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

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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.

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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

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

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 (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

August 2003 Source	Numerator d.f.	Denominator d.f.	Percentage herbivory[a]		Number of leaves[b]		Plant dry weight (g)[b]	
			F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Intercept	1	Plot	355.345	**<0.0001**	3962.650	<0.0001	319.9612	**<0.0001**
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	**<0.0001**	22.1559	**<0.0001**
Insecticide treatment	1	Subplot	8.572	**0.0044**	21.082	**<0.0001**	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.
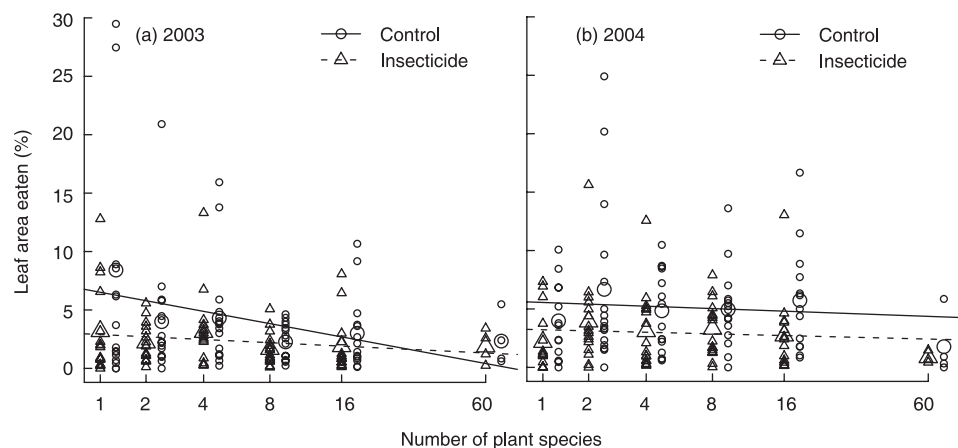
Results

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
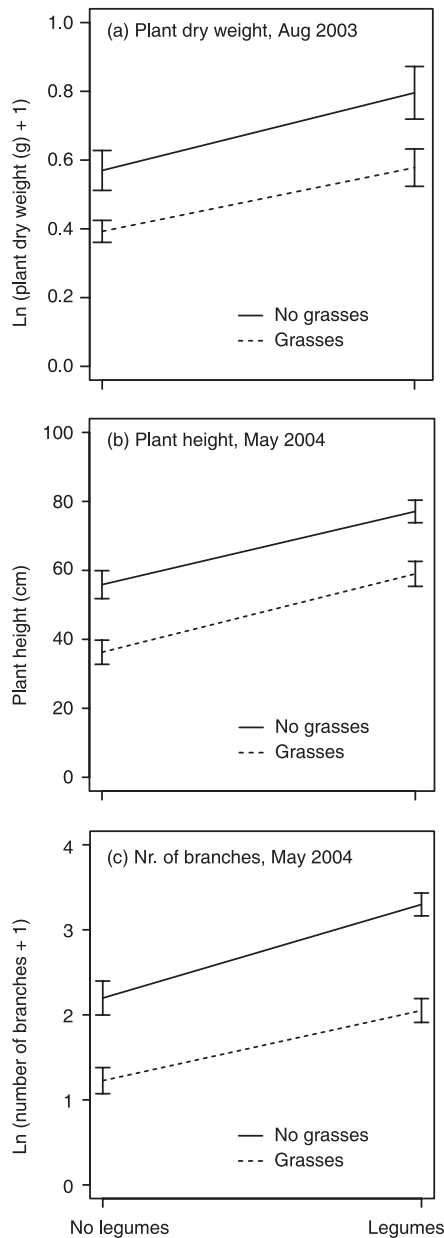

Fig. 1 Relationship between number of plant species and leaf area eaten (%) in *R. acetosa* in (a) 2003 and (b) 2004. Open circles and solid lines, control plants; open triangles and dashed lines, insecticide-treated plants. Lines show mean squares fits. r^2 values for 2003 are 0.017 (insecticide) and 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 Source	Numerator d.f.	Denominator d.f.	Percentage mortality[a]		Percentage herbivory[a]		Plant height (cm)		Number of branches[b]		Number of main axes[c]	
			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	**<0.0001**	523.2211	**<0.0001**	719.8119	**<0.0001**	630.9144	**<0.0001**	1201.156	**<0.0001**
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	**<0.0001**	32.208	**<0.0001**
Legumes	1	Plot	4.3943	**0.0393**	Excluded	Excluded	25.2185	**<0.0001**	22.6474	**<0.0001**	23.341	**<0.0001**
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	**<0.0001**
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	**<0.0001**	19.080	**<0.0001**
Number of observations			164		159		NA		NS		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.
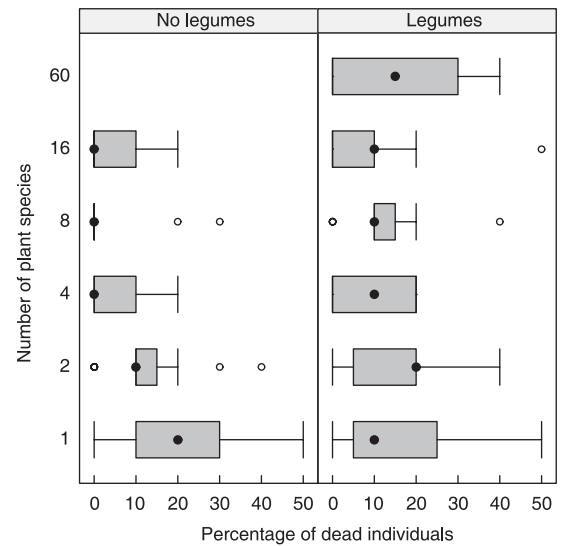
Fig. 2 Effects of particular functional groups on (a) plant dry weight, (b) maximum inflorescence length, and (c) number of first-order inflorescence branches in *R. acetosa*. Solid (dashed) lines indicate plots without (with) grasses. Lines connect means ± 1 SE.

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).

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.

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).
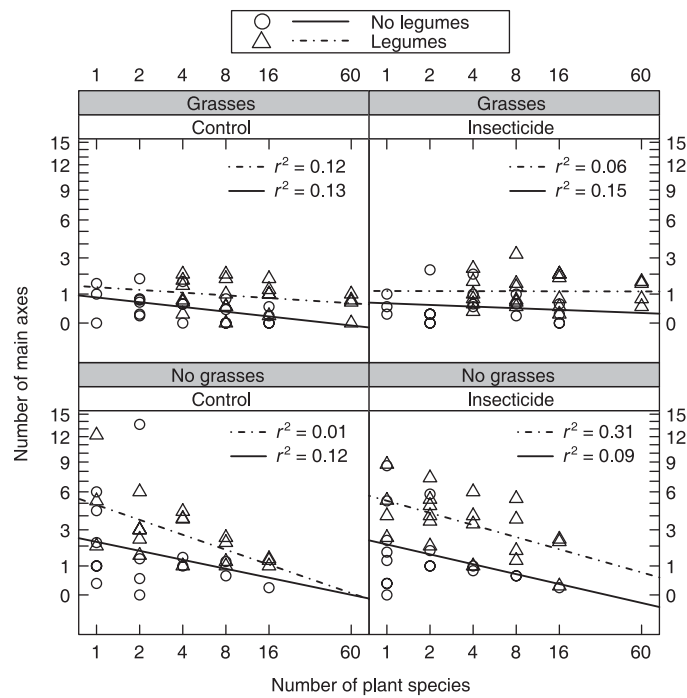
PLANT DRY WEIGHT

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

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; r^2 values are for these mean squares fits only.

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.

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).

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.

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.

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