and spring 2004 respectively. By contrast, *F. pratense* performed well in its own monocultures

Table 3 Summary of analyses of variance of summer 2003 data for home-vs.-away effect on the four phytometer species using plots with resident communities consisting of species from only one functional group. Significance levels are * P < 0.05; ** P < 0.01; *** P < 0.001.

		Biomass		Leaves		Height	
Source	d.f.	MS	F	MS	F	MS	F
Block	3	11.63	4.09*	2.33	2.08	553.61	8.43
Resident Functional Group (RFG)	3	3.11	1.09	0.26	0.23	962.34	14.66***
Monoculture vs. Mixture (Mono)	1	22.02	7.75*	9.16	8.19**	49.64	0.76
RFG x Mono	3	0.53	0.19	0.06	0.06	6.44	0.10
Composition	23	2.84	4.19***	1.12	5.61***	65.66	1.68*
Phytometer species (PS)	3	3.36	4.95**	5.99	30.08***	1818.79	46.65***
Home vs. Away (RFG x PS main diag.)	1	4.25	6.26*	1.59	7.98**	169.93	4.36*
RFG x PS (residual interact.)	8	0.84	1.24	0.22	1.13	191.49	4.91***
Phytometer species x Mono	3	2.47	3.64*	0.28	1.40	42.37	1.09
Home vs. Away x Mono	1	0.19	0.28	0.38	1.89	8.51	0.22
Residual	79	0.68		0.20		38.99	
Total	128	1.68		0.63		128.91	

especially in 2004 (i.e. 19.6 g in its own and 13.5 g in others).

Discussion

Effects of species richness and functional richness

Using native species to eliminate the possible confounding effect of traits of exotic species, we have shown that plant diversity enhances competitive suppression of newly arriving individuals during the invasion process. This supports the proposition that species-rich communities contain less empty niches that can be occupied by extra individuals. In our experimental plots, soil nitrogen decreased with species richness (Oelmann unpublished data) while aboveground biomass (Roscher *et al.*, 2005) and LAI increased. Functional group richness, which had smaller effects on invasion resistance, also had smaller effects on the LAI of resident communities ($F_{1,68}$=3.37, *p*=0.071) and soil nitrogen (Oelmann unpublished data). Our results agree with several previous findings (e.g. Levine, 2000; Prieur-Richard *et al.*, 2002), but in our case, the response of the test invaders can be attributed purely to invasion resistance. With one of the most balanced designs in terms of species and functional diversity achieved so far in biodiversity experiments (Roscher *et al.*, 2004), our results show that in contrast to previous suggestions (Diaz & Cabido, 2001) species richness was a better predictor of invasion resistance than was functional group richness. Nevertheless, stronger effects of functional group richness on invasion resistance have been found in cases where an effect of functional diversity on resource pre-emption is more likely (Prieur-Richard *et al.*, 2000; Symstad, 2000; Dukes, 2001).

Since functional groups are aggregations of species, three observations (that can apply in natural communities) may explain why the effect of species richness is stronger than that of functional group richness. First, aggregating several species into few functional groups makes species richness have a wider range (1–60 species) than functional richness (1–4 functional groups). Second, differential effects of functional richness on different phytometer species leads to an averaging of the overall effect of functional richness in a balanced design, where each functional group is represented equally among the four phytometer species and among the resident plant communities. A case in point is the positive effect of resident legumes on non-legume test-invaders and the negative effect on the legume test-invader. Third, contrasting effects of different functional groups on resources may weaken the overall effect of functional group richness. For example, while legumes enrich soil with nitrogen, grasses deplete this resource (Tilman *et al.*, 1997; Scherer-Lorenzen *et al.*, 2003). These counteracting effects of functional groups on resource pre-emption weakens the overall effect of functional group richness on suppression of test-invaders, supporting resource pre-emption as one

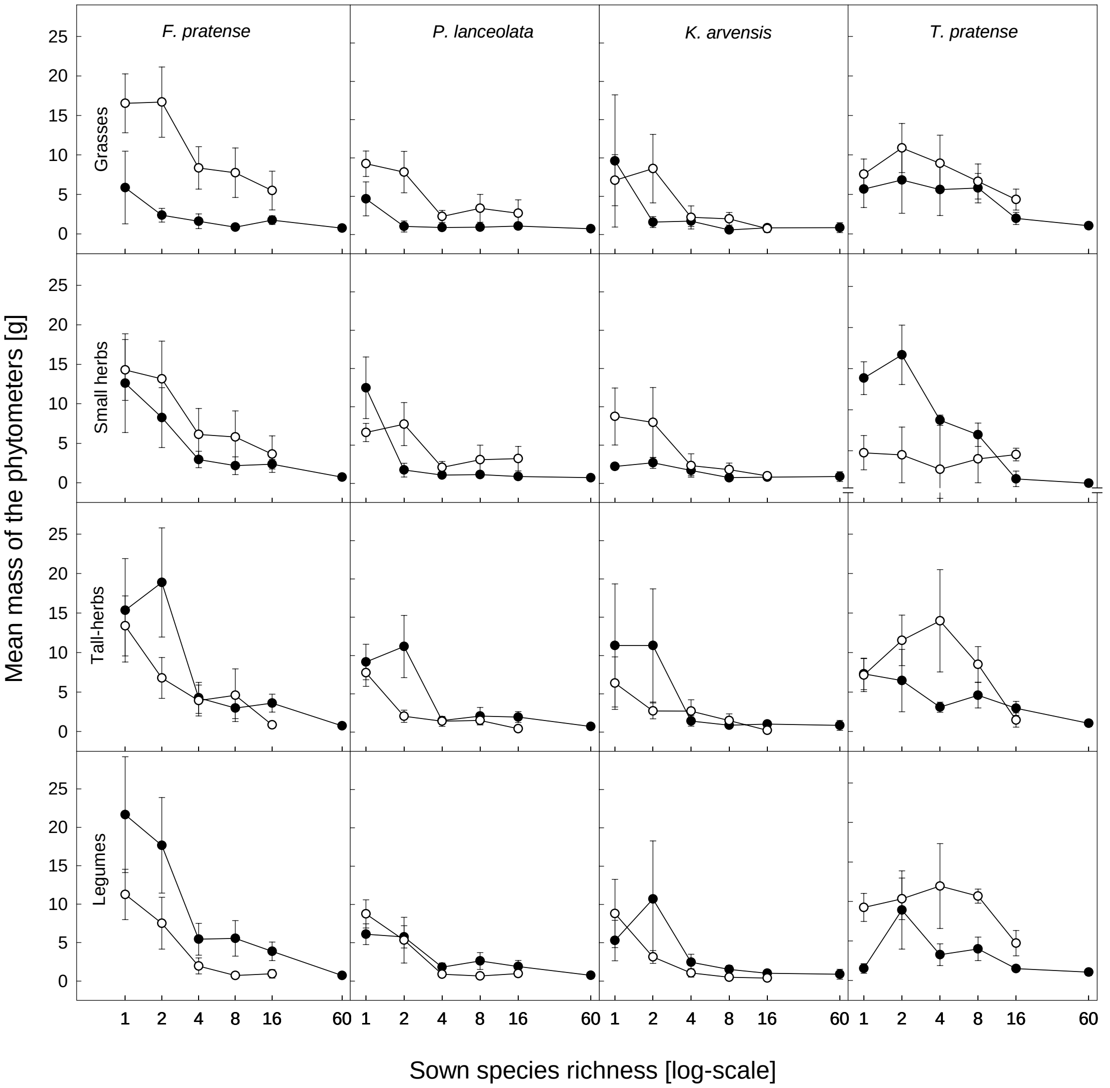


Fig. 5. Effects of plant species richness and presence/absence of different functional groups on biomass of four transplanted phytometer species in the second season (spring 2004). Columns represent phytometers species *F. pratensis, P. lanceolata, K. arvensis* and *T. pratense* from left to right and rows represent presence/absence of grasses, small herbs, tall herbs and legumes from top to bottom. Closed and open symbols indicate, respectively, presence and absence of functional groups in the resident communities. Points and vertical bars represent means ± 1 standard error.

mechanism of invasion resistance in plant communities.

Nonetheless, as in the study by Symstad (2000), functional group richness can enhance invasion resistance, if considered alone. However, Symstad (2000) also observed little effect of functional group richness on resource use and therefore could not attribute increased invasion resistance by functionally rich communities to niche pre-emption. Our study showed that pure grass mixtures can be most resistant to invasion, underlying the importance of traits of specific groups (see next section). It remains debatable, of course, if the a *priori* definitions of functional groups that we adopted in The Jena Experiment are adequate to understand the relationship between functional diversity and invasion resistance. Nevertheless, the fact that species richness increased invasion resistance even within plant assemblages consisting of a single functional group further exemplifies the importance of species richness *per se* in this particular case of an ecosystem function.

Effects of the presence of particular functional groups

The negative, positive and neutral effect of legumes, grasses and herbs, respectively, on invasion resistance in our study reflects their known patterns of resource use (Fargione *et al.*, 2003; Fargione & Tilman, 2005). In our experiment, better performance of the phytometers in communities with legumes corresponded with findings that they actually benefited from nitrogen fixed by legumes (Temperton *et al* submitted). It is well documented that legumes, by adding nitrogen to the soil, can promote invasion in nitrogen-limited environments (Yelenik *et al.*, 2004 and reference therein). By actively fixing atmospheric nitrogen, legumes do not rely on soil-nitrogen pools. Thus, a related effect of legumes is the reduction of competition for soil nitrogen. Some previous studies, however, have reported increased invasion resistance due to presence of legumes (Hector *et al.*, 2001; Fargione *et al.*, 2003). It is notable that positive effects of legumes usually correlate with their effect on belowground resources, mainly soil nitrogen (Maron & Connors, 1996; Prieur-Richard *et al.*, 2002) while their negative effects usually correlate with their effect on aboveground resources (Hector *et al.*, 2001; Fargione *et al.*, 2003). One can therefore propose that legumes enhance invasion resistance in fertile soils but promote invasion in poor soils.

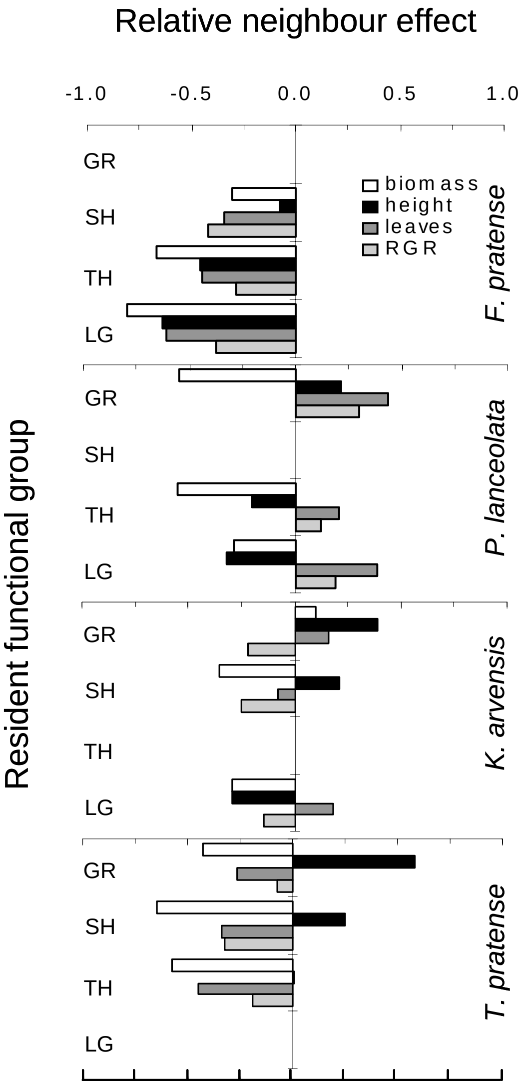


Fig. 6. Performance of phytometers transplanted into single-functional-group assemblages of grasses (GR), small herbs (SH), tall herbs (TH) and legumes (LG) relative to performance in mono-functional group assemblages of their own functional group. Negative values indicate negative "home" effects, i.e. that resident species belonging to a functional group different from that of the phytometer were less inhibitive, while positive values indicate positive home effects.

Suppression of all phytometers was particularly strong in resident communities containing grasses. Due to their extensive root systems, grasses are efficient in taking up resources from the upper soil layers (Fargione *et al.*, 2003), thereby diminishing resources for potential invaders. Other studies have also reported grasses as a keystone functional group reducing the success of invaders (Crawley *et al.*, 1999; Dukes, 2002; Prieur-Richard *et al.*, 2002). Crawley *et al.* (1999) found that an assembly of 80 herbaceous species was more vulnerable to invasion than were assemblies composed of 1–4 grass species. A weak effect of grasses on LAI did not explain the strong negative effect of their presence on invasion resistance, suggesting that their contribution to invasion resistance is mainly through their effect on belowground resources. From these results we can conclude that with regard to functional diversity, functional group identity may be more important than pure number of functional groups (Schmid *et al.*, 2002). This was also observed in the same experiment by Scherber *et al.* (2006), investigating herbivory on a different phytometer species, *Rumex acetosa*.

Effects of the functional group of the phytometer species

With regard to the identity of the invader, experimental communities were particularly resistant to a phytometer species if they contained species belonging to the same functional group. For example, although non-legume herbs had no effect on invasion resistance in general, their presence in the resident communities enhanced suppression of their respective phytometers. Likewise, despite notable facilitation by legumes, communities containing only this functional group strongly inhibited the legume test invader, *T. pratense*. Our results and a previous observation that legume monocultures were most resistant to invasion by legumes (Turnbull *et al.*, 2005) indicate that resident legumes also pre-empt other resources that limit legumes, most likely phosphorus, water and light (Vitousek & Howarth, 1991). This is consistent with high niche overlap along several resource-use axes between resident and invading legumes, and further supports niche pre-emption as a mechanism of invasion resistance. Thus high niche overlap between newly arriving individuals and resident species can reduce chances of an invasion, rate of colonisation or even success of restoration. This corresponds to findings of Fargione *et al.* (2003) in a seed addition experiment, where they concluded that high invasion resistance was due to similar patterns of resource use between the resident species and the invaders. Xu *et al.* (2004) also found that the presence of a functionally similar herb in a resident community increased resistance to invasion by Alligator weed (*Alternanthera philoxeroides*), which was also attributed to niche overlap.

This study confirms that, first, communities that are more diverse confer high resistance to invasion independent of invasiveness of the introduced species. Secondly, presence of grasses enhances invasion resistance while legumes may promote invasion due to their influence on nitrogen dynamics. Thirdly, communities are more resistant to invaders belonging to functional groups already present among the resident species.

Acknowledgement

We are grateful to the gardeners and numerous helpers who assisted in weeding and maintenance of experimental plots. Thanks to Christina Fischer, Anne Froehlich and Anja Dassler for their assistance in the field and lab. Ernst-Detlef Schulze guided the development of the Jena Experiment and of this project. This research was supported by grants from the German Science Foundation (grant no. FOR 456 - WE 2618/6-1 to W.W.W. and B.S.) and the Swiss National Science Foundation (grant no. 31–65224.01 to B.S.) and by the Friedrich-Schiller-University of Jena and the Max-Planck-Institute for Biogeochemistry, Jena.

References

Bossdorf, O., Prati, D., Auge, H., & Schmid, B. (2004) Reduced competitive ability in an invasive plant. *Ecology Letters*, 7, 346-353.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A., & MacIsaac, H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721-733.

Crawley, M.J., Brown, S.L., Heard, M.S., & Edwards, G.R. (1999) Invasion-resistance in experimental grassland communities: species richness or species identity? *Ecology Letters*, 2, 140-148.

Diaz, S. & Cabido, M. (2001) Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution*, 16, 646-655.

Diemer, M. & Schmid, B. (2001) Effects of biodiversity loss and disturbance on the survival and performance of two Ranunculus species with differing clonal architectures. *Ecography*, 24, 59-67.

Dimitrakopoulos, P.G. & Schmid, B. (2004) Biodiversity effects increase linearly with biotope space. *Ecology Letters*, 7, 574-583.

Dukes, J.S. (2001) Biodiversity and invasibility in grassland microcosms. *Oecologia*, 126, 563-568.

Dukes, J.S. (2002) Species composition and diversity affect grassland susceptibility and response to invasion. *Ecological Applications*, 12, 602-617.

Elton, C.S. (1958) The ecology of invasions by animals and plants. *Methuen, London, UK.*

Fargione, J., Brown, C.S., & Tilman, D. (2003) Community assembly and invasion: An experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 8916-8920.

Fargione, J.E. & Tilman, D. (2005) Diversity decreases invasion via both sampling and complementarity effects. *Ecology Letters*, 8, 604-611.

Garnier, E., Cortez, J., Billes, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C., & Toussaint, J.P. (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, 85, 2630-2637.

Harper, J.L. (1977) *Population biology of plants* Academic Press, London.

Hector, A., Dobson, K., Minns, A., Bazeley-White, E., & Lawton, J.H. (2001) Community diversity and invasion resistance: An experimental test in a grassland ecosystem and a review of comparable studies. *Ecological Research*, 16, 819-831.

Heemsbergen, D.A., Berg, M.P., Loreau, M., van Haj, J.R., Faber, J.H., & Verhoef, H.A. (2004) Biodiversity effects on soil processes explained by interspecific functional dissimilarity. *Science*, 306, 1019-1020.

Hierro, J.L., Maron, J.L., & Callaway, R.M. (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology*, 93, 5-15.

Hutchinson, G.E. (1957) Population Studies - Animal Ecology and Demography - Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, 22, 415-427.

Joshi, J., Matthies, D., & Schmid, B. (2000) Root hemiparasites and plant diversity in experimental grassland communities. *Journal of Ecology*, 88, 634-644.

Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R., Hector, A., Huss-Danell, K., Jumpponen, A., Minns, A., Mulder, C.P.H., Pereira, J.S., Prinz, A., Scherer-Lorenzen, M., Terry, A.C., Troumbis, A.Y., & Lawton, J.H. (2001) Local adaptation enhances performance of common plant species. *Ecology Letters*, 4, 536-544.

Kennedy, T.A., Naeem, S., Howe, K.M., Knops, J.M.H., Tilman, D., & Reich, P. (2002) Biodiversity as a barrier to ecological invasion. *Nature*, 417, 636-638.

Knops, J.M.H., Tilman, D., Haddad, N.M., Naeem, S., Mitchell, C.E., Haarstad, J., Ritchie, M.E., Howe, K.M., Reich, P.B., Siemann, E., & Groth, J. (1999) Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters*, 2, 286-293.

Levine, J.M. (2000) Species diversity and biological invasions: Relating local process to community pattern. *Science*, 288, 852-854.

Levine, J.M. & D'Antonio, C.M. (1999) Elton revisited: a review of evidence linking diversity and invasibility. *Oikos*, 87, 15-26.

MacArthur, R. (1970) Species-packing and competitive equilibrium for many species. *Theor. Popul. Biol., 1, 1-11.*

Markham, J.H. & Chanway, C.P. (1996) Measuring plant neighbour effects. *Functional Ecology*, 10, 548-549.

Maron, J.L. & Connors, P.G. (1996) A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia*, 105, 302–312.

Meiners, S.J., Cadenasso, M.L., & Pickett, S.T.A. (2004) Beyond biodiversity: individualistic controls of invasion in a self-assembled community. *Ecology Letters*, 7, 121-126.

Naeem, S., Knops, J.M.H., Tilman, D., Howe, K.M., Kennedy, T., & Gale, S. (2000) Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos*, 91, 97-108.

Payne, R.W., Baird, D.B., Gilmour, A.R., Harding, S.A., Lane, P.W., Murray, D.A., Soutar, D.M., Thompson, R., Todd, A.D., Tunicliffe Wilson, G., Webstar, R., & Welham, S.J. (2002) *GenStat Release 6.1 Reference Manual* VSN International, Wilkinson House, Jordan Hill Road, Oxford, UK.

Petchey, O.L., Hector, A., & Gaston, K.J. (2004) How do different measures of functional diversity perform? *Ecology*, 85, 847-857.

Pfisterer, A.B., Joshi, J., Schmid, B., & Fischer, M. (2004) Rapid decay of diversity-productivity relationships after invasion of experimental plant communities. *Basic and Applied Ecology*, 5, 5-14.

Prieur-Richard, A.-H., Lavorel, S., Dos Santos, A., & Grigulis, K. (2002) Mechanisms of resistance of Mediterranean annual communities to invasion by Conyza bonariensis: effects of native functional composition. *Oikos*, 99, 338-346.

Prieur-Richard, A.H., Lavorel, S., Grigulis, K., & Dos Santos, A. (2000) Plant community diversity and invasibility by exotics: invasion of Mediterranean old fields by Conyza bonariensis and Conyza canadensis. *Ecology Letters*, 3, 412-422.

Pysek, P., Jarosik, V., & Kucera, T. (2002) Patterns of invasion in temperate nature reserves. *Biological Conservation*, 104, 13-24.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W.W., & al., e. (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology*, 5, 107-121.

Roscher, C., Temperton, V.M., Scherer-Lorenzen, M., Schmitz, M., Schumacher, J., Schmid, B., Buchmann, N., Weisser, W.W., & Schulze, E.D. (2005) Overyielding in experimental grassland communities - irrespective of species pool or spatial scale. *Ecology Letters*, 8, 419-429.

Scherber, C., Milcu, A., Partsch, S., Scheu, S. & Weisser, W.W. (2006) The effects of plant diversity and insect herbivory on performance of individual plant species in experimental grassland. *Journal of Ecology*, Online Early, doi:10.1111/j.1365-2745.2006.01144.

Scherer-Lorenzen, M., Palmborg, C., Prinz, A., & Schulze, E.D. (2003) The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology*, 84, 1539-1552.

Schmid, B., Hector, A., Huston, M.A., Inchausti, P., Nijs, I., Leadley, P.W., & Tilman, D. (2002). The design and analysis of biodiversity experiments. In *Biodiversity and Ecosystem Functioning. Synthesis and Perspectives* (eds M. Loreau, S. Naeem & P. Inchausti), pp. 61-75. Oxford University Press, Oxford, UK.

Shea, K. & Chesson, P. (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution*, 17, 170-176.

Stadler, J., Trefflich, A., Klotz, S., & Brandl, R. (2000) Exotic plant species invade diversity hot spots: the alien flora of northwestern Kenya. *Ecography*, 23, 169-176.

Stohlgren, T.J., Binkley, D., Chong, G.W., Kalkhan, M.A., Schell, L.D., Bull, K.A., Otsuki, Y., Newman, G., Bashkin, M., & Son, Y. (1999) Exotic plant species invade hot spots of native plant diversity. *Ecological Monographs*, 69, 25-46.

Stohlgren, T.J., Chong, G.W., Schell, L.D., Rimar, K.A., Otsuki, Y., Lee, M., Kalkhan, M.A., & Villa, C.A. (2002) Assessing vulnerability to invasion by nonnative plant species at multiple spatial scales. *Environmental Management*, 29, 566-577.

Symstad, A.J. (2000) A test of the effects of functional group richness and composition on grassland invasibility. *Ecology*, 81, 99-109.

Tilman, D. (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology*, 78, 81-92.

Tilman, D. (1999) The ecological consequences of changes in biodiversity: A search for general principles. *Ecology*, 80, 1455-1474.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997) The influence of functional diversity and composition on ecosystem processes. *Science*, 277, 1300-1302.

Troumbis, A.Y., Galanidis, A., & Kokkoris, G.D. (2002) Components of short-term invasibility in experimental Mediterranean grasslands. *Oikos*, 98, 239-250.

Turnbull, L.A., Rahm, S., Baudois, O., Eichenberger-Glinz, S., Wacker, L., & Schmid, B. (2005) Experimental invasion by legumes reveals nonrandom assembly rules in grassland communities. *Journal of Ecology*, 93, 1062-1070.

Vila, M. & Weiner, J. (2004) Are invasive plant species better competitors than native plant species? evidence from pair-wise experiments. *Oikos*, 105, 229-238.

Vitousek, P.M. & Howarth, R.W. (1991) Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13, 87-115.

Xu, K., Ye, W., Cao, H., Deng, X., Yang, Q., & Y., Z. (2004) The role of diversity and functional traits of species in community invasibility. *Botanical Bulletin of Academia Sinica*, 45, 149 - 157.

Yelenik, S.G., Stock, W.D., & Richardson, D.M. (2004) Ecosystem Level Impacts of Invasive *Acacia saligna* in the South African Fynbos. *Restoration Ecology*, 12, 44-51.

Ecology, in preparation.

Running head: Selective herbivory, perturbation, and plant diversity

Chapter 6

Selective herbivory affects stability and composition of plant communities, irrespective of plant species richness

Christoph Scherber[1], Juliane Specht[1], Günter Köhler[1], Nadine Mitschunas[2] & Wolfgang W. Weisser[1]

[1]Institute of Ecology, University of Jena, Dornburger Str. 159, D-07743 Jena; Phone: ++49 3641 949406; Fax: ++49 3641 949402; Email: Christoph.Scherber@web.de; [2]Department of General and Theoretical Ecology, Centre for Environmental Research and -Technology, University of Bremen, Leobener Str., 28359 Bremen, Germany

Manuscript type: Article

Abstract

While theoretical studies on the relationship between biodiversity and stability have a long-standing tradition in ecology, field experiments have so far been scarce. Here, we used a plant-herbivore system to study the effects of selective herbivory on resistance and resilience of vegetation parameters in 81 experimental grassland communities. These communities contained 1-60 plant species of 1-4 functional groups experimentally established from seed in 2002. In 2004, we introduced each 10 male and 10 female subadult individuals of the grasshopper *Chorthippus parallelus* into eighty-one 1 m tall cylindrical cages with 0.5 m diameter. The cages were placed on top of the vegetation in each plot, leaving another 81 control cages empty. The herbivory treatment lasted over a period of >50 days, and system resistance and resilience were measured over a 1-year period. Resistance and resilience were calculated as differences between herbivory and control cages, log response ratios, and relative interaction indices.

Grasshoppers consumed about 30% of aboveground biomass. Total vegetation biomass resistance and resilience were mainly influenced by grasshopper survival and grass presence, but independent of plant species richness and number of functional groups. Grass biomass was more severely affected in species-poor than species-rich communities, with grass monocultures experiencing about 60% herbivory. After one year, herb abundance had increased significantly as a response to the decline in grass abundance. We conclude that selective herbivory changes the functional composition of plant communities, leading to decreased monoculture performance. Species identity rather than species richness determine the overall resistance and resilience of plant communities under intense herbivory.

Keywords

biodiversity, resilience, stability, resistance

Introduction

Ecosystems may respond differently to perturbations, depending on the type, intensity and duration of the perturbation (O´Neill 1999), but also depending on ecosystem properties, such as biodiversity (McCann 2000). There has been an intensive debate about the relationship between ´biodiversity´ and ´stability´ (reviewed, e.g., in Holling 1973; Goodman 1975; Schmitz 1997; McCann 2000; Loreau *et al.* 2002; Hooper *et al.* 2005; Thebault & Loreau 2005). Because current regional and global rates of species extinctions are unprecedented in geological history (Thomas *et al.* 2004), the scientific community needs to address if and how changes in diversity affect an ecosystem´s response towards external disturbances. And because the diversity-stability issue is important both from a theoretical (May 1973) and an applied (Murdoch 1975; Andow 1991) point of view, experimental tests need to be performed in ways that allow a separation of true effects of species richness from other confounding factors covarying with diversity (Wardle & Grime 2003; Hooper *et al.* 2005). This has so far only rarely been possible under field conditions (but see Pfisterer & Schmid 2002; Caldeira *et al.* 2005); yet, even the work by Pfisterer & Schmid (which found a positive relationship between diversity and stability) has been criticized for not having accounted for these confounding factors (Wardle & Grime 2003), and Hooper *et al.* (2005) even write that "[t]heoretical work on stability has outpaced experimental work, especially field research."

While responses of one trophic level to changes in species richness under different disturbance treatments have been frequently studied (e.g. Tilman & Downing 1994; Pfisterer & Schmid 2002), there is still only limited knowledge about how multitrophic systems react towards external disturbances across a wide range of species richness, i.e., where plant species richness is an explanatory variable (Schläpfer & Schmid 1999; Schmitz *et al.* 2000). Thébault & Loreau (2006) state that these aspects deserved to be analysed experimentally to gain better knowledge of the impacts of biodiversity changes on ecosystem functioning in multitrophic systems.

In the experiment presented here, we study diversity-stability relationships in a plant-herbivore system using experimental grassland plant communities where both the species richness of terrestrial plants and their functional identity are varied as independently as possible. We use an insect herbivore preferentially feeding on one of four functional groups (FG) present in the system, and study its effects on plant productivity and species composition. We thus set a system-specific perturbation by simulating the outbreak of an insect herbivore (Pimentel 1961) in grassland, where plant species richness is varied experimentally. The experimental factors we vary are (1) +/- addition of an insect herbivore (2) number of plant species; (3) number of FG; and (4) identity of plant FG. We measure aboveground biomass, vegetation cover and height before, during and after a ´press´ perturbation (Schmitz 1997) of ≤55 days duration. This allows us to simultaneously assess system resistance and resilience, as well as plant compositional changes induced by selective herbivory (e.g. Bach 2001).

In contrast to many previous studies, the ´disturbance´ treatment imposed by us is itself expected to change in its intensity across the gradient in plant species richness, e.g. due to changed rates of consumption or survival in the insect herbivore used. Thus, the interactions involved may be considerably complex, and we offer a detailed theoretical analysis of several null models, both excluding or including herbivore survival, proportional herbivory, or constant herbivory (Appendix D). We compare the outputs from these models with our experimental findings, and in such a way aim for a suitable explanation for the patterns we find.

We hypothesize that (1) Herbivore survival will be positively correlated with plant species richness; (2) Herbivore selectivity will lead to a change in plant functional group composition; (3) Plots with low species richness will be less resistant and recover more slowly from selective herbivory than species-rich plots; thus, stability will increase with plant species richness.

Material and Methods

Study organism

In terms of biomass turnover, Orthopterans are the most important group of phytophagous insects in temperate grasslands (e.g. van Hook 1971; Mitchell & Pfadt 1974; Köhler *et al.* 1987) and include some of the most voracious pests (Lockwood 1998). The meadow grasshopper, *Chorthippus parallelus* ZETT (Orthoptera: Acrididae), is one of the most abundant and widespread grasshopper species in Central Europe (Ingrisch & Köhler 1998), inhabiting mesic grasslands and predominantly feeding on grasses and, to a lesser extent, on forbs (e.g. Gangwere 1961; Bernays & Chapman 1970a, b; Ingrisch & Köhler 1998). *C. parallelus* is univoltine and passes through four nymphal stages. Adults occur at the field site mainly between July and August.

C. parallelus was chosen because: (1) its preference for grasses makes it suitable for a study on the effect of selective herbivory on plant communities; (2) its biology is well known (e.g. Richards & Waloff 1954; Bernays & Chapman 1970a; Reinhardt & Köhler 1999); (3) it is the most abundant grasshopper species on meadows around the field site (Pratsch 2004), and an important chewing invertebrate herbivore in terms of the amount of plant biomass removed (cf. Köhler *et al.* 1987).

General experimental design

The experiment was installed in spring 2001 on former arable land and consisted of 82 plots 20x20 m in size, allocated to four blocks in a randomized complete block design (Plate 1A; Roscher *et al.* 2004). Each plot was seeded in May 2002 with 1, 2, 4, 8, 16 or 60 plant species from a combination of 1-4 plant functional groups. Species for each plot were drawn from a pool of 60 plant species of Central European *Arrhenatherum* meadows using randomization constrained on block and functional group identity. Functional groups were defined a priori using cluster analysis of a trait matrix. Thus, each mixture contained either grasses, legumes, small herbs, tall herbs, or possible combinations of these (1-4 FG). Presence of each group was coded as 0=absent, 1=present. For details see Roscher *et al.* (2004) and Scherber *et al.* (2006). Plots are mown (June, September) and weeded (April, July) every year to maintain species composition. A 2 x 4 m subplot within each 20 x 20 m plot was selected at random along the West-East axis of each plot to set up the grasshopper cages as described below.

Installation of cages and addition of grasshoppers

Between 14 and 25 June 2004, we installed each two cylindrical cages per 2 x 4 m subplot at a distance of 1.4 m from each other. In total, there were 162 cages on 81 plots; a plot with a monoculture of *Bellis perennis* L. (Asteraceae) was excluded due to its plant density being too low. Each cage was 1 m in height and 0.5 m in diameter and consisted of a drum-shaped galvanized aluminium frame welded from 8 mm-diameter rods, covered with 2-mm aluminium mesh (Plate 1B-F). Cages had a 12-cm aluminium sheet metal base of 3-mm thickness that was sunk in soil, to which the aluminium mesh was strapped using polypropylene strapping (Rajapack, Birkenfeld, Germany). One of the two cages per subplot was selected at random to serve as the herbivory cage, the other as a control cage without grasshoppers. 7-9 days before adding the grasshoppers, we removed all other invertebrates from both cages, using a 1400-W vacuum cleaner (Kärcher A2801 plus, Alfred Kärcher GmbH, Winnenden, Germany). At the same time, we measured mean vegetation height to the nearest cm using a metering rule, and visually estimated grass, legume, and total vegetation cover, as a measure of the initial conditions of the experiment.

Between 6-15 July 2004, about 1,600 fourth-instar nymphs of *C. parallelus* were caught from three *Arrhenatherum* meadows in the vicinity of the field site using sweep nets. The nymphs were separated by sex, weighed, and transferred to the herbivory cages block-wise in groups of each five males and five female nymphs. Every herbivory cage received a total of 10 male and 10 female nymphs. For every block, two days were needed until all cages had received grasshoppers. Grasshopper initial weights did not differ significantly between the diversity treatments.

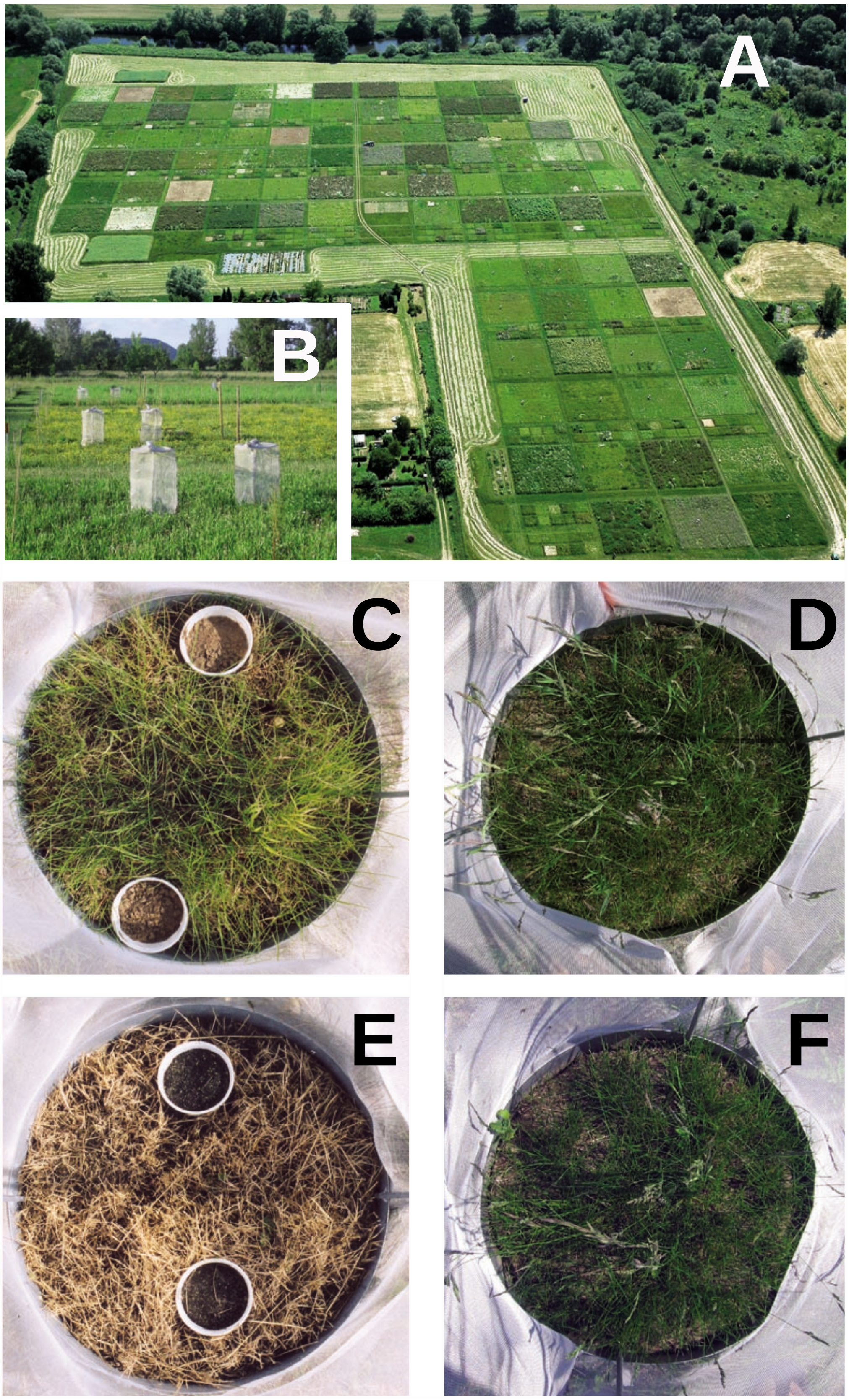


PLATE 1 (A) Aerial view of the Jena Experiment, 14 June 2006 © The Jena Experiment; (B) Overview of three of the 81 plots with two cages each, © C.Scherber, 4 June 2004; (C,E) Effects of grasshopper herbivory on a monoculture of *Festuca rubra* in August 2004 (D,F) Recovery of the monoculture in May 2005. (C) and (D) show control cages, (E) and (F) show herbivory cages. The white pots in C and E were used to measure deposition of oothecae in another experiment.

Measurement of plant parameters

The caging experiment had three different stages (Appendix A); in stage 1, we measured initial conditions before adding the grasshoppers (´reference state´); in stage 2, we measured the conditions while grasshoppers were feeding, or shortly after their removal from the cages; and in stage 3 we measured the conditions one year after grasshopper addition to the cages.

Total vegetation cover and relative cover of grasses and legumes was visually estimated at all stages using an integer cover scale. Percentage cover of every plant species was estimated in the same way, but this was only done in stages 2 and 3. Mean vegetation height and mean height of grasses and legumes was measured using a metering rule.

The initial above-ground plant community biomass was harvested between 27 May and 10 June 2004 at 3 cm above ground using two aluminium frames of 20x50 cm diameter at the positions on which the cages were installed later. The samples were oven-dried at 70° C for 48 hours and weighed. To give a representative estimate, these initial biomass values were summed for every plot, and initial herbivory and control cage biomasses were assumed equal ($H_1=C_1$, see below).

Plant community biomass at all later stages of the experiment was measured by harvesting all living plant material inside each cage 1 cm above ground. The material was sorted into species, dried for 48h at 70°C, and weighed using balances (Mettler-Toledo PB 303 S, Mettler-Toledo Ltd., Leicester, UK, and Kern 470-36, Gottlieb Kern & Son GmbH, Balingen-Frommern, Germany).

Measurement of grasshopper parameters

Grasshopper nymphs (4th instar) were weighed in groups of each five individuals, separately for each sex, using a portable balance (Sac 51, Scaltec Instruments GmbH, Heiligenstadt, Germany). The survival of the grasshoppers was assessed every two days between 09 July - 01 September 2004 by counting the number of female and male individuals alive per cage and correcting for individuals overlooked at previous observation times. In this manuscript, we use ´grasshopper days´ (GD) as a measure of survival, calculated as the cumulative survival (in days) of all individuals per cage, irrespective of sex (see the ´mantid-days´ in Fagan *et al.* 2002 for comparison). GD were linearly correlated with Kaplan-Meier estimates of survival ($\rho=0.897$, $P<0.001$), but a better predictor of herbivore damage. A more detailed analysis of data on grasshopper survival and fecundity will be presented elsewhere.

Calculation of stability properties

There are two basic ways in which resistance (*sensu* Schläpfer & Schmid 1999) and resilience (sensu Grimm & Wissel 1997) can be calculated. First, biomass differences between herbivory and control cages can be computed separately for each stage of the experiment. Second, the biomass differences between each two stages of the experiment can be computed, for example "before" and "after" herbivore addition. Note that all measurements conducted in this study are discrete-time and plant compensation is not explicitly accounted for. We define H_i and C_i (i=1,2,3) as the vegetation biomass in herbivory vs. control cage at stages i of the experiment; further, R_i shall denote biomass differences between herbivory and control cage biomass (H_i, C_i) at stage i of the experiment, where δ_i is used as an estimate of biomass consumption, equalling $-R_i$ for all $R_i<0$. R_{21} and R_{31} are biomass differences in the herbivory cages between stages 2 and 1, or 3 and 1, respectively. Log-response ratios (LRR_i) have frequently been used by other authors in diversity-stability studies (e.g. Tilman & Downing 1994; Pfisterer & Schmid 2002; Zhang & Zhang 2006) as a comparatively unbiased index of experimental effect with an approximately normal sampling distribution (Hedges *et al* 1999). We also calculate relative interaction indices (RII_i), as suggested in a recent publication by Armas *et al.* (2004). Both LRR_i and RII_i are negative when herbivory cages have lower biomass than control cages, i.e. when herbivores have consumed parts of the biomass in the herbivory cages. When referring to grass, legume or herb biomass instead of total vegetation biomass, we use superscripts G, L and H, e.g. R_2^G. Resistance and resilience at stage 1 are assumed to be 0 and $H_1 = C_1$. Using the definitions given above, we calculate resistance (1a-1e) and resilience (2a-2e) as follows:

Resistance:

(1a) $R_2=H_2-C_2=-\delta_2$

(1b) $R_{21}=H_2-H_1$

(1c) $LRR_2=\ln(H_2)-\ln(C_2)=\ln\frac{H_2}{C_2}$

(1d) $LRR_{21}=\ln(H_2-H_1)-\ln(C_2-C_1)=\ln\frac{H_2-H_1}{C_2-C_1}$

(1e) $RII_2=\frac{H_2-C_2}{H_2+C_2}$

Resilience:

(2a) $R_3=H_3-C_3=-\delta_3$

(2b) $R_{31}=H_3-H_1$

(2c) $LRR_3=\ln(H_3)-\ln(C_3)=\ln\frac{H_3}{C_3}$

(2d) $LRR_{31}=\ln(H_3-H_1)-\ln(C_3-C_1)=\ln\frac{H_3-H_1}{C_3-C_1}$

(2e) $RII_3=\frac{H_3-C_3}{H_3+C_3}$

Statistical analysis

Data analysis was carried out using R 2.3.0 (R Development Core Team 2006). Data for each response variable (e.g. vegetation biomass in herbivory and control cages) were always analysed in a two-step procedure: First, we calculated R_n, LRR_n and RII_n for each pair of cages per plot. We then analysed these variables using linear analysis of covariance models. Using differences rather than absolute values is necessary because both samples per plot are *paired* samples, and absolute comparisons between herbivory and control cages would possibly overestimate effect sizes. This type of analysis is also more parsimonious than using nested models, and allows model simplification. We started by fitting a maximal model in the following sequence:

y~Block+GD+Logdiv+Logdiv²+Funcgr+Grass+Leg+Block:Grass+Block:GD+GD:Grass+GD:Logdiv+GD:Logdiv²+GD:Funcgr+Grass:Leg,

where y is the response variable, Logdiv is log-linear species richness, and Grass and Leg are binary variables indicating grass or legume presence. Small and tall herb presence was not included because they were assumed to have minor influence on herbivory (Ingrisch & Köhler 1998). GD was always used as a primary covariate, but not included into models for stage 1 or where GD itself was the response variable. When functional group-specific variables were analysed (e.g. grass biomass), we restricted the datasets to those plots only containing at least one species of the respective functional group (e.g. Grass>0), removing all terms containing that functional group in the model. Starting from the maximal model, we sequentially deleted non-significant terms from it, starting with highest-order interactions, and compared each resulting model with its predecessor using AIC (Burnham & Anderson 1998) and conditional F-tests (Crawley 2002). Model simplification was followed until no further deletions of terms were possible, i.e. models differed significantly. In addition, we constructed null models (only consisting of the intercept) to test whether the differences between herbivory and control cages in the response variables were significantly different from zero (using student´s t tests), i.e. whether the herbivory treatment itself lead to significant increases or decreases in total plant community parameters or functional group abundances. For each model, we inspected the residuals for constant mean function, constant variance function and normality, and removed outliers as indicated by leverage and Cook´s distance (Weisberg 2005). Additional methods used for calculation of expected herbivory under different theoretical scenarios are described in Appendix D.

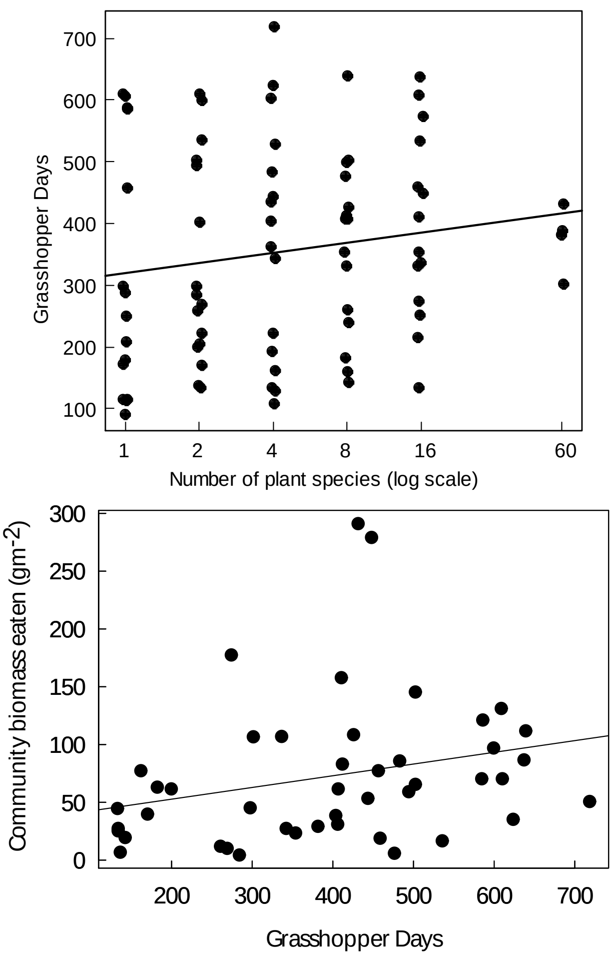


FIG. 1 Grasshopper survival. (A) Relationship between log-linear number of plant species and grasshopper survival (expressed as GD); R^2=0.02 (B) Relationship between GD and the biomass differences between herbivory and control cages in August 2004; R^2=0.10

Results

Initial conditions prior to the start of the experiment

Before the grasshoppers were added to the cages, mean vegetation height across all 162 cages was approximately 13±0.5 cm, and initial aboveground plant community biomass averaged 498.7 ± 25.8 g m^{-2}. Total vegetation cover, grass, legume and herb cover averaged 60±2%, 20±2%, 11±2%, and 29±2%, respectively. Vegetation cover was always higher when grasses were present than without grasses ($F_{1,72}$=7.48, P=0.007); this means that grass presence was associated with *higher* vegetation cover before grasshoppers were added to the cages. Consequently, if vegetation cover in later stages of the experiment is lower, then this effect is likely to have been caused by grasshopper feeding.

Grass cover did not differ significantly between herbivory and control cages, but there were significant differences between blocks ($F_{3,40}$=3.82, P=0.017). When both grasses and legumes were present, herb cover was lower in the herbivory cages than in the control cages. This means, there was a significant interaction between legume and grass presence on herb cover ($F_{1,58}$=8.26, P=0.006). Neither legume cover nor mean vegetation height were significantly affected by any of the explanatory variables.

Herbivore survival and amount of herbivore damage

Plant species richness *per se* had no significant effect on survival of *C. parallelus* (in terms of *GD*) (Fig. 1a), but survival increased significantly with number of FG ($F_{1,74}$=7.17, P=0.009). The most important factor that significantly increased herbivore survival was presence of grasses ($F_{1,74}$=37.77, P<0.001). Presence of legumes decreased survival ($F_{1,74}$=4.56, P=0.036), and there was a significant difference between blocks ($F_{3,74}$=5.06, P=0.003).

With increasing grasshopper survival, the amount of biomass consumed in stage 2 (for plots with δ>0) increased significantly (Fig. 1b; intercept: 18.17±29.66 gm^{-2}, slope = 0.15±0.06 $gm^{-2}d^{-1}$, $F_{1,44}$=5.02, P=0.03; R^2=0.10), and total herbivory was about 26.7%. Survival-independent herbivory values are calculated in Appendix D.

Resistance of vegetation biomass

The biomass across all 182 cages at stage 2 of the experiment averaged 214.9±12.5 g m^{-2}. The proportion of total biomass consumed by the grasshoppers averaged 26.7 % (N=46, based on geometric means, excluding plots with higher biomass values in herbivory than control cages). R_2 across all plots (N=81) averaged -3.41±14.31 g m^{-2} (about 1.9% of H_2). With increasing grasshopper survival, significantly more biomass was consumed, but independently of log-linear plant species richness, grass or legume presence (Table 1), although some grass monocultures experienced severe herbivore damage (Plate 1 C, E). LRR_2 decreased significantly with increasing grasshopper survival, and number of FG (Appendix B).

Table 1 Analysis of covariance table showing effects of the explanatory variables listed in the left column on differences in vegetation biomass, log-response ratios, and relative interaction indices.

	Resistance, Vegetation biomass									
	R_2		LRR_2		RII_2		R_{21}		LRR_{21}	
Explanatory Variables	F	P	F	P	F	P	F	P	F	P
Block	Excl.	Excl.	Excl.	Excl.	Excl.	Excl.	5.11	**0.003**	Excl.	Excl.
GD	6.72	**0.011**	19.61	**<0.001**	22.32	**<0.001**	10.68	**0.001**	7.62	**0.007**
Diversity	1.62	0.207	0.45	0.504	0.001	0.975	2.17	0.145	0.49	0.485
Diversity (q)	Excl.	Excl.	Excl.	Excl.	Excl.	Excl.	4.03	**0.048**	Excl.	Excl.
Funct. Groups	Excl.	Excl.	4.62	**0.035**	5.17	**0.025**	Excl.	Excl.	Excl.	Excl.
Grasses	1.66	0.200	10.36	**0.002**	10.85	**0.001**	9.02	**0.003**	4.72	**0.033**
Legumes	3.54	0.063	Excl.	Excl.	Excl.	Excl.	0.88	0.350	6.29	**0.014**
Block:Grasses	Excl.	Excl.	Excl.	Excl.	Excl.	Excl.	3.15	**0.030**	Excl.	Excl.
GD:Grasses	2.77	0.099	3.18	0.078	3.98	**0.049**	Excl.	Excl.	Excl.	Excl.
GD:S	Excl.	Excl.	4.22	**0.043**	Excl.	Excl.	0.98	0.323	3.66	0.059
GD:S (q)	Excl.	Excl.	Excl.	Excl.	Excl.	Excl.	1.26	0.265	Excl.	Excl.
GD:Funct. Groups	Excl.	Excl.	Excl.	Excl.	3.85	0.053	Excl.	Excl.	Excl.	Excl.
Grasses:Legumes	Excl.	Excl.	Excl.	Excl.	Excl.	Excl.	2.06	0.156	3.11	0.082
Residual D.f.	75		74		74		66		67	

Notes: Significant P-values are indicated by bold font. “Excl.” indicates that the respective term was excluded during model simplification.

Thus, herbivore effects on the plant community were stronger when grasshoppers lived longer or when more plant functional groups were present in the system.When grasses were present (absent), LRR_2 was 0.41±0.13 (-0.11±0.07), and there was a slightly significant interaction between GD and log-linear plant species richness (Table 1). Similarly to LRR_2, RII_2 decreased significantly with increasing GD (Table 1), and decreased significantly with increasing number of FG (Table 1, Appendix B). When grasses were present (absent), RII_2 was -0.16±0.04 (0.05±0.03; Table 1). This means, herbivore effects on the plant communities were stronger when grasses were present, indicating that grasses were the preferred food plants for the grasshoppers.

With RII_2, as the response variable, there was a slightly significant interaction between GD and grass presence: The relationship had a negative slope when grasses were present, and had a slope of about zero in absence of grasses. This, again, shows that herbivore effects on the plant community were stronger when grasses were present.

Resistance, expressed as R_{21}, differed significantly between blocks. High grasshopper survival was associated with lower values of R_{21}, indicating that biomass decline from stage 1 to stage 2 of the experiment was more pronounced when grasshoppers survived longer (Table 1). The overall mean of R_{21} was -242.98±24.42 g m^{-2}. The relationship between log-plant species richness and R_{21} was U-shaped, with highest values of R_{21} in monocultures and 60-species mixtures, indicated by a significant quadratic term of log-species richness in the model (Table 1). When grasses were present (absent), R_{21} was -330.45±27.34 g m^{-2} (-138.92±35.87 g m $^{-2}$; Table 1), and there was a significant interaction between blocks and grasses (Table 1).

Resistance, expressed as LRR_{21}, was highly significantly correlated with GD: With increasing number of GD, LRR_{21} significantly decreased (Table 1), indicating that vegetation biomass in herbivory cages grew more slowly than vegetation biomass in control cages when grasshopper survival was high. When grasses were present (absent), LRR_{21} was -0.22±0.07 (0.28±0.14; Table 1). For legumes, the relationship was the opposite (0.19±0.13 vs.-0.17±0.08).

Resilience of vegetation biomass

The biomass across all 182 cages at stage 3 of the experiment averaged 347.2±21.9 g m^{-2}. For all plots with lower biomass in herbivory than in control cages, biomass in herbivory cages was about 115.2 g m^{-2} lower than biomass in control cages (N=42, geometric mean), i.e. there was still a difference of about 30% in total vegetation biomass between the two cages, even one year after the start

Table 2 Analysis of covariance table showing effects of the explanatory variables listed in the left column on differences in vegetation biomass, log-response ratios, and relative interaction indices.

	Resilience, Vegetation biomass					
	LRR_3§		RII_3		R_{31}†	
Explanatory Variables	F	P	F	P	F	P
Block	Excl.	Excl.	Excl.	Excl.	2.52	0.065
GD	7.93	**0.006**	7.39	**0.008**	4.51	**0.037**
Diversity	Excl.	Excl.	Excl.	Excl.	0.19	0.664
Diversity (q)	Excl.	Excl.	Excl.	Excl.	3.23	0.076
Grasses	Excl.	Excl.	Excl.	Excl.	12.25	**<0.001**
Legumes	Excl.	Excl.	Excl.	Excl.	12.81	**<0.001**
GD:Block	Excl.	Excl.	Excl.	Excl.	4.18	**0.009**
Residual D.f.	78		78		69	

Notes: §For LRR_{31}, the minimal adequate model was the null model. †For R_3, GD was marginally significant, and retained in the minimal adequate model ($F_{1,78}$=3.44, P=0.067). Significant P-values are indicated by bold font. "Excl." indicates that the respective term was excluded during model simplification

of the experiment. Yet, when all plots were taken into account (N=81), R_3 was slightly positive and averaged 2.9 g m^{-2}, but with a high standard error of 22.2 g m^{-2}. Though positive, R_3 was not significantly different from zero (t=0.247, d.f.=77, P= 0.805) and was also not significantly influenced by any of the other explanatory variables. Thus, in terms of absolute biomass differences, herbivory effects were not as pronounced as in stage 2. Even severely affected grass monocultures recovered remarkably well (Plate 1 D, F).

LRR_3 decreased highly significantly with increasing GD (Table 2). This means, although the non-transformed differences, R_3, were not significantly influenced by GD, there was still a significant influence of grasshopper feeding on the linearized and normalized metric provided by the use of log-response ratios.The same was true for RII_3, which was also highly significantly negatively correlated with GD, even one year after the start of the experiment (Table 2).

Herbivory cages in stage 3 had about 21.1±5.2 g m^{-2} lower vegetation biomass than in stage 1 (i.e., R_{31} was negative), and this effect became stronger with increasing grasshopper survival, indicating that longer grasshopper survival had long-term effects on the resilience of the system. When grasses were present (absent), R_{31} was -35.53±5.64 g m^{-2} (-3.95±8.34 g m^{-2}; Table 2). The opposite was true for legume presence (-5.38±7.36 g m^{-2} vs. -38.90±6.11 g m^{-2}; Table 2). There was also a highly significant interaction between GD and block (Table 2); in all blocks except block 1, the relationship had a negative slope.

Differences in plant community height induced by herbivory

Mean plant community height in stage 1 (i.e. a few weeks after mowing), stage 2 and stage 3 was 13.1±0.4 cm, 28.6 ±1.6 cm and 39.6±1.7 cm, respectively. Before the grasshoppers were added, vegetation in herbivory cages was about 0.2±0.4 cm shorter than in control cages, but this difference was not significant. Looking at plots containing at least one grass species, herbivory cages had a higher mean vegetation height than control cages before the addition of grasshoppers (0.1±0.5 cm; not significantly different from zero).

In stage 2, plant community height was clearly affected by grasshopper herbivory, and vegetation was about 4.01±1.05 cm shorter in herbivory than in control cages (t=-3.86, P<0.001, d.f.=80). There was a slight but non significant recovery in stage 3, where herbivory cages had only 1.57±1.21 cm shorter vegetation than control cages. The effects of grasshopper herbivory were even more pronounced when looking at grass instead of plant community height differences in stage 2 (-

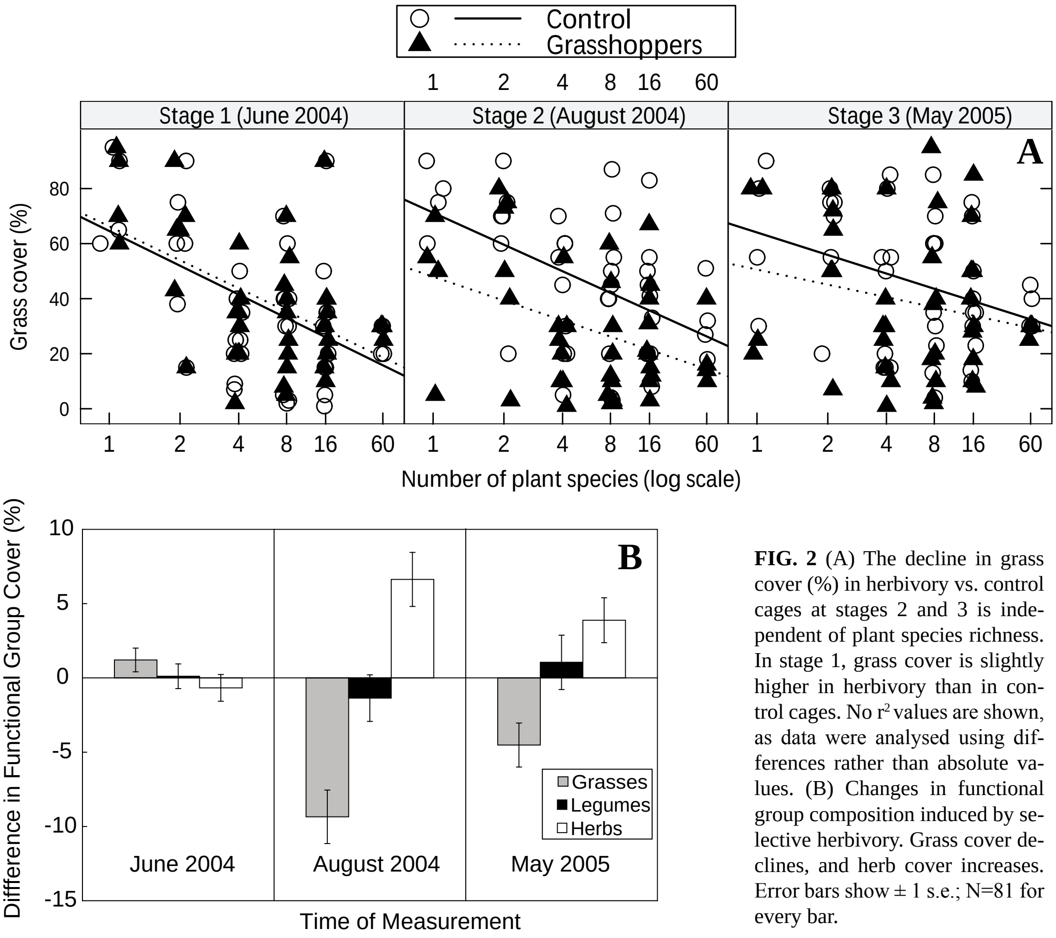


FIG. 2 (A) The decline in grass cover (%) in herbivory vs. control cages at stages 2 and 3 is independent of plant species richness. In stage 1, grass cover is slightly higher in herbivory than in control cages. No r^2 values are shown, as data were analysed using differences rather than absolute values. (B) Changes in functional group composition induced by selective herbivory. Grass cover declines, and herb cover increases. Error bars show ± 1 s.e.; N=81 for every bar.

5.65±1.01 cm; t=-6.85,P<0.001,d.f.=43) and stage 3 (-4.05±1.12 cm; t=-3.86, P<0.001,d.f.=43). This means, both plant community and grass height in herbivory cages were still smaller than in the control cages, even one year after the start of the experiment.

The higher the grasshoppers´ survival, the greater was the difference in height between herbivory and control cages, i.e. vegetation was shorter under intense grasshopper herbivory,

both in stage 2 ($F_{1,76}$=8.84, P=0.004, Appendix C) and stage 3 ($F_{1,69}$=4.88, P=0.030, Appendix C). In addition to the effects of grasshopper survival, total plant community height in stage 3 was also significantly affected by the quadratic term of log-linear plant species richness ($F_{1,69}$=5.06, P=0.028), and by a GD: Block interaction ($F_{3,69}$=3.99, P=0.011). Grass height in stage 2 was significantly affected by the quadratic term of log-linear plant species richness ($F_{1,38}$=6.60, P=0.014; Appendix C). In stage 3, there was a significant GD:block interaction effect on grass height ($F_{3,36}$=3.36, P=0.029). All other terms in the models were not significant.

Differences in functional group composition induced by herbivory

In stage 2, total vegetation cover was about 4±2 percent lower in herbivory than in control cages (t=-2.05, P=0.043, d.f.=80), and there was a significant interaction between grasshopper survival (GD) and block on total cover ($F_{3,66}$=3.15, P=0.031). When grasses were present (absent) in the plant community, the differences in total cover averaged -7.7±2.8% (0.22±2.66%). This means that when grasses were present, herbivory effects were more pronounced than without grasses. While grass cover strongly and significantly decreased as a consequence of selective herbivory (t=-6.09, P<0.001, d.f.=43; Fig. 2a, b), herb cover increased significantly (t=3.72, P<0.001, d.f.=63; Fig. 2b), and this increase was highly positively correlated with GD ($F_{1,60}$=4.40, P=0.040; Fig. 3a). Thus, the relative loss in terms of grass cover was compensated by another functional group, depending on the number of grasshoppers present, and on their survival.

In stage 3, total cover was 0.4±1.8% higher in herbivory than in control cages, while grass cover was still significantly negatively affected (t=-3.20, P=0.002, d.f.=43; Fig. 2a,b). Total cover was significantly affected by an interaction between GD and number of FG ($F_{3,76}$=4.56 P=0.036). Herb cover was still higher in herbivory than in control cages (t=2.60, P=0.011, d.f.=63) and highly positively correlated with GD (F=$_{1,49}$=5.57 P=0.022; Fig. 3b). When grasses were present in the plant community ($F_{1,49}$=5.02, P=0.029), the herbivory cages had 7.8±2 % higher herb cover than the control cages, while herb cover in absence of grasses was 0.6±2% higher in herbivory than control cages. Thus, grasshopper feeding not only had a negative influence on grass cover, but also lead to a compensatory increase in herb cover.

Herb cover was also significantly affected by an interaction between GD and block ($F_{3,49}$=4.12, P=0.010) and between GD and number of FG ($F_{3,49}$=8.52, P=0.005).

The differences in terms of functional group cover induced by herbivory were also evident when looking at biomass values per functional group: plots containing grasses, grasshoppers removed about 67.1% of control grass biomass at stage 2. While grass biomass in herbivory cages was significantly lower than in control cages at stage 2 (t=-6.49, P<0.001, d.f.=43; Fig. 4), dependent on GD ($F_{1,37}$=9.00, P=0.005) and plant species richness ($F_{1,37}$=7.54, P=0.009; Fig. 4), herb biomass was significantly higher in herbivory than in control cages (t=2.67, P=0.009, d.f.=63), and, in addition, increased significantly with plant species richness ($F_{1,60}$=11.09, P=0.001) and number of FG ($F_{3,60}$=6.27, P=0.015).

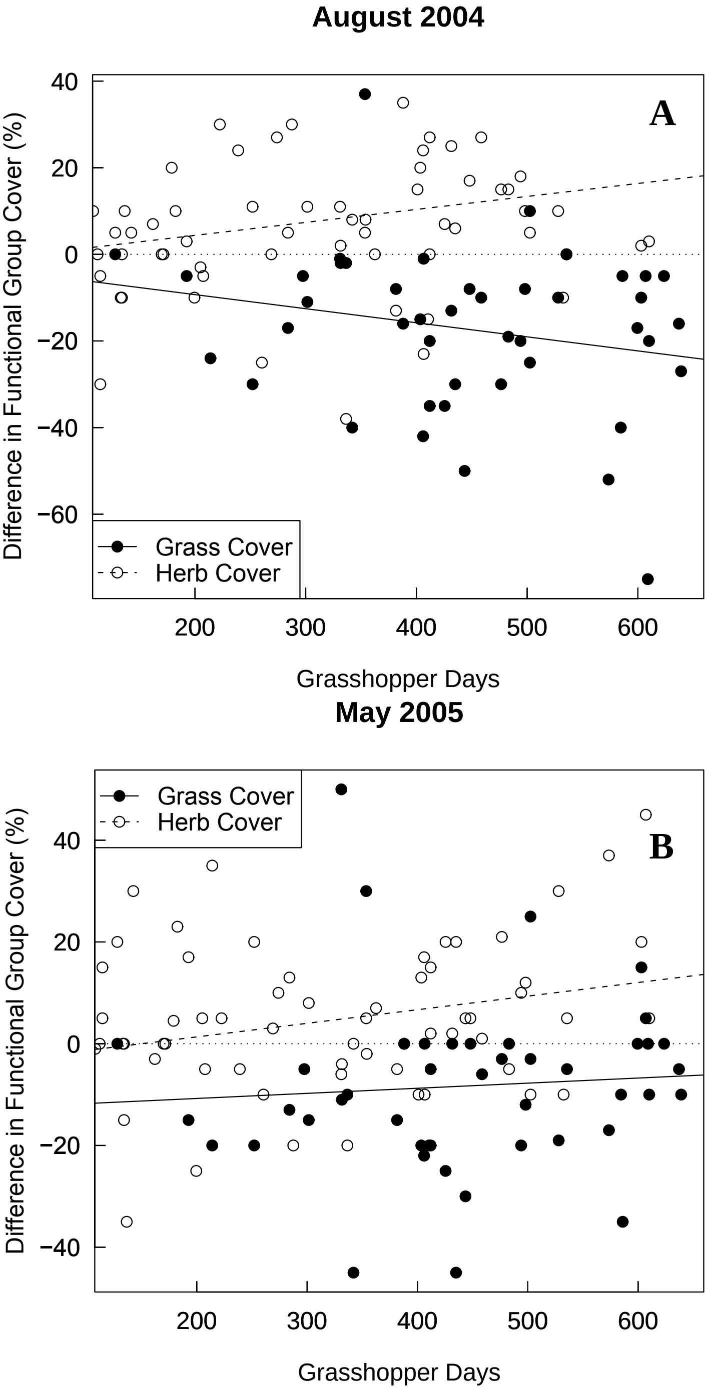


FIG. 3 Relationship between grass and herb cover, and grasshopper survival (GD) in (A) August 2004 and (B) May 2005. Lines show mean squares fits. R^2 values are 0.05 vs. 0.06 for grass- and herb cover in 2004, and 0.005 vs. 0.07 for grass- and herb cover in 2005.

In stage 3, for plots containing grasses, herbivory cages had still 47.2% lower grass biomass than control cages. Overall, grass biomass was still significantly lower on herbivory than control cages (t=-2.87, P=0.006, d.f.=43; Fig. 4), and herb biomass had significantly increased (t=2.72, P=0.008, d.f.=62). Legume biomass in stage 2 and 3 was significantly higher (lower) in herbivory than in control cages when plant species richness was low (high) ($F_{1,38}$=7.98, P=0.007 for August 2004, and $F_{1,32}$=4.79, P=0.036 for May 2005).

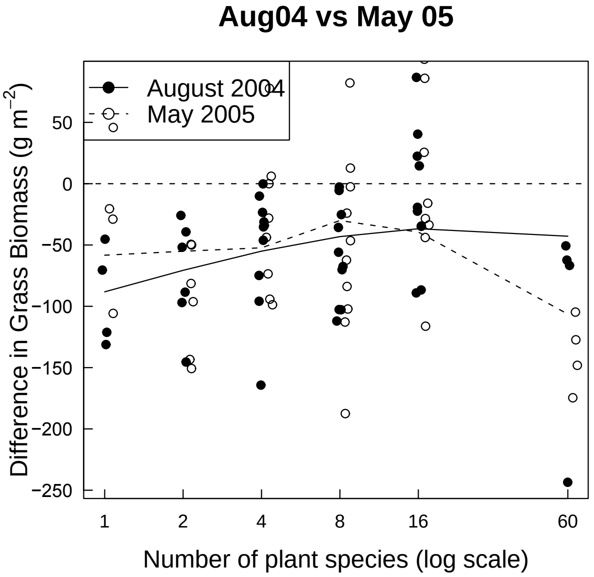


FIG. 4 Grass Biomass Differences August 2004 vs May 2005. Points are jittered along the X axis by a factor of 0.2; Lines were created using a LOWESS scatterplot smoother (Cleveland 1981) with span 0.8.

Modelling herbivore effects with and without survival

A detailed description and results from these models can be found in Appendix D.

Discussion

The results presented in this manuscript clearly show that, at least in terms of total vegetation biomass, resistance and resilience are independent of plant species richness. Several authors (reviewed by Loreau *et al*. 2002; see, e.g., Wardle *et al.* 2000) have so far come to similar conclusions, but the design of previous experiments with terrestrial herbaceous plant communities has often not been specifically designed to separate species richness effects from other components of diversity (Spehn *et al*. 2005). For example, Pfisterer *et al.* (2003), who used an experimental approach comparable to our study, did not take into account the effects of grass presence (because grasses were present in almost all mixtures, see Wardle & Grime 2003), and they also did not analyse interactions between herbivore load and their explanatory variables. Hence, they tended to focus on the main effects of plant species richness in the discussion of their results, although species richness had no significant effects on vegetation biomass consumed by the grasshoppers in their experiment.

Our results point out that presence of the preferred resource (grasses) and herbivore survival are the most important variables determining the recovery of the systems after a selective ′press′ perturbation. This means, whenever grasses are present in a plant community, and grasshoppers survive for a sufficiently long time, both resistance and resilience of the whole plant community are negatively affected – at least under the scenario imposed by us, i.e. using a rather high herbivore density. Especially the grass monocultures suffered severely and over a period of more than two years (own observations from June 2006) from intense herbivory. Importantly, the decline in the preferred resource (grasses) is counteracted by an increase in a different plant functional group (obviously only in mixtures with >1 species), leading to a significant change in plant community composition induced by selective herbivory. Similar changes in plant community composition after perturbation have frequently been reported (e.g. Danell & Ericson 1990; Bach 2001; Klanderud & Totland 2005). Pfisterer *et al.* (2003) have suggested that “consumers can change the relative biomasses and cover proportions of different species and functional groups in the plant community”, but they did not provide an explicit proof for this assertion. Our experiment shows that selective herbivory can induce changes in plant communities that persist for at least one year, and that these changes depend on the number of plant species present. While grass biomass was most severely affected in low-diversity mixtures, herb biomass increased concomitantly. We suggest that selective herbivory changes competitive hierarchies in plant communities (c.f. Suding & Goldberg 2001) – in our case at least at the level of plant functional groups.

Looking at initial cover and height differences, it is worth noting that grass cover and height had initially been higher in herbivory than in control cages, while this relationship was reversed in the later stages of the experiment. The differences between blocks that existed already from the onset of the experiment can be explained by (i) the time difference between the dates at which each block was populated with grasshoppers, and (ii) by the different proportions of plots per block containing grasses (which is why the block:grass interaction was included into the models). The significant grass:legume interaction effect on initial differences in herb cover is difficult to explain, but this effect was small and vanished away over time.

Contrary to expectation, we could not find a positive correlation between plant species richness and grasshopper survival. The positive effect of number of FG on grasshopper survival could be due to several factors, *inter alia* changes in microclimate, nitrogen availability (Berner *et al.* 2005), or beneficial effects of a mixed diet (Bernays *et al.* 1994). The negative effect of legume presence is, to our opinion, mainly attributable to the presence of some very tall-growing legumes (e.g. *Onobrychis viciifolia*, *Medicago* x *varia*) that in most cases tended to fill a large proportion of the cage volume and tended to outcompete grasses; such legume species also might have indirect effects on grasshopper survival, e.g. via changes in microclimate, affecting many aspects of grasshopper behavior, including thermoregulation and food searching efficiency, but this remains speculative.

The overall relationship between grasshopper survival and amount of biomass difference between herbivory and control cages may seem trivial, but the linearity of this relationship can be interpreted as indicating a constant or proportional consumption of biomass per individual and day, rather than more complicated herbivore functional responses that might also be expected (Crawley 1997). The observed proportion of total biomass removed by the grasshoppers is in line with other studies; Quinn *et al.* (1993), for example, estimate that 15-20 grasshoppers of the polyphagous *Melanoplus sanguinipes* (F.) remove about 20% of grass biomass, and Mitchell & Pfadt (1974) come to almost the same value for vegetation biomass consumption in a shortgrass prairie system under field conditions. In our experiment, biomass removal resulted in consistent decreases in LRR and RII, showing that both indices can be of use in the comparison of control and treatment cages; yet, we point out that such indices have been criticized in the recent literature (e.g. Jasienski & Bazzaz 1999; Oksanen *et al.* 2006). We have used these indices as one of several tools to study resilience and resistance, and we conclude from our study that a direct analysis of the differences between treatments and controls yields more insight into biological processes than hiding that information in less informative indices.

Pre-versus post-experimental resistance is more difficult to interpret than R_2, because several processes are overlapping to produce a given value; namely, differences in spring vs. summer vegetation biomass (productivity in spring is on average higher than in late summer). Although the relationship with species richness was significant, resistance is mainly driven by grasshopper survival.

Even one year after the onset of the experiment, herbivory cages still had a much lower biomass, LRR and RII than control cages, indicating that resilience was not as quick as could be expected from the high regrowth capabilities of grasses. All variables used to estimate resilience showed this relationship, notably independent of species richness.

A comparison with theoretical predictions (Appendix D) showed that the observed patterns in total plant community resilience and resistance can be sufficiently well explained by assuming a linear proportional consumption of grass biomass, modulated by herbivore survival.

Conclusions

While our experiment showed no significant diversity effects on plant community resistance and resilience, plant species richness did have significant effects at the level of the preferred resource: mixtures containing grasses were highly and consistently affected by herbivory, particularly if only a few plant species were present. In mixtures containing more than one species, the loss caused by herbivory was completely counteracted by an increase in herb biomass, resulting in an overall non-significant difference in total vegetation biomass.

Our experiment is therefore is the first to experimentally demonstrate under which conditions plant species richness will influence system resilience and recovery: If only parts of the whole system are perturbed (here: grass biomass), plant species richness will affect resilience and resistance, but only in those systems that contain these parts. Hence, the effects of a selective perturbation are system-specific. Systems will recover more quickly if the perturbation can be counteracted by compensation, which is, in our case, the increased growth of herbs at the expense of grasses.

To our opinion, these results also apply to other kinds of experimental perturbations, such as drought (Kahmen *et al.* 2005; Pfisterer & Schmid 2002; Tilman & Downing 1994), because susceptibility of a plant species to drought is species-specific, and hence some parts of the system may be more severely perturbed than others.

The strong effects of plant functional group presence on system recovery may indicate that species´ functional identity, or even species identity itself (Steiner *et al.* 2006), are important drivers of a system´s response to perturbations.

References

Andow, D.A. (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology*, 36, 561-586.

Armas C., Ordiales R. & Pugnaire, F. (2004) Measuring plant interactions: A new comparative index. *Ecology*, 85, 2682-2686.

Bach, C.E. (2001) Long-term effects of insect herbivory and sand accretion on plant succession on sand dunes. *Ecology*, 82, 1401-1416.

Bernays, E.A., Bright, K.L., Gonzalez, N., & Angel, J. (1994) Dietary Mixing in a Generalist Herbivore - Tests of 2 Hypotheses. *Ecology*, 75, 1997-2006.

Bernays, E.A. & Chapman, R.F. (1970a) Experiments to Determine Basis of Food Selection by *Chorthippus parallelus* (Zetterstedt) (Orthoptera - Acrididae) in the Field. *Journal of Animal Ecology*, 39, 761-775.

Bernays, E.A. & Chapman, R.F. (1970b) Food Selection by *Chorthippus parallelus* (Zetterstedt) (Orthoptera:Acrididae) in the field. *Journal of Animal Ecology*, 39, 383-394.

Berner, D., Blanckenhorn, W.U., & Korner, C. (2005) Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos*, 111, 525-533.

Burnham, K.P. & Anderson, D.A. (1998) *Model Selection and Inference - A Practical*

Information-Theoretic Approach. Springer, Berlin.

Caldeira, M.C., Hector, A., Loreau, M., & Pereira, J.S. (2005) Species richness, temporal variability and resistance of biomass production in a Mediterranean grassland. *Oikos*, 110, 115-123.

Cleveland, W.S. (1981) LOWESS: A program for smoothing scatterplots by robust locally weighted regression. *The American Statistician*, 35, 54.

Crawley, M.J. (1997). Plant-Herbivore Dynamics. *Plant Ecology* (ed M.J. Crawley). Blackwell Science Ltd., Oxford.

Crawley, M.J. (2002) *Statistical Computing. An Introduction to Data Analysis using S-Plus*. John Wiley & Sons, Ltd., Chichester, West Sussex, UK.

Danell, K. & Ericson, L. (1990) Dynamic Relations between the Antler Moth and Meadow Vegetation in Northern Sweden. *Ecology*, 71, 1068-1077.

Fagan, W.F., Moran, M.D., Rango, J.J., & Hurd, L.E. (2002) Community effects of praying mantids: a meta-analysis of the influences of species identity and experimental design. *Ecological Entomology*, 27, 385-395.

Gangwere, S.K. (1961) A Monograph on Food Selection in Orthoptera. *Transactions of the American Entomological Society*, 87, 67-230.

Goodman, D. (1975) Theory of Diversity-Stability Relationships in Ecology. *Quarterly Review of Biology*, 50, 237-266.

Grimm, V. & Wissel, C. (1997) Babel, or the ecological stability discussions: An inventory and analysis of terminology and a guide for avoiding confusion. *Oecologia*, 109, 323-334.

Hedges, L.V., Gurevitch, J. & Curtis, P.A. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150-1156.

Holling, C.S. (1973) Resilience and Stability of Ecological Systems. *Annual Review of Ecology and Systematics*, 4, 1-23.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A.J., Vandermeer, J., & Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75, 3-35.

Ingrisch, S. & Köhler, G. (1998) *Die Heuschrecken Mitteleuropas*. Westarp Wissenschaften, Magdeburg.

Jasienski, M. & Bazzaz, F.A. (1999) The fallacy of ratios and the testability of models in biology. *Oikos*, 84, 321-326.

Kahmen, A., Perner, J., & Buchmann, N. (2005) Diversity-dependent productivity in semi-natural grasslands following climate perturbations. *Functional Ecology,* 19, 594-601.

Klanderud, K. & Totland, O. (2005) Simulated climate change altered dominance hierarchies and diversity of an alpine biodiversity hotspot. *Ecology*, 86, 2047-2054.

Köhler, G., Brodhun, H.P., & Schaller, G. (1987) Ecological Energetics of Central-European Grasshoppers (Orthoptera, Acrididae). *Oecologia*, 74, 112-121.

Limpert, E., Stahel, W.A., & Abbt, M. (2001) Lognormal Distributions across the Sciences: Keys and Clues. *BioScience*, 51, 341-352.

Lockwood, J.L. (1998) Management of orthopteran pests: a conservation perspective. *Journal of Insect Conservation*, 2, 253-261.

Loreau, M., Downing, A., Emmerson, M., Gonzales, A., Hughes, J., Inchausti, P., Joshi, J., Norberg, J. & Sala, O. E. (2002). A new look at the relationship between stability and diversity. In:. *Biodiversity and ecosystem functioning: synthesis and perspectives.* (eds S. Naeem, M. Loreau & P. Inchausti), pp. 79-91. Oxford University Press, Oxford.

May, R.M. (1973) *Stability and Complexity in Model Ecosystems*. Princeton University Press, Princeton.

McCann, K.S. (2000) The diversity-stability debate. *Nature*, 405, 228-233.

Mitchell, J.E. & Pfadt, R.E. (1974) A Role of Grasshoppers in a Shortgrass Prairie Ecosystem. *Environmental Entomology,* 3, 358-360.

Murdoch, W.W. (1975) Diversity, Complexity, Stability and Pest Control. *Journal of Applied Ecology*, 23, 795-807.

O´Neill, R.V. (1999) Recovery in Complex Systems. *Journal of Aquatic Ecosystem Stress and Recovery*, 6, 181–187.

Oksanen, L., Sammul, M., & Magi, M. (2006) On the indices of plant-plant competition and their pitfalls. *Oikos*, 112, 149-155.

Pfisterer, A.B., Diemer, M., & Schmid, B. (2003) Dietary shift and lowered biomass gain of a generalist herbivore in species-poor experimental plant communities. *Oecologia*, 135, 234-241.

Pfisterer, A.B. & Schmid, B. (2002) Diversity-dependent production can decrease the stability of ecosystem functioning. *Nature*, 416, 84-86.

Pimentel, D. (1961) Species diversity and insect population outbreaks. *Annals of the Entomological Society of America*, 54, 76-86.

Pratsch, R. (2004) *The Consumer Community (Arthropoda) of selected cultivated grasslands in the surroundings of the Jena biodiversity experiment*. M.Sc. Thesis (in German), University of Jena, Jena.

Quinn, M.A., Johnson, P.S., Butterfield, C.H., & Walgenbach, D.D. (1993) Effect of Grasshopper (Orthoptera, Acrididae) Density and Plant Composition on Growth and Destruction of Grasses. *Environmental Entomology*, 22, 993-1002.

R Development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reinhardt, K. & Köhler, G. (1999) Costs and benefits of mating in the grasshopper *Chorthippus parallelus* (Caelifera : Acrididae). *Journal of Insect Behavior*, 12, 283-293.

Richards, O.W. & Waloff, N. (1954) Studies on the biology and population dynamics of British grasshoppers. *Anti-Locust Bulletin*, 17, 1-182.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W.W., Schmid, B., & Schulze, E.D. (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology*, 5, 107-121.

Scherber, C., Mwangi, P.N., Temperton, V.M., Roscher, C., Schumacher, J., Schmid, B., & Weisser, W.W. (2006) Effects of plant diversity on invertebrate herbivory in experimental grassland. *Oecologia*, 147, 489-500.

Schläpfer, F. & Schmid, B. (1999) Ecosystems effects of biodiversity: a classification of hypotheses and exploration of empirical results. *Ecological Applications*, 9, 893-912.

Schmitz, O.J. (1997) Press Perturbations and the Predictability of Ecological Interactions in a Food Web. *Ecology*, 78, 55-69.

Schmitz, O.J., Hambäck, P.A., & Beckerman, A.P. (2000) Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *The American Naturalist*, 155, 141-153.

Spehn E.M. *et al*. (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecological Monographs* 75, 37-63.

Steiner, C.F., Long, Z.T., Krumins, J.A., & Morin, P.J. (2006) Population and community resilience in multitrophic communities. *Ecology*, 87, 996-1007.

Suding, K.N. & Goldberg, D. (2001) Do disturbances alter competitive hierarchies? Mechanisms of change following gap creation. *Ecology*, 82, 2133-2149.

Thebault, E. & Loreau, M. (2005) Trophic interactions and the relationship between species diversity and ecosystem stability. *American Naturalist*, 166, E95-E114.

Thébault, E. & Loreau, M. (2006) The relationship between biodiversity and ecosystem functioning in food webs. *Ecological Research*, 21, 17-25.

Thomas, J.A., Telfer, M.G., Roy, D.B., Preston, C.D., Greenwood, J.J.D., Asher, J., Fox, R., Clarke, R.T., & Lawton, J.H. (2004) Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science*, 303, 1879-1881.

Tilman, D. & Downing, J.A. (1994) Biodiversity and stability in grasslands. *Nature*, 367, 363-365.

van Hook, R.I. (1971) Energy and Nutrient Dynamics of Spider and Orthopteran Populations in a Grassland Ecosystem. *Ecological Monographs*, 41, 1-26.

Venables, W.N. & Ripley, B.D. (1997) *Modern Applied Statistics with S-Plus*. 2 edn. Springer, New York.

Wardle, D. A., Bonner, K. I. & Barker, G. M. (2000) Stability of ecosystem properties in response to above-ground functional group richness and composition. *Oikos*, 89, 11–23.

Wardle, D.A. & Grime, J.P. (2003) Biodiversity and stability of grassland ecosystem functioning. *Oikos*, 100, 622-623.

Weisberg, S. (2005) *Applied linear regression*. 3 edn. John Wiley & Sons, Inc., Hoboken, New Jersey.

Zhang, Q.G. & Zhang, D.Y. (2006) Species richness destabilizes ecosystem functioning in experimental aquatic microcosms. Oikos, 112, 218-226.

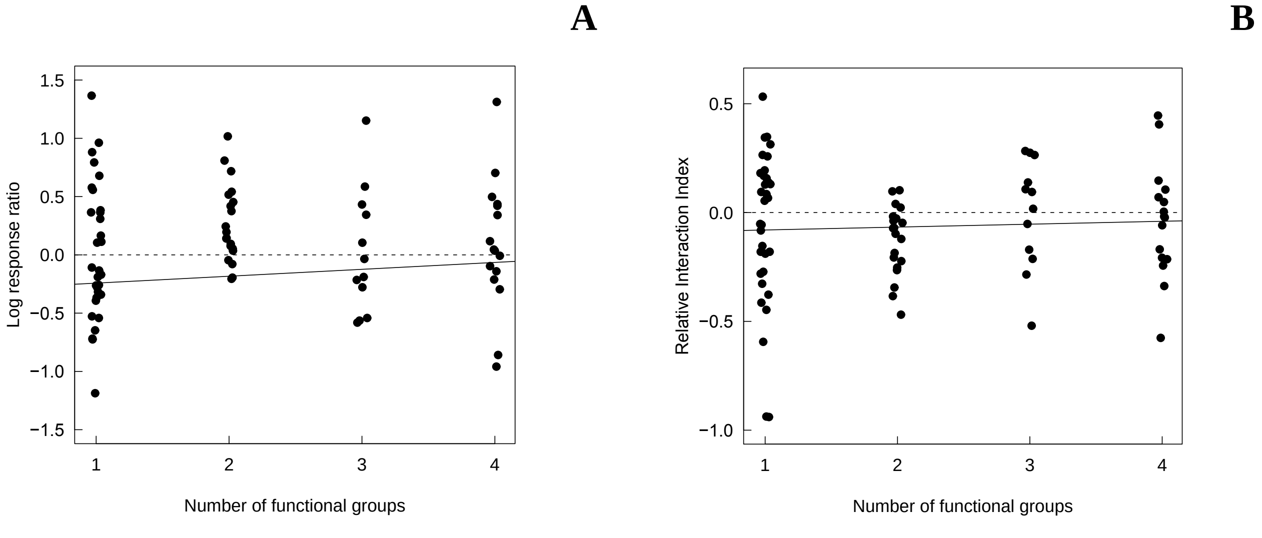
APPENDIX A Stages of the experiment, response variables, and when they were measured

Time of Measurement	Experimental Procedures	Stage	Response Variables measured
27.05. - 10.06.2004	Before installation of cages	1	Plant Biomass
14.06. - 25.06.2004	Installation of cages	1	None
28.06. - 01.07.2004	Pre-experimental conditions	1	Cover, height
06.07. - 15.07.2004	Addition of grasshoppers	2	Initial weight of grasshoppers
03.08. - 09.08. 2004	Peak of grasshopper feeding	2	Cover, height†
31.08. - 07.09.2004	Directly After grasshopper removal	2	Plant Biomass
26.05. - 04.06.2005	One year after grasshopper removal	3	Cover, height
20.06. - 01.07.2005	One year after grasshopper removal	3	Plant Biomass

Notes: †Grasshopper survival was assessed every 2 days between 09.07.-27.08.2004

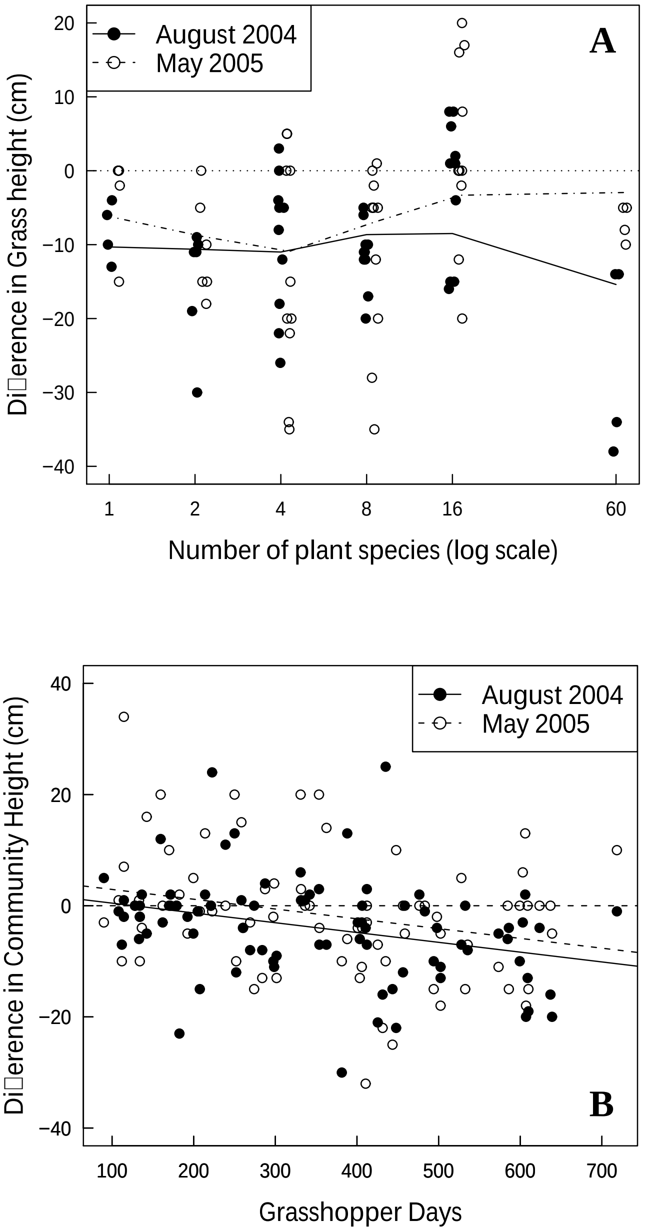
APPENDIX B Resistance in August 2004

Scatterplots showing (A) the relationship between LRR_2 and the number of FG; and (B) the relationship between RII_2 and the number of FG. Data points are jittered along the X axis by a factor of 0.2



APPENDIX C

Plant community height and grass height



Appendix C: (A) Relationship between GD and the difference in plant community height between herbivory and control cages; (B) Relationship between log-linear plant species richness and the difference in grass height between herbivory and control cages. Lines were created using a LOWESS scatterplot smoother (Cleveland 1981) with span 0.8.

APPENDIX D Modelling herbivore effects

In contrast to studies on the effects of rather homogeneous disturbances (such as drought) on ecosystem processes, our herbivory treatments impose a kind of perturbation that is very selective and does not affect all plant species equally. In particular, the perturbation intensity depends on herbivore survival: Unsuitable host plants will reduce grasshopper survival, and hence the plant community will be less severely affected than if the preferred food plants are sufficiently abundant.

We therefore use a computer modelling approach to predict what would have happened if all grasshoppers had survived for the total of 57 possible days. In addition, we test if herbivory is dependent on the amount of plant material available (proportional herbivory), or if constant amounts of biomass are consumed irrespective of plant abundance (fixed amount eaten). For this purpose, we use the following scenarios:

(1a) All grasshoppers per cage consume a fixed amount δ of biomass over the experimental period, independent of their survival, and also independent of the actual amount of biomass present.

(1b) All grasshoppers per cage consume a fixed amount δ of biomass over the experimental period, but some die before the end of the experiment; thus, δ depends on GD.

(2a) All grasshoppers per cage consume a constant proportion p of biomass over the experimental period, calculated from the actual amount present in the control cages at stage 2 of the experiment, and independent of GD.

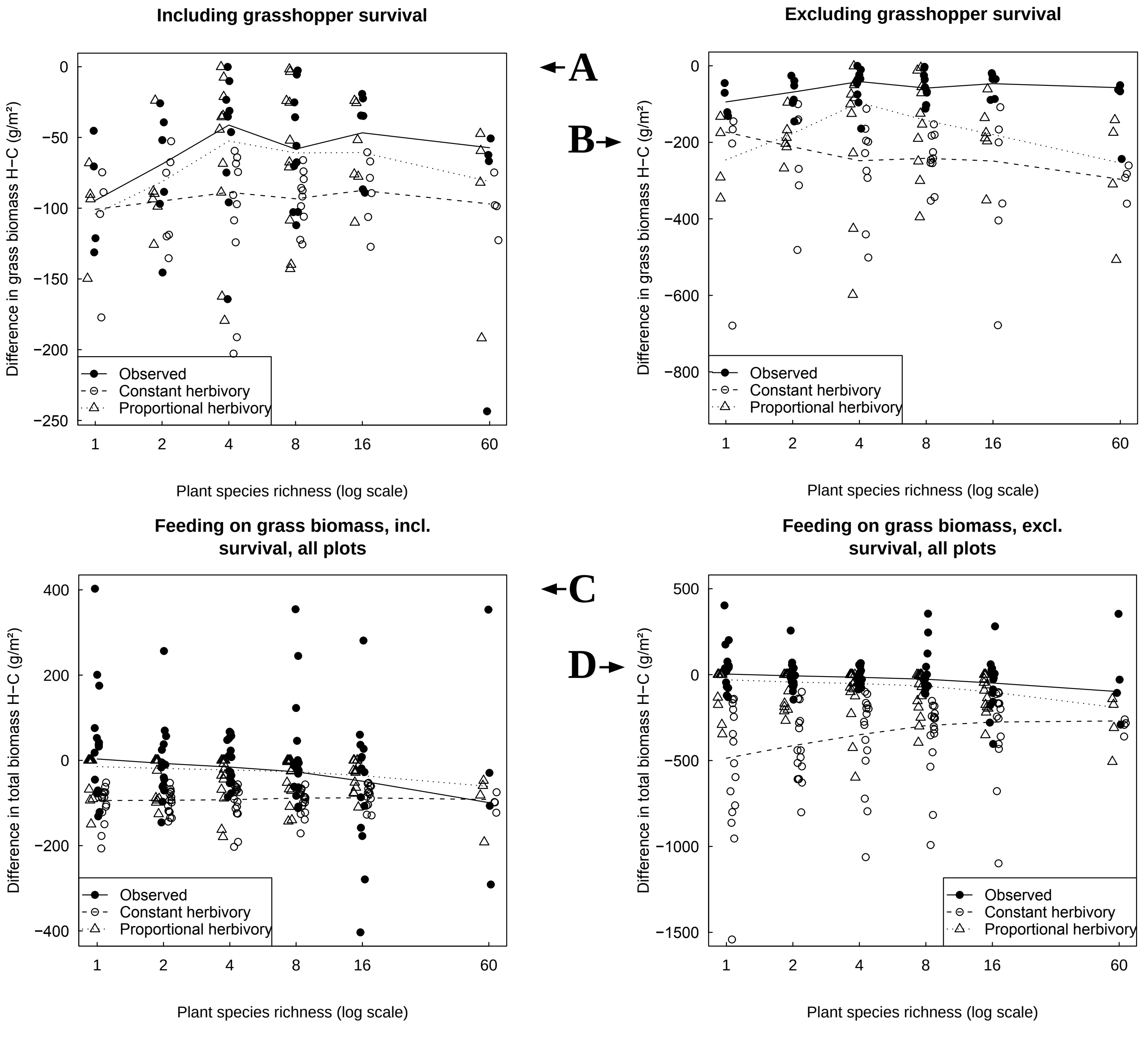
(2b) All grasshoppers per cage consume a constant proportion p of biomass over the experimental period, calculated from the actual amount present in the control cages at stage 2 of the experiment. Some grasshoppers die before the end of the experiment; thus, p depends on GD.

It is assumed that plant compensation for herbivore damage is zero, herbivores feed until the resource is depleted, consumption can never be negative, and proportional consumption can never be >1. We assume a linear (type 1) functional response between plant availability and herbivory (Crawley 1997).

We used computer modelling to simulate constant vs. proportional herbivory with and without grasshopper survival, for stage 2 of the experiment, as described below. For all these modelling exercises, it was assumed that feeding occurred only on grasses.

(1) Fixed consumption of grass biomass

We used the observed amounts of grass biomass eaten by the grasshoppers, δ_2^G, as the input variable (N=40) to generate simulated values of grass biomass consumption. This was done by fitting a lognormal distribution to the frequency distribution of (Crawley 2002; Limpert *et al.* 2001). Mean and standard deviation of this distribution were estimated using maximum likelihood (Venables & Ripley 1997). We then randomly sampled 30 numbers per plot (for N=81 plots) from this distribution (30 random deviates x 81 plots = 2430 random numbers), and aggregated the data again across all 81 plots. The resulting values are the amounts of grass biomass eaten by the number of surviving grasshoppers in each cage; thus, it was necessary to calculate another set of values for a hypothetical scenario of "infinite" grasshopper survival; to find out how much grass biomass



Appendix D: Comparison between observed and predicted values of (A) grass biomass, including grasshopper survival; (B) grass biomass, excluding grasshopper survival; (C) vegetation biomass, including grasshopper survival, and (D) vegetation biomass, excluding grasshopper survival. All biomass values are per m². Points are jittered along the X axis by a factor of 0.2; Lines were created using a LOWESS scatterplot smoother (Cleveland 1981) with span 0.8.

had been consumed if every herbivory cage had contained 20 grasshoppers over the whole course of time ("infinite survival"), we divided each of the simulated values by the cumulative number of days that the grasshoppers had survived in each cage (GD), and multiplied by the maximum possible value of GD (20 individuals x 57 days).

(2) Proportional consumption of grass biomass

The rationale for this approach is exactly the same as for fixed consumption of grass biomass, except that sampling is not done from the frequency distribution of , but from a distribution of the ratio of and control cage grass biomass. This calculation yields p, the proportion of grass biomass consumed in herbivory vs. control cage. Again, we fitted a lognormal distribution to these values (other statistical distributions did not produce a meaningful fit to the data). Infinite survival was again explicitly incorporated as described above.

Calculation of simulated total cage biomass differences

The last step in the simulation process was to use the simulated amounts of grass biomass eaten and (i) subtract them from the observed control cage biomasses, C_2, or (ii) subtract the product of p and control grass biomass from control cage biomass, resulting in a simulated herbivory cage biomass, $H_{2\ sim}$.

Assessing model quality

To compare observed and simulated biomass values, and hence to find out which scenario had the best explanatory power, we constructed a factor with three levels (observed, predicted from constant herbivory, and predicted from proportional herbivory), concatenated all observed and simulated cage biomass values into a single vector, and used the interactions between the three-level factor, log-linear and quadratic plant species richness as explanatory variables in analyses of covariance. Tests for differences between slopes and intercepts were used to assess which of the models fitted the observed values best. The results of these analyses are shown below.

Results

The observed amount of grass biomass consumed for plots with $\delta_2^G > 0$ averaged 65.53±49.47 gm^{-2} (about 66%). Under the assumption of infinite grasshopper survival, this value would have averaged 422.82±319.51 gm^{-2} (about 280%). Incorporating survival, simulated consumption values averaged 96.79±34.27 gm^{-2} (67%).

In all cases, assuming proportional consumption of grass biomass, incorporating survival, yielded the best fit to the observed data. This means, the values of grass biomass consumption reported in this study are significantly better explained by proportional consumption than by a constant removal of biomass.

Excluding grasshopper survival, neither constant nor proportional herbivory were adequate in describing the data: Constant herbivory overestimated the amount of grass biomass eaten by 244.44±40.47 g m^{-2} ($t=-6.040, P<0.001$), while proportional herbivory over-estimated grass biomass consumption by 124.70±40.47 g m^{-2}($t=-3.081$, $P=0.002$).

Incorporating grasshopper survival, the intercept for constant herbivory was still significantly different from the observed value (-33.30±9.81 g m^{-2}; $t=-3.394$, $P<0.001$). The simulated values, assuming proportional herbivory, however, did not differ significantly from the data any more. This means, assuming proportional and survival-dependent feeding of the grasshoppers is sufficient to explain the observed patterns in grass biomass differences between herbivory and control patterns.

For the observed differences in total biomass, the same principles apply; excluding survival, the assumption of constant herbivory massively over-estimated the actual difference by over 500 gm^{-2} ($t=-10.00, P<0.001$) while proportional herbivory did not differ significantly from the data. Incorporating grasshopper survival, the constant herbivory simulation over-estimated consumption by almost 130 gm^{-2} ($t=-6.01, P<0.001$), while proportional herbivory over-estimated this value by only about 50 g ($t=-2.38$, $P=0.018$).

7 General Discussion

It is an assumption, but not an established fact, that **human-caused species extinctions** in ecosystems occur at random; Lawler *et al*. (2001) even state that “it should be obvious to any observer of the modern world that humans do not imperil species at random” . Assuming a **random extinction scenario**, however, has been the basis for many previous biodiversity experiments (Schmid *et al*. 2002), and this assumption has also formed the basis for the experiments presented in this study; while being a reasonable point to start, such an approach should – at least with regard to future experiments – be developed further, to incorporate extinction scenarios such as removal of dominants, removal of top predators, reduction of diversity through pollution, nutrient enrichment, altered disturbance regimes, removal of attractive or other species of aesthetic or nutritive value to human beings, and others (Lawler *et al*. 2001). This is not to say that the experiments conducted within the framework of the Jena Experiment have failed to address realistic extinction scenarios[1], but the overall main experiment has been designed using repeated random draws of species.

A second extinction scenario, assuming losses of whole groups of organisms with similar functional characteristics - the **functional groups**-, is a further simplification that may or may not apply to ´natural´ grassland ecosystems.

Further, it is an assumption that experimentally assembled ecosystems will be a good **approximation to reality**. Philosophically, it is also questionable under which circumstances an ecosystem will be regarded as “natural”, because even most of the “naturally occurring” grassland ecosystems in Central Europe owe their existence to human activities (Küster 1995), and even the most “pristine” ecosystem will in one way or the other receive its atmospheric **nitrogen fertilization** through wet and dry deposition, amounting to about 50 kg N ha^{-1}yr^{-1} (Berendse *et al*. 1993, cited in Bakker and Berendse 1999). Hence, even naturally occurring grassland ecosystems will be indirectly influenced by human activities, shifting their equilibrium positions away from what would be expected under a **potential natural vegetation scenario** (*sensu* Ricotta *et al*. 2002). With regard to the experiments presented in this thesis, it is necessary to critically assess if the scenarios simulated by the experiment, and the combinations of species used, will be a **working approximation** to reality. In summary, the experiments presented in this study will strictly only apply to systems with the following characteristics:

Figure 1: One of the four 60-species mixtures used in the Jena Experiment. Note the pale pink inflorescences of *Onobrychis viciifolia*. Photograph © Christoph Scherber, 13 June 2006.

- grassland communities established on former arable land with a **Eutric Fluvisol** characterized by a Ap-Go-Gr horizontation (Schachtschabel *et al*. 1992) on river alluvial parent material;

- plant communities that are created using a **replacement series design** (Gibson 2002), with constant initial sowing density, and a target community of which all other experimental communities are sub-components; the target community approximated by this experiment is an ***Arrhenatheretum elatioris*** BRAUN community (**Figure 1**).

---

[1] In fact, some experiments presented in this thesis go one step further; for example, adding a selective herbivore (or removing it) is one step into the simulation of a new kind of extinction scenario; likewise, the so-called dominance experiment (Roscher *et al*. 2005), and the nutrient enrichment and mowing experiments (installed 2006), are new approaches to more realistic simulations of species extinctions.

- plant communities are **weeded** and **mown** twice a year

However, one should not over-emphasize these limitations; many important advances in biology and the natural sciences have been made using **models of reality**, and the Jena Experiment has been set up in a way that should come as close as possible to naturally managed grasslands in the study region.

In the following sections, the general approach followed in this study shall be considered in the light of already existing experiments.

7.1 General experimental approach

The experimental plots used in this study were installed on **former arable land** to form grassland plots with different experimental plant communities. It is important to note that this is not an unrealistic scenario. In several regions across Germany, **agri-environment schemes** have been introduced that promote the conversion of arable land into extensively managed hay meadows. Specifically, the KULAP (e.g. Falconer and Whitby 2000) subprogram B4[2] ("Umwandlung von Ackerflächen in Grünland und dessen extensive Bewirtschaftung") subsidises the conversion of arable land into grassland.

Another, much more noteworthy question is whether the **combinations** of (plant) species used in the Jena Experiment can be termed **´realistic´**. In particular, several of the species used as part of the overall species pool are not typical elements of *Arrhenatheretum elatioris* Braun communities. For example, Strobel and Hölzel (1994) list *Arrhenatherum elatius* L. and *Geranium pratense* L. as (weakly) differential species of this plant community, but **agriophytic species** such as *Onobrychis viciifolia* Scop., that are present in many of the experimentally sown plots (Figure 1), may be seen as rather unfortunate choices. Other species that may be regarded atypical are, for example, the cultivars *Medicago* x *varia* Martyn and *Trifolium hybridum* L., and the saltmarsh-inhabiting *T. fragiferum* L.[3]. Some of the cultivars are mainly used in **crop-rotation systems** and not naturally occurring members of the target plant species community. It is left to the reader´s discretion whether or not the inclusion of these (partly highly productive[4]) species will have overriding effects on community-level variables. If this were the case, then these effects would appear in the "legume" term in statistical models, and interpretations of overall legume effects would then possibly be influenced by the presence or absence of these species.

Another point that has often been criticized in grassland biodiversity experiments is the appearance of **´unrealistic´ species combinations**. It is true that these combinations will appear, but it is also true that at least some of the previous biodiversity experiments (e.g. Spehn *et al.* 2005) have tried to use more realistic compositions of species, for example by including grasses in most of the multi-species mixtures. As the results from these experiments, compared with the Jena Experiment, have pointed into a similar direction so far (for example when looking at aboveground plant community biomass), one could conclude that unrealistic species combinations do not matter to the overall results obtained using grassland biodiversity experiments constructed by means of randomly assembled plant communities.

There are further aspects of the overall experimental design used in the Jena Experiment, that deserve thorough discussion; for example, the overall experiment is **unreplicated**. Although the 20 x 20-m plots have been replicated at a smaller scale, own observations (Scherber *et al.*, unpublished), have shown that, e.g., herbivory values obtained from small plots differ systematically from those obtained in large plots, making comparisons between both plot sizes a difficult task. Further, **species identity effects** could, strictly speaking, only

---

[2] Source: „Programm zur Förderung von umweltgerechter Landwirtschaft, Erhaltung der Kulturlandschaft, Naturschutz und Landschaftspflege in Thüringen (KULAP 2000)", published in „Thüringer Staatsanzeiger 38/2004, p. 2222-2259, with recent changes published in „Thüringer Staatsanzeiger" 49/2005, p. 2346-2349

[3] which is a typical member of *Armerion maritimae* Br.-Bl. et De Leeuw (1936) saltmarsh communities

[4] For example, Roscher *et al.* 2005 have stated that *Onobrychis viciifolia* belonged to the five most productive species in monocultures in the second year of the experiment.

be tested if the overall experimental setup were replicated at least three times. A replication of the overall experimental setup, however, would have been an impossible task in terms of available time, money and personnel.

Another point that could have been cured at the setup phase of the experiment is that treatments are not equally represented within blocks. This, however, would have been a prerequisite for an analysis of the experiment as a **randomized complete block** design. Yet, the current experimental design is characterized by an **unequal representation** of plots containing grasses vs. legumes (block three has more plots containing grasses than the other blocks). There is no way to cure this afterwards, but it nevertheless needs to be mentioned in an overall discussion of the experimental design.

In summary, generalizations from The Jena Experiment to larger scales or to other types of ecosystems may be limited due to (i) the **lack of replication**, (ii) the **unequal representation** of treatments within blocks, (ii) the cyclic and non-homogeneous **disturbances** introduced by mowing and weeding, and (iv) the **land-use history** at the field site.

7.2 Specific experiments conducted

The specific experiments conducted in this study can be classified as follows: **Phytometer** experiments, **plant community** measurements, **insecticide** applications, and **caging** experiments.

The **phytometer** approach, using transplanted plant individuals, has a long-standing tradition in ecology (e.g. Gibson 2002). With regard to the Jena Experiment, such an approach offers the opportunity to test diversity effects at the level of **individual species**; due to the random assignment of species to mixtures, effects at the individual plant species level across all plots are only possible using such a phytometer approach, because even the most abundantly present plant species only occur at a maximum of about 12 out of 82 experimental plots[5]. Of course, it is questionable whether the transplantation of plant individuals into an already established community will result in meaningful conclusions about the performance of individual plants in plant communities differing in diversity. However, the more ′realistic′ approach – namely, **sowing** individual species into every plot (Turnbull *et al.* 2000; Turnbull *et al.* 2005) – was not possible because the experiments presented in this thesis started after installation of the Jena experimental field plots.

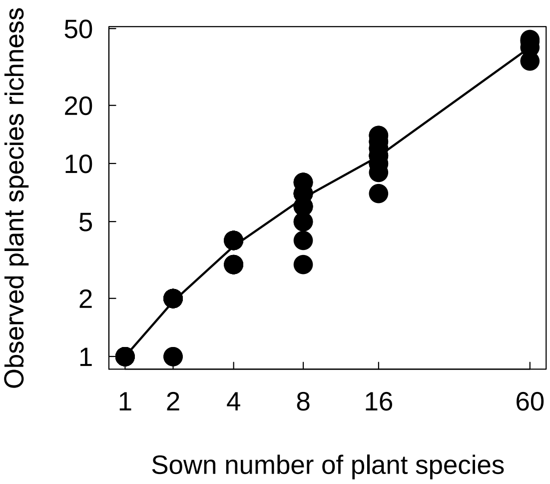


Figure 2 When sown and realized species richness are plotted on a double-logarithmic scale, the relationship is almost linear, as indicated by a Lowess non-parametric smoothing function with span 0.4 fit through the data. Data on the y axis are based on species numbers determined from vegetation cover measurements. Cover was measured in 2x2 m quadrates in May 2004 by Christoph Scherber, Alexandru Milcu and Stephan Partsch. Graph © Christoph Scherber.

It would have been interesting to sow thin strips of the plant species later used as phytometers into every single plot, right from the beginning of the Jena Experiment. This would have made it possible to test **invasion resistance** directly from the establishment phase, and the "sown phytometers" would have been true members of the plant communities. Consequently, the **differences in performance** between transplanted phytometers, and their conspecific sown neighbours, as observed – for example – by Unsicker *et al.* 2006, would not have occurred if plants had been sown rather than transplanted into the experimental plant communities. Such an approach would also have made it possible to link the results obtained better to gen-

---

[5] This is not to say that such an undertaking would be completely unfruitful; for some species, a replication of N=12 large plots can be regarded as sufficient to test single-species responses in different experimental communities, and such an approach is also followed by several other scientists within the Jena Experimental research group.

eral ecological theory, for example in comparison with **lottery models** (Chesson and Warner 1981) and models incorporating **competition/colonization trade-off** mechanisms (reviewed in Turnbull *et al.* 2000). However, the overall introduction of individual plant species into all experimental plots has the great advantage of making comparisons at the single-species level possible – and this advantage outweighs the disadvantages of omitting the seedling stage.

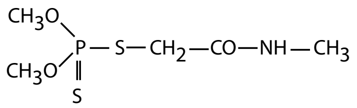
Such a single-species approach should at best be complemented by measurements at the **community level**, and we tried this, for example, using herbivory **transects**, measurements of **leaf area index**, and by harvesting plant **community biomass**. All these approaches rest on the assumption that **small-scale patterns** transfer linearly to each whole 20x20-m plot; i.e., overall **homogeneity** of the plant communities is assumed. An indication that this assumption holds true comes from the approximately linear correlation between sown species richness, and measured number of plant species in subplots within plots (**Figure 2**). Homogeneity is also likely because seed mixtures for overall establishment of the plant communities were homogenously sown.

The **herbivory transects** we used sample species according to their **proportional abundance**. This means, species were not drawn at random from the communities (Crawley 2002). But because all that was needed was an estimate of community herbivory, even a random draw of plant species would later on have needed to be corrected for individual plant species´ abundances. The **increased herbivory** values obtained for those communities containing **legumes** can be explained by two processes: (i) the inclusion of legumes into the herbivory measurements and (ii) increased levels of herbivory on non-legumes due to legume presence. Disentangling these two processes would be an interesting task for further analyses.

Whether or not **biomass** values obtained from a small area of 1x1 m² are representative for the whole community is a question that has been addressed, for example, by Roscher *et al.* (2005), who compared productivity estimates obtained in one, two or four randomly selected sample areas of 20x50 cm in small vs. large plots; these authors found that the differences between biomasses of 20 x 20-m plots and 3.5 x 3.5-m plots (that had been sampled using different numbers of sample frames) was not significant.

The measurements of **leaf area index** used in this study (which are correlates of aboveground productivity) were not corrected for leaf angles, and hence the obtained values may be biased in communities that are characterized by overall small leaf angles (e.g. grass mixtures; Schulze, *personal communication*). But as LAI only served as a covariate in some of the statistical models, it can be assumed that the obtained values have sufficient precision to serve this purpose.

Another point that shall be briefly discussed here is the use of **biocides** to manipulate different components of ecosystems. The aboveground semi-systemic insecticide, Dimethoate, used by us (**Figure 3**) has been shown to be at least weakly **growth-stimulating** for plants: Hector *et al.* (2004) reported "a minor stimulation of growth in three species of forb" when plants were tested in laboratory trials where herbivory was absent.



$(CH_3O)_2P(=S)-S-CH_2-CO-NH-CH_3$

Figure 3 Semi-structural chemical formula of the insecticide used in the experiments presented in chapter 4. In plants, the sulfur atom is quickly replaced by oxygen, and an oxygen carboxy derivative is formed. The final step in the dimethoate metabolization is the formation of phosphoric acid. © Christoph Scherber, from various sources cited in the text.

This stimulatory effect on plants may, for example, be attributable to the uptake of **phosphorous**- or **nitrogen**-containing components via the cuticula, and their subsequent metabolization and hydrolysis to **phosphoric acid** (Banasiak *et al.* 2004). Microorganisms can use Dimethoate as a source of phosphorus (Rosenberg and Alexander 1979), and it is not unlikely that processes such as **soil respiration** (Getenga *et al.* 2000) or plant phosphorus metabolism, are changed after Dimethoate application, resulting in an overall stimulation of plant growth. However, we accounted for such possible direct effects of insecticide treatment because we measured herbivory in both treatment and control plants (Siemann *et al.* 2004); because herbivory differed significantly between the two levels of insecticide treatment, we are confident that at least part of the resulting positive effects of insect herbivore exclusion on individual plant species is due to reduced levels of insect herbivory. Further, we present analyses of single-species herbivory only, because **blanket herbivore exclusion** may affect both insect herbivores and abundance patterns across several plant species, making it difficult to attribute observed differences in community biomass to one general mechanism (Siemann *et al.* 2004). Further, we tried to hold the overall number of insecticide applications per year as low as possible, such that even if any kinds of indirect fertilization effects had occurred, these would have been negligible in quantity.

Note that the general use of biocides to manipulate the abundance or diversity of organisms within an ecosystem can be seen as analogous to the **application of antibiotics** in medicine: There will always be **side-effects**, but these will be unimportant as long as the overall treatments are successful.

One method to overcome the possible drawbacks of pesticide use is to manipulate consumer diversity using **exclosures** or – as has been the case in this study – **cages**. The strengths and weaknesses of such an approach in general have been extensively discussed in Schmitz (2004). The cages used by us have the advantage of (i) being tall enough to include even the tallest plant species present in the experimental communities, (ii) consisting of aluminium mesh with a mesh size that minimizes microclimatic differences and (iii) allowing herbivores to sufficiently forage and move within the cage area. Bernays and Chapman (1970) state that about 70-80% of marked individuals of *Chorthippus parallelus* cover a distance of less than 50 cm per day. While the **grasshopper densities** used by us are clearly higher than can be expected under local field conditions (Behrens and Fartmann 2004, for example, list a maximum of 5.3 individuals m^{-2}), and movement was restricted over a several-month period, it needs to be emphasized that it was not the aim of this experiment to mimic natural herbivore densities; instead, our aim was to set a system-specific, yet intense, **herbivory perturbation** Hooper *et al.* 2005; Schmitz 1997) to the system, and such high grasshopper densities are typical for outbreaks of polyphagous grasshoppers under north-temperate climatic conditions (Joern and Gaines 1990).

In the following sections, the experimental results presented in this thesis shall be discussed in greater detail, and an attempt shall be made to develop generalizations across the systems studied. For the purpose of clarity, the **null hypotheses** formulated in chapter 2 shall be repeated here, together with a discussion on whether any given hypothesis has been rejected.

Initial null hypotheses and their falsification

> Hypothesis A: There is no effect of plant species richness on invertebrate herbivory at the level of whole plant communities, individual plant species, or individual herbivore species.

The results presented in chapters 3, 4 and 5 allow a rejection of all three sub-hypotheses (referring to plant communities, individual plant species, or individual herbivore species, respectively): At the level of the entire **plant community**, there was a significant **positive** relationship between plant species richness and community herbivory in May and August 2004; at the level of **individual plant species**, this relationship was significant for *Rumex acetosa* in August 2003, but **negative** rather than positive. At the level of **individual herbivore species**, plant species richness only had significant effects when analyses were restricted to plots **containing grasses** (i.e. the preferentially consumed resource). However, although any evidence for a statistically significant influence of species richness on herbivory justifies a rejection of hypothesis A, it needs to be noted that plant species richness in general was a **weak explanatory variable**, especially in comparison with the overall overriding effects of **functional group identity** in the statistical models used.

How, then, could the significant species richness effect on plant community herbivory be explained? Clearly, herbivory as measured especially at the plant community level incorporates both insect and mollusc feeding, both generalist and specialist herbivory, and is furthermore an integration over time not accounting for green leaf fall or early leaf abscission (Schulze, *personal communication*), and it also does not include plant compensatory growth responses (McNaughton 1983; Tiffin 2000). With such a rather crude way of estimating the effects of herbivorous organisms on plant communities differing in the number of plant species present, it is of course difficult to find general rules determining the relationships between herbivory and plant diversity. Nevertheless, the herbivory measurements presented in this thesis are pioneering for several reasons: (i) herbivory has never been measured simultaneously on so many experimental plots (for comparison, see, e.g., Mulder *et al.* 1999); (ii) the wide range of plant species richness covered (1-60 species) is unprecedented in the ecological literature; (iii) this study is the first to allow a clear separation of plant species richness effects from other ecosystem properties, such as plant community biomass and plant functional composition (Huston 1997). Thus, it is now possible to make inferences about the exact nature of the processes involved, and not just a "pro or con" statement on effects of plant species richness.

With regard to **plant diversity**, one could formulate the following **alternative hypothesis**: *Invertebrate herbivores tend to consume more photosynthetically active plant tissue per unit area and time when plant species richness increases*. Or, formulated as a simple statement:

> Invertebrate herbivory in the experimental plant communities studied tends to slightly increase with plant species richness.

This finding is in accordance with **previous studies** in experimental grassland (e.g. Mulder *et al.* 1999), and is also reflected in a general increase in abundance and diversity of several groups of insect herbivores with increasing plant species richness (Haddad *et al.* 2001; Knops *et al.* 1999; Koricheva *et al.* 2000). In a study conducted in extensively managed grasslands, Unsicker *et al.* (2006) have demonstrated that community leaf area loss due to invertebrate herbivory tended to significantly decrease with increasing plant species richness. To the author´s opinion, a reconciliation of these two diverging findings is possible if one allows a more complex explanation than the simple "species richness or not"-view. An **alternative hypothesis** might, for example, be:

> Invertebrate herbivory at the community level is determined by **plant functional identity** and **plant community composition**.

Unsicker *et al* (2006) themselves write that "*herbivory levels in plant communities cannot simply be explained as a consequence of the effects of plant species richness on specialist herbivore abundances*"; the authors further note that plant community biomass and –composition "*were selected in multiple regressions […] more often than plant species richness*".

From the experiments presented in this thesis, it seems that **plant species richness** as an explanatory variable in diversity-herbivory studies has such **low explanatory power**, that slopes of regression lines will be positive, negative or zero just by chance alone, especially if there exists a significant correlation between plant community composition, or the abundance of plant functional groups present in a community, and plant species richness. The overall inconclusive effects of plant species richness further solidify that explanatory variables other than plant species richness are the ´true´ drivers of invertebrate herbivory in experimental grassland.

Very similar explanations apply to the observed herbivory values at the level of **individual plant species**: In our study, herbivory was either completely not influenced by plant species richness at all, or – as in the case of *R. acetosa* – **negatively** correlated with plant species richness. For *T. pratense* and *P. lanceolata*, our findings are consistent with Unsicker *et al.* (2006), except for August 2003, where herbivory tended to increase with species richness in *T. pratense*. Again, the author tends to conclude that species richness as the sole explanatory variable has been over-emphasized in previous studies, and the following alternative hypothesis may be formulated:

Invertebrate herbivory at the level of individual plant species is determined by plant functional identity and plant community composition, rather than species richness.

Looking now at herbivory in a simplified system consisting of just one (graminivorous) **insect herbivore** and experimental plant communities, we find that if the preferred resource is present, monocultures and low-diversity mixtures will be significantly more severely affected by herbivory, than high-diversity mixtures. This effect may, for example, be due to higher **resource quality** and lower **ingestion rates** in high-diversity mixtures (cf. **dietary mixing** *sensu* Bernays *et al.* 1994). This finding would be in accordance with a re-formulated resource concentration hypothesis: For insect herbivores specialized on a specific functional group of plants, biomass consumption per unit area and time will decrease with increasing plant species richness. An in-depth discussion of further aspects follows below.

Hypothesis B: Number of plant functional groups and plant functional group identity are not better predictors of invertebrate herbivory than plant species richness.

Based on the results presented in this thesis, hypothesis B can clearly be rejected. Plant **functional group identity** was the single most important factor influencing **invertebrate herbivory** in all datasets – exemplified by the much higher sums of squares or effect sizes in comparison with other explanatory variables such as plant species richness. In general, **legume presence** tends to positively influence invertebrate herbivory, while **grass presence** tends to have the opposite effect. Positive **legume effects** on levels of invertebrate herbivory may be explained by (i) changes in soil respiration (Milcu, personal communication); (ii) increased herbivory in legumes compared with non-leguminous plants; (iii) transfer of nitrogen from legumes to other plant species. **Figure 4** shows that transplanted plant individuals indeed have higher nitrogen contents if they grow together with legumes, than in absence of legumes; higher leaf nitrogen might, in turn, lead to greater rates of herbivory, for example because of herbivore nitrogen limitation (Crawley 1983).

Grasses have been shown to have lower levels of invertebrate herbivory than other plants (Tscharntke and Greiler 1995); this may explain the lower herbivory levels in communities containing grasses. On the other hand, grasses may also exert a strong **competitive effect** on growth of other plants, e.g. via root competition, and these plant species (as in the case of *T. pratense* phytometers) may then experience higher levels of herbivory in those plots containing grasses. **Figure 5** shows that with increasing proportion of grasses in a mixture, soil water content in the upper 20 cm of the soil significantly decreases.

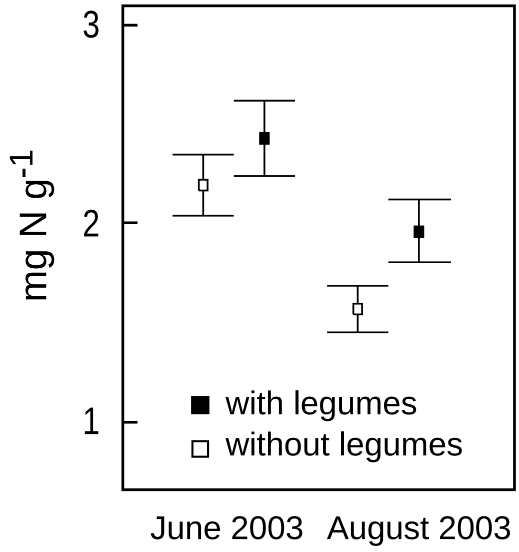


Figure 4 *Plantago lanceolata* phytometers tend to have higher leaf nitrogen contents (expressed per g dry weight) in plots containing legumes than without legumes. Data from harvested and dried plant material. Graph © Y. Oelmann, the data are © P.N. Mwangi & V.M. Temperton. Reproduction kindly permitted by B. Schmid (Zurich).

At the level of **individual herbivore species**, presence of grasses was crucial for the outcome of all plant-herbivore interactions; grass presence thus will influence (i) grasshopper survival, (ii) the relationship between herbivory and plant species richness and (iii) the overall amount of herbivore damage experienced by the community.

In summary, the following alternative hypothesis may be formulated: ***Plant functional identity** determines the outcome of plant-herbivore interactions in experimental grassland. **Specific interactions** between individual plant species and their herbivores are governed by the **identity of the interacting partners**, and not solely by their taxonomic diversity.*

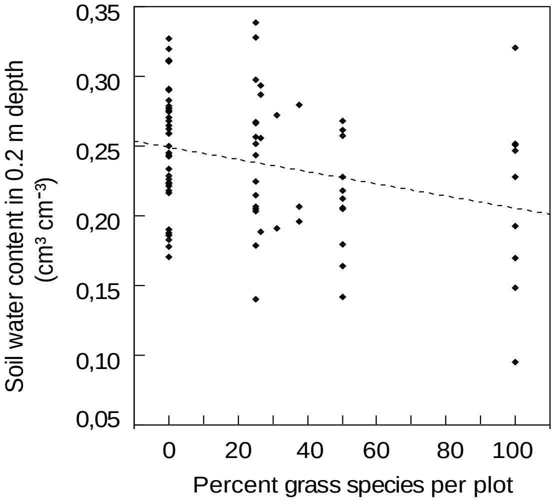


Figure 5 With increasing proportion of grasses per plot (calculated from sown number of grass species), volumetric soil water content in 0.2 m depth significantly decreases. If plants compete for soil water (e.g. Casper & Jackson 1997), then a high proportion of grasses may decrease the performance of individual non-grass plant species. Graph adapted from Y. Kreutziger, © Y. Kreutziger, reprinted with kind permission.

Hypothesis C: Plant community biomass and related parameters do not significantly influence invertebrate herbivory.

The experiments presented in this thesis clearly indicate that aspects other than plant diversity may influence invertebrate herbivory to a considerable extent. Hypothesis C, therefore, is to be rejected and replaced by an alternative hypothesis. Plant **community biomass** or community **leaf area index** did have significant **positive** effects on herbivory (i) in *R. acetosa*[6], (ii) in *P. lanceolata* and (ii) at the level of whole pant communities. Thus, it is important to include such correlates of plant community productivity as **covariates** into statistical models in diversity-herbivory studies. Only of community biomass or related parameters are included as *primary* covariates, and **sequential sums of squares** are used in statistical modelling, will it be possible to analyze the remaining plant species richness effects independently of primary productivity (Schmid *et al.* 2002). Note that Unsicker *et al.* (2006) came to a similar conclusion – herbivory in their study was also correlated with community biomass in many cases; notably, the relationship between invertebrate herbivory and community biomass they reported was also **positive**, giving support to the assertion that variables other than plant species richness might be better mediators for a reconciliation of study results obtained in experimental vs. extensively managed grasslands. In conclusion, the following **alternative hypothesis** may be formulated:

Plant community biomass and related parameters are significantly positively correlated with invertebrate herbivory in grassland ecosystems.

Hypothesis D: The performance of individual plant species is not significantly influenced by plant species richness, plant functional group richness, identity of plant functional groups, or plant community biomass.

This hypothesis mainly refers to the results presented in chapters 5 and 6, and can be extended to incorporate all processes acting at the level of individual plant species, e.g. **invasion resistance** (which will be treated in a **separate section** below). Hypothesis D consists of four **sub-hypotheses** relating the performance of individual plants to (i) plant species richness, (ii) plant functional group richness, (iii) identity of plant functional groups, and (iv) plant community biomass. The evidence presented in this thesis suggests a clear rejection of hypothesis D: **plant species richness** significantly affected reproductive parameters and survival in *R. acetosa* phytometers, and it was negatively correlated with biomass, number of leaves or ramets, and growth rate in *Trifolium pratense*, *Plantago lanceolata*, *Knautia arvensis* and *Festuca pratense*, and this effect remained significant even when functional group richness was fitted first in the statistical models. Functional group richness (sub-hypothesis 2) was only significant if fitted first. As in the hypotheses presented above,

---

[6] This was only true for the individuals not treated with insecticide; the mixed effects models presented in chapter 4 did not show significant biomass or LAI effects in *R. acetosa*.

the presence of **particular plant functional groups** – especially grasses and legumes – was an important explanatory variable affecting growth and reproductive parameters at the level of individual plant species, with legumes generally having positive, **grasses** generally having **negative** effects on transplant performance. The **positive** effects of **legumes** on performance of neighbouring plant species may be explained by a transfer of symbiotically fixed nitrogen from legumes to other plant species (Figure 4; see also Spehn *et al.* 2002). The study by Spehn *et al.* showed that the specific composition of the communities and the presence of legumes, had major effects on nitrogen accumulation in plant community biomass – again pointing at the relative importance of community composition – and hence plant functional identity – in determining the performance of plants within a community.

7.3 Diversity and invasion resistance

A plant community is **invasible** when a given plant species introduced to the community fulfils the **invasion criterion**, i.e. when the invading species is able to increase when rare (Crawley 1987; Shea and Chesson 2002). **Invasion** should be seen as an **ecosystem process** that is in principle independent of the **identity** of the invading species; this means, it should not matter whether a given species is native to the invaded plant community or not[7]. Gross *et al.* (2005) state that “the composition of any local community results from the interplay of within-community interactions and the dispersal of species between communities”. Many studies have used **exotic plants[8]** as invasion phytometers; for example, Scherber *et al.* (2003) transplanted individuals of the invasive alien plant *Senecio inaequidens* DC (Asteraceae) into mature grassland communities at Silwood Park (UK). Prieur-Richard *et al.* (2002) studied herbivory, survival, growth and reproduction in *Conyza canadensis* (L.) CRONQUIST and *C. bonariensis* (L.) CRONQUIST in plant communities differing in plant species richness. In the experiments presented here, plant species **already present** in the species pool of the experimental plant communities were used to test how different components of plant diversity affect growth, reproduction, and – at least for *Rumex acetosa* – herbivory and survival. This study system offers the opportunity to simultaneously test principal mechanisms of community assembly, without confounding **invasiveness** of a given plant species, and **invasion resistance** of a given plant community. A similar approach, but using seed sowing experiments rather than transplanted individuals, has been followed, for example, by Turnbull *et al.* (2005).

The results presented in this thesis only apply to the **late establishment phase** of invasion processes (Shea and Chesson 2002); clearly, a proper test of invasion resistance would have needed to involve **seed-sowing** experiments (see above), because processes acting upon germination and seedling establishment (e.g. seedling herbivory; Hulme 1994) will act as a first barrier preventing invasion already upon arrival of a propagule within a community. Yet, the transplanted individuals used by us were just four weeks old, and in order to test the **invasion criterion**, the experiments conducted are fully sufficient. The much more important question is which weight is given to the clearly existing **role of plant species richness** in comparison with **plant functional identity** or even plant species identity (Crawley *et al.* 1999). Undoubtedly, our results show that **species richness** reduces growth and reproductive parameters in the phytometer species studied; this result is also supported by theoretical models (e.g. Shea and Chesson 2002). But what our results also show is that the degree of **niche overlap**[9] between invading and resident species is an important driver of invasion resistance. Or, put into more concrete terms: The probability of successful establishment of a plant species within a plant community is determined by the f**unctional identity** of that plant species, and by the **functional composition** of the plant community into which it establishes. Species-rich communities will gener-

---

[7] Of course, invasion resistance is often treated with regard to “invasive alien” species (e.g. Rejmanek 1989; Rejmanek 2000; Richardson *et al.* 2000), but the process of invasion and subsequent establishment of any given species within a community of course follows the same principle mechanisms.

[8] For terminological issues of this term, see Colautti and MacIsaac 2004; Rejmanek 2000; Richardson *et al.* 2000

[9] We define a niche according to Whittaker *et al*. 1973 as a species´ population response within its niche hypervolume; niche overlap, then, is any overlap between niche variables in any given niche hypervolume.

ally have an increased probability of containing several functionally dissimilar species, hence promoting invasion resistance in its broadest sense. In nature, invasion resistance will be mediated not only by species richness, but also by processes such as competition from resident plants, herbivory by resident animals, soil fungal communities, or diseases (Levine *et al.* 2004). Such processes will need to be taken into account in future studies relating biodiversity to invasion resistance, if an integrative view is to be obtained.

7.4 Invertebrate communities

Patterns of invertebrate herbivory in plant communities can not be interpreted without knowing the identity of the **herbivorous organisms** responsible for the observed patterns. Because collection, determination and identification of invertebrate herbivores across a total of 82 plots several times per year is an enormous task, the invertebrate communities were studied by collaborating scientists (e.g. Kowalski 2005).

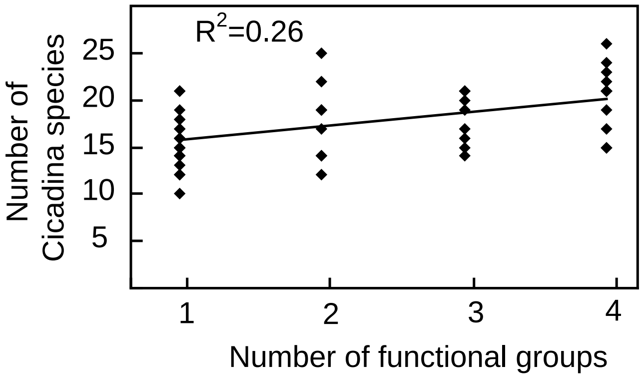


Figure 6 The species richness of leafhoppers (Cicadina) increases significantly with plant functional diversity. Other herbivorous insect groups have not been analyzed yet. Graph © E. Kowalski, reproduced with kind permission from W. Voigt.

The results obtained so far indicate that (i) herbivorous **Gastropoda** (Mollusca) are important and abundant herbivores at the field site (Bahr 2006); (ii) the abundance and species richness of sap-sucking **Cicadina** (Homoptera) is significantly positively correlated with plant species richness and plant functional group richness (Kowalski 2005; **Figure 6**); (iii) abundance and species richness of **Staphylinidae**[10] (Coleoptera) increases significantly with plant species richness (Lauterbach 2005); (iv) abundance and species richness of **Carabidae**[10] (Coleoptera) are not significantly correlated with plant species richness; (v) grasshoppers of the family **Acrididae** (e.g. *Chorthippus parallelus* Zett.) are abundant chewing insect herbivores at the field site.

While patterns of **leaf damage** observed at the **community level** will be caused by several of the above-mentioned groups, it is much more straightforward to attribute observed herbivory in **phytometers** to specific herbivore species. For example, leaf beetles and curculionid beetles are the most abundant insect herbivores feeding on *Rumex acetosa, Plantago lanceolata,* and *Trifolium pratense*, with herbivorous molluscs mainly restricted to *R. pratense* (Scherber *et al.*, own observations, unpublished) In summary, knowledge of the exact links between the plant species present at the field site, and their specific invertebrate herbivores, is still limited; future studies could, for example, use sticky traps or stable isotope marking techniques (Unsicker *et al.* 2005) to infer more details about interactions across several trophic levels.

7.5 Performance of a single invertebrate herbivore

To partly overcome the difficulties in studying whole invertebrate herbivore communities mentioned above, a **caging experiment** with one single insect herbivore, the acridid grasshopper *Chorthippus parallelus*, was conducted. The results presented in chapter 6 clearly indicate that **survival** of *C. parallelus* was independent of plant species richness, but strongly influenced by the presence of the **preferred resource** (grasses). Other parameters, such as the **weight** of male and female imagines, were also significantly higher in plots containing grasses; for example, the percentage cover of grasses in the communities was linearly and significantly related with adult weight (Specht 2006; **Figure 7**). In a similar experiment, but using a different grasshopper species, and using an experimental design with all multi-species mixtures containing grasses, Pfisterer *et al.* (2003) claimed to have found evidence for strong relationships between **grasshopper performance** and plant species richness; yet, their study was limited

---

[10] These groups are secondary consumers and are mentioned here because of their potentially predominant role in determining abundances of herbivorous invertebrates.

to a period of 13 days, and grasshoppers fed on plant communities that had been freshly cut down to 5 cm height. The results we have presented in this thesis are, therefore, much more robust: Not only did our experimental design allow a **separation** of **species richness** effects from effects of **other diversity components**, but also did we conduct our experiments over more than one year (including experiments with 1st instar stages in 2005), and using plant communities that were allowed to grow to a height of up to one meter.

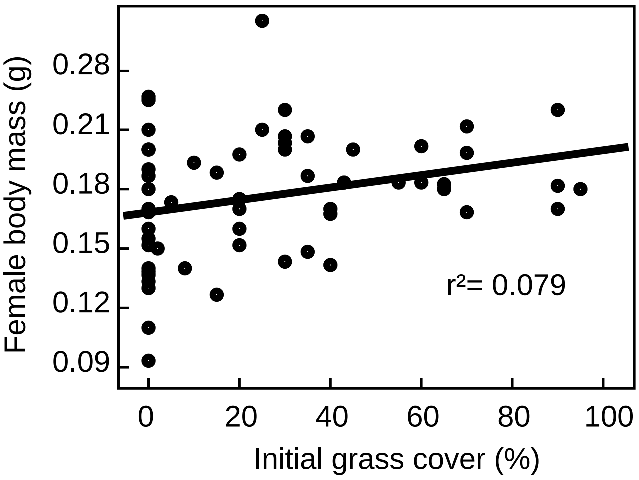


Figure 7 The performance of *Chorthippus parallelus*, the insect herbivore used in the caging experiment, is significantly positively correlated with the cover of grasses per cage at stage 1 of the experiment. Performance is indicated by female body mass (measured nondestructively) at stage 2 of the caging experiment (see Appendix in chapter 6). Grasshopper body mass was determined by J. Specht; initial grass cover was measured by C. Scherber. Graph modified from © J. Specht, with kind permission.

In conclusion, the results obtained so far indicate a predominant role of **plant functional identity** for the **performance** of individual herbivore species in experimental grassland.

7.6 Selective herbivory and stability

Likewise, studying herbivory in simplified systems consisting of just one single insect herbivore species and whole plant communities using experimental enclosure cages (e.g. Pfisterer *et al.* 2003; Schmitz 2004) offers the opportunity to observe **specific trophic interactions** to a greater detail than would be possible at the level of whole invertebrate herbivore assemblages. The results obtained so far clearly show that (i) selective herbivory changes plant **functional identity**, (ii) **stability**, as measured by **resilience** and **resistance** of community biomass, is in general only weakly correlated with plant species richness, (iii) yet, if analyses are confined to those plots containing the preferred resource, then a strong dependence on **plant species richness** can be observed, with grass monocultures being much more severely affected by selective herbivory than mixtures containing grasses. This finding is in accordance with results presented by Pfisterer *et al.* (2003), who found that vegetation cover in high-diversity mixtures increased by up to 4% in comparison with pre-experimental cover values, whereas monocultures increased by a significantly smaller amount. These authors also found indications for changes in functional group composition, but did not show an explicit proof for that.

> In summary, resistance and resilience of aboveground plant community biomass after a selective herbivory perturbation will increase with plant species richness if and only if the herbivores´ preferred resource is present.

7.7 Prerequisites for a general theory of plant diversity and invertebrate herbivory

Having presented results from experiments with single plant species, single herbivore species, and whole plant and invertebrate herbivore communities, it is now possible to take the next step. All that is necessary is to write down the relevant alternative hypotheses stated above, and to formulate general conclusions from these:

1. Invertebrate herbivory in **plant communities** tends to slightly increase with plant species richness, if environmental parameters are held constant and only components of plant diversity are experimentally manipulated.

2. Invertebrate herbivory in **plant communities** is determined by plant functional identity and plant community composition.

3. Invertebrate herbivory in **plant communities** significantly increases with plant community biomass and community leaf area index.

4. Invertebrate herbivory at the level of **individual plant species** is determined by plant functional identity and plant community composition, rather than species richness.

5. For **insect herbivores** specialized on a specific functional group of plants, biomass consumption per unit area and time will decrease with increasing plant species richness.

6. **Specific interactions** between individual plant species and their herbivores are governed by the identity of the interacting partners, and not solely by their taxonomic diversity.

These statements can form the basis of a general theory of invertebrate herbivory and plant diversity. Such a theory would need to incorporate:

- the relative palatabilities and competitive abilities of all plant species present
- the relative electivities (feeding preferences) of all herbivore species present
- the functional response curves of all herbivore species present
- mortality rates and per capita fecundity of all species present

The output variable from a mathematical model incorporating these parameters could, for example, be the number of individuals of all plant and herbivore species, or other measures of their relative abundances. A decision would also have to be made as to how **temporal** vs. **spatial** dependencies should be incorporated into such a model; Siemann & Weisser (2004), for example, have presented niche-based models on the effects of herbivores on ecosystem productivity, but herbivore diversity was not explicitly considered. On the other hand, Pacala and Crawley (1992) have started with a two-species lottery model, but what would be needed is a multi-species temporally explicit model – a task that can of course not be fulfilled within the framework of this thesis. Clearly, if the model were to match closely with reality, modelling should involve different scenarios for the initial abundances of plant and herbivore species:

- single herbivore and plant species
- single plant species and multiple herbivores
- multiple plant species and single herbivores
- multiple plant species and multiple herbivores

The thesis presented here has laid the basis for an in-depth theoretical investigation into this subject, providing the baseline data with which theoretical models could be validated, especially with regard to the relative importances of plant species richness versus plant functional identity, and plant species identity in experimentally assembled grassland ecosystems.

7.8 Specific biotic interactions, species richness and species identity

The experiments presented in thesis have aimed to elucidate the relationships between different components of **plant species richness**, **invertebrate herbivory**, and **ecosystem processes**. It has been shown that **community herbivory** increases with plant species richness, but that presence of particular functional groups is more important; **single plant-species herbivory** is mainly governed by community biomass, and presence of particular functional groups. The performance of **individual plants** is influenced especially by insect herbivory, by the functional groups present in the surrounding community, and by plant species richness. The same principles hold for community **invasion resistance**, which is influenced mainly by the functional characteristics of invader and community, and to a lesser extent by plant species richness. The performance of **single herbivore species** depends on presence of the preferred resource, and on plant species richness only if that preferred resource is present. Finally, **resistance** and **resilience** of plant communities towards a selective herbivory perturbation are governed by herbivore survival, and by the presence of the preferred resource.

Across all experiments conducted so far, and across all datasets presented, analyzed and discussed in this thesis, **plant functional identity** seems to be of fundamental importance, whereas **plant species richness** seems not to be as important as anticipated by previous authors. Specific biotic interactions seem to be governed by species identity, rather than by the diversity of interaction partners. If a specific functional group, defined *a priori* by analyses of relevant traits, is present in a community, then community properties will be altered to a greater extent than when the number of taxonomically or functionally distinct members present in that community changes.

7.9 Outlook

Knowing that species diversity is neither constant in space, nor in geologic time, what can we conclude from the findings obtained thus far? What implications does it have if one of the main conclusions from this work is “species functional identity matters”? Should we discard species richness as a concept to explain patterns of invertebrate herbivory? The answer is: Of course not. To the contrary, if it is really species functional identity that matters, then the implications are immediately linked to species richness again – because the number of species present in a community will be positively correlated with the functional diversity of the species in that community (Petchey and Gaston 2002; Roscher *et al.* 2004).

If **species identity** and **community composition** are the prime determinants of patterns of consumption in grasslands, and if species identity also is the prime driver of invasion resistance and plant community stability, then we really have to divert our course and face complexity again.

Maybe, it has been too early to see species richness as the sole determinant of the patterns we observe in nature. While focusing on diversity is ethically appealing as a conservationist concept, we should probably allow more diversity also in the explanatory variables we use, and we should carefully look not only for significant P-values, but also for the explanatory power attributable to each variable we use to explain patterns in nature.

Only then will we arrive at the predictive power we need for a sustainable future on this planet.

References for chapter 7

Bahr C (2006) Effects of plant species diversity, identity and architectural density on the density of slugs in an experimental grassland. Diploma Thesis (in German), University of Jena, Jena.

Bakker JP, Berendse F (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. Trends in Ecology & Evolution 14:63-68

Banasiak U, Dutra Caldas E, Funk S, Hamilton DJ, Ossendorp BC, Yamada Y, Boobis AR, Davies L, Dellarco VL, Hakansson H, Moretto A, Solecki R, Tasheva M (eds) (2004) Pesticide Residues in Food. World Health Organization, Food and agriculture organization of the United Nations, Rome

Behrens M, Fartmann T (2004) Are high population densities the cause of macropterism in the meadow grasshopper (*Chorthippus parallelus*; Caelifera: Acrididae)? [in German]. Articulata 19:91-102

Berendse F, Aerts R, Bobbink R (1993) Atmospheric nitrogen deposition and its impact on terrestrial ecosystems. In: Voss CC, Opdam P (eds) Landscape Ecology in a Stressed Environment. Chapman & Hall

Bernays EA, Bright KL, Gonzalez N, Angel J (1994) Dietary Mixing in a Generalist Herbivore - Tests of two Hypotheses. Ecology 75:1997-2006

Bernays EA, Chapman RF (1970) Food Selection by *Chorthippus parallelus* (Zetterstedt) (Orthoptera:Acrididae) in the field. Journal of Animal Ecology 39:383-394

Casper BB, Jackson RB (1997) Plant competition underground. Annual Review of Ecology and Systematics 28:545–570

Chesson PL, Warner RR (1981) Environmental Variability Promotes Coexistence in Lottery Competitive Systems. The American Naturalist 117:923-943

Colautti RI, MacIsaac HJ (2004) A neutral terminology to define 'invasive' species. Diversity and Distributions 10:135-141

Crawley MJ (1983) Herbivory. Blackwell Scientific Publications, Oxford.

Crawley MJ (1987) What makes a community invasible? In: Gray AJ, Crawley MJ, Edwards PJ (eds) Colonization, Succession and Stability. The 26th symposium of the British Ecological Society. Blackwell Scienctific Publications, Oxford

Crawley MJ (2002) Statistical Computing. An Introduction to Data Analysis using S-Plus. John Wiley & Sons, Ltd., Chichester, West Sussex, UK

Crawley MJ, Brown SL, Heard MS, Edwards GR (1999) Invasion-resistance in experimental grassland communities: species richness or species identity? Ecology Letters 2:140-148

Falconer K, Whitby M (2000) Untangling red tape: Scheme administration and the invisible costs of European agri-environmental policy. European Environment 10:193–203

Getenga ZM, Jondiko JIO, Wandiga SO, Beck E (2000) Dissipation behavior of Malathion and Dimethoate residues from the sole and their uptake by Garden Pea (*Pisum sativum*). Bulletin of Environmental Contamination and Toxicology 64:359-367

Gibson DJ (2002) Methods in comparative plant population ecology. Oxford University Press, Oxford

Gross KL, Mittelbach GG, Reynolds HR (2005) Grassland invasibility and diversity: Responses to nutrients, seed input, and disturbance. Ecology 86:476-486

Haddad NM, Tilman D, Haarstad J, Ritchie ME, Knops JMH (2001) Contrasting effects of plant richness and composition on insect communities: a field experiment. The American Naturalist 158:17-35

Hector A, Wilby A, Latsch OG, Brown VK (2004) Phyto-activity of biocides used to manipulate herbivory: tests of three pesticides on fourteen plant species. Basic & Applied Ecology 5:313-320

Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setala H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75:3-35

Hulme PE (1994) Seedling herbivory in grassland: relative impact of vertebrate and invertebrate herbivores. Journal of Ecology 82:873-880

Huston MA (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110:449-460

Joern A, Gaines SB (1990) Population dynamics and regulation in grasshoppers. In: Chapman RF, Joern A (eds) Biology of grasshoppers. John Wiley & Sons, New York, pp 415-482

Knops JMH, Tilman D, Haddad NM, Naeem S, Mitchell CE, Haarstad J, Ritchie ME, Howe KM, Reich PB, Siemann E, Groth J (1999) Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecology Letters 2:286-293

Koricheva J, Mulder CPH, Schmid B, Joshi J, Huss-Danell K (2000) Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grassland. Oecologia 125:271-282

Kowalski E (2005) Density patterns of invertebrate herbivores (Auchenorrhyncha and Heteroptera) along a diversity gradient in an experimental grassland.Diploma Thesis (in German), University of Jena, Jena.

Küster H (1995) Geschichte der Landschaft in Mitteleuropa. C.H.Beck

Lauterbach D (2005) Abundance and diversity of secondary consumers (Staphylinidae) along a gradient in plant species richness. Diploma Thesis (in German), University of Jena, Jena.

Lawler SP, Armesto JJ, Kareiva P (2001) How relevant to conservation are studies linking biodiversity and ecosystem functioning? In: Kinzig AP, Pacala SW, Tilman D (eds) The Functional Consequences of Biodiversity. Princeton University Press, Princeton and Oxford, pp 294-313

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecology Letters 7:975-989

McNaughton SJ (1983) Compensatory plant growth as a response to herbivory. Oikos 40:329-336

Mulder CPH, Koricheva J, Huss-Danell K, Högberg P, Joshi J (1999) Insects affect relationships between plant species richness and ecosystem processes. Ecology Letters 2:237-246

Pacala SW, Crawley MJ (1992) Herbivores and plant diversity. The American Naturalist 140:243-260

Petchey OL, Gaston KJ (2002) Functional diversity (FD), species richness and community composition. Ecology Letters 5:1-29

Pfisterer AB, Diemer M, Schmid B (2003) Dietary shift and lowered biomass gain of a generalist herbivore in species-poor experimental plant communities. Oecologia 135:234-241

Prieur-Richard A-H, Lavorel S, Linhart YB, Dos Santos A (2002) Plant diversity, herbivory and resistance of a plant community to invasion in Mediterranean annual communities. Oecologia 130:96-104

Rejmanek M (1989) Invasibility of Plant Communities. In: Drake JA, *et al.* (ed) Biological Invasions: a Global Perspective (SCOPE report). John Wiley & Sons Ltd., pp 369-388

Rejmanek M (2000) Invasive plants: approaches and predictions. Austral Ecology 25:497-506

Richardson DM, Pysek P, Rejmanek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6:93-107

Ricotta C, Carranza ML, Avena G, Blasi C (2002) Are potential natural vegetation maps a meaningful alternative to neutral landscape models? Applied Vegetation Science:271-275

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze ED (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology 5:107-121

Roscher C, Temperton VM, Scherer-Lorenzen M, Schmitz M, Schumacher J, Schmid B, Buchmann N, Weisser WW, Schulze ED (2005) Overyielding in experimental grassland communities - irrespective of species pool or spatial scale. (vol 8, pg 419, 2005). Ecology Letters 8:576-577

Rosenberg A, Alexander M (1979) Microbial cleavage of various organophosphorus insecticides. Applied and Environmental Microbiology 37:886-891

Schachtschabel P, Blume H-P, Brümmer G, Hartge K-H, Schwertmann U (1992) Scheffer/Schachtschabel, Lehrbuch der Bodenkunde, 13 edn. Ferdinand Enke Verlag, Stuttgart

Scherber C, Crawley MJ, Porembski S (2003) The effects of herbivory and competition on the invasive alien plant *Senecio inaequidens* (Asteraceae). Diversity and Distributions 9:415-426

Schmid B, Hector A, Huston MA, Inchausti P, Nijs I, Leadley PW, Tilman D (2002) The design and analysis of biodiversity experiments. In: Loreau M, Naeem S, Inchausti P (eds) Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxford University Press, Oxford, pp 61-75

Schmitz OJ (1997) Press Perturbations and the Predictability of Ecological Interactions in a Food Web. Ecology 78:55-69

Schmitz OJ (2004) From mesocosms to the field: The role and value of cage experiments in understanding top-down effects in ecosystems. In: Weisser WW, Siemann E (eds) Insects and Ecosystem Function. Springer, Heidelberg, pp 277-302

Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution 17:170-176

Siemann E, Carson WP, Rogers WE, Weisser WW (2004) Reducing herbivory using insecticides. In: Weisser WW, Siemann E (eds) Insects and ecosystem function. Springer, Heidelberg

Siemann E, Weisser WW (2004) Testing the role of insects in ecosystem functioning. In: Weisser WW, Siemann W (eds) Insects and Ecosystem Function. Springer, Berlin & Heidelberg

Specht J (2006) The relationship between plant diversity and the fitness of the common grasshopper *Chorthippus parallelus* ZETT. (Orthoptera: Acrididae). Diploma Thesis, University of Jena, Jena.

Spehn EM, Hector A, Joshi J, Scherer-Lorenzen M, Schmid B, Bazeley-White E, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Jumpponen A, Koricheva J, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Palmborg C, Pereira JS, Pfisterer AB, Prinz A, Read DJ, Schulze ED, Siamantziouras ASD, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (2005) Ecosystem effects of biodiversity manipulations in European grasslands. Ecological Monographs 75:37-63

Spehn EM *et al.* (2002) The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. OIKOS 98:205-218

Strobel C, Hölzel N (1994) Lebensraumtyp Feuchtwiesen.- Landschaftspflegekonzept Bayern, Band II.6. Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen (StMLU) und Bayerische Akademie für Naturschutz und Landschaftspflege (ANL), Munich (Germany)

Tiffin P (2000) Mechanisms of tolerance to herbivore damage: what do we know? Evolutionary Ecology 14:523-536

Tscharntke T, Greiler H-J (1995) Insect communities, grasses, and grasslands. Annual Review of Entomology 40:535-558

Turnbull LA, Crawley MJ, Rees M (2000) Are plant populations seed-limited? A review of seed sowing experiments. Oikos 88:225-238

Turnbull LA, Rahm S, Baudois O, Eichenberger-Glinz S, Wacker L, Schmid B (2005) Experimental invasion by legumes reveals non-random assembly rules in grassland communities. Journal of Ecology 93:1062-1070

Unsicker S, Renker C, Kahmen A, Spindler S, Buchmann N, Weisser WW (2005) Testing the efficiency of three ^{15}N-labeled nitrogen compounds for indirect labeling of grasshoppers via plants in the field. Entomologia Experimentalis et Applicata 116: 219–226

Unsicker SB, Baer N, Kahmen A, Wagner M, Buchmann N, Weisser WW (2006) Invertebrate herbivory along a gradient of plant species diversity in extensively managed grasslands. Oecologia DOI: 10.1007/s00442-006-0511-3

Whittaker JB, Levin SA, Root RB (1973) Niche, Habitat, and Ecotope. The American Naturalist 107:321-338

Summary

Human-caused rates of species extinctions are unprecedented in geologic history. There is growing concern within the scientific community about the effects of anthropogenic species losses on ecosystem processes. One process of interest especially from an applied point of view is herbivory by invertebrates, because the role of invertebrate primary consumers as a dominant component of biodiversity in terrestrial ecosystems is still largely unexplored. Hence, it is unclear if and how invertebrate herbivory is correlated with components of plant diversity.

This thesis uses experimentally sown grassland ecosystems installed near Jena (Germany) to test several hypotheses relating components of plant diversity to (i) **invertebrate herbivory**, (ii) **performance of individual plants** and (iii) **aspects of plant community stability**. The main hypotheses tested in this thesis, formulated as null hypotheses, are:

Hypothesis A: There is no effect of plant species richness on invertebrate herbivory at the level of whole plant communities, individual plant species, or individual herbivore species.

Hypothesis B: Number of plant functional groups and plant functional group identity are not better predictors of invertebrate herbivory than plant species richness.

Hypothesis C: Plant community biomass and related parameters do not significantly influence invertebrate herbivory.

Hypothesis D: The performance of individual plant species is not significantly influenced by plant species richness, plant functional group richness, identity of plant functional groups, or plant community biomass.

To test these hypotheses, a total of 82 plant communities were experimentally sown in 2002 using a gradient of 1-60 plant species belonging to 1-4 functional groups that were defined *a priori*, and using a species pool of 60 species of Central European *Arrhenatherum* grasslands. The overall experimental design was a randomized block design. Specific experiments conducted involved (i) single herbivore species (**cage experiments**), (ii) single plant species (**phytometer experiments**), (iii) multiple insect herbivore species (**insecticide treatments**) and (iv) multiple plant species (e.g. community **herbivory transects**, biomass harvests).

The results, presented in four manuscripts, allow a clear rejection of all four main hypotheses, and the formulation of **alternative hypotheses**:

(1) Invertebrate herbivory at the level of **whole plant communities** is weakly positively correlated with plant species richness. Plant functional identity is the most important determinant of community herbivory. Legumes increase, while grasses decrease community herbivory.

(2) Invertebrate herbivory at the level of **individual plant species** is not consistently linked to plant species richness. Plant functional identity is the most important determinant of single-plant species herbivory.

(3) Herbivory by a **single invertebrate herbivore**, studied in a caging experiment, is significantly negatively correlated with plant species richness if the preferred resource is present. Again, plant functional identity is the most important explanatory variable.

(4) **Plant community biomass** and leaf area index are in some cases positively correlated with invertebrate herbivory.

(5) The **performance of individual plant species** is significantly negatively correlated with plant species richness and plant functional group richness. Plant functional group identity, and the functional identity of each individual plant species under study, are the most important determinants of the performance of individual plants. This finding

may have important implications for the **invasion resistance** of experimental plant communities.

(6) The **stability of plant communities**, expressed as **resistance** and **resilience** after a selective herbivory perturbation, is mainly influenced by herbivore survival, and by the functional composition of plant communities; stability will be most severely affected, if the herbivores´ preferred resource is present in the plant community.

The findings presented in this thesis clearly show that the **functional identity** of the plant species within experimental grassland communities can be an important determinant of invertebrate herbivory, invasion resistance, and aspects of community stability. Species richness *per* se may be of less importance than previously thought.

It is hypothesized that data from biodiversity experiments in general should be analyzed with regard to possible effects of plant community composition, as well as plant functional and plant species identity, instead of solely concentrating on the significance of plant species richness as a single explanatory variable.

Such an approach could then serve to provide the predictive power that is needed to relate the findings from model systems to the real world, and to society.

Zusammenfassung

Noch nie sind im Verlauf der Erdgeschichte so viele Tier- und Pflanzenarten in so kurzer Zeit ausgestorben, wie dies gegenwärtig aufgrund menschlicher Aktivitäten der Fall ist.

Unter Wissenschaftlern wächst daher die Besorgnis darüber, wie sich anthropogen bedingte Artenverluste auf in Ökosystemen stattfindende Prozesse auswirken könnten.

Von besonderem Interesse ist dabei, wie sich der Artenrückgang auf Primärkonsumenten, und dabei insbesondere auf herbivore Wirbellose, auswirken wird; denn die Bedeutung herbivorer Invertebraten für Prozesse in Ökosystemen ist bisher – verglichen mit ihrem vergleichsweise hohen Artenreichtum in terrestrischen Ökosystemen – noch kaum erforscht. Demzufolge ist es nach wie vor unklar, ob und auf welche Weise Invertebraten-Herbivorie mit verschiedenen Aspekten pflanzlicher Artendiversität korreliert ist.

In der vorliegenden Arbeit wurden künstliche Wiesenökosysteme in der Nähe von Jena angelegt, um verschiedene Hypothesen zu testen, welche den Zusammenhang zwischen Pflanzendiversität und (i) **Invertebratenherbivorie**, (ii) **Wachstum und Entwicklung einzelner Pflanzenarten** und (iii) der **Stabilität von Pflanzengemeinschaften** betreffen. Die zentralen Hypothesen wurden dabei in Form von Nullhypothesen wie folgt formuliert:

Hypothese A: Es gibt keinen Zusammenhang zwischen Pflanzenartenreichtum und Invertebratenherbivorie, weder auf der Ebene ganzer Pflanzengemeinschaften, noch auf Ebene einzelner Pflanzenarten, oder bei Betrachtung einzelner Herbivorenarten.

Hypothese B: Invertebratenherbivorie lässt sich nicht besser vorhersagen, wenn man die Anzahl funktioneller Gruppen von Pflanzen oder deren funktionelle Identität anstelle von Artenreichtum als erklärende Variablen verwendet.

Hypothese C: Invertebratenherbivorie ist nicht signifikant mit der Biomasse einer Pflanzengemeinschaft, oder mit vergleichbaren Variablen, korreliert.

Hypothese D: Wachstum und Entwicklung einzelner Pflanzenarten werden nicht signifikant durch Pflanzenartenreichtum, Anzahl funktioneller Gruppen, funktionelle Identität von Pflanzenarten, oder durch die Vegetations-Biomasse beeinflusst.

Um diese Hypothesen zu testen, wurden im Jahre 2002 insgesamt 82 experimentelle Pflanzenartengemeinschaften angelegt, die sich in ihrem Pflanzenartenreichum sowie der Anzahl anwesender funktioneller Gruppen von Pflanzen unterschieden (1-60 Pflanzenarten, 1-4 funktionelle Gruppen). Die funktionellen Gruppen wurden *a priori* festgelegt, und die Pflanzenarten wurden zufällig aus einer Liste von 60 Vertretern mitteleuropäischer *Arrhenatherum*-Gemeinschaften ausgewählt. Die im Einzelnen durchgeführten Experimente umfassten (i) Manipulationen einzelner Herbivoren (**Käfigexperimente**), (ii) Studien an einzelnen Pflanzenarten (**Phytometerexperimente**), (iii) Manipulation von Insektengemeinschaften (**Insektizidbehandlungen**), und (iv) Studien auf Ebene ganzer Pflanzengemeinschaften (z.B. **Herbvorietransekte**, Biomasseernte).

Die in vier Manuskripten vorgelegten Ergebnisse dieser Arbeit erlauben eine klare Falsifikation aller vier Nullhypothesen. Die folgenden **Alternativhypothesen** werden vorgeschlagen:

(1) Invertebratenherbivorie auf Ebene **ganzer Pflanzengemeinschaften** ist schwach positiv mit Pflanzenartenreichtum korreliert. Die funktionelle Identität von Pflanzenarten ist der bedeutendste Faktor in Hinblick auf Invertebratenherbivorie. Die Anwesenheit von Leguminosen führt zu einem Anstieg, wohingegen die Anwesenheit von Gräsern zu einer Reduktion von Herbivorie führt.

(2) Invertebratenherbivorie auf Ebene **einzelner Pflanzenarten** hängt nicht auf konsistente Weise mit Pflanzenartenreichtum zusammen. Die funktionelle Identität anwesender Pflanzenarten ist der bedeutendste Faktor, der Herbivorie auf Ebene

einzelner Pflanzenarten bestimmt.

(3) Herbivorie, welche durch **eine einzelne**, im Rahmen eines Käfigexperiments untersuchte **Insektenart** hervorgerufen wird, ist nur dann negativ mit Pflanzenartenreichtum korreliert, wenn die vom Herbivoren bevorzugte Nahrungsressource vorhanden ist. Wiederum stellt die funktionelle Identität von Pflanzenarten die bedeutendste erklärende Variable dar.

(4) Die **Biomasse von Pflanzengemeinschaften**, sowie deren Blattflächenindex, sind in einigen Fällen positiv mit Invertebratenherbivorie korreliert.

(5) Wachstum und Entwicklung **einzelner Pflanzenarten** sind signifikant negativ mit Pflanzenartenreichtum sowie mit der Anzahl funktioneller Gruppen korreliert. Die funtionelle Identität von Pflanzenarten innerhalb einer Gemeinschaft, sowie die funktionelle Identität der jeweils betrachteten Pflanzenart, sind die bedeutendsten Determinanten von Wachstum und Entwicklung einzelner Pflanzenarten. Dieser Befund könnte große Bedeutung für die **Invasionsresistenz** experimenteller Pflanzengemeinschaften aufweisen.

(6) Die **Stabilität von Pflanzengemeinschaften**, gemessen anhand der **Resistenz** und **Resilienz** nach einer Störung in Form selektiver Herbivorie, ist in erster Linie durch das Überleben des Herbivoren und durch die funktionellen Charakteristika der jeweiligen Pflanzengemeinschaften beeinflusst; die Stabilität von Pflanzengemeinschaften wird insbesondere dann vom Pflanzenartenreichtum abhängen, wenn die vom betrachteten Herbivoren präferierte Nahrungsressource in einer Pflanzengemeinschaft vorhanden ist.

Die im Rahmen dieser Arbeit vorgelegten Ergebnisse verdeutlichen, dass die funktionelle Identität von Pflanzenarten innerhalb experimenteller Wiesenpflanzengemeinschaften einen bedeutenden Einfluss auf Invertebratenherbivorie, Invasionsresistenz, und Stabilität von Pflanzengemeinschaften haben kann. Die Anzahl anwesender Pflanzenarten *per se* dürfte demnach von geringerer Bedeutung sein, als man dies bisher angenommen hat.

Demnach lässt sich die Hypothese aufstellen, dass Daten von Biodiversitätsexperimenten ganz generell im Hinblick auf mögliche Effekte der Variablen Pflanzenartenzusammensetzung, Funktionelle Identität und Artidentität von Pflanzenarten analysiert werden sollten – anstatt sich ausschließlich auf die Signifikanz des Faktors Pflanzenartenzahl zu konzentrieren.

Durch eine derartige Vorgehensweise könnte man letztlich dann jene Vorhersagekraft erhalten, die nötig ist, um in Modellsystemen erhaltene Befunde auf die Realität, und nicht zuletzt auch auf die Gesellschaft, übertragbar zu machen.

CURRICULUM VITAE

EDUCATION

Nov 02-Aug 06	PhD Student, supervised by Prof. Dr. Wolfgang W. Weisser, University of Jena, DFG Forschergruppe Biodiversität; Thesis title „Invertebrate herbivory, plant diversity and ecosystem processes in experimental grassland“
2003	Professional Development Course “Advances in Ecology”, Imperial College London, UK
2002	Diploma work with Prof. Dr. Michael J Crawley, Imperial College London, Silwood Park, UK; Thesis title “The effects of herbivory and competition on *Senecio inaequidens* DC. (Asteraceae), an invasive alien plant”
Feb 2002	Professional course „Generalized Linear Models and Statistical Computing, Prof. Dr. Michael J. Crawley, Imperial College London, UK
Nov 99- Oct 02	Studies of biology (Hauptstudium), University of Rostock, with distinction
Nov 97- Oct 99	Studies of biology, University of Regensburg, grade A („sehr gut“)
1996	Abitur, Max-Planck-Gymnasium München-Pasing, grade A (Note 1,3)

AWARDS AND DISTINCTIONS

2003-2006	Studienstiftung des deutschen Volkes, PhD Scholarship
2004-2006	International Max Planck Research School, membership
2001-2006	E-Fellows.net Career Network Scholarship
1998-2002	Studienstiftung des deutschen Volkes, Scholarship
2002	Studienstiftung des deutschen Volkes, Scholarship for foreign studies
2002	Deutscher Studienpreis, (German students award), 2nd prize
2000	Deutscher Studienpreis (German students award), 1st prize
1997	Jugend forscht, nationwide 1st prize (Biology section)

Acknowledgements

I acknowledge the support of the following people, who have been very helpful for the completion of this thesis:

I would wish to express my special gratitude to **Prof. Dr. Wolfgang W. Weisser** (Jena, Germany) for supervising all experiments and the whole process of writing and completion of this thesis.

Further, I thank **Prof. Dr. Stefan Halle** (Jena, Germany) and **Prof. Dr. Teja Tscharntke** (Göttingen, Germany) for refereeing this thesis.

Prof. Dr. Michael J. Crawley (Silwood Park, UK), has always taken his precious time to advise me in statistical data analysis, and his way of thinking has been very inspiring for my work.

Of the many people who have been invaluable during all my work, I want to especially say thank you to **Sylvia Creutzburg**, who has always assisted me during all phases of this work. Further special thanks go to **Sybille Unsicker**, **Juliane Specht**, **Peter Mwangi**, **Ilka Egerer**, and **Ingrid Jakobi**, for their help out in the field and for their inspiration. Thanks also to **Denise Göpfert** for her support in administrative questions.

In addition, the following people have helped and assisted me in various ways:

Prof. Dr. Markus S. Fischer (Potsdam, Germany), Prof. Dr. Stefan Halle (Jena, Germany), Prof. Dr. Michael Scherer-Lorenzen (Zurich, Switzerland), Prof. Dr. Stefan Scheu (Darmstadt, Germany), Prof. Dr. Bernhard Schmid (Zurich, Switzerland), Prof. Dr. Ernst-Detlef Schulze (Jena, Germany).

Dr. Karin Groten (Jena), Dr. Elke Grunow (Jena), Dr. Günter Köhler (Jena), Dr. Grit Kunert (Jena), Dr. Alexandru Milcu (Silwood Park, UK), Dr. Yvonne Oelmann (Mainz), Dr. Christiane Roscher (Jena), Dr. Jens Schumacher (Jena), Dr. Vicky M. Temperton (Jülich), Dr. Winfried Voigt (Jena), Dr. Alexandra Weigelt (Jena).

Alexander Sabais, Alexandra Franzke, Andrea Schmittke, Anett Oswald, Anja Wildner, Anne Richter, Aristides Moustakas, Carolin Bergmann, Caroline Henkel, Claudia Bahr, Claudia Heise, Daniela Reich, David Wildner, Diana Fricke, Ding Wang, Doreen Köhler, Gerlinde Kratzsch, Heike Scheffler, Holger Merker, Ingrid Jakobi, Inka Riccius, Jana Petermann, Janine Hofmann, Katja Kunze, Katrin M. Meyer, Lars W. Clement, Linda Jung, Lisa Marquard, Lisann Deringer, Marlen Gubsch, Martin Schmitz, Melanie Marr, Michaela Knauer, Nadine Mitschunas, Nico Eisenhauer, Raphael Marché, René Müller, Sabine Hänniger, Sabine Reinsch, Sandra Lechowski, Silke Grohe, Silke Hengelhaupt, Steffen Eismann, Stephan Partsch, Sylvia Junghans, Tanja Rottstock, Tim Walther, Ulrike Wehmeier, Yvonne Kreutziger.

Finally, I would wish to thank my parents, **Gertraude and Jörg Scherber**, and my girlfriend **Julia Wolf**, for all their love and support for so many years.

List of scientific publications and talks
(Wissenschaftliche Publikationen und Vorträge des Autors)

Peer-reviewed:

Scherber, C., Milcu, A., Partsch, S., Scheu, S., Weisser, W.W. (2006) *The effects of plant diversity and insect herbivory on performance of individual plant species in experimental grassland*. **Journal of Ecology** 94: 922-931, Blackwell Publishing, Oxford, doi: 10.1111/j.1365-2745.2006.01144.x

Scherber, C., Mwangi, P.N., Temperton, V. M., Roscher, C., Schumacher, J., Schmid, B., Weisser, W.W. (2006) *Effects of plant diversity on invertebrate herbivory in experimental grassland*. **Oecologia** 147: 489-500, Springer Berlin, Heidelberg, New York. DOI: 10.1007/s00442-005-0281-3

Scherber, C., Crawley M.J., Porembski S. (2004) *Insect herbivory and plant competition studies on the invasive alien plant* Senecio inaequidens *(Asteraceae) under laboratory conditions*. In: Kühn, I. & Klotz, S. (eds.) Biological Invasions – Challenges for Science. **Neobiota** 3: 41-52, Berlin.

Scherber C., Crawley M.J., Porembski S. (2003) *The effects of herbivory and competition on the invasive alien plant* Senecio inaequidens *(Asteraceae)*. **Diversity and Distributions** 9: 415-426, Blackwell Publishing, Oxford.

Further Publications (Weitere Publikationen):

Scherber, C., Weisser, W.W. (2005) *Plant diversity and invertebrate herbivory in experimental grassland ecosystems*. Proceedings of the Annual Meeting of the British Ecological Society, University of Hertfordshire, UK, 5 - 7 September 2005.

Scherber, C., Weisser, W.W. (2005) *Effects of plant diversity on invertebrate herbivory in grasslands*. Proceedings of the GFÖ **35**, 147.

Specht, J., Scherber, C., Köhler, G., Weisser, W.W. (2005) *The relationship between plant diversity and fitness of the Common Grasshopper,* Chorthippus parallelus *ZETT. (Orthoptera: Acrididae)*. Proceedings of the GFÖ **35**, 149.

Scherber, C. (2003) *Biologische Kontrolle des Neophyten* Senecio inaequidens *(Asteraceae) durch ein einheimisches Insekt, Longitarsus jacobaeae (Coleoptera: Chrysomelidae). Neozoen-Newsletter*, Allgemeine & Spezielle Zoologie, Universität Rostock.

Scherber, C. (2002) *The effects of herbivory and competition on* Senecio inaequidens *DC (Asteraceae), an invasive alien plant*. Proceedings of the 2nd International Conference of the German Working Group on Biological Invasions “Neobiota” – Biological Invasions: Challenges for Science: p. 53, October 10th-12th, 2002

Scherber, C. (2002) *The effects of herbivory and competition on* Senecio inaequidens *DC (Asteraceae), an invasive alien plant*. Diplomarbeit, Online-Veröffentlichung, http://www.diplomarbeiten24.de/vorschau/8982.html

Scherber, C. (2002): *Schönheit siegt. Ursachen und Folgen physischer Attraktivität*. Traditio et Innovatio 4, Universität Rostock.

Scherber, C. (2000) *Gentechnisch veränderte Lebensmittel und Öffentlichkeitsarbeit – Ergebnisse einer interdisiplinären Studie*. Tagungsband Innovative und Nachhaltige Agrobiotechnologie in Mecklenburg-Vorpommern, Rostock.

Scherber, C. (2000) *Artenturnover – ein Maß für die zeitliche Dynamik in Biozönosen*. Online-Veröffentlichung eines Seminarbeitrages, http://www.grin.com/de/preview/3238.html

Scherber, C. (2000) *Fleischfressende Pflanzen*. Online-Veröffentlichung eines Seminarbeitrages, http://www.grin.com/de/preview/3238.html

Scherber, C. (1998) *Die Tier- und Pflanzenwelt von Hochmooren am Beispiel des Naturschutzgebiets "Bernrieder Filz" (Südbayern), mit besonderer Berücksichtigung gefährdeter Arten und deren Mobilität*. In: Das Taublatt 33, 1/1998, Sonderheft Lebensraum Hochmoor. ISSN 0942-959X

Scherber, C. (1998) *Charakteristische Tierarten in Hochmoorbiotopen*. In: Das Taublatt 33, 1/1998, Sonderheft Lebensraum Hochmoor. ISSN 0942-959X

Scherber, C. (1998) *Anpassungen von Tieren an die Lebensbedingungen im Hochmoor*. In: Das Taublatt 33, 1/1998, Sonderheft Lebensraum Hochmoor. ISSN 0942-959X

Scherber, C. (1998) *Pflanzenwelt im Moor*. In: Das Taublatt 33, 1/1998, Sonderheft Lebensraum Hochmoor. ISSN 0942-959X

Scherber, C. (1998) *Tiere und Pflanzen im Lebensraum Hochmoor*. In: Das Taublatt 33, 1/1998, Sonderheft Lebensraum Hochmoor. ISSN 0942-959X

Scherber, C. (1998) *Allgemeine Definition und Abgrenzung des Lebensraumes "Hochmoor" von anderen Moortypen*. In: Das Taublatt 33, 1/1998, Sonderheft Lebensraum Hochmoor. ISSN 0942-959X

Scherber, C. (1998) *Moore – eine Einführung*. In: Das Taublatt 33, 1/1998, Sonderheft Lebensraum Hochmoor. ISSN 0942-959X

Scherber, C. (1997) *Leben im Hochmoor*. Veröffentlichung der Jugend forscht-Bundeswettbewerbs-Arbeit, http://www.grin.com/de/fulltext/bik/16819.html

Scherber, C. (1997) *Renaturierungsmaßnahmen im Naturschutzgebiet "Bernrieder Filz"*. Ausfertigung als Auftragsarbeit für das Gemeindearchiv der Gemeinde Seeshaupt, Starnberger See, Bayern.

Scherber, C. (1996) *Renaturierungsmaßnahmen im Naturschutzgebiet "Bernrieder Filz"*. Ausfertigung als Auftragsarbeit für das Bayerische Landesamt für Umweltschutz, Bibliothek des Bayerischen Landesamts für Umweltschutz.

Talks (Vorträge):

Scherber, C. (2005) *Insect herbivory in grasslands: New insights from The Jena Experiment*. Seminar in plant population biology and biodiversity research, Institut für Umweltwissenschaften, Universität Zürich, 14. Dezember 2005.

Scherber, C. (2005*) Invertebrate herbivory and plant diversity in The Jena Experiment*. Institutsseminar, Institut für Ökologie, Friedrich-Schiller-Universität Jena, 27. April 2005.

Scherber, C., Weisser W.W (2005) *Invertebrate herbivory*. Internal progress report, DFG research group biodiversity, 4th -5th April 2005.

Scherber, C. (2005) *Insect herbivory and plant diversity in* Rumex acetosa *L. (Polygonaceae).* International Max Planck Research School 2nd Biannual Symposium 2005, Jena, Germany.

Scherber, C. (2005) *Two years of experimental research on herbivory and plant diversity*. International Max Planck Research School 1st Biannual Symposium 2005, Jena, Germany.

Scherber, C. (2004) *Invertebrate Herbivory, Invertebrate Diversity and Ecosystem Functioning in Experimental Grassland Ecosystems*. International Max Planck Research School Biannual Symposium 2004, Jena, Germany.

Scherber, C. (2004) *From Alaska to the lab: Complex systems research in ecology* (in German). German Cancer Research Centre, Heidelberg, Germany, June 2004.

Scherber, C., Weisser, W.W. (2004) *Insect herbivory and plant diversity in experimental grassland ecosystems*. Multitrophic Interactions Meeting, University of Goettingen, 31st March 2004, Germany.

Scherber, C., Weisser, W.W. (2004) *A new subexperiment on biodiversity, herbivory and stability*. Internal project meeting, DFG research group biodiversity, 11th March 2004.

Scherber, C. (2004) *Herbivory and plant diversity: a caging experiment*. Internal progress report, DFG research group biodiversity, 5th Feb 2004

Scherber, C. (2003) *Invertebrate herbivory*. Internal progress report, DFG research group biodiversity, 9th -10th December 2003.

Scherber, C., Weisser W.W. (2003) Biodiversität und das Funktionieren von Ökosystemen: Warum ist Artenvielfalt wichtig? Botanischer Garten Münster, 7.Oktober 2003.

Scherber, C. (2003) Invertebrate herbivory, invertebrate diversity and ecosystem functioning in experimental grasslands. Institutsseminar, Institut für Ökologie, Friedrich-Schiller-Universität Jena, 16. April 2003.

Scherber, C. (2003) *Invertebrate herbivory, invertebrate diversity and ecosystem functioning in experimental grasslands*. Internal planning paper, DFG research group biodiversity, 3rd March 2003.

Scherber, C. (2002) *The effects of herbivory and competition on* Senecio inaequidens *DC. (Asteraceae), an invasive alien plant*. Silwood Seminar Series, 23rd September 2002.

Scherber, C. (1998) *Animals and Plants of the ' Bernrieder Filz' Nature Reserve (Bavaria, Municipality of Seeshaupt).* Proceedings of the 2nd Conference of the International Carnivorous Plant Society, Botanisches Institut der Universität Bonn.

Scherber, C. (1997) *Natur- und Artenschutz im Landkreis Starnberg*. Vortrag und Diskussion gemeinsam mit E. Rohde-von Saldern, Landratsamt Starnberg. Weßling, Landkreis Starnberg, 16. Oktober 1997

Scherber, C. (1997*) Lebensraum Hochmoor. Die Tier- und Pflanzenwelt heimischer Hochmoore – Gefährdung und Schutz*. Dia-Vortrag, Seehäusl, Weßling, Landkreis Starnberg, Bund Naturschutz in Bayern e.V.

Scherber, C. (1997) *Die paar Frösche – Wozu brauchen wir eigentlich Artenschutz?* Podiumsdiskussion mit Christoph Scherber und Dr. Helmut Klein (Bund Naturschutz in Bayern e.V.) sowie Vertretern des Forstamtes Starnberg, 26.März 1997, Fontana die Trevi, Farchach am Starnberger See

Scherber, C. (1997) *Lebensraum Hochmoor*. Vortrag bei der Fachgruppe Steingarten und Alpine Stauden der Gesellschaft der Staudenfreunde e.V., München, 28.Juni 1997

Scherber, C. (1997) *Lebensraum Hochmoor – Gefährdung und Schutz heimischer Hochmoore*. Vortrag beim Landesbund für Vogelschutz in Bayern e.V., Kreisgruppe Starnberg.

Scherber, C. (1997) *Ökologische Untersuchungen im Bernrieder Filz*. Vortrag bei der Mikrobiologischen Vereinigung München, 24. September 1997

Scherber, C. (1996) *Lebensraum Hochmoor*. Vortrag beim Bund Naturschutz in Bayern e.V., Ortsgruppe Tutzing (Starnberger See), Bayern, 7. November 1996

Posters (Poster):

Scherber, C., Weisser, W.W. (2005) *Insect herbivory and plant diversity in Rumex acetosa L. (Polygonaceae)*. International Max Planck Research School 2nd Biannual Symposium 2005, Jena, Germany.

Scherber, C., Weisser, W.W. (2004) *Invertebrate Herbivory, Invertebrate Diversity and Ecosystem Functioning in Experimental Grassland Ecosystems*. International Max Planck Research School Biannual Symposium 2004, Jena, Germany.