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# The effects of long-term nitrogen loading on grassland insect communities

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**Abstract** Just as long-term nitrogen loading of grasslands decreases plant species richness and increases plant biomass, we have found that nitrogen loading decreases insect species richness and increases insect abundances. We sampled 54 plots that had been maintained at various rates of nitrogen addition for 14 years. Total insect species richness and effective insect diversity, as well as herbivore and predator species richness, were significantly, negatively related to the rate of nitrogen addition. However, there was variation in trophic responses to nitrogen. Detritivore species richness increased as nitrogen addition increased, and parasitoids showed no response. Insect abundances, measured as the number of insects and insect biovolume (an estimate of biomass), were significantly, positively related to the rate of nitrogen addition, as were the abundances of herbivores and detritivores. Parasitoid abundance was negatively related to the rate of nitrogen addition. Changes in the insect community were correlated with changes in the plant community. As rates of nitrogen addition increased, plant species richness decreased, plant productivity and plant tissue nitrogen increased, and plant composition shifted from $C_4$ to $C_3$ grass species. Along this gradient, total insect species richness and effective insect diversity were most strongly, positively correlated with plant species richness. Insect biovolume was negatively correlated with plant species richness. Responses of individual herbivores varied along the nitrogen gradient, but numbers of 13 of the 18 most abundant herbivores were positively correlated with their host plant biomass. Although insect communities did not respond as strongly as plant communities, insect species richness, abundance, and composition were impacted by nitrogen addition. This study demonstrates that long-term nitrogen loading affects the entire food chain, simplifying both plant and insect communities.

**Key words** Insect communities · Productivity · Diversity · Abundance · Nitrogen loading

## Introduction

Humans have greatly altered the global nitrogen cycle, increasing the rate and magnitude of nitrogen deposition (Vitousek et al. 1997). Among its many environmental consequences, long-term nitrogen loading decreases plant species richness, increases standing crop biomass, and shifts plant composition to a few dominant species (Bakelaar and Odum 1978; Tilman 1987, 1988; Aerts and Berendse 1988; Inouye and Tilman 1988, 1995; Huenneke et al. 1990; Pysek and Leps 1991). Such plant community responses to nitrogen loading may impact the rest of the food chain, but there are few studies of the consequences of long-term nutrient loading on terrestrial trophic structure. Responses of terrestrial food chains to nutrient loading may be similar to responses of aquatic food chains, where phosphorus loading increases algal productivity and shifts algal, zooplankton, insect, and fish community composition (Mundie et al. 1991; Lathrop and Carpenter 1992; Peterson et al. 1993; Perrin and Richardson 1997; Carpenter et al. 1998).

Here, we test the hypothesis that long-term nitrogen loading impacts insect communities much as it impacts plant communities, causing lower insect species richness, higher insect abundances (both numbers of insects and biomass), and shifts in insect species composition. Because inputs of limiting nutrients simultaneously affect plant species richness, plant productivity, plant composition, and plant tissue quality, separating their independent effects on insect communities can be difficult (Siemann 1998). In some cases, attributes of insect communities re-

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spond similarly to changes in plant species richness, plant productivity, plant composition, and plant tissue quality along nitrogen gradients. For example, high nitrogen input should increase insect abundances because these increase as plant species richness decreases, as plant productivity increases, and as plant composition shifts to a few dominant plant species with high tissue quality (Pimentel 1961; Tahvanainen and Root 1972; Root 1973; Hurd and Wolf 1974; Vince et al. 1981; Strauss 1987; Sedlacek et al. 1988; Andow 1991; Siemann 1998). Numerous studies have shown that nitrogen fertilization increases tissue quality for many insect species by increasing plant tissue nitrogen, soluble nitrogen, and amino acid concentrations, and fertilized plants can then support larger insect population sizes (e.g., McNeill and Southwood 1978; Mattson 1980; Scriber and Slansky 1981; Scriber 1984; Strong et al. 1984; Nordin et al. 1998).

The effects of nitrogen addition on insect diversity are more complicated because changes in the plant community may have opposing effects on insect species richness. When nitrogen input is high, the resulting lower plant species richness should decrease insect species richness because the diversity of resources for insect specialists is lower (Murdoch et al. 1972; Nagel 1979; Siemann et al. 1998; Knops et al. 1999). But nitrogen inputs should also increase plant productivity, which should increase the availability of insect resources and increase the number of insect individuals and possibly the number of insect species (Hurd et al. 1971; Hurd and Wolf 1974; Kirchner 1977; Vince et al. 1981; but see Prestidge 1982; Sedlacek et al. 1988; Siemann 1998). In addition, fertilized plants with higher tissue quality should be resources for a greater number of generalist herbivore species and individuals (Mattson 1980; Prestidge and McNeill 1983a; Strauss 1987; Nordin et al. 1998). Thus, the effects of nutrient addition on insect communities are potentially complex and interrelated.

Results of previous studies along experimental nitrogen gradients illustrate difficulties in predicting insect community responses to nitrogen loading. In these studies, data were collected 1–2 years after nitrogen manipulations (Hurd et al. 1971; Hurd and Wolf 1974; Kirchner 1977; Sedlacek et al. 1988; Siemann 1998). Each reported that higher nitrogen input increased insect species richness. These results are consistent with the prediction that nitrogen input increases plant productivity and tissue quality, which increases insect species richness, but contrast with the prediction that nutrient addition decreases plant species richness, which would then decrease insect species richness. Even when plant productivity is held constant, insect species richness may increase as plant species richness decreases due to the effects of nutrient addition on predator-prey interactions (Siemann 1998). However, plant community responses to nutrient addition occur over many years (Carson and Barrett 1988; Goldberg and Miller 1990; Tilman 1993). Previous studies may have been analyzing insect responses to short-term changes in plant productivity, rather than to changes in plant species richness that occur after long-term nutrient loading.

To better understand insect responses to long-term nutrient addition, we evaluated the diversity and abundance of insects along a nitrogen addition gradient that had been experimentally maintained for 14 years (Tilman 1987, 1988, 1996). We analyzed the responses of insect species richness and abundance (both the number of insects and biovolume, an approximation of biomass) to the rate of nitrogen input, which was the direct experimental manipulation. In addition, to gain insights into the factors that might have contributed to insect responses to nitrogen addition, we used multiple regressions to try to identify the influences of plant species richness, plant productivity, plant composition, and plant tissue nitrogen on insect communities. Clearly, such analyses are not as definitive as analyses based solely on manipulated variables, but they might provide insights into responses of the insect community. We controlled for spatial effects on local diversity that might result from the small size and proximity of our experimental plots. Because nutrient addition may alter trophic interactions between insects (Siemann 1998), we also controlled for the effects of trophic interactions on species richness and abundance at each trophic level.

## Methods

### Nitrogen addition experiment

The experiment was conducted at Cedar Creek Natural History Area, which is located in Isanti and Anoka Counties in east-central Minnesota, in field C described in Tilman (1987, 1988). The field was abandoned from agriculture in 1935. Beginning in 1982, the field was fenced above- and belowground to exclude non-insect herbivores and divided into 54 4×4 m plots, each separated from adjacent plots by 1 m on each side and by aluminum flashing buried to a depth of 25 cm. The completely randomized design included nine treatments – six replicates each of a control (no nutrients added), and eight treatments that received a mixture of P, K, Ca, Mg, S, and trace metals (achieved by adding 10 $g \cdot m^{-2}$ $P_2O_5$ and $K_2O$, 15 $g \cdot m^{-2}$ $MgSO_4$, 20 $g \cdot m^{-2}$ $CaCO_3$, and 0.0625 $ml \cdot m^{-2}$ trace metals), and nitrogen in amounts of 0.0, 1.0, 2.0, 3.4, 5.4, 9.5, 17.0, or 27.2 $g \cdot m^{-2} \cdot year^{-1}$.

### Plant community responses

Detailed descriptions of plant species richness, plant productivity, plant composition, and plant community stability in these plots are presented in Tilman (1987, 1988, 1993, 1996), Inouye and Tilman (1988, 1995), and Tilman and Downing (1994). Vegetation in low-nitrogen plots was composed of perennial bunch grasses, primarily *Schizachyrium scoparium*, and forbs, including *Artemisia ludoviciana*, *Aster azureus*, and *Solidago rigida*. High-nitrogen plots were dominated by perennial $C_3$ grasses, including *Poa pratensis* and *Agropyron repens*.

As had been done every year since 1982, a 10×300 cm strip of vegetation was clipped in each plot at ground level in early July of both 1995 and 1996. Samples were sorted to litter or individual living species, dried, and weighed. A different area within the plot was clipped each year. Plant variables used in statistical analyses of insect species richness and abundance included plant species richness, total plant productivity, total litter biomass, plant functional group composition, and plant tissue nitrogen. Plant productivity was estimated as total standing crop biomass. Plants were separated into one of five functional groups: $C_3$ grasses, $C_4$ grasses, forbs, legumes, and woody plants. To determine the effects of plant composition on insects, the proportion of total biomass of $C_3$ grasses (the most productive group) was included as an independent variable. Plant tissue nitrogen was measured in the experimental plots as part of another study in 1993 (Wedin and Tilman 1996). Although tissue nitrogen varied within plants among years, the 1993 measures characterized differences among plants that would be retained across years and would most influence insect communities. Plot tissue nitrogen was estimated by summing percent tissue nitrogen for each species in each plot in 1993 multiplied by species average biomass in 1995 and 1996. This measure of plant tissue quality was not ideal; however, foliar tissue nitrogen was assumed a good correlate of other characteristics, like soluble nitrogen, amino acid concentration, and the C:N ratio, which are known to influence herbivores (Prestidge and McNeill 1983a; Brodbeck and Strong 1987; Nordin et al. 1998). Values of plant variables from the 2 study years were averaged, except in analyses of numbers of individual insect species that occurred in only 1 of the 2 years.

### Insect surveys

On 31 August 1995, 28 June 1996, and 30 July 1996, each of the 54 plots was sampled for insects and other arthropods, including mites and spiders. Each sample consisted of 25 sweeps with a standard 15-inch-diameter light muslin net. While sweep netting does not sample all insects in the community, community measures obtained from sweep netting have been shown to be highly correlated with insects sampled by other methods at Cedar Creek, particularly suction sampling (Siemann 1998; N.M. Haddad, unpublished data). Net contents were transferred to a 4-l plastic bag and frozen. Samples were subsequently thawed, dried, and examined under a Bausch and Lomb ×10 scope. All individuals were identified to species or morphospecies and counted. Counts were exact for all insects except aphids, which, when counts were high, were approximated to the nearest 10. Voucher specimens were stored in an insect collection at Cedar Creek Natural History Area, Bethel, Minn.

### Analysis

We analyzed the responses of insect abundance, insect species richness and effective diversity ($e^{H'}$, where $H'$ is the Shannon index, measured as $-\Sigma p_i \times \log(p_i)$ and $p_i$ is the proportion of individuals represented by species i). Effective diversity controlled for differences in species richness caused by differences in numbers of insects sampled (Ricklefs and Miller 2000); other diversity measures, including Fischer's α and Simpson's index, were also analyzed, but results did not differ from effective diversity and are not presented. We used two measures of insect abundance, including number of individuals and total biovolume, an estimate of biomass. Biovolumes were calculated as the average product of the maximum length, width, and thickness of each species (Siemann et al. 1996) and were measured for five individuals or, if fewer were collected, for all individuals collected. When possible, we used biovolumes determined by Siemann et al. (1996), supplementing measurements for species new to this study.

Insect data were analyzed in three stages. First we used simple regressions to examine the dependence of insect species richness, effective diversity, and abundance on the rate of nitrogen addition. Second, we performed backwards elimination multiple regressions that examined insect responses to nitrogen, plant species richness, plant productivity, plant composition, and plant tissue nitrogen. Third, we performed backwards elimination multiple regressions to assess the response of each insect trophic level. Analyses with plant community variables were strictly correlative, since only nitrogen was manipulated and the plant variables were often correlated with each other. However, we tested for collinearity (Freund and Littell 1991), and if two variables were collinear we dropped one variable from the final model.

To determine spatial effects on insect species richness and abundance in our small experimental plots, we performed a multiple regression with the rate of nitrogen addition and a neighborhood index, calculated as the mean insect species richness or number of insects in the four plots surrounding the focal plot or, for plots on the edge of the experiment, in the two or three neighboring plots. Other neighborhood indices using more distant plots were never significant, and are not reported. We also analyzed how differences in insect community composition between target and nearby plots (measured as the proportion of shared species between all plot pairs using the Jaccard index) were influenced by differences in (1) rates of nitrogen addition (measured as the difference in rank order of experimental treatment) and (2) spatial distance.

Insects were classified into one of five trophic categories, based on field observations and a literature review. Herbivores fed on live vascular plant tissue; parasitoids fed on and developed within other animals; predators killed and consumed other insects; detritivores fed on fungi or dead plant or animal tissues or byproducts, and omnivores, all ants, consumed both plant and animal tissues. A small number of individuals of a small number of species with aquatic larval stages that could not be classified into one of the five categories were excluded from trophic analyses. When species occupied different trophic levels in different stages of their life cycles, we assumed that adults were in plots searching for larval hosts and classified species based on larval feeding preferences, unless we knew that adults were feeding within the plots. A separate analysis was performed using a second trophic categorization based on adult feeding preferences, and the results did not differ qualitatively. For each trophic level, we performed simple regressions on the rate of nitrogen addition, and multiple regressions to determine those variables that most strongly correlated with species richness and abundance. These regressions excluded the level of nitrogen added (because insects were not consuming nitrogen) and included plant species richness, plant productivity, and plant composition. Analyses of herbivores and detritivores also included variables quantifying plant tissue nitrogen, litter biomass, and predator and parasitoid species richness and abundance. Analyses of predators and parasitoids included variables quantifying herbivore and detritivore species richness and abundance.

Finally, we analyzed responses of the 18 most abundant herbivore species in simple regressions against the rate of nitrogen addition. We report best-fit regressions from linear, logarithmic, or quadratic models. In addition, we performed multiple-regression analyses to assess responses of these herbivore species to nitrogen and the biomass of their host plant. We identified host plants from the literature or from personal observation.

## Results

### Responses to nitrogen

### *Plant community*

Plant species richness, plant productivity, litter biomass, and plant composition changed along the nitrogen gradient much as has been reported previously (Tilman 1987, 1988, 1993, 1996; Inouye and Tilman 1988, 1995). Plant species richness decreased as the rate of nitrogen addition increased (Fig. 1A). Plant productivity, estimated as peak, living, aboveground biomass (Fig. 1B), litter biomass (Fig. 1C), and aboveground plant tissue nitrogen ($n$=54, $r^2$=0.73, $P$=0.001) increased as the rate of nitrogen addition increased.

Plant community composition changed along the nitrogen gradient (Inouye and Tilman 1988, 1995; Tilman 1988). Of the two dominant plant species, *P. pratensis*

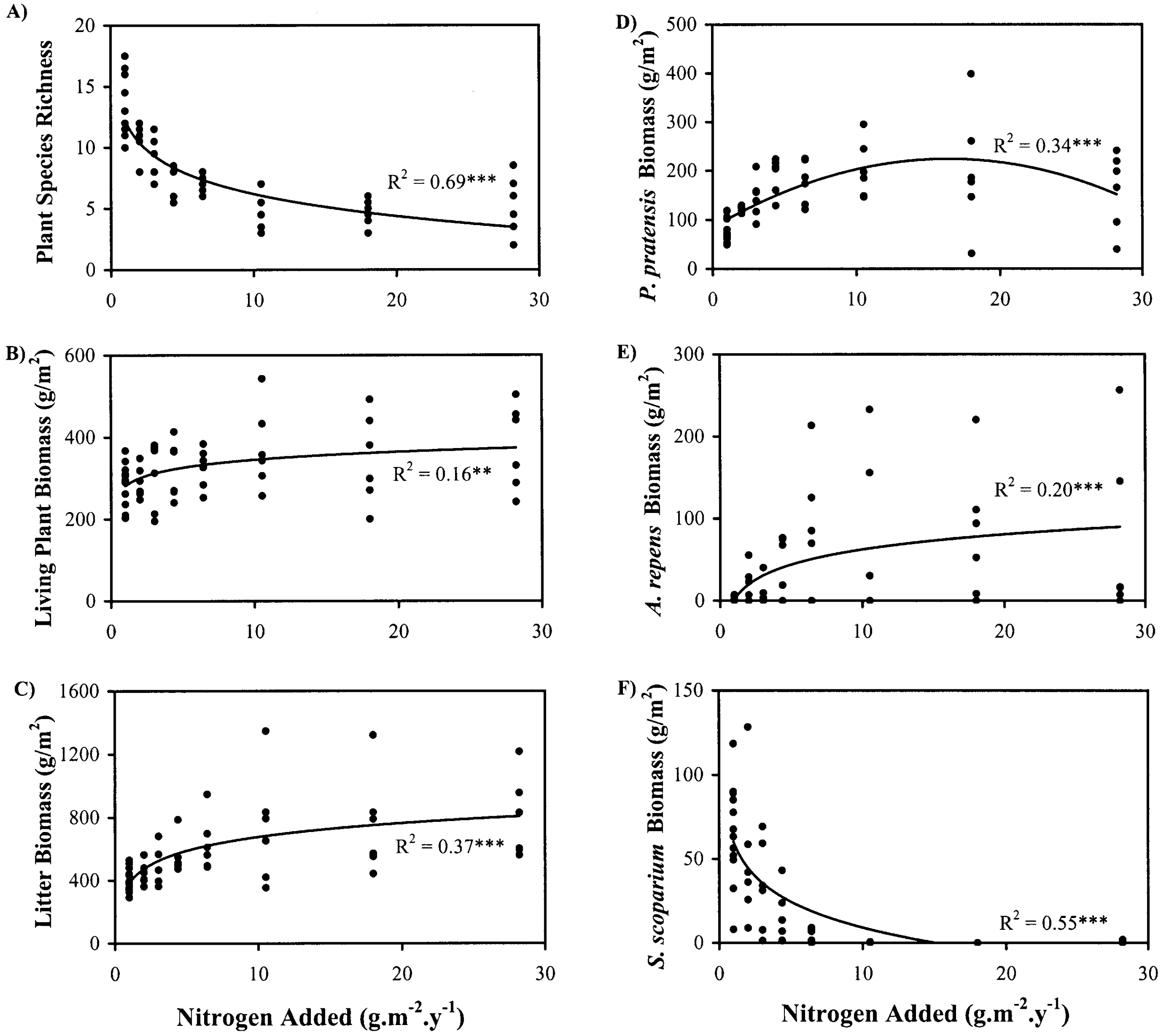

**Fig. 1A–F** Changes in vegetation along the nitrogen gradient (*x*-axis is the rate of nitrogen addition plus 1). Each *point* represents the average of data collected in 1995 and 1996. Patterns are similar to those reported previously (Tilman 1987, 1988; Inouye and Tilman 1988, 1995). Fitted curves are best fit linear, log, or quadratic (** $P<0.01$, *** $P<0.001$)

reached its peak abundance at intermediate rates of nitrogen addition (Fig. 1D), and *A. repens* reached its peak abundance at high rates of nitrogen addition (Fig. 1E). The dominant grass species in control and low-nitrogen plots, *S. scoparium*, decreased in abundance with increasing rates of nitrogen addition (Fig. 1F), as did a forb, *A. azureus*, that was a host for several abundant herbivores.

### *Insect diversity*

In total, we collected 43,942 individual insects and terrestrial arthropods representing 554 species (Table 1). In plots where no nitrogen was added, insect species richness (ANOVA, $n$=12, $F$=1.42, $P$=0.26) and the number of insects ($n$=12, $F$=0.00, $P$=1.00) did not differ significantly between plots with and without addition of other nutrients.

As the rate of nitrogen addition increased, total insect species richness significantly decreased (Fig. 2A). Because this relationship was found for two of three sampling dates when considered separately ($n$=54; June: $r$=–0.32, $P$=0.02; July: $r$=–0.16, $P$=0.23; August: $r$=–0.36, $P$=0.007), data from the three dates were pooled for all further analyses. Effective insect diversity was strongly, negatively related to the logarithm of nitrogen added (Fig. 2B).

We detected spatial influences on diversity in our plots. Insect species richness was positively correlated with mean insect species richness in adjacent plots (Fig. 2C). When the rate of nitrogen addition and the neighborhood index were combined in a multiple regres-

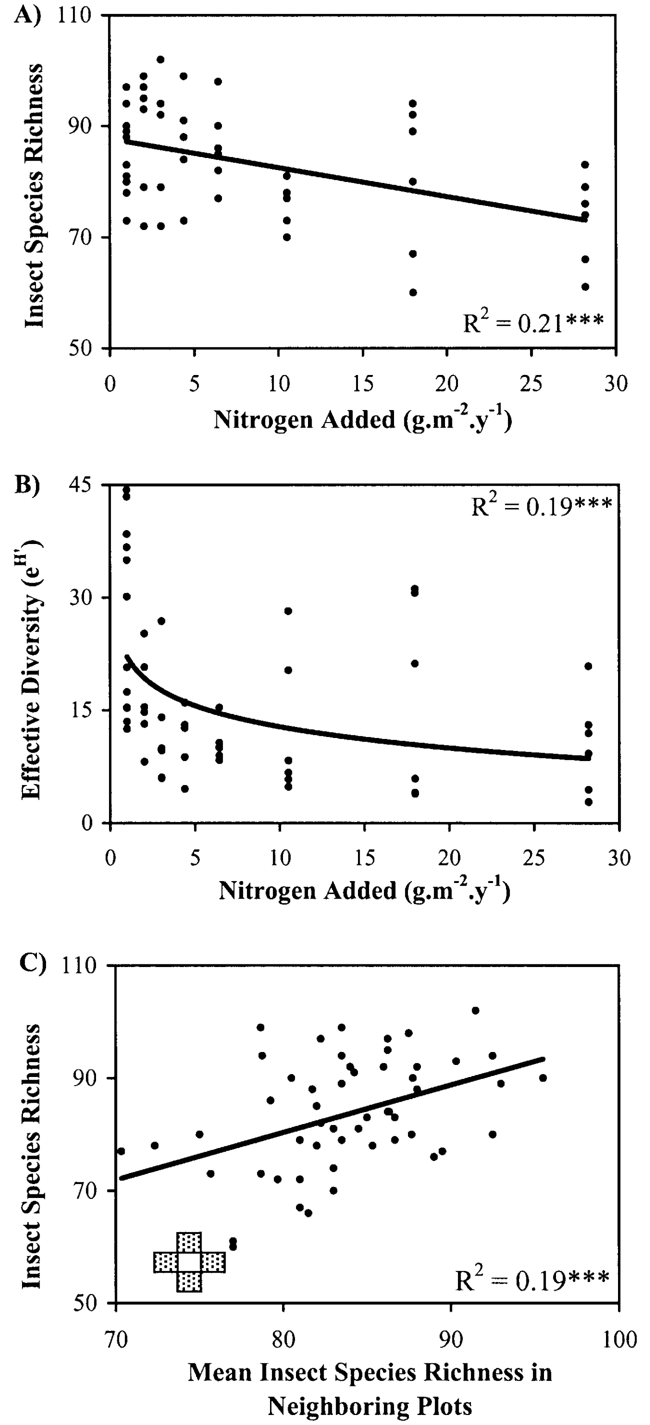

**Fig. 2** Linear regressions of insect species richness against the rate of nitrogen addition plus 1 ($n$=54, $P$=0.001, $y$=–0.52$x$+87.14) (**A**); effective insect diversity ($e^{H'}$) against the rate of nitrogen addition plus 1 ($n$=54, $P$=0.001, y=–4.06log$x$+22.12) (**B**), and insect species richness against the neighborhood index, calculated as the average species richness in the four adjacent plots (see diagram; $n$=54, $P$=0.001, $y$=0.84$x$+13.47) (**C**) (***$P$<0.001)

**Table 1** Insects collected during three samples of the nitrogen addition experiment

| Order | Families | Species | Count |
|---|---|---|---|
| Araneida | 13 | 33 | 543 |
| Collembola | 1 | 2 | 8 |
| Ephemeroptera | 1 | 1 | 1 |
| Odonata | 3 | 4 | 155 |
| Phasmida | 1 | 1 | 5 |
| Orthoptera | 4 | 19 | 703 |
| Hemiptera | 11 | 44 | 1,512 |
| Homoptera | 10 | 62 | 30,345 |
| Thysanoptera | 1 | 7 | 684 |
| Neuroptera | 1 | 2 | 30 |
| Coleoptera | 19 | 59 | 443 |
| Diptera | 44 | 127 | 3,925 |
| Lepidoptera | 14 | 53 | 398 |
| Hymenoptera | 34 | 140 | 5,190 |
| Total | 157 | 554 | 43,942 |

sion, both were significant predictors of insect species richness ($n$=54, $F$=15.93, $r^2$=0.38, $P$<0.001). In pairwise comparisons between plots, insect species composition diverged with larger differences in rates of nitrogen addition and with greater physical distances between plots. The proportion of shared species between plot pairs, measured by the Jaccard index, decreased with increasing differences between nitrogen treatments ($n$=1,430, $r^2$=0.20, $P$<0.001) and with increasing distances between plots ($r^2$=0.04, $P$<0.001). In a multiple regression, both the difference in rate of nitrogen addition and distance between plots were significant predictors of the number of shared insect species ($F$=240.49, $r^2$=0.25, $P$<0.001). The results of this regression should be interpreted cautiously, because the degrees of freedom are inflated by pairwise comparisons between all plots. Nevertheless, the effects of nitrogen on differences in insect community composition were stronger than the effects of distance.

Trophic-group responses to the nitrogen gradient varied. Herbivore and predator species richness decreased significantly as the rate of nitrogen addition increased (Fig. 3A,B). Detritivore species richness increased significantly as the rate of nitrogen addition increased (Fig. 3D). Parasitoid and omnivore species richness showed no relationship to the rate of nitrogen addition (Fig. 3C,E).

### *Insect abundances*

The number of insects and insect biovolume increased significantly with increasing rates of nitrogen addition (Fig. 4). As with insect species richness, this relationship was found for 2 of 3 months when considered separately ($n$=54; June: mean number of insects/plot=327.83, $r$=0.31, $P$=0.02; July: mean number of insects/plot=301.39, $r$=0.35, $P$=0.01; August: mean number of insects/plot=186.30, $r$=0.11, $P$=0.42), and data were combined for all further analyses. One potential outlier was retained because removing it had no effect on the interpretation. There was no significant influence of

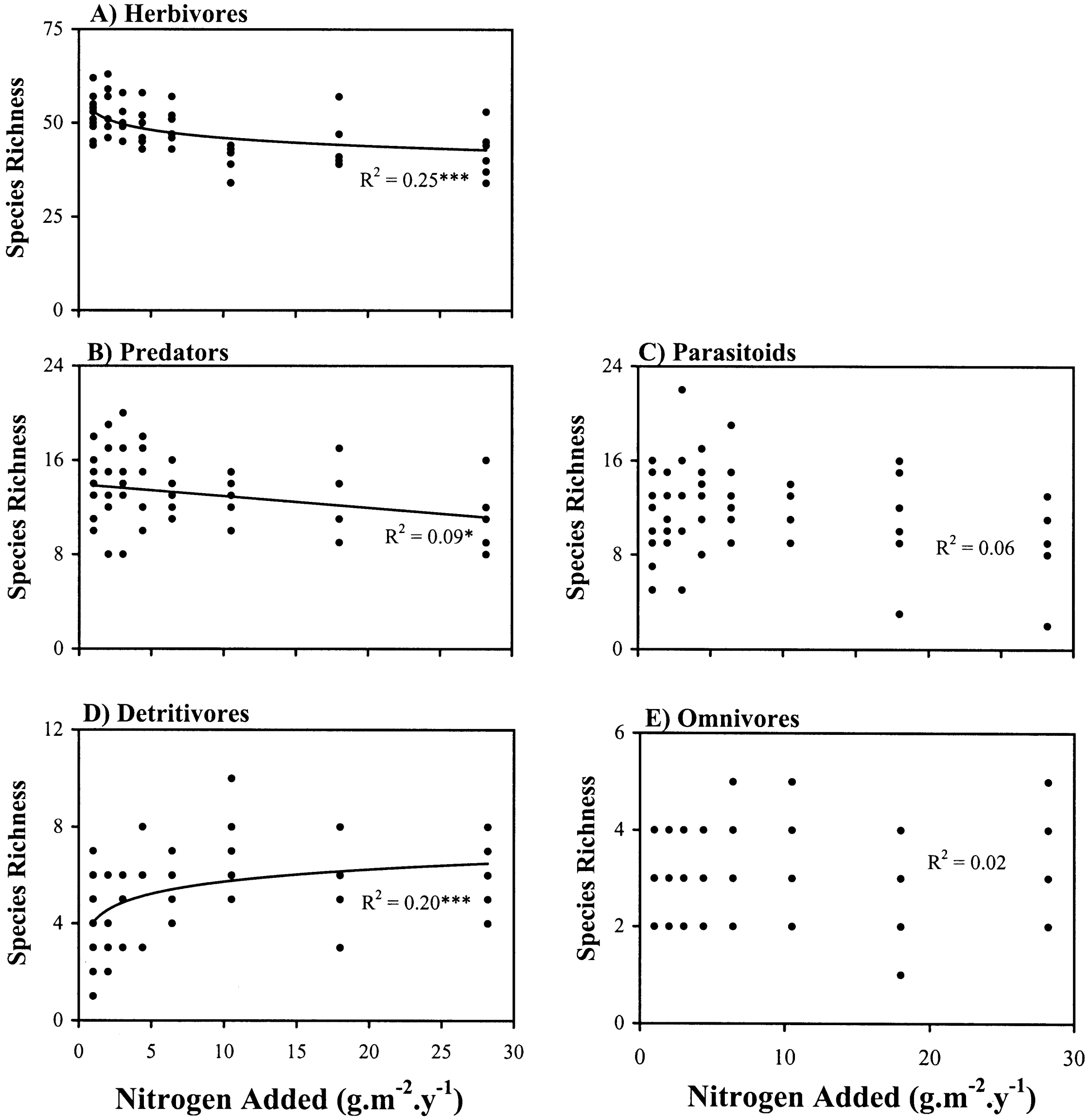

**Fig. 3** Species richness at each trophic level against rate of nitrogen addition plus 1: herbivores ($n$=54, $P$=0.001, $y$=–3.07log$x$+53.08) (**A**); predators ($n$=54, $P$=0.03, $y$=–0.0983$x$+13.926) (**B**); parasitoids ($n$=54, $P$=0.06) (**C**); detritivores ($n$=54, $P$=0.001, $y$=–0.52log$x$+87.14) (**D**); omnivores ($n$=54, $P$=0.29) (**E**) (*$P$<0.05, ***$P$<0.001)

neighboring plots on insect abundances ($n$=54, $r^2$=0.06, $P$=0.07). As the rate of nitrogen addition increased, the abundance of herbivores and detritivores increased significantly, the abundance of parasitoids decreased significantly, and the abundance of predators and omnivores showed no relationship (Fig. 5). The 18 most abundant herbivores varied in their responses to the rate of nitrogen addition (Fig. 6; see below).

## Possible causes of nitrogen responses

We conducted multiple-regression analyses to gain insights into the most important factors influencing insect community responses to the nitrogen gradient. Insect species richness was significantly, positively correlated with plant species richness (the proportion of $C_3$ grasses remained in the final model, but was dropped due to collinearity; Table 2), but not with the rate of nitrogen addition, plant biomass, litter biomass, or plant tissue nitrogen. Effective insect diversity was significantly, positively correlated with plant species richness and litter biomass.

The number of insects was significantly, positively related to the rate of nitrogen addition, but not to plant species richness, plant biomass, litter biomass, the proportion of $C_3$ grasses, or plant tissue nitrogen. Insect biovolume was significantly, negatively correlated with plant species richness, but not with the rate of nitrogen addition, plant biomass, litter biomass, the proportion of $C_3$ grasses, or plant tissue nitrogen (Table 2).

We also conducted multiple-regression analyses on species richness and abundance at each trophic level. Herbivore species richness was significantly, negatively correlated with plant tissue nitrogen, but not with plant

**Table 2** Results of backward elimination multiple regression. Values for retained variables are parameter estimates (– variables that were not included in the final model)

| Independent variables | Dependent variables | | | |
|---|---|---|---|---|
| | Insect species richness | Insect effective diversity | Number of insects | Insect biovolume |
| Overall regression $r^2$ | 0.18*** | 0.33*** | 0.14** | 0.23*** |
| Intercept | 65.40*** | –8.55 NS | 647.70*** | – |
| Nitrogen | – | – | 23.01** | – |
| Plant species richness | 8.95***[a] | 1.96*** | – | –461.97*** |
| Plant biomass | – | – | – | – |
| Litter biomass | – | 0.01* | – | – |
| Proportion $C_3$ grasses | –[b] | – | – | – |
| Plant tissue nitrogen | – | – | – | – |

[a] Log(plant species richness)

[b] Dropped from final model due to collinearity

*$P<0.05$; **$P<0.01$; ***$P<0.001$

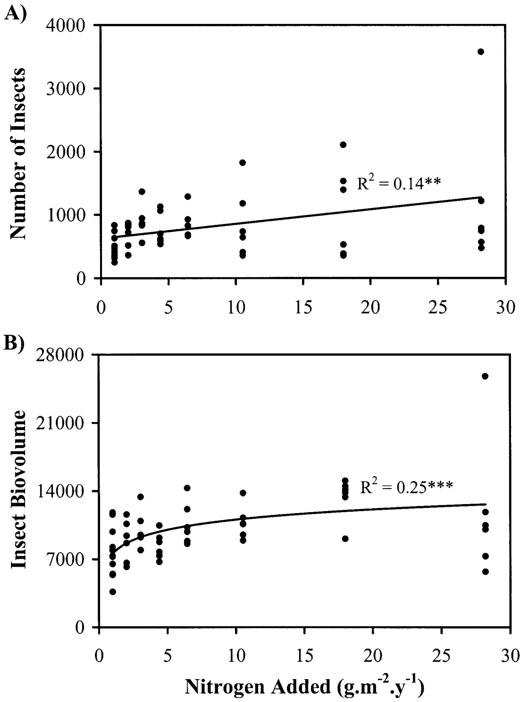

**Fig. 4** Regressions of insect abundance against rate of nitrogen addition plus 1. Abundance was measured as number of individuals ($n=54$, $P=0.005$, $y=23.02x+647.7$) (**A**) and biovolume ($n=54$, $P=0.001$, $y=1511.8\log x+7{,}577.4$) (**B**) (**$P<0.01$, ***$P<0.001$)

species richness, plant biomass, litter biomass, the proportion of $C_3$ grasses, or the species richness or abundance of predators and parasitoids (Table 3). Predator species richness was significantly, positively correlated with the diversity of herbivores and detritivores, but not with plant species richness, plant biomass, the proportion of $C_3$ grasses, or herbivore and detritivore abundances. Parasitoid species richness was significantly, negatively correlated with plant biomass, but not with plant species richness, the proportion of $C_3$ grasses, or herbivore and detritivore species richness or abundance. Detritivore species richness was significantly, negatively correlated with plant species richness and significantly, positively correlated with litter biomass, but was not correlated with plant biomass, the proportion of $C_3$ grasses, plant tissue nitrogen, or the species richness or abundance of predators and parasitoids (Table 3).

Herbivore abundance was significantly, positively correlated with plant tissue nitrogen, but not with plant species richness, plant biomass, litter biomass, the proportion of $C_3$ grasses, or the number of predators and parasitoids (Table 3). Predator abundance was not correlated with any variable that we measured. Parasitoid abundance was significantly, positively correlated with plant species richness, but not with plant biomass, the proportion of $C_3$ grasses, or the diversity or abundance of herbivores and detritivores. Detritivore abundance was significantly, positively correlated with litter biomass and the number of predators and parasitoids, significantly, negatively correlated with plant species richness, and not correlated with plant biomass, litter biomass, or the proportion of $C_3$ grasses.

In multiple regressions with the rate of nitrogen addition and host plant biomass, the abundances of 13 of the 18 most abundant species were significantly, positively related to the biomass of their host plant (host plants were known for only 15 of the species). Species that increased in abundance with increasing rates of nitrogen addition fed on $C_3$ grass species that dominated high-nitrogen plots (Fig. 6). The abundances of *Delphacodes* sp. (Homoptera: Delphacidae; $n=54$, $F=22.65$, $r^2=0.47$, $P=0.001$), *Delphacodes campestris* (Homoptera: Delphacidae; $F=6.95$, $r^2=0.21$, $P=0.002$), *Athysanus argentarius* (Homoptera: Cicadellidae; $F=10.08$, $r^2=0.28$, $P=0.001$), and *Chorthippus curtipennis* (Orthoptera: Acrididae; $F=3.82$, $r^2=0.13$, $P=0.028$) were positively dependent on *A. repens* biomass. The abundances of *Doratura stylata* (Homoptera: Cicadellidae; $F=10.80$, $r^2=0.30$, $P=0.001$) and *Malloweia* sp. (Diptera: Chloropidae; $F=8.31$, $r^2=0.33$, $P=0.001$) were positively dependent on *P. pratensis* biomass. The abundance of *Oscinella* sp. (Diptera: Chloropidae) was positively dependent on the abundance of both *P. pratensis* and *A. repens* ($F=21.05$, $r^2=0.56$, $P=0.001$). However, two insects that fed on *P. pratensis* or *A. repens*, *Diplocolenus configuratus* (Homoptera: Cicadellidae; $F=1.65$, $r^2=0.09$, $P=0.19$) and *Quantas sayi* (Homoptera: Cicadellidae; $F=0.57$, $r^2=0.03$, $P=0.64$), showed no significant relationship to host biomass.

Herbivores that decreased in abundance as the rate of nitrogen addition increased fed on forbs or $C_4$ grasses (Fig. 6). Abundances of *Lygus* sp. (Homoptera: Miridae; $F=4.02$, $r^2=0.14$, $P=0.02$), *Macrosteles fascifrons*

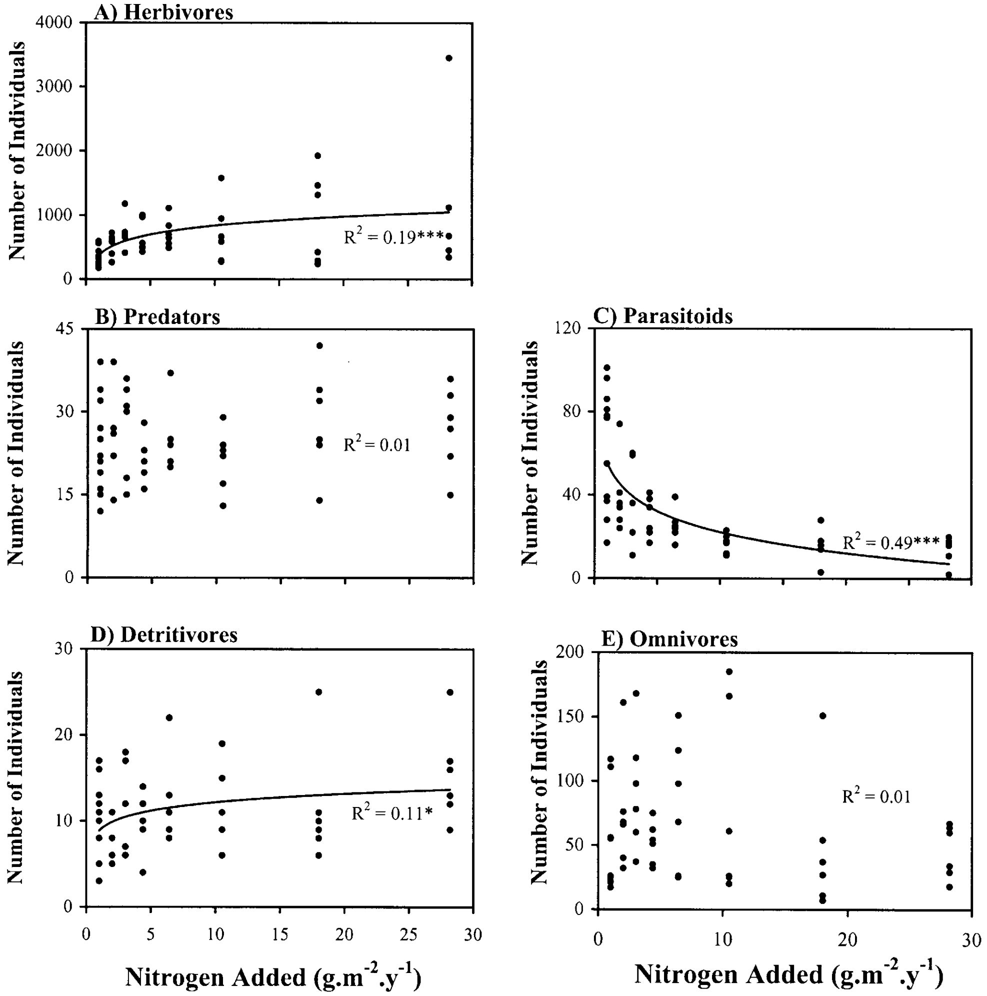

**Fig. 5** Insect abundance at each trophic level against rate of nitrogen addition plus 1: herbivores ($n$=54, $P$=0.001, $y$=203.07log$x$+369.99) (**A**); predators ($n$=54, $P$=0.46) (**B**); parasitoids ($n$=54, $P$=0.001, $y$=–14.02log$x$+80.05) (**C**); detritivores ($n$=54, $P$=0.02, $y$=1.44log$x$+8.88) (**D**); omnivores ($n$=54, $P$=0.44, $y$=–0.56$x$+66.36) (**E**) (*$P$<0.05, ***$P$<0.001)

**Table 3** Results of backward elimination multiple regression by trophic level. Values for retained variables are parameter estimates (– variables that were not included in the final model; blank cells indicate that a parameter was never entered in the regression)

| Independent Variables | Dependent variables | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Herbivores | | Predators | | Parasitoids | | Detritivores | |
| | Species richness | Number | Species richness | Number | Species richness | Number | Species richness | Number |
| Overall regression $r^2$ | 0.33*** | 0.16** | 0.10* | – | 0.10* | 0.42*** | 0.31*** | 0.45*** |
| Intercept | 62.38 | –66.59 NS | 5.72 NS | – | 16.44*** | –1.80 NS | 5.33*** | 5.38 NS |
| Plant species richness | – | – | – | – | – | 4.28*** | –0.18* | –0.73*** |
| Plant biomass | – | – | – | – | –0.01* | – | – | – |
| Litter biomass | – | – | | | | | 0.002* | 0.009*** |
| Proportion $C_3$ grasses | – | – | – | – | – | – | – | – |
| Plant tissue nitrogen | –9.14*** | 487.49** | | | | | – | – |
| Herbivore and detritivore species richness | | | 0.14* | | | | | |
| Predator and parasitoid species richness | – | | | | | | – | |
| Number of herbivore and detritivores | | | – | – | – | – | | |
| Number of predators and parasitoids | – | | | | | | – | 0.11*** |

*$P$<0.05, **$P$<0.01, ***$P$<0.001

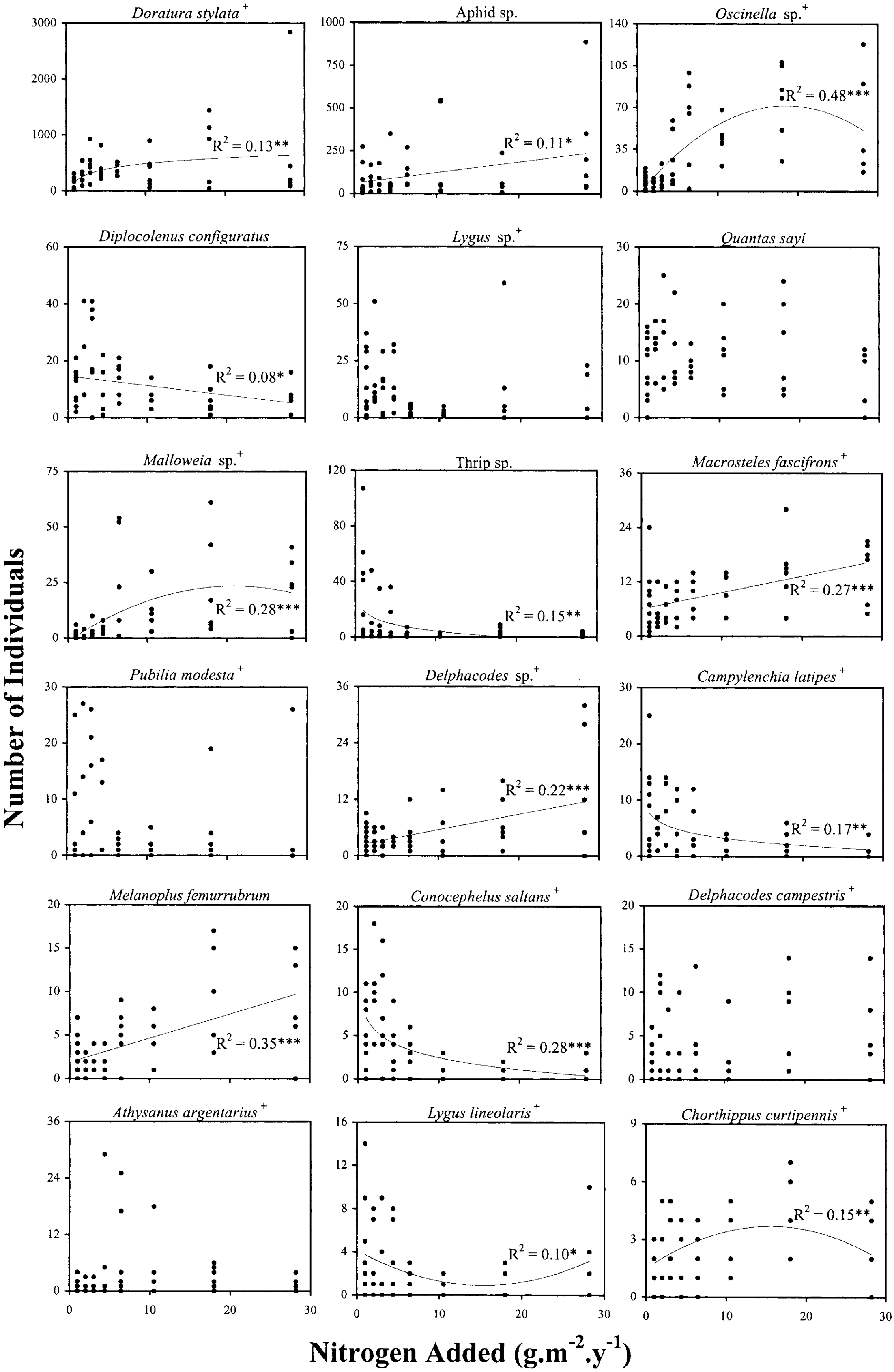

**Fig. 6** Abundances of the 18 most abundant herbivores against rate of nitrogen addition plus 1 ($n$=54 for all graphs). Graphs are presented in order of species abundance. Species whose names are marked + were significantly, positively correlated with the abundance of their host plant. Fitted curves are best fit linear, log, or quadratic (*$P$<0.05, **$P$<0.01, ***$P$<0.001)

(Homoptera: Cicadellidae; *F*=12.46, $r^2$=0.33, *P*=0.001), *Campylenchia latipes* (Homoptera: Membracidae; *F*=7.37, $r^2$=0.22, *P*=0.002) and *Lygus lineolaris* (Homoptera: Miridae; *F*=3.36, $r^2$=0.12, *P*=0.04) were positively dependent on *A. azureus* biomass. The abundance of *Publilia modesta* (Homoptera: Membracidae) was positively dependent on *A. ludoviciana* biomass (measured in 1995; *F*=3.27, $r^2$=0.11, *P*=0.05). The abundance of *Conocephalus saltans* (Orthoptera: Tettigoniidae; *F*=12.58, $r^2$=0.33, *P*=0.001) was positively dependent on *S. scoparium* biomass.

## Discussion

Human domination of the nitrogen cycle has greatly increased rates of nitrogen deposition, contributing to the simplification of terrestrial and aquatic ecosystems (Vitousek et al. 1997). This study, based on data collected after 14 years of nitrogen addition, shows that insect communities respond to nitrogen deposition much as plant communities do, resulting in lower total insect species richness and higher insect numbers and biomass.

### Nitrogen loading and insect species richness

Our result that nitrogen loading decreases insect species richness is consistent with two related conclusions from other studies: (1) at moderate to high levels of nitrogen addition, plant species richness decreases (Bakelaar and Odum 1978; Tilman 1988, 1993, 1996; Theodose and Bowman 1997) and (2) insect species richness is positively related to plant species richness (Murdoch et al. 1972; Nagel 1979; Szentkiralyi and Kozar 1991; Siemann et al. 1998; Knops et al. 1999). This result contrasts with previous short-term studies that report a positive relationship between nitrogen supply rate and insect species richness (Hurd et al. 1971; Hurd and Wolf 1974; Kirchner 1977; Sedlacek et al. 1988; Siemann 1998). At Cedar Creek, a 2-year study that separated the effects of plant species richness and plant productivity found that insect species richness was negatively correlated with plant species richness, although indirect effects may have hidden a positive relationship between plant species richness and insect species richness (Siemann 1998). However, nutrient addition has both short- and long-term effects on plant species richness. Nitrogen addition initially either does not change or increases plant species richness (e.g., Carson and Barrett 1988; Goldberg and Miller 1990), while long-term nitrogen addition consistently decreases plant species richness (discussed in Tilman 1993). Studies in which insect data were gathered 2–3 years after nitrogen manipulations reported an initial increase in insect species richness followed by little to no increase in subsequent years (Hurd and Wolf 1974; Kirchner 1977). Although we found the impact of nitrogen on insects to be much lower than on plants, our results demonstrate that 14 years of nitrogen loading reduced both plant species and insect species richness.

Because of the small size of the experimental plots and of the potential for spatial gradients to confound observed effects on insect species richness, we controlled for “spillover effects” from neighboring plots. Spatial effects were only weakly evident beyond immediately adjacent plots. Differences in insect species composition between all plots, even adjacent ones, were large (overlap in insect species composition between plots was typically less than 35%). Moreover, differences in species composition increased more strongly as differences in nitrogen treatments increased, strengthening our conclusion that our observed response of insect species richness was caused by nitrogen addition and was not confounded by the proximity of plots.

To test the hypothesis that nitrogen loading decreases plant species richness, which decreases insect species richness, we included nitrogen supply rate, plant species richness, plant productivity, plant composition, and plant tissue nitrogen as independent variables in analyses of insect species richness (Table 2). We emphasize that these results are strictly correlative, since we did not directly manipulate any aspect of the plant community. In addition, nitrogen supply, plant species richness, plant composition, and plant tissue nitrogen were highly correlated, and in one case (the model of total insect species richness), the proportion of $C_3$ grasses was removed from the final model due to collinearity. However, the best correlate of insect species richness and effective insect diversity, as determined by backward regression, was plant species richness.

Although herbivore and predator species richness did decrease in response to nitrogen addition, not all trophic groups showed negative responses to the rate of nitrogen addition. Detritivore species richness increased as nitrogen loading increased, and was positively correlated with litter biomass (but negatively correlated with plant species richness; Table 3). In part, the small magnitude of the response of total insect species richness along the nitrogen gradient may have been caused by the opposing responses of different trophic levels to nutrient loading.

In our analyses, $r^2$ values were generally low, suggesting that other factors, such as unmeasured effects of nitrogen addition on plant quality other than foliar nitrogen, changes in plant structural diversity (Lawton 1983), unquantified effects of predators (Siemann 1998), or the presence of transient species, may have influenced insect species richness. Plots with higher plant species richness were much more visually complex than plots with low plant species richness, which were often uniform mats of one to two plant species. Although we observed a direct correlation between the diversity of herbivores and detritivores and predator diversity, nitrogen addition may have had additional indirect effects on herbivores through predators (Siemann 1998). Our results do not preclude top-down effects; however, we did not detect them in our analyses that included predator and parasitoid abundance as an independent variable. Mobile, transient species (such as the one Ephemeroptera) were counted in plots and may have inflated measures of insect species richness and abundance, and increased variance in insect community responses to nitrogen addition. However, these species probably did not influence our interpretation, because they were either not targeting resources in the plots (and could be assumed to have a similar affect on all plots), or were targeting specific treatments and thus should be considered among the responses to nitrogen addition.

### Nitrogen loading and insect abundances

The positive response of insect abundances to long-term nitrogen loading was consistent with previous, short-term studies that also reported increased abundances (Hurd et al. 1971; Hurd and Wolf 1974; Kirchner 1977; Sedlacek et al. 1988; Siemann 1998). The number of insects was positively correlated with the rate of nitrogen addition, and insect biovolume was negatively correlated with plant species richness. Changes in insect numbers were driven primarily by herbivore abundances, which were positively correlated with plant tissue nitrogen. A positive relationship between herbivore abundance and plant tissue nitrogen (or other measures of plant quality related to nitrogen, such as plant soluble nitrogen, and amino acid concentration) has been found for many species (Mattson 1980; Scriber 1984), particularly homopterans (Prestidge 1982; Prestidge and McNeill 1983b; Spencer and Port 1988), which were among the species that showed the strongest, positive responses to nitrogen in this study (Fig. 6). The negative relationship between plant species richness and total insect biovolume agrees with numerous studies that have demonstrated negative correlations between total herbivore abundance (especially the abundance of insect pests) and plant diversity (e.g., Pimentel 1961; Tahvanainen and Root 1972; Root 1973; Andow 1991). The positive correlation between parasitoid abundances and plant species richness may have been caused by greater availability of nectar resources or higher abundances of host species in diverse plots (Russell 1989; Andow 1991; Siemann 1998). We found no evidence for direct effects of predators and parasitoids limiting herbivore abundances.

Changes in plant community composition may have contributed to higher insect abundances in high-nitrogen plots. $C_3$ grasses dominated total biomass in high-nitrogen plots and, because of their high tissue quality, they are often more palatable than $C_4$ grasses or non-leguminous forbs that dominated low-nitrogen plots (Caswell et al. 1973). The effects of plant composition were demonstrated by changes in the abundances of dominant herbivore species along the nitrogen gradient. Nearly three-quarters of the most abundant species were positively dependent on the biomass of their host plant. Although the responses of individual insects varied, the positive relationship between total insect community abundance and nitrogen addition was dominated by the positive responses of the three most numerous species, and by one of the largest species, *Melanoplus femurrubrum*, a grasshopper (Fig. 6).

This study showed that long-term nitrogen addition may impact the entire food chain. At higher rates of nitrogen addition, plant species richness decreased, plant productivity increased, plant tissue nitrogen increased, and plant composition shifted from $C_4$ to $C_3$ grasses. These and perhaps other changes in the plant community may have contributed to lower herbivore species richness and higher herbivore abundances. Changes in the plant community and in herbivore and detritivore species richness, in turn, likely contributed to decreases in predator species richness and parasitoid abundance. Although the impact of nitrogen loading on insect communities was weaker than on plant communities, the results of this study confirm for insects what has been demonstrated for plants and aquatic ecosystems – that long-term nutrient loading leads to simplified communities.

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