

## LETTER

# Plant species loss decreases arthropod diversity and shifts trophic structure

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## Abstract

Plant diversity is predicted to be positively linked to the diversity of herbivores and predators in a foodweb. Yet, the relationship between plant and animal diversity is explained by a variety of competing hypotheses, with mixed empirical results for each hypothesis. We sampled arthropods for over a decade in an experiment that manipulated the number of grassland plant species. We found that herbivore and predator species richness were strongly, positively related to plant species richness, and that these relationships were caused by different mechanisms at herbivore and predator trophic levels. Even more dramatic was the threefold increase, from low- to high-plant species richness, in abundances of predatory and parasitoid arthropods relative to their herbivorous prey. Our results demonstrate that, over the long term, the loss of plant species propagates through food webs, greatly decreasing arthropod species richness, shifting a predator-dominated trophic structure to being herbivore dominated, and likely impacting ecosystem functioning and services.

## Keywords

Arthropods, biodiversity, consumers, ecosystem function, herbivores, insects, long-term, plant diversity, predators, trophic structure.

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## INTRODUCTION

As biodiversity loss accelerates, ecologists have devoted increasing effort to understand how these declines will affect ecosystem functioning (Chapin *et al.* 1997; Tilman 1999; Loreau *et al.* 2001). Most effort has focused on plant biodiversity (Balvanera *et al.* 2006), and how loss of plant species or functional groups impacts processes such as ecosystem productivity (Hector *et al.* 1999; Tilman *et al.* 2006a; Cardinale *et al.* 2007), nutrient cycling (Hooper & Vitousek 1998), and ecosystem stability (Tilman *et al.* 2006b). What is less understood is if and how the loss of diversity at the producer level impacts associated consumer species (Haddad *et al.* 2001; Balvanera *et al.* 2006; Crutsinger *et al.* 2006; Johnson *et al.* 2006), and whether the effects of

plant species extinctions are dampened or magnified across trophic levels (Cardinale *et al.* 2006; Duffy *et al.* 2007). In this study, we show that, during more than a decade of study, the loss of plant diversity propagates through the food web, causes dramatic losses of arthropod diversity at multiple trophic levels, and alters the trophic structure of the arthropod community.

We address several prevailing hypotheses underlying the positive relationship between plant and consumer diversity. First, many herbivores exhibit some degree of feeding specialization (Bernays & Graham 1988; but see Novotny *et al.* 2002), such that, in comparison with a simple community, a diverse plant community should provide a greater diversity of resources for a greater number of herbivore species (the Resource Specialization Hypothesis,

Hutchinson 1959; Southwood *et al.* 1979; Strong *et al.* 1984). Second, diverse plant communities are often more productive than simple plant communities (i.e. Tilman *et al.* 2001). Higher productivity provides a greater quantity of resources for consumers, thereby increasing the number of consumer individuals, and, concomitantly, the number of consumer species (the More Individuals Hypothesis, Srivastava & Lawton 1998). Third, and in contrast to the More Individuals Hypothesis, the Resource Concentration Hypothesis predicts that specialist herbivores are attracted to and remain on high concentrations of their host plants (Root 1973). Therefore, plant communities with few plant species should show higher herbivore abundances, particularly of specialist herbivores, than diverse plant communities where host plants are more dispersed.

While the above hypotheses predict strong 'bottom-up' consequences of plant diversity for herbivore richness and abundance, predators have also been positively linked to plant diversity (Haddad *et al.* 2001). Predator species may simply be responding to increased diversity and/or productivity of resources provided by herbivore species in diverse plant communities (i.e. Resource Specialization or More Individuals Hypotheses at the predator trophic level). Predators may also be responding positively to structural habitat diversity in diverse and productive plant communities (Strong *et al.* 1984). As a consequence, there is the potential for plant diversity to alter the structure of associated animal communities, with stronger 'top-down' effects of predators in more diverse plant communities (Paine 1966; Lubchenco 1978). For example, the Enemies Hypothesis predicts higher abundances of predators in more productive and structurally diverse areas will limit overall herbivore abundances (Root 1973).

Taken together, the Resource Specialization and More Individuals Hypotheses make similar predictions that increasing the number of plant species in a community should lead to more herbivore and predator species. These predictions have been supported by some studies, mainly experiments (Siemann *et al.* 1998; Haddad *et al.* 2001; Wimp *et al.* 2004; Crutsinger *et al.* 2006; Johnson *et al.* 2006). However, a number of observational studies (Currie 1991; Wright & Samways 1998; Hawkins & Porter 2003; Jetz *et al.* 2009) and some experimental studies (Koricheva *et al.* 2000) have found no effect of plant diversity on consumer diversity. Moreover, species richness patterns are often strongly linked to abundance patterns in ecological communities (Gotelli & Graves 1996) and these hypotheses make different predictions about how plant diversity should affect consumer abundances. For example, herbivore abundances may either increase in diverse plant communities because of increased plant productivity (More Individuals Hypothesis), or decrease because of higher preferences for more concentrated resources (Resource Concentration

Hypothesis) or increased consumption by more abundant predators (Enemies Hypothesis).

In this study, we test the effects of plant diversity on herbivorous and predatory/parasitoid arthropods in an 11-year study from the longest running experiment to manipulate the species richness of plants, the Cedar Creek Ecosystem Science Reserve biodiversity experiment (details in Tilman *et al.* 2001). Specifically, we asked (1) does higher plant species richness increase arthropod species richness across trophic levels? (2) Are individual plant species or functional groups responsible for the effects of plant diversity on consumers? and (3) does plant species richness affect the trophic structure within arthropod communities by altering the relative abundances of herbivores and predators?

## METHODS

The Cedar Creek Ecosystem Science Reserve biodiversity experiment was created in 1994 by seeding 168, 13 × 13 m plots with 1, 2, 4, 8 or 16 plant species drawn at random from a pool of 18 plant species that represented five different plant functional groups (C3 grasses, C4 grasses, forbs, legumes and woody species). Details of the experimental design have been reported in numerous publications, including Tilman *et al.* (2001, 2006b). Plots have been weeded manually 3–4 times per year since 1997, and the actual number of plant species was strongly, positively correlated with the intended number of plant species each year (Tilman *et al.* 2001). Many characteristics of plants were measured once or annually and allowed us to address hypotheses about effects of plant diversity on consumers, including actual plant species richness (Resource Specialization Hypothesis), and plant biomass or percent cover (More Individuals Hypothesis, details in Tilman *et al.* 2001) through their inclusion as covariates. Plots were burned annually, and measures of total aboveground biomass include that year's litter production. In 2000, plot sizes were reduced, by mowing, to 9 × 9 m.

Arthropod species richness and abundance were measured annually from 1996 to 2006. Each plot annually received 25 'sweeps' with a 38 cm diameter muslin net while walking for 10 m *c.* 2–3 m from the plot's edge, where a 'sweep' is defined as a rapid *c.* 2 m long movement of the net through vegetation. Although sweep samples miss part of the arthropod community, particularly species living on the ground or within plant tissues, our previous work has shown similar responses of arthropod communities to plant diversity in samples taken with sweep and vacuum samples (Knops *et al.* 1999). Samples were taken on one day in August each year except 1996, when samples were taken in July. We sampled plots in August at approximately the time of peak plant biomass, and we restricted sampling to

1 day year<sup>-1</sup> because our previous work has shown consistent patterns with respect to our treatments across months within a year (Haddad *et al.* 2001) and because of the large effort involved in identifying species and individuals in a diverse community.

Samples from each plot were sorted to species or morphospecies and individuals were counted. In addition, nearly all arthropods were assigned to one of five trophic categories, herbivores, parasitoids, predators, detritivores, or omnivores, based on mouth parts, knowledge of their natural histories, and consultation with the literature. For the purposes of this manuscript, we focus on herbivore, predator, and parasitoid responses (predators and parasitoids were grouped). In total, we sampled 112 238 individuals of 733 arthropod species, of which 50% of species and 56% of individuals were herbivores, and 36% of species and 27% of individuals were predatory or parasitoid (referred to, for brevity, as 'predators'). We further classified herbivores as specialists or generalists based on the literature and our knowledge of their specific feeding associations at Cedar Creek. We did this for the one hundred most abundant herbivore species, making up 97% of all herbivore individuals.

## Analysis

We conducted two sets of repeated measures analyses of annual herbivore and predator species richness and abundance using general linear models. First, we analysed responses to our specific treatment variables, plant species richness and number of plant functional groups, plus plant biomass, which is known to be an important predictor of arthropod diversity and abundance. Second, we analysed effects of plant composition. We did this by evaluating additional measures of plant diversity averaged across all years of the study, including actual (rather than planted) plant species richness or actual Shannon-Wiener Index, which accounts for the variable abundances of each plant species. For number of plant functional groups, we substituted a binary variable for the presence or absence of four of the five most important groups by species number and biomass – C3 grasses, C4 grasses, legumes and forbs. Woody plants were not included because burning effectively removed them from multi-species plots.

Because so many species of herbivorous or predatory arthropods were rare, often represented by one or a few individuals for the entire study, a sample collected in a given plot in a given year likely missed many of these rare species. To overcome this limitation and to allow us to get a better estimate of the total cumulative effect of plant species richness on arthropod species richness, we focus much of our analyses on the cumulative number of arthropod species and individuals observed, in total, across the full 11-year

time series (summed number of species or individuals for each plot). To do this, we tested the effects of plant species richness and composition on cumulative herbivore and predator species richness and abundance using multiple linear regression. We conducted additional tests on herbivore load, defined as the number of herbivores/plant biomass, and, separately, on herbivore generalists and specialists. As with year-to-year analyses, we created two general sets of models, one with treatment variables and one with other measures of plant species diversity and composition. All analyses were based on Type-III sums of squares.

To test whether the response of cumulative arthropod richness to plant richness was the result of sampling limitations, we computed cumulative rarefied richness, estimated richness, and inter-annual species turnover for both herbivores and predators. We used individual-based rarefaction to correct for biases in species richness that arise from differences in the number of individuals among plots (Gotelli & Colwell 2001). In our case, we rarefied arthropod species abundances in all plots down to the abundance in the plot that has the fewest individuals (122 individuals for herbivores; 32 individuals for predators; Gotelli & Graves 1996). While rarefaction scaled down the observed numbers of arthropod species in our plots, we also used the Chao 1 species richness estimator to scale up the estimated number of species that occurred in each plot annually and during the entire study. Chao 1 is particularly useful for datasets, such as ours, skewed toward low-abundance classes because it adds a correction factor to observed richness based on the number of rare species (singletons and doubletons) in a plot (Chao 1984; Magurran 2004). We analysed the effects of plant species richness and plant functional group richness on rarefied richness and estimated richness as in our analysis of species richness using multiple linear regression.

Finally, a strong factor governing cumulative arthropod richness in a given plot is the year-to-year turnover in arthropod species composition. To address the long-term effects of plant species richness on herbivore and predator cumulative richness, we calculated species turnover, defined as:

$$t = \frac{b + c}{S_1 + S_2}$$

where  $b$  = the number of species present in a plot that are unique to year 1;  $c$  = the number of species present in a plot that are unique to year 2;  $S_1$  = the total number of species present in a plot in year 1; and  $S_2$  = the total number of species present in a plot in year 2 (Magurran 2004). Turnover was calculated separately for herbivores and predators for every plot and between each of the 11 years. We then analysed the effects of plant species richness on inter-annual herbivore and predator turnover averaged across the time series.

### Additivity analysis

We tested for sampling effects (Huston 1997) in two different ways. First, we determined whether the presence of particular plant species or functional groups accounted for diversity effects. Second, we conducted an ‘additivity’ analysis in which we predicted arthropod species richness in plant mixtures based on each arthropod species’ occurrence in monocultures. We sketch the calculation of this predicted arthropod species richness here for the annual analyses and provide a more detailed derivation in the Supporting Information. Define  $\lambda_{k,l}$  as the Poisson rate (in units of number of individuals per plot) at which arthropods of species  $l$  occur on monocultures of plant species  $k$  in a given year, and let  $\hat{\lambda}_{k,l}$  denote the maximum likelihood estimate of this rate. If, in polycultures from the same year, individuals of each arthropod species occur as independent Poisson processes with the same rate as in monocultures, then it can be shown that the expected arthropod richness in a polyculture is given by the formula

$$\sum_l \left\{ 1 - \exp \left( - \sum_k \hat{\lambda}_{k,l} n_k \right) \right\} \quad (1)$$

where  $n_k$  is the proportion of the mixture planted in plant species  $k$ . This expected arthropod richness is nearly, but not exactly, comparable with the observed arthropod species richness in mixtures. To allow an exact comparison, we then repeated this calculation using the observed arthropod abundance in each polyculture in place of the sum  $\sum_k \hat{\lambda}_{k,l} n_k$  in eqn 1. We then calculated the percentage difference between the expected and (adjusted) observed arthropod species richness for each polyculture, and averaged these percentage differences over plant species richness treatments. Additivity analyses for cumulative and rarefied richness were performed using similar methods.

### RESULTS

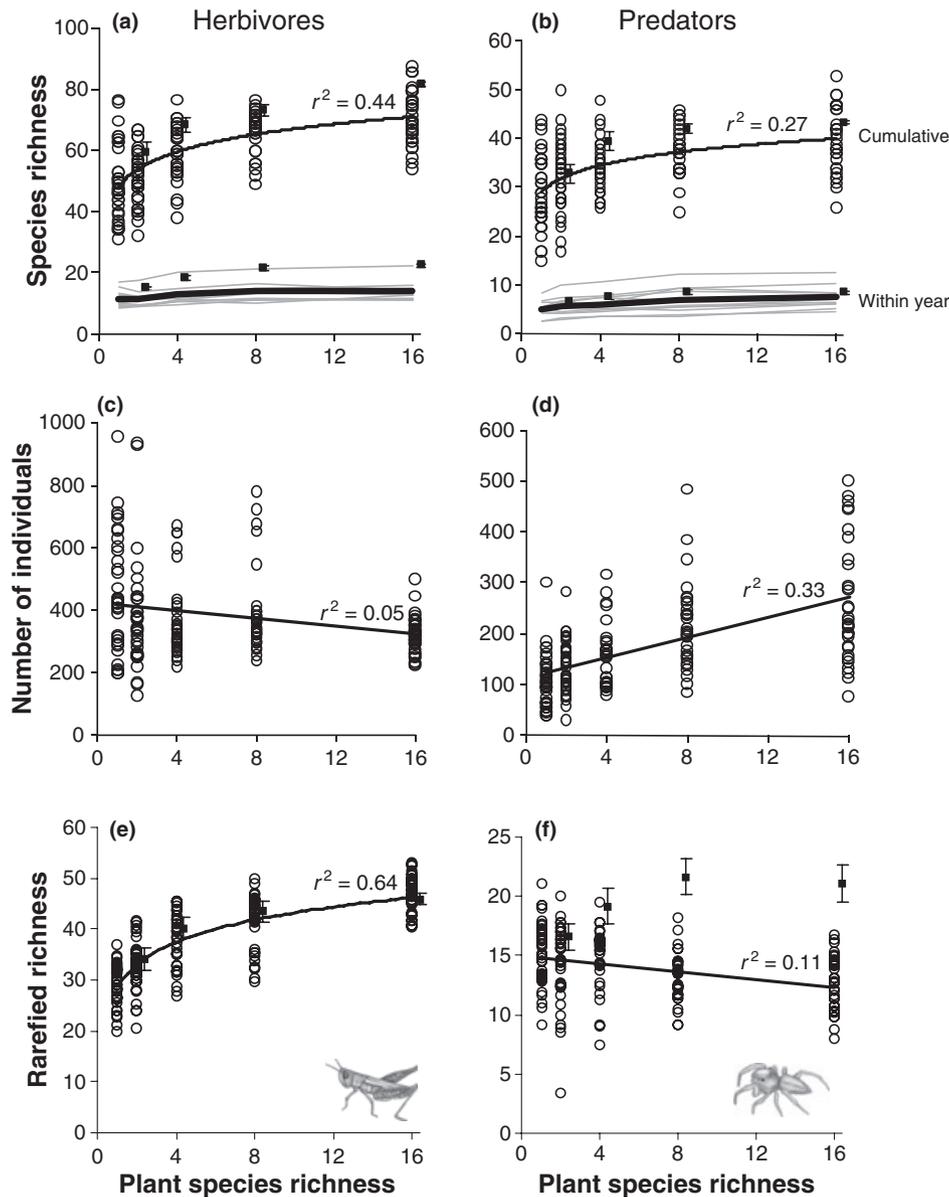
Within each individual year, higher plant species richness significantly increased herbivore species richness (Fig. 1a: lower data and curves; Table S1 and S2). As the number of plant species increased from 1 to 16, herbivore species richness within a year increased by an average of 22%. Responses were relatively weak, with plant species richness explaining, on average, 7% (year range 0–23%) of the variation in herbivore species richness, with the relationship strengthening over time (average  $r^2 = 0.03$  from 1996 to 1999, average  $r^2 = 0.10$  2000–2006). Similarly, predator richness within years was positively related to plant species richness (Fig. 1b: lower data and curves; Table S5). On average, predator richness increased

by 47% with the number of plant species, which explained *c.* 11% (year range 2–30%) of the variation in predator species richness. Both herbivore and predator species richness were positively related to plant biomass (Tables S1 and S5).

While annual arthropod responses were relatively weak, we found strong responses of cumulative richness of both herbivores and predators to plant species richness (Fig. 1a,b: upper data and curves). Herbivore richness increased by 43% and predator richness increased by 35% as the number of plant species increased from 1 to 16. We observed 18% higher interannual turnover in herbivorous species composition with increasing plant species richness (Fig. 2). In contrast, interannual turnover of predators actually declined by 11% with increasing plant species richness. Higher herbivore turnover may have been caused by the 46% increase in the number of rare herbivore species (singletons and doubletons, Fig. S2) with the number of plant species.

We used both species rarefaction and richness estimators (Chao 1) to test for the possibility of sampling limitations causing observed effects of plant species richness on cumulative herbivore and predator richness. Both estimated and rarefied cumulative herbivore richness showed strong, positive relationships with plant species richness (Fig. 1e, Table 1, Supporting Information), indicating that the relationships we observed were not sampling artefacts. For herbivores, the effects of plant species richness did not saturate, as plots with 16 plant species had significantly higher rarefied herbivore species richness than plots with eight plant species (Fig. S1). In contrast, cumulative rarefied predator richness decreased by 18% with plant species richness (Fig. 1f; Table 2). Moreover, increased herbivore turnover with plant species richness was not caused by successional dynamics in establishing plots. When we considered only the final 6 years of data (that is, once plant communities had become well established; Tilman *et al.* 2001), we found similar strong, positive relationship between plant species richness and cumulative herbivore and predator species richness (data not shown).

We found opposing responses of herbivore and predator abundances to plant richness, which suggests richness responses are caused by different underlying mechanisms. Cumulative herbivore abundance declined by 30% with increasing plant species richness ( $r^2 = 0.05$ ,  $P = 0.003$ ; Fig. 1c). Contrary to the Resource Concentration Hypothesis, we found declines in abundances of both specialist ( $r^2 = 0.04$ ,  $P = 0.01$ ) and generalist ( $r^2 = 0.06$ ,  $P = 0.002$ ) herbivores. This negative response of herbivore abundance to plant species richness was intensified when we accounted for changes in plant biomass: because higher plant species richness increased plant biomass (Tilman *et al.* 2001) and decreased herbivore abundances, herbivore load (number of

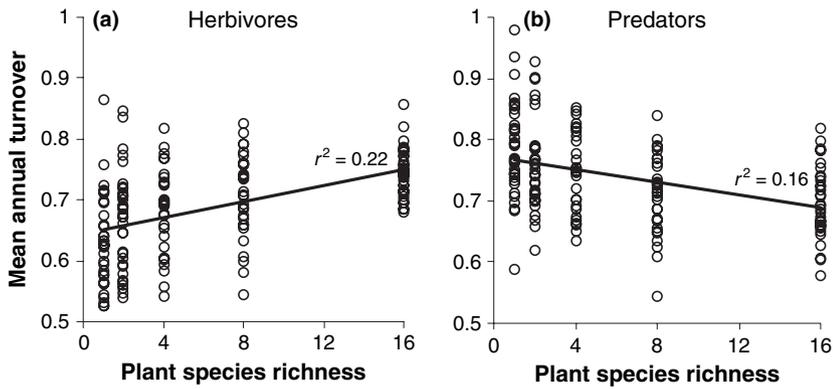


**Figure 1** Effects of plant species richness on (a) herbivore and (b) predator species richness, (c) herbivore and (d) predator cumulative abundance, and cumulative rarefied (e) herbivore and (f) predator richness. In (a) and (b), upper data points and the upper dark line are for cumulative richness (when pooled across years). Lower grey lines are for each of the 11 years of data, and the dark line running through them is the means across all years. In (a), (b), (c), and (f), solid squares show the predicted richness ( $\pm$  95% confidence interval) based on additive contributions of each monoculture for the cumulative data set (the lower squares in (a) and (b) are for yearly data). Rarefaction accounts for effects of arthropod abundance on diversity. For predator abundance (d), one outlying point is not shown for which 1303 spiderlings were sampled on one day in a two species plot.  $r^2$  values in all figures are for simple least squares regression.

insects/plant biomass, Root 1973) was significantly, negatively related to the log of plant species richness ( $r^2 = 0.31$ ,  $P = 0.001$ ). In contrast to herbivores, as plant species richness increased from 1 to 16 species, predator abundance increased by 41% ( $r^2 = 0.17$ ,  $P = 0.001$ ; Fig. 1d). So, although the proportion of herbivore species relative to predator species declined slightly across our diversity

gradient (mean = 0.6,  $r^2 = 0.05$ ,  $P = 0.004$ ), the proportion of predator individuals increased from a ratio of 0.26 to a ratio of 0.81 (Fig. 3).

When examining the effects of particular functional groups on arthropods, we found that the presence of key functional groups had some influence on arthropod species richness across trophic levels. Cumulative herbivore



**Figure 2** Effects of plant species richness on the interannual turnover in (a) herbivore and (b) predator species composition. We present mean plot-level turnover (averaged across all years).

**Table 1** Full multiple linear regression model predicting effects of plant species richness, functional group composition, and plant biomass on rarefied herbivore richness of cumulative data

Source	d.f.	Sum of squares	F-value	P-value
Model	6	7891	84.47	0.001
Error	156	2429		
Corrected total	162	10 320		
$R^2 = 0.76$				

Variable	Parameter estimate	Standard error	t-value	P-value
Intercept	23.005	0.999	23.03	0.001
Ln (plant species richness)	2.038	0.916	2.23	0.028
Presence C3 grasses	4.016	1.014	3.96	0.001
Presence C4 grasses	3.214	0.850	3.78	0.001
Presence forbs	-0.601	0.973	-0.62	0.538
Presence legumes	3.905	0.975	4.00	0.001
Mean plant biomass	0.022	0.004	5.30	0.001

The four variables for functional group composition denote the presence or absence from a plot of any species of that functional group (coded '0' if absent, '1' if present). Type-III sums of squares are reported.

richness increased in the presence of C3 grasses and legumes (Table S9). Cumulative herbivore abundance decreased in the presence of C4 grasses (Table S10). Rarefied herbivore richness increased in the presence of C3 and C4 grasses and legumes (Table 1). Cumulative predator abundance increased in the presence of C3 and C4 grasses (Table S15). Rarefied predator richness decreased in the presence of C4 grasses (Table 2). The effects of functional groups observed within-years were similar to cumulative results (Table S2, S4, and S7). These responses may be explained in part by variation across functional groups in percent tissue nitrogen of above-ground biomass (averaged across 1996–2000, 2002, 2006;  $F = 81.59$ ,  $r^2 = 0.67$ ,  $P = 0.001$ ), which likely affects plant tissue quality for insect herbivores. Percent tissue nitrogen

**Table 2** Full multiple linear regression model predicting effects of plant richness, functional group composition, and plant biomass on rarefied predator richness of cumulative data

Source	d.f.	Sum of squares	F-value	P-value
Model	6	371	10.07	0.001
Error	156	958		
Corrected total	162	1329		
$R^2 = 0.25$				

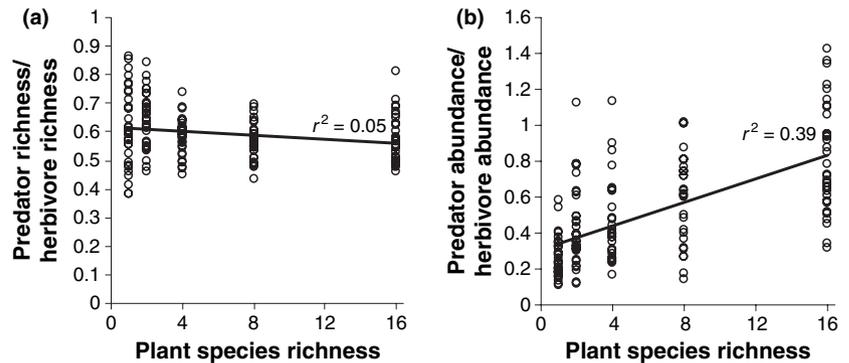
Variable	Parameter estimate	Standard error	t-value	P-value
Intercept	16.399	0.581	28.25	0.001
Ln (plant species richness)	-0.043	0.068	-0.63	0.530
Presence C3 grasses	-0.244	0.516	-0.47	0.638
Presence C4 grasses	-2.685	0.470	-5.72	0.001
Presence forbs	-0.077	0.509	-0.15	0.879
Presence legumes	0.731	0.556	1.32	0.190
Mean plant biomass	-0.003	0.002	-1.38	0.169

The four variables for functional group composition denote the presence or absence from a plot of any species of that functional group (coded '0' if absent, '1' if present). Type-III sums of squares are reported.

increased strongly when plots contained legumes, and decreased when plots contained C4 grasses, C3 grasses, or forbs.

When testing whether arthropod species richness in plant mixtures could be predicted based on arthropod species richness in monocultures, we found fewer herbivore and predator species in polycultures than additive predictions (i.e. underyielding). Underyielding was stronger within-years (36–63% for herbivores; 14–31% for predators) than in cumulative analyses (11–16% for herbivores; 3–14% for predators; Fig. 1a,b). Rarefied herbivore richness was equivalent to richness predicted from monocultures (Fig. 1e); rarefied predator richness was lower than richness predicted from monocultures (Fig. 1f).

**Figure 3** Effects of plant species richness on cumulative arthropod trophic structure. Plant species richness slightly reduced the relative number of predator species (a), but dramatically shifted the ratio of predator to herbivore individuals (b).



## DISCUSSION

Our results show that, over more than a decade, the diversity of arthropods was determined by the diversity of plants. The effects were evident across trophic levels, although they were generated by different mechanisms. Our results indicate that herbivore species richness responded mainly to the diversity of resources provided by different host plants (Table 1). In particular, species richness of herbivores was positively related to plant species richness and strongly affected by the functional groups of species represented. These results provide support for the Resource Specialization Hypothesis. Despite an increase in primary productivity with the number of plant species, we found that herbivore abundance did not drive the increases in herbivore richness, thereby opposing the predictions of the More Individuals Hypothesis for herbivores. Furthermore, and contrary to the predictions of the Resource Concentration Hypothesis, we found similar qualitative responses of both generalist and specialist herbivores. The strong effect of plant species richness was also supported by the non-saturating increase in rarefied herbivore richness through the entire gradient of plant species richness.

In contrast to herbivore responses, the increase in predator species richness with plant species richness was driven by concurrent increases in predator abundances (in support of the More Individuals Hypothesis), and rarefied predator richness actually declined with plant species richness (Fig. 1f). Predator abundance was positively related to higher plant biomass and the presence of grasses, indicating that predators might be responding to the structural complexity created by plant diversity. Our long-term results regarding effects of plant species richness on herbivore and predator species richness were consistent with, but greater in magnitude than shorter term experiments (Siemann *et al.* 1998; Haddad *et al.* 2001; Crutsinger *et al.* 2006; Johnson *et al.* 2006).

Whereas our results show that the loss of plant diversity will have one conservation impact on the loss of rare arthropod species at higher trophic levels, they show that

loss of plant diversity will also affect ecosystem functioning. The strongest and most surprising result observed in our experiment was the change in arthropod community structure, with a shift in the relative abundances of herbivorous vs. predatory arthropods with increasing plant species richness. In particular, in our most diverse plant mixtures predator abundance was 81% that of herbivores, whereas in monocultures predator abundance was just 26% that of herbivores (Fig. 3b). Thus, the abundance of predators relative to herbivores was 3.1 times greater in high-diversity plots than monocultures. Moreover, there was a strong linear dependence of the relative abundance of predators on plant species richness (Fig. 3b). Although our results are consistent with patterns observed in agroecosystem experiments (Russell 1989; Andow 1991), our long-term results are stronger and unsaturating, and extend over a larger range of native plant diversity than has been previously observed.

Furthermore, herbivore abundance was weakly related to primary productivity, when we expected a strong positive relationship. Strikingly, this relationship did not change with time, even though the positive plant species richness–productivity relationship became stronger over the decade-long experiment (Tilman *et al.* 2006a). In light of observed shifts in trophic structure, it is likely that predators and parasitoids are controlling herbivore populations to a much greater extent in communities with higher plant species richness. The causes of this shift in relative abundances of herbivores to predators are uncertain, as we were unable to separate the effects of plant diversity, complexity in habitat structure, and trophic effects in this study. These possibilities, and alternative explanations, merit further exploration. Regardless of mechanism, by greatly reducing the relative abundance of predators, loss of plant diversity may alter the ability of natural communities to control herbivore populations that can become pests. If our findings extend to larger scales, biodiversity maintenance in nearby natural areas may provide an important service of biological control, particularly in agricultural landscapes (Tscharrntke *et al.* 2005; Isaacs *et al.* 2009).

A key issue in biodiversity studies is the relative importance of plant species diversity vs. functional group diversity in controlling community and ecosystem responses. Functional groups in our study had important effects on the richness and abundance of arthropods (Tables 1 and 2), as they have in similar plant diversity studies (Koricheva *et al.* 2000). For example, the presence of C4 grasses in our study had strong effects across trophic levels, and particularly on predators. These grasses are productive, increasing the biomass and habitat structure of plots, and supported more individual predators. But C4 grasses also have low tissue quality, and likely reduced the abundance of herbivores (Tables 1 and 2). Legumes had high tissue nitrogen content, and plots with legumes had higher abundances of herbivores. Other plant functional groups also had significant effects on arthropod species richness and abundance (Table S9, S10, S13 and S15).

Yet, no single plant species or functional group explained arthropod community responses across all trophic levels. This addresses a contentious issue in biodiversity experiments, that observed responses to increased plant species richness are caused by the higher likelihood of a plant mixture containing a particularly important host-plant species (e.g. a highly productive or palatable plant species, Huston 1997; Hughes *et al.* 2008). The conclusion that our results are not caused by the inclusion of one or a small group of plant species in polycultures is supported by our result that rarefied herbivore richness of every plot with 16 plant species was higher than for any plot with one plant species (conversely, 28% of monocultures had higher rarefied predator richness than plots with 16 plant species).

We additionally tested whether arthropod richness in plant mixtures could be predicted based on the arthropods associated with component plant species growing in monoculture. For the most part they could not; in fact, observed herbivore and predator richness were generally lower than predicted richness (Fig. 1a,b). Interestingly, underyielding of arthropod species richness in high-diversity plant mixtures corresponded with higher relative abundances of predators in these plots, and suggests that top-down control of arthropod communities could strengthen with increasing plant diversity (Fig. 3b). Predicting bottom-up effects of plant diversity on the cumulative richness of herbivores in plant mixtures was only possible after richness in monocultures was controlled for changes in abundances using rarefaction (Fig. 1e). Overyielding is typically thought of as a positive aspect of biodiversity, such as in the case of primary productivity responses that are usually attributed to factors such as niche complementarity or facilitation among plant species (Hooper *et al.* 2005). However, our results provide strong support for the

Enemies Hypothesis that predicts higher abundances of predators in more productive and structurally diverse areas will limit herbivore abundances (Root 1973). Our results point to an entirely different explanation for the higher than expected productivity in field experiments with plant polycultures. The increased abundances of predatory and parasitoid arthropods that we observed at higher plant species richness, and the concomitant decreased relative abundance of herbivorous arthropods, could contribute to observed overyielding in primary productivity in this experiment (Tilman *et al.* 2001).

From a conservation perspective, our results show that short-term biodiversity studies greatly underestimate the importance of plant diversity for higher trophic levels, both in terms of the strength of the relationship between plant and arthropod species richness, and also whether the relationship saturated (within each individual year, it did at *c.* 4 plant species; in cumulative analyses it did not saturate). Discrepancies can be explained by the fact that high plant diversity ensured habitat and food resources for numerous rare species that, although present, only occurred in our samples once or twice over the course of a decade (i.e. singletons and doubletons). More rare herbivore species could be caused by rare host plant species in polycultures, or by incomplete sampling of the herbivore community in plant polycultures. Yet, our results were not just caused by incomplete sampling within years; sampling based diversity estimators for cumulative herbivores (Chao 1) as well as for cumulative rarefied richness also increased as plant species richness increased. Simply measuring the diversity of arthropods within just one or a few years – the duration of most studies – is unlikely to detect the full effects of loss of plant diversity on higher trophic levels.

Taken together, our results demonstrate that different mechanisms drive positive trophic responses to plant diversity, the Resource Specialization Hypothesis for herbivores and the More Individuals Hypothesis for predators. Moreover, we found support for the Enemies Hypothesis that predators control herbivore abundances in plant polycultures, and we provide a novel top-down explanation of predators' contribution to overyielding of primary productivity in biodiversity experiments. Finally, we show that even small losses of biodiversity at one trophic level may cause considerable long-term losses throughout the food chain. These losses of plant diversity will have two different conservation impacts, including on the loss of rare consumer species, and on ecosystem functioning as trophic structure shifts toward higher numbers of herbivores. Our findings heighten the need for biodiversity conservation to prevent the long-term disassembly of ecological communities and changes in ecosystem functioning and ecosystem services.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1** 95% CI of rarefied herbivore and predator richness. (a) For herbivores, significant differences were in the following order relative to plant species richness: 1 species < 2 species < 4 species = 8 species < 16 species. (b) For predators, significant differences: 1 species = 2 species = 4 species > 16 species.

**Figure S2** Effects of plant species richness on the number of (a) herbivore and (b) predator and parasitoid species observed one or two times (i.e. singletons or doubletons) within a plot over the entire duration of the experiment.

**Table S1** Repeated measures analysis, using a General Linear Model, of effects of plant species richness, plant functional group richness, and plant biomass on *herbivore species richness within years*.

**Table S2** Repeated measures analysis, using a General Linear Model, of effects of plant diversity, plant functional group composition, and plant biomass on *herbivore species richness within years*.

**Table S3** Repeated measures analysis, using a General Linear Model, of effects of plant species richness, plant functional

group richness, and plant biomass on *herbivore abundance within years*.

**Table S4** Repeated measures analysis, using a General Linear Model, of effects of plant species richness, plant functional group composition, and plant biomass on *herbivore abundance within years*.

**Table S5** Repeated measures analysis, using a General Linear Model, of effects of plant species richness, plant functional group richness, and plant biomass on *predator and parasitoid species richness within years*.

**Table S6** Repeated measures analysis, using a General Linear Model, of effects of plant species richness, plant functional group richness, and plant biomass on *predator and parasitoid abundance within years*.

**Table S7** Repeated measures analysis, using a General Linear Model, of effects of plant species richness, plant functional group composition, and plant biomass on *predator and parasitoid abundance within years*.

**Table S8** Multiple linear regression model predicting effects of plant species richness, plant functional group richness, and plant biomass on *cumulative herbivore species richness*.

**Table S9** Multiple linear regression model predicting effects of plant diversity, functional group composition, and plant biomass on *cumulative herbivore species richness*.

**Table S10** Multiple linear regression model predicting effects of plant diversity, functional group composition, and plant biomass on *cumulative herbivore abundance*.

**Table S11** Multiple linear regression model predicting effects of plant species richness, plant functional group richness, and plant biomass on *rarefied herbivore richness*.

**Table S12** Multiple linear regression model predicting effects of plant species richness, plant functional group richness, and plant biomass on *cumulative predator and parasitoid species richness*.

**Table S13** Multiple linear regression model predicting effects of plant diversity, functional group composition, and plant biomass on *cumulative predator and parasitoid species richness*.

**Table S14** Multiple linear regression model predicting effects of plant species richness, plant functional group richness, and plant biomass on *cumulative predator and parasitoid abundance*.

**Table S15** Multiple linear regression model predicting effects of plant species richness, functional group composition, and plant biomass on *cumulative predator and parasitoid abundance*.

**Table S16** Multiple linear regression model predicting effects of plant species richness, plant functional group richness, and plant biomass on *cumulative detritivore species richness*.

**Table S17** Multiple linear regression model predicting effects of plant species richness, functional group composition, and plant biomass on *cumulative detritivore species richness*.

**Table S18** Multiple linear regression model predicting effects of plant species richness, plant functional group richness, and plant biomass on *cumulative detritivore abundance*.

**Table S19** Multiple linear regression model predicting effects of plant species richness, functional group composition, and plant biomass on *cumulative detritivore abundance*.

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