

DIRECT AND INDIRECT EFFECTS OF CO₂, NITROGEN, AND COMMUNITY DIVERSITY ON PLANT–ENEMY INTERACTIONS

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Abstract. Resource abundance and plant diversity are two predominant factors hypothesized to influence the amount of damage plants receive from natural enemies. Many impacts of these environmental variables on plant damage are likely indirect and result because both resource availability and diversity can influence plant traits associated with attractiveness to herbivores or susceptibility to pathogens. We used a long-term, manipulative field experiment to investigate how carbon dioxide (CO₂) enrichment, nitrogen (N) fertilization, and plant community diversity affect plant traits and the amount of herbivore and pathogen damage experienced by the common prairie legume *Lespedeza capitata*. We detected little evidence that CO₂ or N affected plant traits; however, plants growing in high-diversity treatments (polycultures) were taller, were less pubescent, and produced thinner leaves (higher specific leaf area). Interestingly, we also detected little evidence that CO₂ or N affect damage. Plants growing in polycultures compared to monocultures, however, experienced a fivefold increase in damage from generalist herbivores, 64% less damage from specialist herbivores, and 91% less damage from pathogens. Moreover, within diversity treatments, damage by generalist herbivores was negatively correlated with pubescence and often was positively correlated with plant height, while damage by specialist herbivores typically was positively correlated with pubescence and negatively associated with height. These patterns are consistent with changes in plant traits driving differences in herbivory between diversity treatments. In contrast, changes in measured plant traits did not explain the difference in disease incidence between monocultures and polycultures. In summary, our data provide little evidence that CO₂ or N supply alter damage from natural enemies. By contrast, plants grown in monocultures experienced greater specialist herbivore and pathogen damage but less generalist herbivore damage than plants grown in diverse communities. Part of this diversity effect was mediated by changes in plant traits, many of which likely are plastic responses to diversity treatments, but some of which may be the result of evolutionary changes in response to these long-term experimental manipulations.

Key words: carbon dioxide; disease; diversity; herbivory; *Lespedeza capitata*; nitrogen deposition; plant–herbivore; plant–pathogen; resource availability.

INTRODUCTION

Resource availability and the community context in which plants grow can have large effects on the amount of damage plants experience from natural enemies. Resource availability primarily influences attack from natural enemies indirectly by altering susceptibility to natural enemies via effects on plant traits. For example, the relative availability of carbon (C) vs. nitrogen (N) often alters plant secondary chemistry, leaf nutrient concentrations, and leaf toughness (Lindroth et al. 1993, Bezemer and Jones 1998, Diaz et al. 1998, Throop and Lerdau 2004, Berner et al. 2005), all of which may affect

herbivore feeding. Similarly, plants grown in high N (or lower C) environments may be more susceptible to disease because increased foliar nitrogen concentrations increase resources available to pathogens (the “nitrogen–disease hypothesis”; Mitchell et al. 2003). Indeed, many of the early hypotheses on the evolution of plant defenses point to a central role of resource availability (particularly C and N) in mediating costs of and allocation to defense (Bryant et al. 1983, Coley et al. 1985).

In contrast to resource availability, some of the effects of plant community diversity on damage from natural enemies likely are due to direct effects on herbivore population sizes. For example, monocultures may support greater numbers of herbivores because they provide greater quantities of a preferred food source or because they support lower populations of higher-order predators than more diverse communities (Elton 1958, Root 1973; see also Agrawal et al. 2006). Plant community composition, however, can also influence

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damage levels indirectly by influencing plant quality (He et al. 2002, Pennings et al. 2005; J. Strengbom, P. B. Reich, and M. E. Ritchie, *unpublished manuscript*). These effects on plant quality likely result because competition alters water and nutrient availability, which in turn can affect plant chemistry and morphology.

Plant resource availability is rapidly changing on a global scale with increases in atmospheric CO₂ concentration and soil N deposition. Empirical studies have revealed that elevated atmospheric CO₂ concentrations (eCO₂; see Bezemer and Jones 1998, Coviella and Trumble 1999, Mitchell et al. 2003 for review) and N addition (reviewed in Throop and Lerdau 2004) have a wide range of effects on herbivore and pathogen damage and abundance. There appears to be no consistent response to eCO₂ or N, even within a given feeding guild (Coviella and Trumble 1999, Lawton 2000). Furthermore, in several cases the response of a single herbivore species to eCO₂ or N addition varies from positive to negative as a function of host plant identity (e.g., Sudderth et al. 2005, Cleland et al. 2006). Changes in community composition also have an array of effects on natural enemy damage. Plants grown in low-diversity plots (e.g., monocultures) typically experience more herbivory (Root 1973, Andow 1991, Scherber et al. 2006) and pathogen attack (Mitchell et al. 2002, Mitchell et al. 2003) than plants grown in high-diversity environments, but the opposite pattern also has been observed (e.g., Mulder et al. 1999).

The lack of consistent effects that nutrient enrichment and community diversity have on plant damage may be due to a multitude of factors, including the effects these environmental variables have on plant traits and the specific responses of different enemy species to these plant traits. The effects that diversity and resource availability have on plant growth and physiological traits that may affect plant resistance or attractiveness to enemies are often species specific. For example, most plant species grow larger in eCO₂ environments, yet some plant species show negative growth responses (Berntson et al. 1998, Reich et al. 2001, He et al. 2002). Similarly, there is substantial interspecific variation in responses of leaf chemistry to eCO₂ (Bazzaz 1990, Lindroth et al. 1993, Bezemer and Jones 1998). Likewise, different enemy species often show a wide range of responses to variation in plant traits, and the effects that environmentally induced changes in plant traits have on enemy damage may depend on the host range, feeding strategy (e.g., specialists vs. generalists and foliar feeders vs. phloem feeders), and nutrient requirements of the enemy.

We used two years of data from manipulative field experiments to examine how atmospheric CO₂ concentration, soil N availability, and plant community diversity independently and interactively impact the damage caused by generalist herbivores, specialist herbivores, and pathogens to the common prairie legume *Lespedeza capitata*. We also examined how these

changes in resource availability and community diversity affect plant traits that may mediate plant-enemy interactions, including height, pubescence, and specific leaf area, and quantified the relationships between these traits and enemy damage. We then investigated whether the damage levels predicted by these plant-mediated effects are consistent with the observed effects of our experimental treatments on enemy damage. We uncoupled direct from indirect effects of the treatments by combining data on plant trait responses with data on the relationships between plant traits and enemy damage. Because increasing atmospheric CO₂ concentrations and increased N deposition are two of the most important environmental changes occurring globally, these data also provide insight into the effects that global changes may have on plant populations and interactions between plants and their natural enemies.

MATERIALS AND METHODS

Study system and experimental design

We measured the amount of herbivore and pathogen damage received by *Lespedeza capitata* growing in ambient or eCO₂ (560 ppm) treatments, ambient or elevated soil nitrogen (N) treatments, and monoculture or polyculture (16 plant species) treatments in 2004 and 2005. In 2005 we also collected data on the height, leaf pubescence, and specific leaf area (SLA) of plants on which we surveyed damage. The experimental plants were part of a long-term experiment examining the ecological effects of plant biodiversity, CO₂, and N ("BioCON"; Reich et al. 2001) at Cedar Creek Natural History Area, Minnesota, USA. In this experiment, atmospheric CO₂ concentrations, soil N, and biodiversity (plant species richness) are manipulated in a modified split-plot design. CO₂ is manipulated at the whole-plot level using free air CO₂ enrichment (FACE) technology. The two CO₂ treatments (elevated and ambient) are applied to six 20 m diameter open-air rings (three rings per treatment). The eCO₂ treatment is maintained by blowing concentrated CO₂ through a vertical array of pipes surrounding the perimeter of the ring. The ambient CO₂ (aCO₂) rings are surrounded by a similar pipe structure, but the air blown through these pipes is not enriched in CO₂. In other field environments, manipulating atmospheric CO₂ concentrations in this way has only minor effects on microclimate or light conditions (Hendrey et al. 1993). The CO₂ treatments were applied during daylight hours throughout each growing season (~April-September of each year).

The N and biodiversity treatments were applied to 2 × 2 m plots within each of the six rings. Plots were randomly assigned to N treatments (0 or 4 g N·m⁻²·yr) and biodiversity treatments (1, 4, 9, or 16 plant species). The N addition treatments were maintained by applying slow-release anhydrous ammonium nitrate three times each growing season. Only *L. capitata* monoculture plots and the 16 species (hereafter "polyculture") treatment plots were included in this study. For each



PLATE 1. Example of a heavily damaged *Lespedeza capitata* plant. Photo credit: J. Strengbom.

of the four possible $\text{CO}_2 \times \text{N}$ treatments, there were two monoculture and 12 polyculture plots. All other species in the polyculture plots were perennial grassland species native or naturalized to the site. Plots were planted in 1997 with 12 g/m^2 of seed, divided equally by mass among the planted species. CO_2 and N treatments have been applied annually since 1998, and diversity treatments have been maintained by hand weeding. For more details on BioCON design, see Reich et al. (2001).

Plant measurements

In 2004 and 2005, in the seventh and eighth full year of treatments, we collected data on the most common types of herbivore and pathogen damage on 50 haphazardly selected *L. capitata* plants per monoculture plot and all available *L. capitata* plants in the polyculture plots (1–17 plants per polyculture plot, 64–109 plants per $\text{CO}_2 \times \text{N}$ treatment), growing in a $50 \text{ cm} \times 2 \text{ m}$ area along one edge of each of 39 plots (2004) or 54 plots (2005). We censused three types of herbivore damage on each plant: damage from (1) generalist chewing herbivores (primarily grasshoppers), (2) *Pachyschelus laevigatus* (Coleoptera: Buprestidae), and (3)

Tortriodon sp. (Lepidoptera). All three herbivore taxa are folivores, but they differ in the pattern of damage (size and location of damage and presence/absence of silken webbing). We assessed the amount of herbivore damage by visually estimating the proportion of leaf area damaged by each type of herbivore on 10 haphazardly selected leaves per plant (individual plants had on average 23 ± 13 leaves [mean \pm SD]). The grasshopper species that inflict the most damage to *L. capitata* at Cedar Creek typically are polyphagous and feed on a mixture of forbs and grasses (Ritchie 2000). In contrast, *P. laevigatus* is more specialized and predominantly feeds on *L. capitata*. These two types of damage accounted for the vast majority of observed herbivore damage (see Plate 1). We measured pathogen damage as present/absent based on the presence of leaf browning and stem curling. The University of Minnesota Plant Disease Clinic identified *Pythium* spp. or *Fusarium* spp. as potential causes of this damage from cultures of diseased individuals. During each year, all damage estimates were made by a single researcher. However, a different researcher estimated damage in 2004 than 2005; therefore, across-year comparisons of damage should be made with caution.

In 2005, to investigate CO_2 , N, and biodiversity effects on plant traits that may influence natural enemy damage, we measured plant height and visually categorized pubescence in July, before herbivores or disease became abundant. Pubescence was assigned to one of four categories based on visual estimates of the number of trichomes on the axial leaf surface: 0, no obvious trichomes; 1, trichomes present but sparse; 2, trichomes abundant; 3, leaf surface entirely covered with trichomes. We also collected one fully expanded leaf from each marked plant to estimate SLA. We aimed to collect the youngest fully expanded leaf with no herbivore damage. In most cases, the leaf was one of the topmost five leaves on the plant. SLA was calculated as the area (cm^2) of a fresh leaf (measured using SCION image analysis software; Scion Corporation, Frederick, Maryland, USA) divided by leaf dry mass (g). Leaves were dried for two days at 60°C before weighing. As part of a larger experiment examining how plant nutrient concentrations are influenced by the BioCON treatments, percent N, percent C, and C:N ratios of *L. capitata* tissue were estimated from harvested aboveground samples from a subset of plots in each treatment (sample sizes ranged from one to three plots per $\text{CO}_2 \times \text{N} \times$ diversity treatment). The samples included all aboveground *L. capitata* plant material in a $10 \times 100 \text{ cm}$ strip in each plot.

Statistical analyses

To determine how damage from natural enemies is affected by CO_2 , N, and diversity, we performed a MANOVA, followed by univariate ANOVAs (Proc GLM and Proc Mixed, Version 8.1, SAS Institute 2001) with generalist chewing damage, *P. laevigatus* damage,

Tortrion sp. damage, and disease incidence (proportion of infected plants per plot) as response variables. Identical analyses were performed on plant traits (height, pubescence, and SLA). For all analyses, CO₂, N, diversity, and all interactions were included as fixed factors, and ring (nested within CO₂ treatment) was included as a random factor. All analyses were performed on nontransformed plot means. Plots were considered split-plot replicates, and rings were considered whole-plot replicates. Different researchers collected the damage data in 2004 vs. 2005; therefore, we analyzed data from the two years separately.

To investigate how CO₂, N, and diversity impact tissue nutrient concentrations (percent N and C:N ratios), we first performed ANOVA that included CO₂, N, diversity, and all interactions as fixed factors, as in the above analyses. However, data on tissue nutrient concentrations was only collected for a subset of plots; therefore, because of low replication, we also performed ANOVA that included only the main effects of CO₂, N, and diversity. No significant effects were detected in the simplified analysis, and only results from the full model that included interactions are presented.

To determine how plant traits are related to natural enemy attack and how these associations vary with CO₂, N, and diversity treatments, we used mixed-model ANCOVAs (herbivore damage) or logistic ANCOVAs (disease incidence) to analyze the 2005 data (all plant traits were not measured in 2004) (Proc MIXED and Proc GENMOD, Version 8.1, SAS Institute 2001). These analyses also allowed us to differentiate between direct effects of the CO₂, N, and diversity treatments on natural enemy attack and indirect effects that occur because these treatments also influence plant traits. CO₂, N, and diversity were included as fixed factors, and the three plant traits that showed some evidence as responding to the treatments (height, SLA, and pubescence) were included as covariates. Plot (nested within the CO₂, N, and diversity treatments) was included as a random factor. Interactions between each plant trait and each fixed factor also were included in the models; significant interactions are evidence that the CO₂, N, and/or diversity treatments alter associations between plant traits and herbivore damage. Only pairwise interactions between traits and treatments were included in the analysis because of difficulty interpreting higher-order interactions. Separate analyses were performed on the four types of enemy damage; the amounts of leaf area damaged by generalist chewing herbivores, *P. laevigatus*, and *Tortrion* sp. were the response variables in the mixed model ANCOVAs, and the presence/absence of disease symptoms was the binomial response variable in the logistic ANCOVA. Herbivore response variables were angular transformed to improve normality.

The correlations between damage and plant traits were typically in the same direction across environments, but the strengths of some relationships differed

significantly (ANCOVA indicated significant treatment × trait interactions, see Appendix). Because initial analyses revealed strong impacts of diversity on both plant traits and damage, additional multiple regressions, which included all three plant traits as predictor variables and damage as the response variables, were performed within each diversity treatment. We used the multiple regression coefficients obtained from the analyses in ambient environments (aCO₂, no N addition, polyculture) to predict how changes in plant traits would influence natural enemy damage in monoculture vs. polyculture treatments.

RESULTS

CO₂, N, and diversity effects on damage and plant traits

We detected little evidence that N addition affected herbivory or disease, although most types of damage tended to be lower in the N addition treatment (Fig. 1A, B, Table 1). Similarly, we detected no evidence that the CO₂ treatments affected herbivore damage (Fig. 1C, D, Table 1). Disease incidence, however, was lower in the eCO₂ environment, although this difference was statistically significant only in 2005 ($P < 0.01$; Table 1B, Fig. 1D).

In contrast to the CO₂ and N treatments, diversity strongly influenced damage from natural enemies, particularly in 2005 (Fig. 1E, F, Table 1). In both 2004 and 2005, damage by generalist chewing herbivores was greater in polyculture than monoculture plots, whereas all other types of herbivore damage were either not significantly affected by diversity or were less in polycultures (e.g., *P. laevigatus* 2005). Disease incidence also was lower in polycultures in 2005. Although the effects of diversity were similar in both years, they were much more pronounced in 2005 when plants growing in polycultures experienced fivefold higher amounts of damage by generalist herbivores, 91% less incidence of disease, and 64% less damage from the specialist herbivore *P. laevigatus*.

For herbivore damage, we detected no statistically significant evidence for interactive effects between CO₂, N, and diversity (Table 1). Pathogen damage in 2005, however, was higher in low-N plots, but only in aCO₂ environments (least-square means ± SE: low N, 0.73 ± 0.08 ; N addition, 0.48 ± 0.08 [proportion of plants with disease symptoms, as in Fig. 1]; $F_{1,46} = 5.75$, $P = 0.02$), resulting in a significant interaction between CO₂ and N. There was also a significant CO₂ × diversity interaction in 2005, with a greater decrease in disease incidence under eCO₂ relative to aCO₂ in monocultures than in polycultures (Table 1). This interaction likely resulted from the extremely low disease incidence, and the associated low variance, in polycultures. The CO₂ × N and CO₂ × diversity interactions were no longer significant after data were log-transformed (both $P > 0.38$), suggesting that the proportional reduction in disease incidence due to N addition was similar across both CO₂ treatments and that the proportional decrease

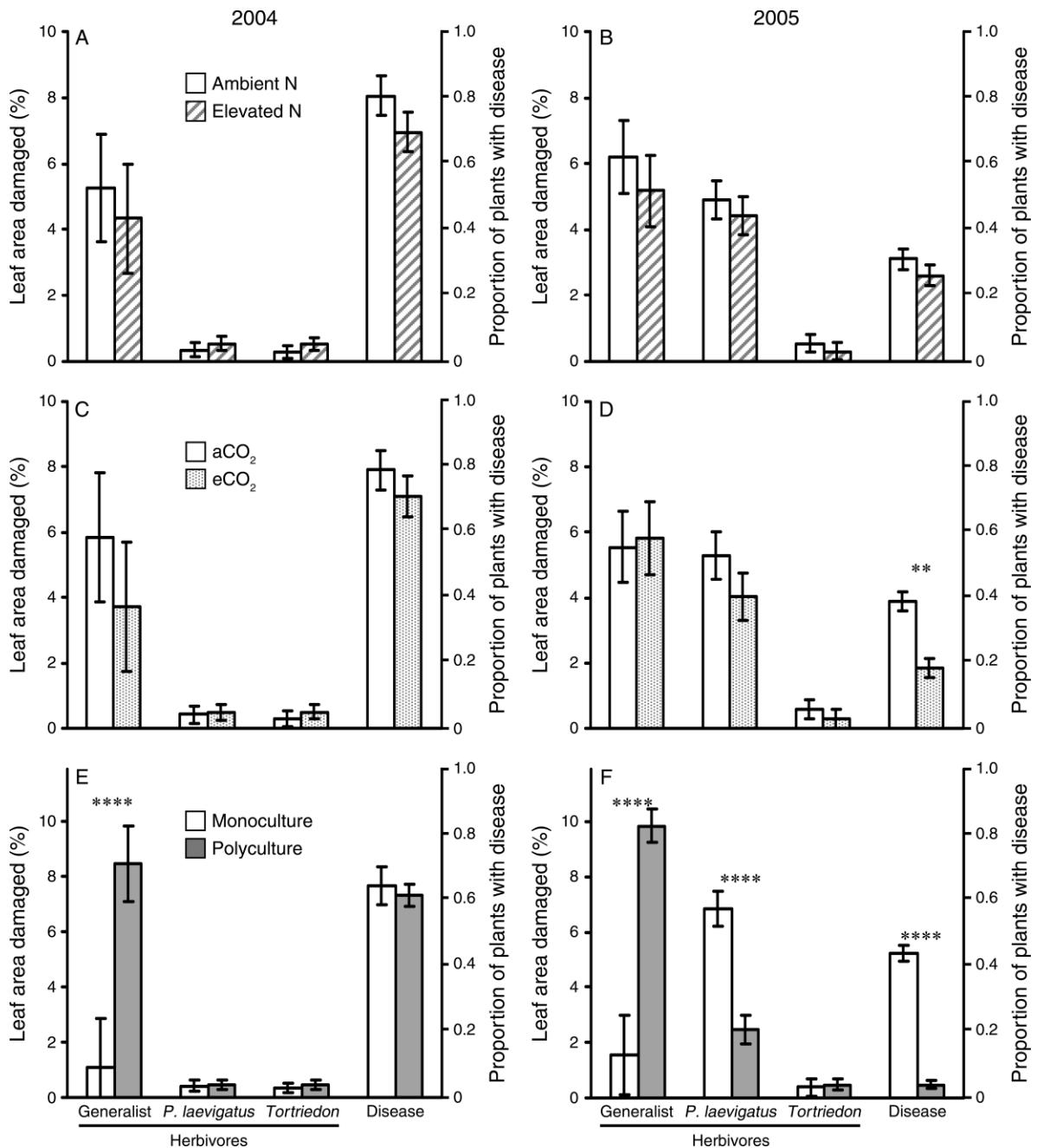


FIG. 1. Mean percentage of leaf material of the prairie legume *Lespedeza capitata* damaged by the three classes of herbivores (generalists [primarily grasshoppers], *Pachyschelus laevigatus* (Coleoptera), and *Tortriedon* sp. (Lepidoptera), and the proportion of diseased plants per plot in 2004 (A, C, E) and 2005 (B, D, F); all values are least-square means \pm SE. (A, B) Low N (ambient) vs. N addition (elevated) treatments; (C, D) ambient (aCO₂) vs. elevated (eCO₂) CO₂ treatments; (E, F) monoculture vs. polyculture treatments. The left-hand vertical axis is the scale for herbivore damage; the right-hand vertical axis is the scale for disease incidence. Significance is indicated as follows: ** $P < 0.01$; **** $P < 0.0001$.

in disease incidence in response to eCO₂ did not differ across diversity treatments. In 2004 disease incidence was uniformly high (Fig. 1E), suggesting that other factors may be important regulators of pathogen abundance and that eCO₂, N, and diversity may have minimal impacts when disease is especially prevalent.

Similar to the generally small effects of CO₂ and N on herbivore and disease damage, the univariate analyses indicated that CO₂ and N did not significantly influence plant traits, although we did detect a significant effect of CO₂ in the multivariate analysis (Table 2). In contrast, the diversity treatment had a large impact on plant

TABLE 1. MANOVA results and *F* statistics from subsequent univariate ANOVAs of the effects of CO₂ (C), N (N), and diversity (D) treatments on the amount of enemy damage in (A) 2004 or (B) 2005.

Factor	MANOVA			Generalist		<i>P. laevigatus</i>		<i>Tortrionedon</i> sp.		Disease	
	Pillai	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>
A) 2004											
C	0.39	4, 1	0.39	1, 4	0.58	1, 4	0.03	1, 4	0.40	1, 4	0.81
N	0.12	4, 23	0.80	1, 30	0.30	1, 30	0.87	1, 30	1.84	1, 23	2.03
D	0.54	4, 23	6.65***	1, 26	27.05****	1, 26	0.08	1, 26	0.66	1, 26	0.19
C × N	0.08	4, 23	0.52	1, 30	0.44	1, 30	0.00	1, 30	0.09	1, 23	0.04
C × D	0.04	4, 23	0.27	1, 26	0.79	1, 26	0.04	1, 26	0.01	1, 26	0.12
N × D	0.11	4, 23	0.68	1, 26	0.01	1, 26	0.69	1, 26	0.40	1, 26	2.34
C × N × D	0.05	4, 23	0.33	1, 26	0.33	1, 26	0.07	1, 26	0.85	1, 26	0.11
Ring(CO ₂)	1.12	16, 104	1.28	$\chi^2 = 4.9^*$		$\chi^2 = 8.4^{**}$		$\chi^2 = 10.2^{***}$		$\chi^2 = 0.3$	
B) 2005											
C	0.98	1, 4	16.32	1, 46	0.03	1, 5	1.49	1, 7	0.41	1, 46	30.59**
N	0.07	4, 39	0.77	1, 46	0.44	1, 43	0.77	1, 43	0.71	1, 46	1.78
D	0.87	4, 39	65.52****	1, 46	28.38****	1, 42	74.82****	1, 43	0.13	1, 46	167.4****
C × N	0.12	4, 39	1.38	1, 46	0.12	1, 43	0.03	1, 43	0.08	1, 46	5.71*
C × D	0.42	4, 39	7.02***	1, 46	0.02	1, 42	0.06	1, 43	1.09	1, 46	30.90****
N × D	0.05	4, 39	0.51	1, 46	0.08	1, 43	0.86	1, 43	0.63	1, 46	0.69
C × N × D	0.11	4, 39	1.15	1, 46	0.04	1, 43	0.40	1, 43	3.24	1, 46	0.89
Ring(CO ₂)	0.58	16, 168	1.79*	$\chi^2 = 0.0$		$\chi^2 = 13.1^{***}$		$\chi^2 = 4.5^*$		$\chi^2 = 0.0$	

Notes: Significant *F* and χ^2 values are in boldface. Pillai refers to Pillai's trace, a MANOVA test statistic (Scheiner 2001). Ring(CO₂) refers to the 20 m diameter open-air rings to which CO₂ treatments were applied. Significance of random factors was determined with likelihood ratio tests. The likelihood ratio test statistic approximately follows a chi-square distribution with one degree of freedom.

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001; **** *P* < 0.0001.

phenotype: plants growing in polycultures were 33% taller, significantly less pubescent, and tended to have thinner leaves (19% higher SLA) than plants growing in monocultures (Table 2, Fig. 2). We detected no evidence for significant interactions between CO₂, N, and diversity treatments on any plant trait (Table 2). Percentage N and C:N ratio did not respond significantly to any treatment (Table 2), and since they were measured at the plot level rather than on individual plants, were not included in further analyses linking plant traits to natural enemy damage.

Relationships between enemy attack and plant traits

Damage was correlated with plant traits (Fig. 3); however, the relationship depended upon the enemy. Averaged across all treatments, generalist herbivore

damage was negatively correlated with pubescence (*r* = -0.27, *P* < 0.0001), *P. laevigatus* damage was positively correlated with pubescence (*r* = 0.11, *P* = 0.008) and negatively correlated with plant height (*r* = -0.32, *P* < 0.0001), and disease incidence was positively correlated with pubescence ($\chi^2 = 17.8$, *P* < 0.0001) and plant height ($\chi^2 = 17.8$, *P* < 0.0001). *Tortrionedon* damage was not significantly correlated with any plant trait.

Because diversity affected both enemy damage and plant traits, the analyses on plot means do not allow us to determine the relative contributions of direct effects vs. indirect effects mediated through changes in plant traits. We used multiple regression to examine the within-diversity environment relationships between damage and plant traits to disentangle these direct vs. indirect effects. With these analyses, we assessed whether

TABLE 2. MANOVA results and *F* statistics from subsequent univariate ANOVAs of the effects of CO₂ (C), N (N), and diversity (D) on plant traits.

Factor	MANOVA			Height		SLA		Pubescence		Percentage N		C:N	
	Pillai	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>
C	0.26	3, 40	4.64**	1, 8	1.33	1, 46	1.06	1, 46	0.38	1, 7	0.09	1, 7	0.00
N	0.05	3, 40	0.68	1, 44	0.62	1, 46	0.96	1, 46	0.01	1, 7	3.05	1, 7	4.79
D	0.62	3, 40	21.71****	1, 43	12.54***	1, 46	3.67	1, 46	8.04**	1, 7	2.30	1, 7	5.54
C × N	0.01	3, 40	0.14	1, 44	0.16	1, 46	0.00	1, 46	0.23	1, 7	0.31	1, 7	0.10
C × D	0.09	3, 40	1.31	1, 43	2.88	1, 46	0.92	1, 46	0.19	1, 7	0.24	1, 7	0.23
N × D	0.04	3, 40	0.62	1, 44	0.06	1, 46	0.66	1, 46	0.45	1, 7	3.55	1, 7	4.25
C × N × D	0.02	3, 40	0.31	1, 44	0.03	1, 46	0.50	1, 46	0.18	1, 7	0.34	1, 7	0.20
Ring(CO ₂)	0.50	12, 126	2.08	$\chi^2 = 1.0$		$\chi^2 = 0.0$		$\chi^2 = 0.0$					

Notes: Percentage N and C:N ratio were not included as response variables in the MANOVA. SLA is specific leaf area. See Table 1 notes for further explanations.

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001; **** *P* < 0.0001.

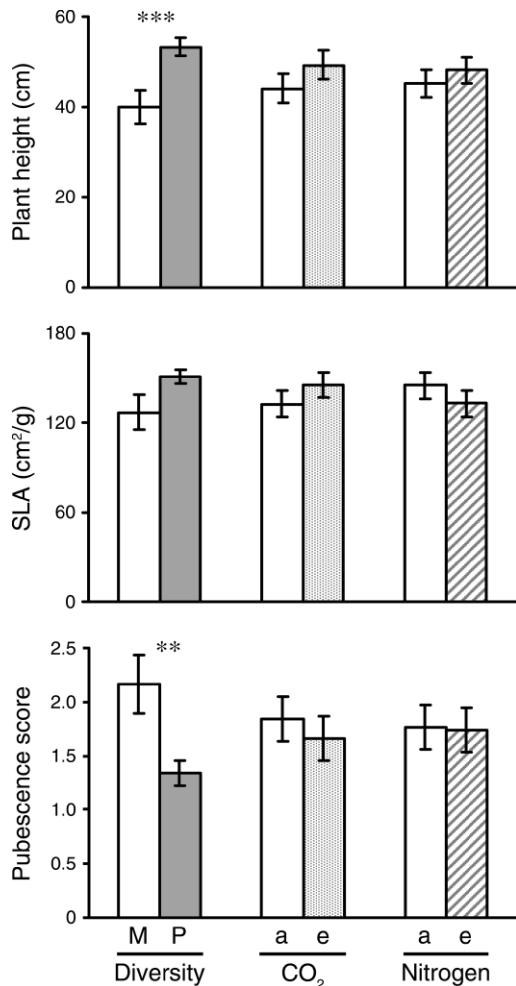


FIG. 2. Characteristics of *L. capitata*: least-square means \pm SE of height, specific leaf area (SLA), and pubescence score in 2005 in monocultures ("M") vs. polycultures ("P"), and under ambient ("a") vs. elevated ("e") CO₂, or ambient/low N ("a") vs. elevated/N addition ("e"). Plants were assigned pubescence scores of 0, 1, 2, or 3, based on the apparent number of trichomes on the leaf surface (0, nonpubescent; 3, densely covered with trichomes).

** $P < 0.01$; *** $P < 0.001$.

plant traits significantly affected enemy damage and whether the direction of the effects of diversity on enemy damage were consistent with expectations based on differences between diversity treatments in these plant traits. Within diversity environments, generalist damage (both amount and likelihood of attack) was negatively correlated with pubescence (in monoculture, partial regression coefficient $\beta = -0.61$, $P < 0.0003$; in polyculture, $\beta = -0.70$, $P < 0.094$). Likelihood of attack by generalists was positively correlated with plant height (in monoculture, $\beta = 0.05$, $P < 0.0001$; in polyculture, $\beta = 0.02$, $P < 0.34$), but there was a negative relationship between height and amount of damage in polycultures ($\beta = -0.18$, $P < 0.0001$). Consistent with the greater amounts of generalist damage in high-diversity environ-

ments being partially mediated through changes in plant traits, plants were less pubescent and taller in polycultures. When we used multiple regressions to predict the amount of damage received in monocultures vs. polycultures due to differences in plant traits, we found that the differences in plant traits contributed to, but did not fully explain, the variation in generalist herbivory between diversity treatments. Based on observed differences in plant traits, the multiple regression analyses predicted that generalist herbivores would remove 26% more leaf area in polycultures than monocultures. Thus changes in measured plant traits explained only 28% of the observed difference in amount of damage from generalist herbivores.

The effect of diversity on *P. laevigatus* damage also was consistent with plant-mediated effects; *P. laevigatus* damage was negatively correlated with height (in monoculture, $\beta = -0.04$, $P < 0.08$; in polyculture, $\beta = -0.036$, $P < 0.0022$) and was positively correlated with pubescence in polycultures ($\beta = 0.33$, $P < 0.04$). Plants were significantly shorter and more pubescent in monocultures (where they also received more *P. laevigatus* damage). In polycultures, plants experienced 64% less damage from *P. laevigatus* than in monocultures, but changes in plant traits explained only 9% of the difference in the amount of specialist herbivory between diversity treatments.

The predicted indirect effects of diversity on disease incidence depended on the particular trait examined. In monocultures, disease incidence was positively and significantly correlated with both plant height ($\beta = 0.03$, $P < 0.003$) and pubescence ($\beta = 0.43$, $P < 0.002$). Disease incidence was also positively correlated with plant height and pubescence in the polycultures, although these correlations were not significant (height $\beta = 0.015$, $P < 0.28$; pubescence $\beta = 0.28$, $P < 0.12$). Because plants were taller but less pubescent in polycultures, these indirect effects are expected to offset one another. The multiple regression analysis based on all three measured plant traits predicted a 51% increase in disease incidence in polycultures relative to monocultures. However, we observed a 91% decrease in polycultures relative to monocultures, indicating either that diversity has a direct effect on disease that is much greater than the indirect effects of plant traits or that variation in unmeasured plant traits that also were affected by the diversity treatments strongly influenced disease incidence.

DISCUSSION

Resource availability and plant community composition are two of the main environmental variables expected to impact the amount of damage plants receive from natural enemies. Two years of data on enemy damage collected from a long-term, manipulative study revealed little evidence that increased resource availability (elevated atmospheric CO₂ concentration, or soil nitrogen addition) altered the amount of herbivore

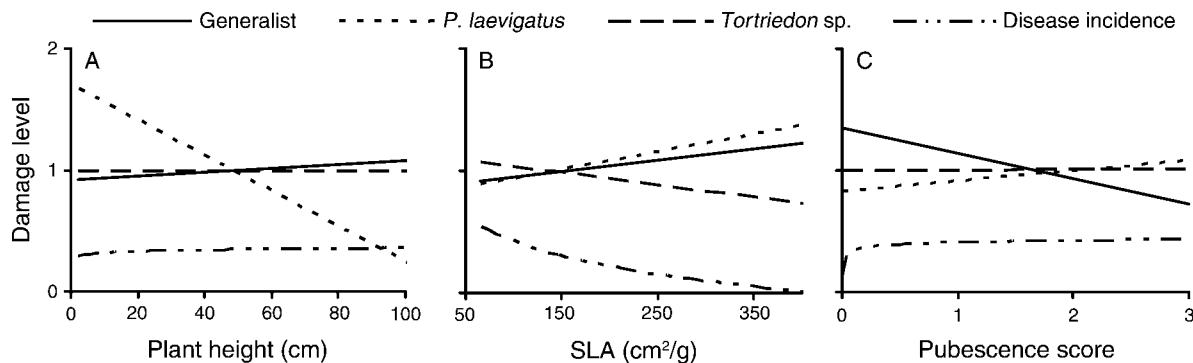


FIG. 3. The relationship between plant traits and natural enemy damage in 2005. So that all classes of natural enemies could be presented on the same figure, we plotted “damage level” rather than raw values. For herbivores, damage level is the proportion of leaf material damaged standardized by the variance. For disease, damage level is a binary variable where 0 signifies not diseased and 1 signifies diseased. Damage from three classes of herbivores is plotted separately, and the logistic curves denoted by the broken dashes indicate disease incidence. While the relationship between traits and damage varied in magnitude across treatments (see Appendix), these panels show the relationship averaged across all environments and illustrate that different traits have different effects, depending on the particular natural enemy observed.

damage experienced by the native perennial legume *Lespedeza capitata*. Disease incidence, while unaffected by N addition, was lower in eCO₂ than aCO₂ environments in 2005. The effects of plant community diversity on damage were more pronounced. *L. capitata* plants growing in polycultures experienced significantly more damage from generalist herbivores in both years of the study and significantly less damage from specialist herbivores and pathogens in 2005.

Given that eCO₂ and N addition can alter plant quality by changing C:N ratios, the quantity of secondary compounds, or physical properties (e.g., leaf toughness) (Lindroth et al. 1993, Bezemer and Jones 1998, Diaz et al. 1998, Throop and Lerdaun 2004, Berner et al. 2005), one might also expect eCO₂ and N addition to alter the amount of herbivore damage plants receive; however, we detected no evidence that CO₂ or N impact herbivore damage. This lack of response is not uncommon; several other empirical studies also have found little or no effect of eCO₂ (e.g., Diaz et al. 1998, Goverde et al. 2002) or N addition (Ritchie 2000, Strengbom et al. 2005) on the amount of herbivore damage plants experience.

In contrast, disease incidence was reduced in eCO₂, although this difference was statistically significant only in 2005. Because >40% of plants in aCO₂ but <20% of plants in eCO₂ were affected by disease in 2005, and because disease caused major reductions in reproductive output (J. Lau, *personal observation*), the effects of CO₂ on disease incidence may be important for *L. capitata* evolution and population dynamics. N addition also tended to reduce disease incidence, but only significantly reduced disease incidence in aCO₂ treatments in 2005. The stronger response of pathogens to resource addition suggests that pathogens may be more sensitive than herbivores to changes in plant quality resulting from eCO₂ and N addition. Strengbom and Reich (2006),

working in the same experimental site from which we collected our data, also found that eCO₂ and N addition both reduced disease incidence on *Solidago rigida*. In contrast, Mitchell et al. (2003), also working at the same experimental site, detected little effect of CO₂ or N on disease incidence, although C₃ grasses experienced increased pathogen loads in response to eCO₂, and C₄ grasses responded to N addition. The leguminous forb functional group, which included *L. capitata*, showed no differences in pathogen loads in response to either eCO₂ or N addition; however, the *L. capitata* data were not analyzed separately, and it is unclear whether this lack of effect was driven by small responses of the other three N-fixing legumes included in the study.

Many empirical studies have found evidence for greater amounts of herbivore damage in low- vs. high-diversity plant communities (Elton 1958, Root 1973, Andow 1991, Scherber et al. 2006). Our data on damage from the specialist herbivore *P. laevigatus* were consistent with these empirical results and with theory predicting that damage is higher in low-diversity environments because of increased availability of preferred food sources (the “resource concentration hypothesis”; Root 1973) and/or decreased densities of higher-order predators that prefer more complex (higher diversity) habitats (the “enemies hypothesis”; Elton 1958). These theories were developed primarily to explain feeding by herbivores in agricultural systems dominated by specialist pests (Root 1973, Andow 1991). Our data on damage by generalist chewing herbivores (primarily grasshoppers) showed the opposite pattern, however, and plants incurred more damage in polycultures than in monocultures. Generalist herbivores may respond to habitat complexity quite differently from specialists. For generalists, diverse plant communities may be more attractive and support higher population sizes because they provide multiple food

sources and allow for diet mixing (e.g., Pfisterer et al. 2003).

Diversity is also expected to impact disease incidence. Previous work in the BioCON experiment documented decreased disease incidence in polycultures relative to monocultures for all sixteen species studied, including *L. capitata* (Mitchell et al. 2003). The effect of diversity on *L. capitata* pathogen loads remained significant even after controlling for differences in host abundance between diversity treatments, suggesting that the diversity effect is not only due to the increased host plant abundance in monocultures (Mitchell et al. 2003). Our results are consistent with these findings; disease incidence was substantially lower in polycultures in 2005.

Effects of resource availability and community composition on plant damage mediated by change in plant traits

Some environmental factors, such as plant community diversity, may influence plant damage from natural enemies both directly (through changes in habitat quality) and indirectly (through changes in plant traits). Although increased resource availability, such as eCO₂ or N deposition, is unlikely to directly affect insect herbivores and fungal pathogens, these factors may affect enemy damage indirectly through their effect on plant traits. Several studies have found that herbivore performance is unaffected by eCO₂ when plant nutrient content is unchanged (e.g., Thompson and Drake 1994, Arnone et al. 1995, Goverde et al. 1999; see also Bezemer and Jones 1998). Similarly, Watt et al. (1993) showed a positive trend between the magnitude of eCO₂ effects on damage (consumption of leaf tissue) and the impact of eCO₂ on leaf N concentrations (i.e., there were only minimal effects of eCO₂ on the amount of plant material consumed when leaf N showed little response to eCO₂). Consistent with these findings, we detected no evidence that CO₂ or N significantly affected any measured plant traits and no evidence that these treatments affected herbivore damage. Nevertheless, we found that pathogen damage was significantly less in eCO₂ than aCO₂ environments in one year of the study, even though we detected no evidence that any of the plant traits we measured were affected by CO₂. Because direct effects of eCO₂ on pathogen abundance are unlikely, this result suggests the possibility that unmeasured traits (e.g., concentrations of defensive chemicals, cuticle thickness) may have mediated changes in disease incidence.

The effects of diversity on herbivore damage were largely consistent with the effects of diversity on plant traits. For example, plants grown in polycultures were less pubescent, and within treatments damage from generalist herbivores was negatively correlated with pubescence. This result suggests that the increased damage from generalist herbivores in polycultures results, at least in part, from herbivore preferences for

less pubescent plants. While changes in plant traits were only able to explain 28% and 9% of the diversity effects on amounts of damage from generalist and specialist herbivores, respectively, these results nevertheless provide evidence that some environmental variables, such as plant community composition, impact damage from natural enemies indirectly by altering plant traits involved in these interactions. For disease incidence, however, the response predicted based on changes in plant height was in the opposing direction of the damage response predicted based on changes in pubescence, and the indirect effects of diversity may have cancelled each other. Regardless, differences between diversity treatments in the measured plant traits failed to explain the patterns of pathogen attack. This suggests that the ultimate impact of diversity (or other environmental variables) on damage will depend on both the magnitude of the effect on the plant trait and the relative importance of the trait to susceptibility to enemies.

The among-treatment differences in plant phenotypes may be due to either phenotypic plasticity or genetic divergence between plants growing in the different treatments that has occurred in the eight years since the BioCON experiment was established. If the differences are plastic responses to the environment, then the environmental cue could be either the experimental treatments or differences in herbivore damage that were correlated with the experimental treatments. Because we measured plant height and pubescence before herbivores were abundant, however, we suspect that our experimental treatments directly influenced the majority of plant traits.

We also suspect that evolutionary changes may have contributed to the among-treatment differences. Evolutionary change is often thought to occur relatively slowly; however, recent review articles have highlighted that selective pressures can cause pronounced evolutionary change in only a few generations (Reznick and Ghalambor 2001, Hairston et al. 2005). Because *L. capitata* is a perennial and because treatments have only been applied for eight years, evolutionary changes would have had to occur within one to a few generations. However, given that a genetically diverse pool of seeds was used to establish the BioCON experiment, the past eight years likely have provided ample opportunity for differential survival and reproductive success, potentially resulting in divergence among treatments. In fact, among-treatment differences in pubescence observed in the experimental plants are also observed among plants grown from seeds collected from the different diversity treatments and grown in a common greenhouse environment (J. Lau and P. Tiffin, *unpublished data*), suggesting that at least some of the differences in plant traits may be due to genetic divergence among treatments.

An important caveat regarding this study (and other research assessing the effects of environmental variables on herbivores) is that the spatial scale of environmental

manipulation used in this experiment is necessarily much smaller than the spatial scale at which this environmental variation naturally occurs. The scale is also smaller than the spatial scale at which herbivore or pathogen populations are regulated and the dispersal distances of most insect herbivores. As a result, some of the effects we see may not scale to nonexperimental environments. For example, in our experiment, herbivores may preferentially move from monoculture to polyculture treatment plots, but the scale of naturally occurring differences in diversity may be larger than herbivore mobility (Agrawal et al. 2006). Problems of spatial scale are particularly important for extrapolating to predicted environmental changes that occur on global scales, such as increases in atmospheric CO₂ concentration, because herbivores cannot and will not be able to choose between different CO₂ environments. Nevertheless, we assume that at least some of the effects we see are due to differences in plant susceptibility, herbivore consumption, and pathogen virulence, mechanisms that will be important over both small and large spatial scales.

In conclusion, our results suggest that the effects of environmental variation on damage from natural enemies are driven, in part, by phenotypic and potentially genetic changes in plant traits that, in turn, influence herbivore feeding or susceptibility to pathogens. Because these natural enemy responses are linked to plant responses to their environment and because plant traits affect various classes of natural enemies differently, the effects of environmental variation in resource availability or plant community composition on higher trophic levels likely will depend on the particular plant-enemy association and specific plant responses to their environment. Thus identifying plant responses to environmental variation and how natural enemy damage is associated with plant traits may help to explain some of the high variability observed in empirical studies investigating how plant-enemy interactions respond to simulated changes in resource availability and diversity. Furthermore, given that resource availability is currently changing rapidly on a global scale with rising atmospheric CO₂ concentrations and increased N deposition, and given that community diversity is also declining, these results also indicate that global environmental changes may have large indirect impacts on plant communities by altering amounts of damage from natural enemies.

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APPENDIX

Summary of results for an ANCOVA testing the effects of CO₂, N addition, diversity, and plant traits on amounts of damage from four classes of natural enemies (*Ecological Archives* E089-012-A1).