PLANT-ANIMAL INTERACTIONS

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Effects of plant diversity on invertebrate herbivory in experimental grassland

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

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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 cm^2 ($i = 1$), 0.065 cm^2 ($i = 2$) and 0.125 cm^2 ($i = 3$), and, for feeding damage on the leaf margins, NM, the number of leaf sites that were damaged with an area of $AM \leq 0.5$ cm^2. The total leaf area eaten per plant (TLD) in centimetre square 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 millimeter square). 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 cm^2 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 block-wise, 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.

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,
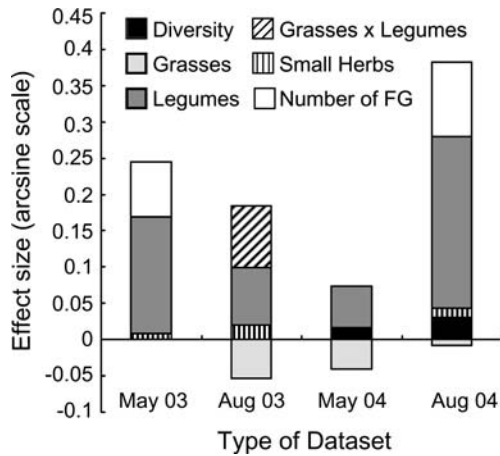
Fig. 1 Community herbivory. *Bars* show factorial effect sizes of explanatory variables retained in the minimal adequate statistical models. Effect sizes are untransformed proportions, p (i.e. arcsin $\sqrt[2]{p}$). Block effects not included. Note the overall minor contribution of plant species richness or number of FG in comparison with legume and grass effect sizes

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. lancolata*: 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 $0.83 \pm 0.11\%$ 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 $1.15 \pm 0.08\%$. 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 ($1.3 \pm 0.12\%$ vs. $0.99 \pm 0.12\%$, 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 $2.61 \pm 0.44\%$. 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 had no significant influence on damage. The minimal adequate model contained presence of grasses, and presence of legumes (AIC = −179.9297, df = 4, $R^2 = 0.15$).

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, May 2003	Herbivory[a] per plant	NA NS	NA NS	$F_{1,70}=1.01,$ $P=0.318$ (0)	NA NS	NA NS	$F_{1,70}=3.42,$ $P=0.068$ (0)	NA NS	NA NS
P. lanceolata, Aug 2003	Herbivory per leaf	$F_{3,74}=3.91,$ $P=0.012$	NA NS	NA NS	NA NS	$F_{1,74}=7.47,$ $P=0.008$ (+)	$F_{1,74}=3.36,$ $P=0.071$ (0)	NA NS	NA NS
T. pratense, Aug 2003	Herbivory per leaf	NA NS	NA NS	NA NS	NA NS	NA NS	$F_{1,76}=6.52,$ $P=0.012$ (+)	$F_{1,76}=7.37,$ $P=0.008$ (−)	NA NS
R. acetosa, Aug 2003	Herbivory per leaf	NA NS	$F_{1,77}=3.08,$ $P=0.08$ (0)	NA NS	$F_{1,77}=5.49,$ $P=0.022$ (+)	NA NS	NA NS	NA NS	NA NS
Transects									
May 2003	Herbivory per plant	$F_{3,74}=5.57,$ $P=0.002$	NA NS	$F_{1,74}=0.62,$ $P=0.43$ (+)	NA NS	NA NS	NA NS	$F_{1,74}=36.76,$ $P<0.001$ (+)	$F_{1,74}=5.92,$ $P=0.017$ (+)
Aug 2003	Leaf size[b] (mm²)	$F_{3,57}=4.47,$ $P=0.006$	$F_{1,57}=8.45,$ $P=0.005$ (+)	NA NS	$F_{1,57}=16.74,$ $P<0.001$ (+)	NA NS	NA NS	NA NS	NA NS
	Leaf damage[c] (mm²)	NA NS	NA NS	NA NS	$F_{1,60}=6.26,$ $P=0.015$ (+)	NA NS	NA NS	$F_{1,60}=10.10,$ $P=0.002$ (+)	NA NS
	Herbivory per leaf	NA NS	NA NS	$F_{1,59}=6.22,$ $P=0.015$ (+)	NA NS	NA NS	$F_{1,59}=4.68,$ $P=0.034$ (−)	$F_{1,59}=7.49,$ $P=0.008$ (+)	NA NS
May 2004	Herbivory per plant	NA NS	$F_{1,73}=5.94,$ $P=0.017$ (+)	NA NS	NA NS	NA NS	$F_{1,73}=4.53,$ $P=0.036$ (−)	$F_{1,73}=9.18,$ $P=0.003$ (+)	NA NS
Aug 2004	Herbivory per plant	NA NS	$F_{1,74}=5.22,$ $P=0.025$ (+)[d]	$F_{1,74}=0.43,$ $P=0.512$ (0)	NA NS	NA NS	$F_{1,74}=0.04,$ $P=0.848$ (0)	$F_{1,74}=19.72,$ $P<0.001$ (+)	$F_{1,74}=4.44,$ $P=0.038$ (+)

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

Rumex acetosa

In August 2003, invertebrate herbivore damage was on average $3.03 \pm 0.3\%$ 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, $df=4$, $R^2=0.10$).

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 $1.33 \pm 0.16\%$ 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, $df=7$, $R^2=0.40$).

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

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
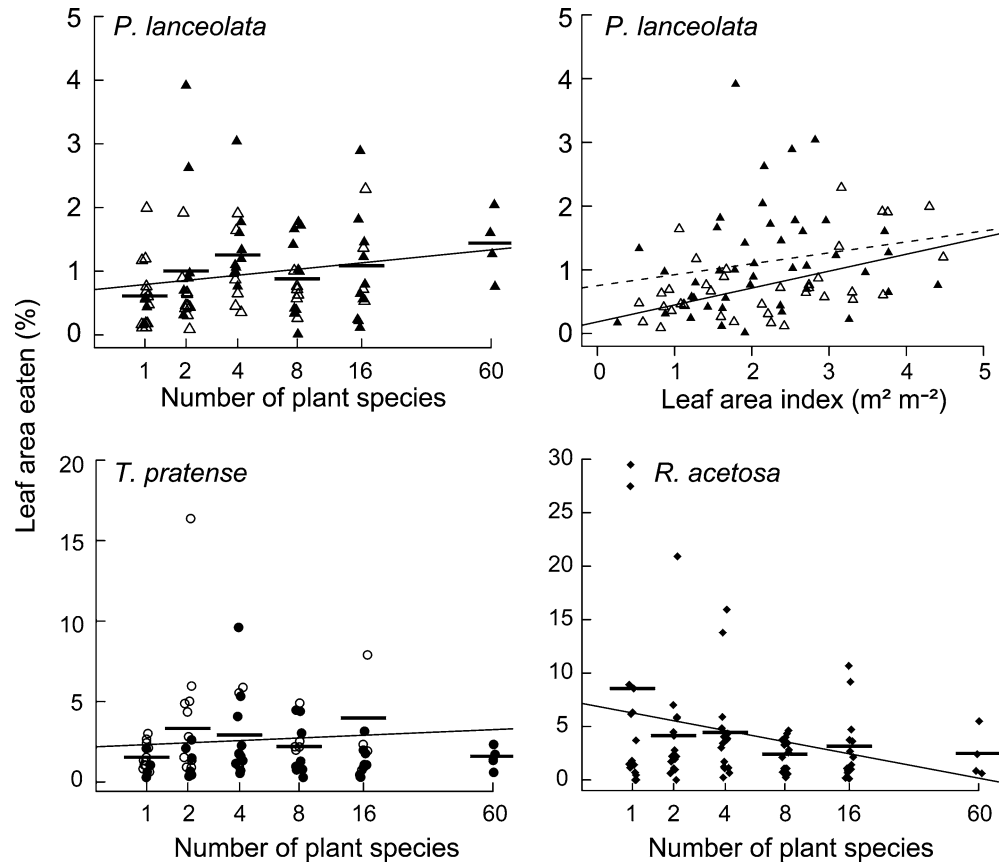
remained constant across the richness gradient (Fig. 3), at a level of $0.65 \pm 0.08\%$, and the effect of species richness on proportional damage was not significant (Table 1). However, the proportion of damaged leaf area increased significantly with increasing number of FG in the community (Table 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 $0.46 \pm 0.08\%$. 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 $2.87 \pm 0.39\%$. 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, $R^2 = 0.29$).

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
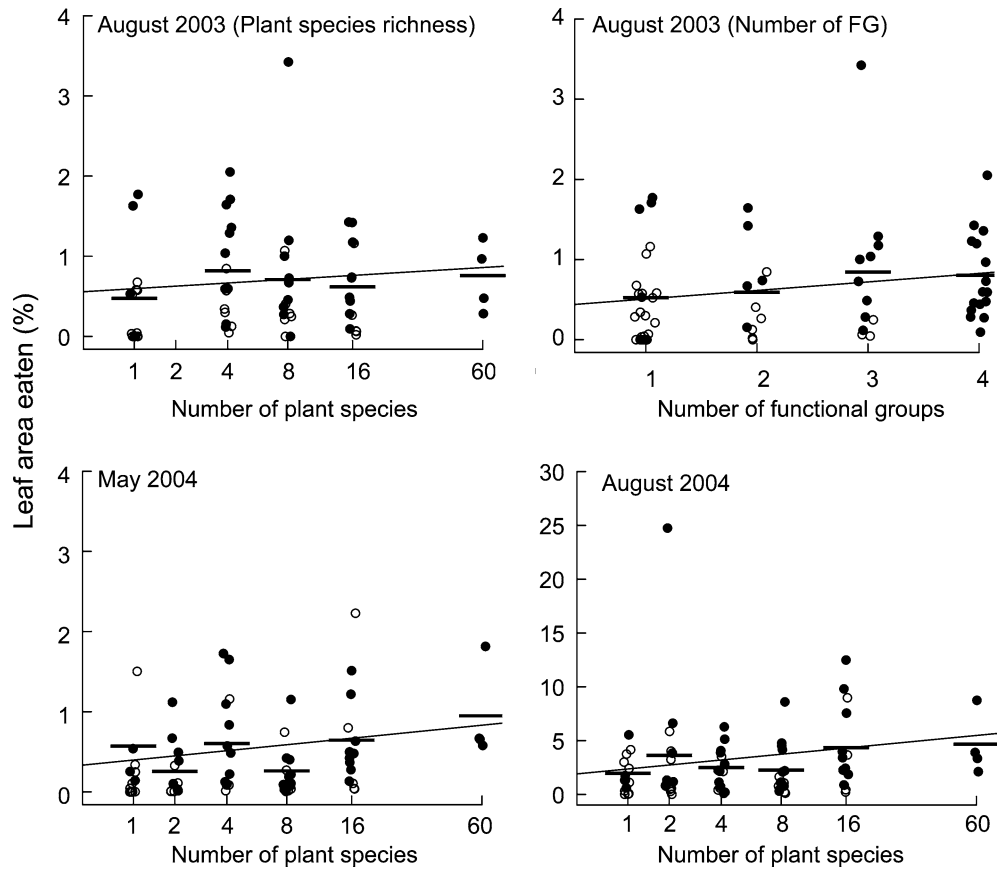
Discussion

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.

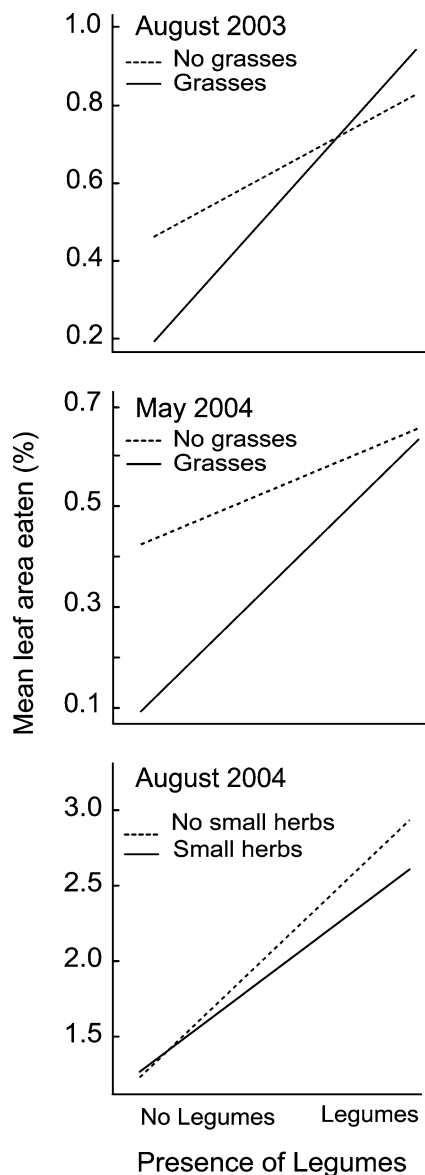Unfortunately, it is difficult to determine the exact links between given plant species and their specific herbivore communities (Unsicker et al. 2005). For this reason, in line with other published studies, we do not differentiate between specialist and generalist herbivores in our analysis even though 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).

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

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

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.

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