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Effects of elevated CO_2, nitrogen deposition, and decreased species diversity on foliar fungal plant disease

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Abstract

Three components of global change, elevated CO_2, nitrogen addition, and decreased plant species richness ('diversity'), increased the percent leaf area infected by fungi (pathogen load) for much to all of the plant community in one year of a factorial grassland experiment. Decreased plant diversity had the broadest effect, increasing pathogen load across the plant community. Decreased diversity increased pathogen load primarily by allowing remaining plant species to increase in abundance, facilitating spread of foliar fungal pathogens specific to each plant species. Changes in plant species composition also strongly influenced community pathogen load, with communities that lost less disease prone plant species increasing more in pathogen load. Elevated CO_2 increased pathogen load of C_3 grasses, perhaps by decreasing water stress, increasing leaf longevity, and increasing photosynthetic rate, all of which can promote foliar fungal disease. Decreased plant diversity further magnified the increase in C_3 grass pathogen load under elevated CO_2. Nitrogen addition increased pathogen load of C_4 grasses by increasing foliar nitrogen concentration, which can enhance pathogen infection, growth, and reproduction. Because changes in foliar fungal pathogen load can strongly influence grassland ecosystem processes, our study suggests that increased pathogen load can be an important mechanism by which global change affects grassland ecosystems.

Keywords: biodiversity, ecosystem, elevated carbon dioxide, nitrogen enrichment, parasites, plant pathogens

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Introduction

Human activities are causing terrestrial ecosystems to simultaneously experience multiple types of environmental change, including decreased species diversity, increased atmospheric CO_2 concentration, and increased nitrogen deposition (Vitousek *et al*., 1997). Each of these components of environmental change can independently increase foliar fungal disease severity, the percent leaf area infected by pathogens (Thompson & Drake, 1994; Nordin *et al*., 1998; Tiedemann & Firsching, 1998; Garrett & Mundt, 1999; Knops *et al*., 1999; Chakraborty *et al*., 2000; Mitchell *et al*., 2002; Strengbom *et al*., 2002). Moreover, these components of environmental change can alter foliar fungal disease severity through the same or related mechanisms, creating the potential for interactive effects of environmental change on disease severity (Thompson *et al*., 1993; Tiedemann & Firsching, 1998). Increased foliar fungal disease severity under environmental change could have large repercussions, because foliar fungal pathogens can regulate ecosystem processes such as net primary production (Mitchell, 2003), and control plant community structure (Strengbom *et al*., 2002). In this paper, we report a test of the interactive and independent effects of decreased plant diversity, increased nitrogen deposition, and elevated atmospheric CO_2 on a grassland guild of 16 foliar fungal pathogens. Our study used a large-scale field experiment in which plant species diversity, atmospheric CO_2 concentration, and nitrogen addition rate were factorially controlled (Reich *et al*., 2001a).

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Species loss

Decreased plant species diversity has long been hypothesized to increase severity of diseases caused by specialist pathogens (the diversity—disease hypothesis; Elton, 1958; van der Plank, 1963; Leonard, 1969). The primary mechanism hypothesized for this effect is that both diversity and disease spread are related to host species abundances (Burdon & Chilvers, 1976; Chapin *et al*., 1997; Mitchell *et al*., 2002), although other mechanisms such as decreased interception of spores by nonhosts, altered microclimate, and decreased host resistance can also be important (Trenbath, 1977; Chin & Wolfe, 1984; Wolfe, 1985; Burdon, 1987; Boudreau & Mundt, 1992, 1994, 1997; Garrett & Mundt, 1999; Zhu *et al*., 2000). Decreased diversity allows remaining species to increase in abundance, on average, as a result of decreased competition. In turn, increased species abundance facilitates spread of pathogens specific to that species by increasing the probability that a given pathogen propagule will reach a host (Anderson & May, 1979; Burdon & Chilvers, 1982; Antonovics *et al*., 1995). In the local grassland community, most foliar fungal pathogen species are specific to one plant species (Mitchell *et al*., 2002). Accordingly, the diversity—disease hypothesis and this mechanism are broadly supported for foliar fungal pathogens of grassland plants (Knops *et al*., 1999; Mitchell *et al*., 2002), as well as crops (Chin & Wolfe, 1984; Boudreau & Mundt, 1997; Garrett & Mundt, 1999; Zhu *et al*., 2000).

As well as decreasing diversity, loss of species from a community necessarily alters the species composition of the community. Because species differ in traits that may influence disease severity across the plant community (community pathogen load, Mitchell *et al*., 2002), altered species composition may influence community pathogen load. One species trait that may cause changes in species composition to influence community pathogen load is disease proneness, or the average disease severity of a species under ambient conditions. If species lost from a community are less disease prone than the community average, then more disease prone individuals should take their place, increasing community pathogen load. Conversely, if species lost are more disease prone than average, then less disease prone individuals should take their place, decreasing community pathogen load. The community characteristic of community disease proneness arises from the species trait of disease proneness and changes with community composition. Community disease-proneness is hypothesized to influence community pathogen load (the species composition—disease hypothesis; Mitchell *et al*., 2002). In the one study to test this hypothesis, changes in species composition caused by species loss explained more variance in community pathogen load than did decreased species diversity (Mitchell *et al*., 2002), although the former clearly contributed to the latter. A second community characteristic, dominant species presence, was found to have little effect on community pathogen load (Mitchell *et al*., 2002).

Elevated CO_2

The effect of elevated CO_2 on foliar fungal disease severity may depend on the plant's photosynthetic pathway (the CO_2 metabolism—disease hypothesis; Thompson & Drake, 1994). This hypothesis predicts that elevated CO_2 will increase C_4 plants' foliar fungal disease severity by decreasing their water stress (Thompson & Drake, 1994; Wand *et al*., 1999), which can increase fungal sporulation (Woolacott & Ayres, 1984). In contrast, it predicts that elevated CO_2 will decrease C_3 plants' disease severity by decreasing their foliar nitrogen concentration (Yin, 2002; Wand *et al*., 1999; Reich *et al*., 2001b), despite any decrease in water stress. Elevated CO_2 has little effect on nitrogen concentration of C_4 plants (Wand *et al*., 1999; Reich *et al*., 2001b). This hypothesis has been supported for three of the five foliar fungal pathogens, including those of crops, for which disease severity has been quantified under elevated CO_2 (Thompson *et al*., 1993; Thompson & Drake, 1994; Tiedemann & Firsching, 1998; Chakraborty *et al*., 2000).

However, C_3 and C_4 plants' responses to elevated CO_2 differ in other ways that can reverse these effects. Specifically, elevated CO_2 increases C_3 plants' growth more than C_4 plants' growth (Wand *et al*., 1999; Reich *et al*., 2001b), which should increase host abundance, canopy humidity, and canopy spore-trapping, all of which increase foliar fungal disease severity (Burdon & Chilvers, 1982; Chakraborty *et al*., 2000; McCartney, 1997). In our experiment, elevated CO_2 increased leaf longevity of C_3, but not C_4, plants (Craine & Reich, 2001), which in turn can increase foliar fungal disease severity (Tiedemann & Firsching, 1998). Additionally, by increasing net photosynthesis in C_3 plants only (Wand *et al*., 1999; Lee *et al*., 2001), elevated CO_2 can increase both C_3 plant resistance to infection and pathogen growth rate after successful infection, thus either increasing or decreasing disease severity (Hibberd *et al*., 1996b).

Among C_3 plants, diseases of grasses, nonleguminous forbs, and leguminous forbs may respond differently to elevated CO_2, making it important to separately quantify the effect of elevated CO_2 on diseases of these groups. For example, elevated CO_2 often increases growth of nitrogen-fixing legumes more than other plants (Luscher *et al*., 1997; Schenk *et al*., 1997), potentially making their diseases most sensitive to elevated CO_2. Also, among nonnitrogen-fixers in our experiment, elevated CO_2 decreased foliar nitrogen concentration much more for C_3

forbs than for C_3 grasses (Reich *et al.*, 2001b), suggesting that elevated CO_2 should have more negative effects on forb than grass disease severity (Reich *et al.*, 2001b).

Nitrogen deposition

Nitrogen addition has been hypothesized to increase foliar fungal disease severity by increasing the concentration of foliar nitrogen available as a resource to the pathogens (the nitrogen—disease hypothesis) (Huber & Watson, 1974; Paul, 1990; Jensen & Munk, 1997; Nordin *et al.*, 1998; Strengbom *et al.*, 2002). Other mechanisms, such as decreased production of defensive compounds (Hartleb & Heitefuss, 1997; Sander & Heitefuss, 1998), increased microclimate humidity (Jenkyn, 1976), decreased rain-splash dispersal (Lovell *et al.*, 1997), and altered host abundance can also increase or sometimes decrease foliar fungal disease severity under nitrogen addition. In agricultural ecosystems, nitrogen addition usually increases crop foliar fungal disease severity (Jenkyn, 1976; Paul, 1990), but can potentially reduce disease severity as well (Huber & Watson, 1974). In non-agricultural ecosystems, higher nitrogen availability has increased the severity of all four foliar fungal diseases that have been reported (Snaydon & Davies, 1972; Jarosz & Burdon, 1988; Nordin *et al.*, 1998; Strengbom *et al.*, 2002). Severity of two of these diseases was correlated with foliar amino acid concentration (Nordin *et al.*, 1998), and experimental addition of glutamine to the leaf surface increased severity of one of these (Strengbom *et al.*, 2002); mechanisms were not tested for the others.

Interactive effects

Most studies of the effects of decreased plant diversity, nitrogen deposition, and elevated CO_2 on foliar fungal disease severity have examined one of these factors in isolation. Yet, multiple drivers may interact to influence disease severity, particularly when multiple drivers influence disease severity through the same or related mechanisms. Considering the diverse potential mechanisms reviewed above, there are numerous interactive effects that could be hypothesized; we will introduce only a few. First, in C_3 plants, if both elevated CO_2 and nitrogen addition influence disease severity by altering foliar nitrogen concentration, then elevated CO_2 may decrease disease severity under nitrogen addition but have no effect in its absence (the C–N resource hypothesis; Thompson *et al.*, 1993). In both C_3 and C_4 plants, if elevated CO_2 and nitrogen addition interact to control the concentration of carbon-based antifungal compounds, then they may interact to control disease severity (the C–N defense hypothesis; Herms & Mattson, 1992; Ayres, 1993). Third, in our experiment, greater plant diversity enhanced the increases in total plant biomass resulting from both elevated CO_2 and nitrogen addition (Reich *et al.*, 2001a). This effect was driven by increases in the biomass of C_3 plants. Because greater plant biomass should increase host abundance, microclimate humidity, and trapping of fungal spores by the plant canopy, elevated CO_2 or nitrogen addition may increase foliar fungal disease severity more at higher plant diversity, particularly for plant species and groups whose abundance increases the most (the diversity enhancement hypothesis).

Comparative effects

The effects of elevated CO_2 on foliar fungal disease severity are expected to vary among plant functional groups (Thompson & Drake, 1994), and two of the three hypothesized interactions all hinge on the response of C_3 plants to elevated CO_2. In contrast, decreased plant diversity is hypothesized to increase community pathogen load by increasing host abundance, on average, across the plant community (Mitchell *et al.*, 2002). Similarly, nitrogen addition should increase community pathogen load by increasing the foliar nitrogen concentration across the plant community. Therefore, the effects of decreased species diversity and nitrogen addition are expected to be qualitatively similar across plant functional groups, while the effects of elevated CO_2 are expected to differ qualitatively among functional groups. Accordingly, we hypothesized that decreased species diversity and nitrogen addition would increase community pathogen load but effects of elevated CO_2 and most interactions involving elevated CO_2 would be limited to certain plant functional groups (the comparative breadth hypothesis).

Approach

Here, we report an experimental test of these eight hypotheses, using ecosystems in which plant species richness, nitrogen addition, and atmospheric CO_2 concentration were factorially controlled (Reich *et al.*, 2001a; Reich *et al.*, 2001b). The experiment was performed in the field, with atmospheric CO_2 concentration controlled by free air CO_2 enrichment (FACE). We quantified the severity (percent leaf area infected) of all 16 foliar fungal diseases found in the experiment in 1999, and thereby estimated community-wide disease severity, or community pathogen load (Mitchell *et al.*, 2002). Because diseases of plant species in different trait-based plant functional groups (i.e. C_4 grasses, C_3 grasses, nonleguminous C_3 forbs, and leguminous C_3 forbs) were hypothesized to respond differently to elevated CO_2, we also tested treatment effects on pathogen load of each functional group. We statistically tested the major proposed mechanisms for each treatment effect observed.

Materials and methods

Experimental system

Our study was performed in the BioCON (Biodiversity, CO_2, and N) experiment (Reich *et al*., 2001a; Reich *et al*., 2001b) (http://www.lter.umn.edu/biocon/) at Cedar Creek Natural History Area, MN, about 50 km north of Minneapolis/St Paul (lat. 45°N, long. 93°W). The site is located on a glacial outwash sandplain, so production is nitrogen limited (Tilman, 1987). The experiment was conducted in a secondary successional oldfield after clearing the existing vegetation. The 16 plant species planted in the experiment were all perennial grassland species native or naturalized to the site. Plots were planted in 1997. By 1998, almost all plant species had been colonized by foliar fungal pathogens, and we sampled in 1999. We focused on foliar fungal pathogens, because they can have strong ecological effects in both agricultural and natural ecosystems (Oerke *et al*., 1994; Mitchell, 2003), and because a knowledge base facilitating their quantification and taxonomic identification exists (Farr *et al*., 1989; Campbell & Madden, 1990).

The experiment was similar to a split plot design, but with replication of split-plot treatments within whole plots; for simplicity we follow standard split-plot terminology. Our study used 296 individual 4 m^2 plots (split plots) distributed among six 20 m diameter rings (whole plots). In three rings, atmospheric CO_2 concentration was maintained at 560 $\mu mol\,mol^{-1}$ using a FACE system, which controls atmospheric CO_2 concentration without altering microclimate or light conditions (Hendrey *et al*., 1993). The three other rings were treated identically except that they received ambient CO_2 levels (368 $\mu mol\,mol^{-1}$). The CO_2 treatment was implemented during daylight hours during the growing season. The use of FACE technology is especially important for studies of plant disease, because open-top chambers can directly decrease foliar fungal disease severity (Thompson & Drake, 1994). Almost all other past studies have used potted plants grown indoors (Thompson *et al*., 1993; Tiedemann & Firsching, 1998; Chakraborty *et al*., 2000), except Karnosky *et al*. (2002). Split plots were pooled within each CO_2 treatment, then randomly assigned both a nitrogen addition treatment (0 or 4 $g\,N\,m^{-1}\,yr^{-1}$) and a species richness treatment (1, 4, 9, or 16 species). For each of the four combinations of CO_2 and N treatment, there were 32 replicates of plots planted with one species, 15 replicates of plots planted with four species, 15 replicates of plots planted with nine species, and 12 replicates of plots planted with 16 species. Thus, in each ring, there were 4–5 plots of a given nitrogen and species richness treatment combination, except for monocultures, for which there were 10–11. Species were assigned to monocultures such that each species was represented by two monocultures under each factorial CO_2/N treatment. In all other plots, species were assigned randomly from the full pool of 16 species. Each plot was planted with 12 $g\,m^{-2}$ of seed, split equally among the planted species. Species composition was maintained by hand weeding. Nitrogen was applied as slow-release anhydrous ammonium nitrate, distributed three times over the growing season. Measured species richnesses and CO_2 concentrations differed little from the treatment levels (Reich *et al*., 2001a).

Sampling

Disease severity, defined as the percent of the leaf surface area visibly covered by fungal lesions, was estimated visually in the field using cards with digitized images of leaves of known disease severity for reference, a standard technique in plant pathology (James, 1971; Campbell & Madden, 1990). For a given foliar fungal pathogen, host, and environment, percent leaf area infected is generally proportional to the pathogen's negative effects on the host (Campbell & Madden, 1990). We collected data on all 16 naturally occurring foliar fungal diseases found in the experiment in 1999 (Table 1). Disease severity was quantified as close to the annual peak of each individual disease as was practical. For each plant species in each plot, 50 leaves were inspected unless this exceeded the abundance of the species. Leaves were blindly chosen for inspection by haphazardly choosing a host plant, then looking away while extending a hand towards the plant, and inspecting the first leaf contacted. Data were collected by two trained personnel standing in the aisles between plots.

Aboveground biomass was measured in each plot by clipping a $1.0 \times 0.1\,m^2$ strip, sorting the clipped vegetation to species, then drying and weighing. We used the average of June and August samples to better estimate abundance over the growing season.

Analysis

We used the disease severity of each species present in a plot to calculate pathogen load for the community and for each functional group. We distinguish between pathogen load and disease severity because pathogen load can be used to compare communities or groups of species differing in composition, but may not be as indicative of the effects of the pathogens on the community or group as disease severity is for individual host-pathogen systems. Pathogen load was calculated as a weighted average of the disease severity of each constituent species, with disease severity of each species weighted by the biomass of that species, following Mitchell *et al*. (2002):

Table 1 Plant species planted in the experiment, plant functional group, observed pathogens and diseases, and disease proneness (see Methods) and dominance (mean aboveground biomass in all plots planted with 16 species)

Plant species name	Group	Associated pathogen species	Disease type(s)	Disease proneness	Dominance
Agropyron repens	C_3 grass	*Bipolaris* sp.	Fungal leaf spot	0.43	12.2
Bromus inermis	C_3 grass	Unidentified Hyphomycetes	Fungal leaf spot	0.38	40.6
Koeleria cristata	C_3 grass	Unidentified fungus	Fungal leaf spot	0.59	16.0
Poa pratensis	C_3 grass	Unidentified Coelomycetes	Fungal leaf spot	0.78	77.8
			Mean:	0.55	36.6
			SEM:	0.090	15.1
Andropogon gerardi	C_4 grass	*Phyllosticta* sp., *Puccinia andropogonis*	Fungal leaf spot, rust	2.7	4.7
Bouteloua gracilis	C_4 grass	*Bipolaris* sp.	Fungal leaf spot	0.22	2.4
Schizachyrium scoparium	C_4 grass	*Colletotrichum* sp.	Fungal leaf spot	0.35	1.3
Sorghastrum nutans	C_4 grass	*Colletotrichum* sp.	Fungal leaf spot	1.13	0.75
			Mean:	1.09	2.3
			SEM:	0.56	0.87
Achillea millefolium	Forb	None	None	0.000	75.2
Anemone cylindrica	Forb	*Mycosphaerella* sp.	Fungal leaf spot	0.001	0.007
Asclepias tuberosa	Forb	*Septoria* sp.	Fungal leaf spot	0.047	0.05
Solidago rigida	Forb	*Septoria* sp.	Fungal leaf spot	0.041	0.11
			Mean:	0.022	18.9
			SEM:	0.012	18.8
Amorpha canescens	Legume	Unidentified	Leaf spot	0.025	0.07
Lespedeza capitata	Legume	*Uromyces lespedezae-procumbentis*	Rust	0.025	1.8
Lupinus perennis	Legume	*Erysiphe polygoni*	Powdery mildew	3.37	67.0
Petalostemum villosum	Legume	Unidentified	Leaf spot	0.003	0.002
			Mean:	0.86	17.2
			SEM:	0.84	16.6

Mean and standard error of the mean of disease proneness and dominance are presented for each plant functional group. All fungi are presumed to be different species. Voucher specimens are deposited in the University of Minnesota Herbarium. For taxonomic authorities, see Farr *et al*. (1989) and Kartesz (1994).

$$l = \frac{\sum_{i=1}^{n} S_i C_i}{\sum_{i=1}^{n} C_i} \quad (1)$$

where l is pathogen load, S_i is disease severity of the ith species, C_i is biomass of the ith species, and n is the number of species planted (of a functional group, if calculating functional group pathogen load) in the plot. Plant functional groups were defined based on species traits, and consisted of C_4 grasses, C_3 grasses, nonleguminous C_3 forbs, and leguminous C_3 forbs.

Within factorial treatments, plots differed in species composition, and plant species differed in traits potentially influencing pathogen load. To test how much variation in pathogen load was explainable by plot-to-plot variation in species composition, we calculated a community level index of a plant trait that can influence pathogen load, disease proneness, following Mitchell *et al*. (2002). Each species' disease proneness was defined as its average disease severity under 'ambient' experimental conditions: in plots planted with 16 species at ambient nitrogen and CO_2 levels. Community disease proneness was calculated as the weighted average of the disease proneness of all species planted in that plot, with each species' disease proneness weighted by its biomass in that plot:

$$p = \frac{\sum_{i=1}^{n} a_i C_i}{\sum_{i=1}^{n} C_i} \quad (2)$$

where p is community disease proneness, a_i is the average disease proneness of the ith species, and other notation is as for pathogen load.

All within-plot measurements were averaged. Plots were considered split plot replicates and rings were considered whole plot replicates. All analyses were based on ordinary unweighted least squares, using Systat for Windows, Version 9. Unless otherwise stated, all statistical models included the independent categorical variables species richness treatment, nitrogen treatment, CO_2 treatment, their four interaction terms, and the whole plot error term. In analyses of three of the 16 individual

diseases, we could not model one or two interaction terms, because random assignment of species composition allowed three plant species to not occur in all 16 factorial treatments. We transformed variables by the natural logarithm or square root to better meet ANOVA assumptions, based on inspection of residual plots. In our split plot analysis, CO_2 treatment was the whole plot factor, and nitrogen and species richness were the split plot factors. Therefore, the CO_2 treatment effect was tested against the random effect of ring nested within CO_2 treatment. Other main effects and interactions were tested against the residual error, because SAS version 8 Proc Mixed confirmed that no treatment by ring interactions were significantly different from zero. The significance of all effects was identical for Systat MGLH and SAS Proc Mixed; we report detailed results from the former for simplicity. Fisher's LSD was used to test cell differences among plant diversity treatments and the factorial treatment combinations of interactions. We used the sequential Bonferroni procedure to determine significance when testing the same hypothesis for each individual disease (Rice, 1989).

In testing whether host plant abundance (aboveground biomass) linked plant diversity and disease severity, it is important to consider not just the effects of host abundance independent of species richness, but also the effects of host abundance due ultimately to plant diversity (Mitchell *et al*., 2002). Therefore, we used Type I sums of squares in this analysis, with host abundance entered in the model first, so that tests of the effects of plant diversity were conditional on host abundance, but not vice versa. In all other analyses, we used Type III sums of squares.

For each model presented, all significant effects are reported here, with two sets of exceptions. We do not report effects of decreased diversity on disease severity of functional groups, because we report the effects of diversity on the severity of each individual disease. Conversely, we do not report effects of nitrogen addition, elevated CO_2, or interactions on severity of individual diseases, because they are redundant to the presented effects on disease severity of each functional group.

Results

The diversity—disease hypothesis

Decreased plant diversity increased community pathogen load, supporting the diversity—disease hypothesis.

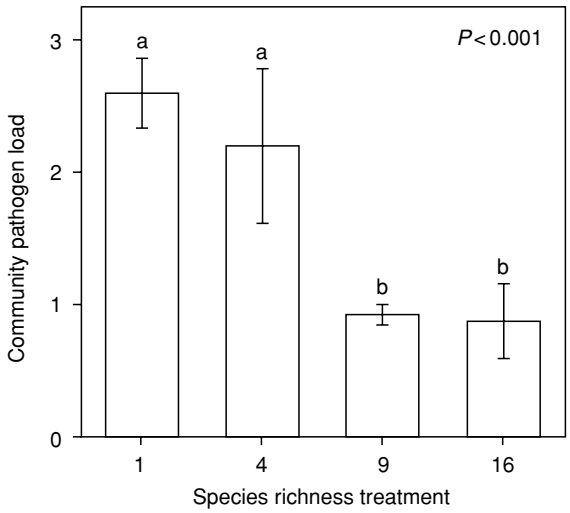

Fig. 1 The effect of plant diversity on community pathogen load (the percent of leaf area infected across the plant community). Data shown are means ± SEM. Different letters indicate significant pairwise differences.

Table 2 Analysis of variance for three general linear models with natural log-transformed community pathogen load, C_3 grass pathogen load, and C_4 grass pathogen load as dependent variables and elevated CO_2 treatment (CO_2), nitrogen addition (N), and species richness treatment (SR) as independent variables

	Whole community		C_3 grasses		C_4 grasses	
Source	Mean-square	*F*-ratio	Mean-square	*F*-ratio	Mean-square	*F*-ratio
CO_2	0.863	$F_{1,4} = 1.400$	1.199	$F_{1,4} = 24.250$**	0.061	$F_{1,4} = 0.031$
Ring (CO_2)	0.618	$F_{4,276} = 1.796$	0.049	$F_{4,159} = 0.781$	1.942	$F_{4,162} = 5.850$***
N	0.342	$F_{1,276} = 0.994$	< 0.001	$F_{1,159} < 0.001$	3.478	$F_{1,162} = 10.478$**
SR	4.010	$F_{3,276} = 11.659$***	3.133	$F_{3,159} = 49.491$***	4.952	$F_{3,162} = 14.922$***
CO_2*N	0.107	$F_{1,276} = 0.312$	0.005	$F_{1,159} = 0.073$	0.952	$F_{1,162} = 2.869$
CO_2*SR	0.296	$F_{3,276} = 0.861$	0.234	$F_{3,159} = 3.697$*	0.716	$F_{3,162} = 2.157$
N*SR	0.127	$F_{3,276} = 0.368$	0.004	$F_{3,159} = 0.065$	0.078	$F_{3,162} = 0.234$
CO_2*N*SR	0.064	$F_{3,276} = 0.185$	0.016	$F_{3,159} = 0.249$	0.195	$F_{3,162} = 0.587$
Error	0.344		0.063		0.332	

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

Natural log-transformed pathogen load increased as plant diversity decreased; plots planted with 1 or 4 species had significantly higher pathogen loads than those with 9 or 16 species (Fig. 1; Table 2; $F_{3,276} = 11.66$; $P < 0.001$). Pathogen load increased from a mean of 0.88 in plots planted with 16 species to a mean of 2.6 in monocultures, a 3.0 fold increase. From high to low diversity, the upper bound of pathogen load increased by an order of magnitude from 1.7 to 17.0 (this upper bound comparison excluded one outlier plot planted with 16 species but dominated by heavily diseased *Lupinus*). However, the coefficient of variation in pathogen load did not show any clear pattern with diversity.

Decreased plant diversity increased the severity of all 16 diseases sampled (Table 3). The rank order of species richness treatment means for disease severity was $1 > 4 > 9 > 16$ for every disease except the leaf spots of *Amorpha*, *Asclepias*, and *Petalostemum*, three of the least disease prone species (Table 1), for each of which the rank order of two treatments was swapped.

The severity of all 16 diseases was strongly positively correlated with host plant species abundance (Table 3), the major factor proposed to link plant diversity and severity of foliar fungal diseases. After controlling for this correlation between host abundance and disease severity (see Methods: analysis), the effect of species richness treatment on disease severity was significant for only six diseases after sequential Bonferroni correction, only 38% of the number without controlling for host abundance (Table 3). The six diseases for which severity was still correlated with diversity after controlling for host abundance were exemplified by the *Poa* leaf spot (Fig. 2a, b), while the ten for which severity was not correlated with diversity were exemplified by the powdery mildew on *Lupinus* (Fig. 2c, d). On average across all diseases, controlling for host abundance reduced the sums of squares explained by diversity by 73%. The decreased significance of plant diversity after controlling for host abundance supports the hypothesis that increased host abundance was the primary mechanism linking decreased plant diversity to increased disease severity and pathogen load.

The species composition—disease hypothesis

Our results also support the species composition—disease hypothesis. The general linear model including all three main effects and four interactions, but not covariates for species composition, explained 13.3% of the variance in log of community pathogen load, of which 81% was uniquely explained by plant diversity treatment. An additional 21% of the variance in log of

Table 3 Effects of plant species diversity and host plant species abundance on severity of each disease

Disease	Diversity	Abundance coefficient	Abundance *F*-ratio	Diversity $\mid_{\text{Abundance}}$
Agropyron leaf spot†	$F_{3,90} = 26.2$*	0.004 ± 0.001	$F_{1,89} = 79.6$*	$F_{3,89} = 3.5$
Amorpha leaf spotY	$F_{3,40} = 54.6$*	0.003 ± 0.001	$F_{1,39} = 73.2$*	$F_{3,39} = 27.5$*
Andropogon leaf spot†	$F_{3,74} = 13.3$*	0.003 ± 0.001	$F_{1,73} = 30.3$*	$F_{3,73} = 3.0$
Andropogon rust‡	$F_{3,74} = 10.2$*	0.007 ± 0.002	$F_{1,73} = 29.5$*	$F_{3,73} = 1.0$
Anenome leaf spotY	$F_{3,29} = 18.5$*	0.021 ± 0.001	$F_{1,28} = 403.7$*	$F_{3,28} = 4.3$
Asclepias leaf spotY	$F_{3,51} = 4.8$*	0.011 ± 0.004	$F_{1,50} = 7.0$*	$F_{3,50} = 1.7$
Bouteloua leaf spotY	$F_{3,78} = 131.6$*	$0.006 \pm < 0.001$	$F_{1,77} = 450.7$*	$F_{3,77} = 2.7$
Bromus leaf spot‡	$F_{3,81} = 13.8$*	0.004 ± 0.001	$F_{1,80} = 27.1$*	$F_{3,80} = 6.5$*
Koeleria leaf spot†	$F_{3,84} = 33.4$*	0.004 ± 0.001	$F_{1,83} = 88.5$*	$F_{3,83} = 10.8$*
Lespedeza rustY	$F_{3,87} = 17.9$*	0.004 ± 0.001	$F_{1,86} = 49.3$*	$F_{3,86} = 9.8$*
Lupinus powdery mildew‡	$F_{3,85} = 6.1$*	0.005 ± 0.001	$F_{1,84} = 18.0$*	$F_{3,84} = 3.6$
Petalostemum leaf spot	$F_{3,18} = 5.0$*	$< 0.001 \pm < 0.001$	$F_{1,17} = 15.2$*	$F_{3,17} = 2.7$
Poa leaf spot†	$F_{3,82} = 20.9$*	$0.002 \pm < 0.001$	$F_{1,81} = 49.6$*	$F_{3,81} = 5.0$*
Schizachyrium leaf spot‡	$F_{3,83} = 15.3$*	0.007 ± 0.001	$F_{1,82} = 30.8$*	$F_{3,82} = 3.5$
Solidago leaf spotY	$F_{3,56} = 37.1$*	$0.003 \pm < 0.001$	$F_{1,55} = 260.1$*	$F_{3,55} = 13.6$*
Sorghastrum leaf spot‡	$F_{3,75} = 19.2$*	0.007 ± 0.001	$F_{1,74} = 52.2$*	$F_{3,74} = 3.5$

For each disease, partial results of two general linear models are shown. First is shown the *F*-statistic and significance after Bonferroni adjustment for the effect of diversity on disease severity without controlling for host abundance. Second are shown the effects of host abundance (slope ± standard error, *F*-statistic and significance after Bonferroni adjustment) and of diversity after controlling for host abundance (*F*-statistic and significance after Bonferroni adjustment) on disease severity. Slopes and standard errors are not back-transformed.

*Significant ($P < 0.05$) after sequential Bonferroni adjustment for multiple comparisons within columns.

†Disease severity was transformed by the square root.

‡Disease severity was transformed by the natural logarithm.

YDisease severity + 1 was transformed by the natural logarithm.

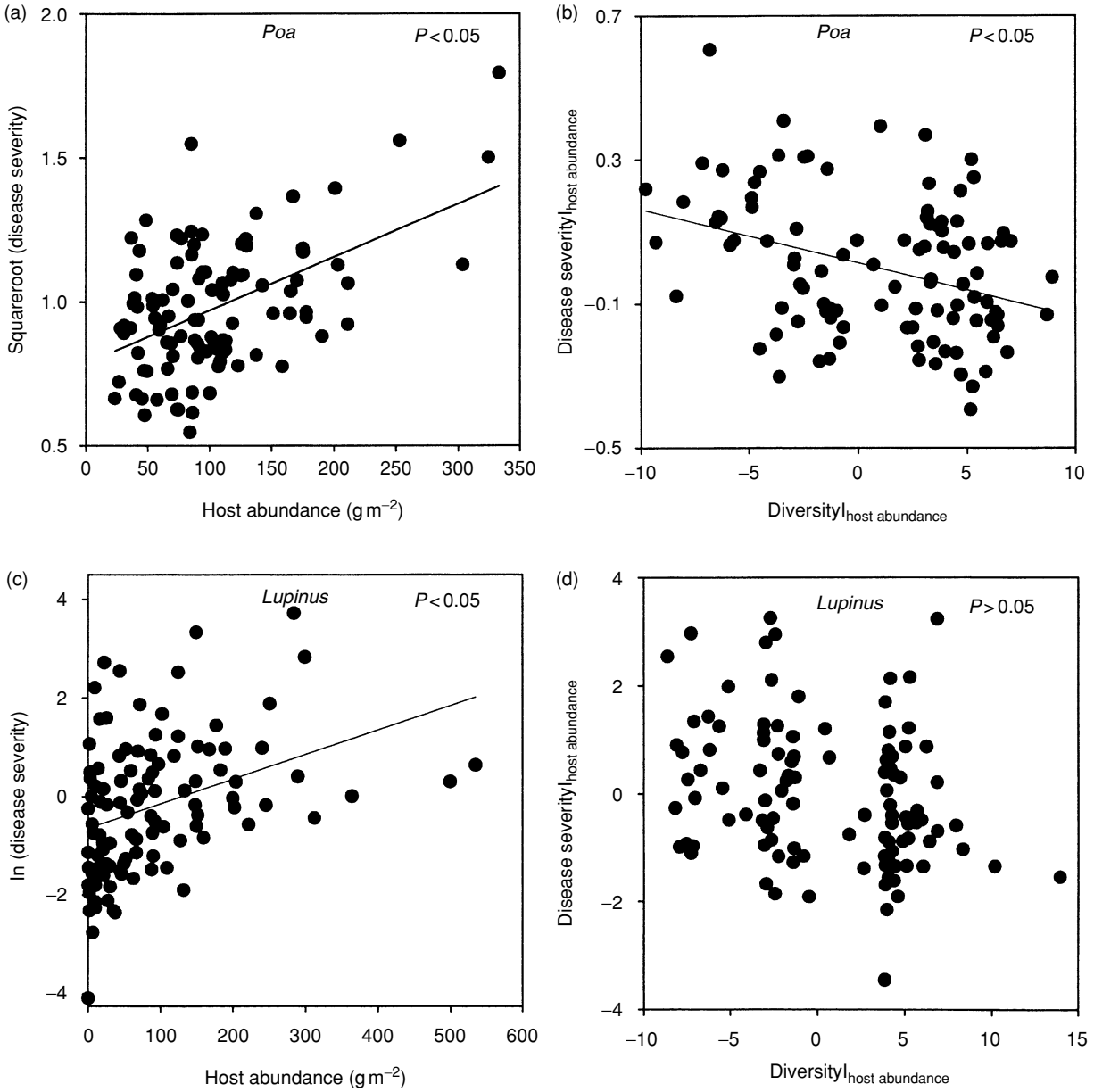

Fig. 2 Illustration of the two major types of relationships between disease severity, host abundance, and species diversity. For the leaf spot on *Poa* (and five other diseases not shown) disease severity was positively correlated with host abundance (a) and, after controlling for this relationship, disease severity was still negatively correlated with species diversity (b). For the powdery mildew on *Lupinus* (and nine other diseases not shown) disease severity was positively correlated with host abundance (c) and, after controlling for this relationship, there was no correlation between disease severity and species diversity (d). Panels b and d are added variable plots with unstandardized residuals as axis units.

community pathogen load was explained by adding disease proneness, a community characteristic resulting from differences in species composition, to this model as a covariate. Thus, community composition explained more variation in community pathogen load than all treatments together. Controlling for disease proneness did not alter the significance of any other variable in the model. Communities with more disease prone species had higher pathogen loads (Fig. 3; $F_{1,275} = 90.48$; $P < 0.001$).

Because disease proneness was determined based on data from the plots planted with 16 species, the correlation between disease proneness and pathogen load could be the result of autocorrelation. Therefore, we also tested a model identical except for excluding the plots used to calculate disease proneness, which

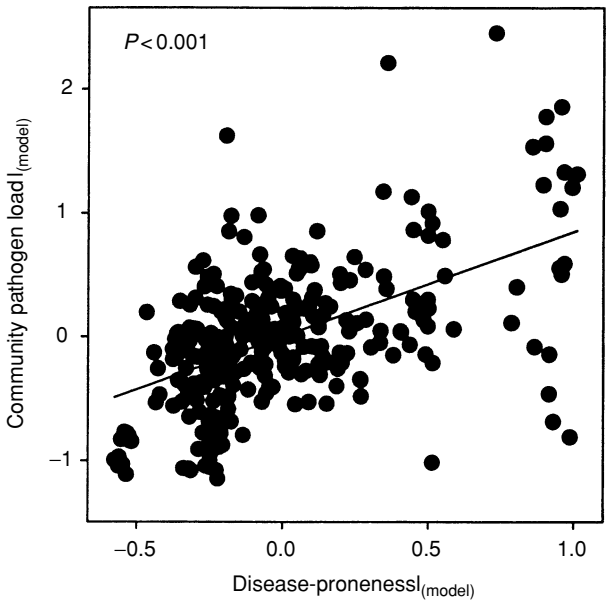

Fig. 3 Added variable plot showing correlation between ln (community pathogen load) and ln (community disease proneness) after controlling for all experimental treatments and interactions. Axis units are unstandardized residuals.

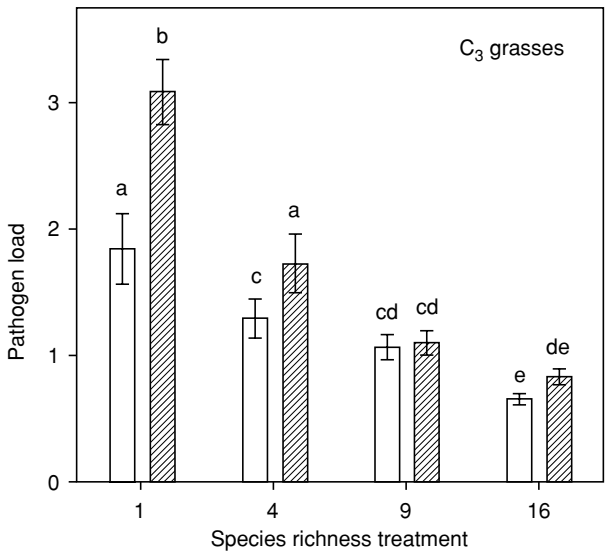

Fig. 4 The effects of elevated CO_2 and plant diversity on C_3 grass pathogen load. Open bars = ambient CO_2 treatment; shaded bars = elevated CO_2 treatment. Data shown are means ± SEM. Different letters indicate significant pairwise differences.

required removing the three-way interaction term. Results from this model (not shown) were quantitatively almost identical to the model using all plots, indicating that autocorrelation did not drive the result reported above.

The CO_2 metabolism—disease hypothesis

Elevated CO_2 did not influence community pathogen load (Table 2; $F_{1,4} = 1.40$; $P = 0.303$). Among plant functional groups, elevated CO_2 increased natural log-transformed pathogen load of C_3 grasses by 33% across all nitrogen and diversity treatments (Fig. 4; Table 2; $F_{1,4} = 24.25$; $P = 0.008$). However, elevated CO_2 did not increase pathogen load of C_4 grasses ($P > 0.6$), nonleguminous C_3 forbs ($P > 0.4$), or leguminous C_3 forbs ($P > 0.2$). These results do not support the CO_2 metabolism—disease hypothesis, which predicted that elevated CO_2 would decrease pathogen load of C_3 plants and increase pathogen load of C_4 plants.

The nitrogen—disease hypothesis

Nitrogen addition did not influence community pathogen load (Tables 2, N: $F_{1,276} = 0.99$; $P = 0.320$). Among plant functional groups, nitrogen addition increased natural log-transformed C_4 grass pathogen load by 58% across all CO_2 and diversity treatments (Fig. 5; Table 2; $F_{1,162} = 10.48$; $P < 0.001$). However, nitrogen addition did not increase pathogen load of C_3 grasses ($P > 0.7$), nonleguminous forbs ($P > 0.4$) or leguminous forbs ($P > 0.2$). These results support the nitrogen—disease hypothesis, but only for C_4 grasses.

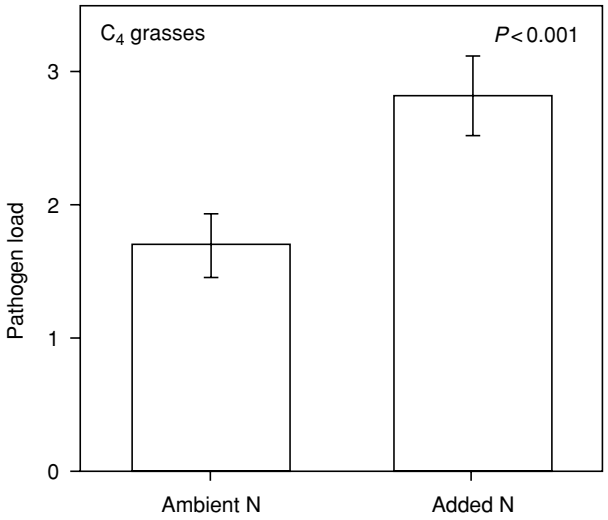

Fig. 5 The effect of nitrogen addition on C_4 grass pathogen load. Data shown are means ± SEM.

Interactions

No factors interacted to influence community pathogen load (Table 2, $P > 0.4$). Elevated CO_2 increased C_3 grass

pathogen load more at lower plant diversity (Fig. 4; $F_{3,159} = 3.70$; $P = 0.013$). Thus, these two elements of global change amplified each other's effects on C_3 grass pathogen load. Specifically, elevated CO_2 increased pathogen load significantly in plots planted with one or four species ($P < 0.05$), but not in plots planted with nine or 16 species ($P > 0.2$). Looked at another way, as diversity decreased from 16 to 1 species, C_3 grass pathogen load increased from 0.7 to 1.8 under ambient CO_2, but from 0.8 to 3.1 under elevated CO_2. This interaction does not support the diversity enhancement hypothesis, which predicts that elevated CO_2 increases pathogen load more at higher plant diversity. For C_4 grasses, elevated CO_2 tended to decrease pathogen load in plots planted with 16 species, but not under decreased diversity ($F_{3,162} = 2.16$; $P = 0.095$), which also is not consistent with the diversity enhancement hypothesis. Elevated CO_2 tended to magnify the effect of nitrogen addition on C_4 grass pathogen load ($F_{1,162} = 2.87$; $P = 0.092$), a pattern consistent with the C–N defense hypothesis. No treatments interacted to influence pathogen load of either nonleguminous or leguminous forbs ($P > 0.4$).

Discussion

The comparative breadth hypothesis

The experimental effect of decreased plant diversity was much broader than that of either nitrogen addition or elevated CO_2. This contrast suggests that losses of plant species will increase foliar fungal pathogen load (percent leaf area infected) across the entire remaining community while nitrogen deposition and elevated CO_2 will only increase pathogen load of C_4 and C_3 grasses, respectively. However, increases in C_4 and C_3 grass pathogen load may still have important consequences, because these functional groups dominate grassland ecosystems. These results also suggest that pathogen load of subdominant forbs will be less affected than pathogen load of grasses by nitrogen deposition and elevated CO_2 unless plant species diversity also decreases. If nitrogen deposition and elevated CO_2 are accompanied by decreased diversity, this combination could potentially allow forbs to increase in abundance at the expense of grasses.

These results partially support the comparative breadth hypothesis. Decreased diversity increased community pathogen load and the effects of elevated CO_2 varied among functional groups, as hypothesized and reported in one previous test of each effect (Thompson & Drake, 1994; Mitchell *et al*., 2002). Contrary to the hypothesis, nitrogen addition only increased C_4 grass pathogen load, not community pathogen load. The specificity of this effect may be explained by the fact that nitrogen addition increased C_4 grass foliar nitrogen concentration by 35%, the most of any functional group (Reich *et al*., 2001b), presumably thus providing the greatest benefit to pathogens of C_4 grasses.

Diversity and disease

Decreased diversity increased community pathogen load and did so primarily by increasing host abundance, supporting the diversity-disease hypothesis in accordance with the few other experimental tests in unmanaged ecosystems (Knops *et al*., 1999; Mitchell *et al*., 2002), and numerous experiments with agricultural mixtures (Chin & Wolfe, 1984; Boudreau & Mundt, 1997; Garrett & Mundt, 1999; Zhu *et al*., 2000). As well as increasing pathogen load, decreased diversity commonly decreases root production, root biomass, and soil respiration (Hector *et al*., 1999; Craine *et al*., 2001; Reich *et al*., 2001a; Tilman *et al*., 2001; Tilman *et al*., 2002). At our site, foliar fungal pathogens decrease root production, root biomass, and soil respiration by decreasing leaf longevity and photosynthetic capacity (Mitchell, 2003), suggesting that increased community pathogen load may be a mechanism linking decreased diversity and these ecosystem processes. For example, experimentally decreasing peak community pathogen load from 8.5% to 0.5% increased root biomass by 47% (Mitchell, 2003). Assuming that root biomass is a linear function of community pathogen load implies that the increase in community pathogen load from 1% at high diversity to 2.5% at low diversity in this experiment (BioCON) would decrease root biomass by 9%. The observed decrease in root biomass from high to low diversity was 53% (Reich *et al*., 2001a), suggesting that increased pathogen load was responsible for almost one-fifth of the total decrease. This value may be conservative, because the effect of increased foliar fungal infection on plant functioning is generally nonlinear with greater marginal effects at lower pathogen load (Bastiaans, 1991; Lopes & Berger, 2001), rather than the linear function assumed in the above calculation. However, it is likely that the effects of pathogens on biomass in BioCON differed quantitatively from those in the experiment manipulating pathogen load as a result of differences in community composition and other factors. Therefore, experiments factorially manipulating plant diversity and pathogen load will be required to better quantify the contribution of disease to the effects of plant diversity on ecosystem processes.

Species composition and disease

Our results demonstrate that species composition is a key determinant of community pathogen load, supporting

the species composition—disease hypothesis and in agreement with its one previous experimental test (Mitchell *et al.*, 2002). Disease proneness, a community characteristic resulting from differences in species composition, explained more variation in community pathogen load than did plant diversity. Thus, the degree to which the loss of a given species from a community will increase community pathogen load will depend on the traits of that species relative to the traits of those species remaining. Losing less disease prone species will increase pathogen load much more than losing an average species. This dependence of pathogen load on community composition suggests that pathogen load will be very sensitive not only to losses of species, but also to additions of species, such as through biological invasions. However, it should be noted that differences in pathogen load among communities differing in species composition may or may not be indicative of the effects of disease on ecosystem processes, because species vary in many traits, such as disease tolerance (the effect on the plant of a given percent leaf area infected), that could affect this relationship.

Carbon dioxide and disease

Contrary to the CO_2 metabolism—disease hypothesis, elevated CO_2 increased pathogen load of C_3 grasses rather than C_4 grasses. Thus, the typical increases in C_3 grass abundance under elevated CO_2 (Poorter, 1993; Wand *et al.*, 1999; Reich *et al.*, 2001b) may be constrained by pathogens. Prior evidence for this hypothesis was based on three pathogens (Thompson *et al.*, 1993; Thompson & Drake, 1994), so the majority of pathogens now studied do not support this hypothesis. The effects of elevated CO_2 on C_3 and C_4 grass pathogen load differed from the prediction of the CO_2 metabolism—disease hypothesis despite the fact that elevated CO_2 altered the physiology of each host group as predicted. In monoculture, elevated CO_2 reduced C_3 grass foliar nitrogen concentration by 23% (Reich *et al.*, 2001b), which is expected to reduce disease severity (Thompson *et al.*, 1993; Thompson & Drake, 1994). In C_4 grass monocultures, elevated CO_2 increased instantaneous water use efficiency by 25% (Lee *et al.*, 2001) and increased soil water concentration by 5% (Reich *et al.*, 2001b), suggesting that it reduced water stress.

Based on data from the monocultures in our experiment, elevated CO_2 could have increased C_3 grass pathogen load through three mechanisms, potentially acting together. First, elevated CO_2 increased C_3 grass instantaneous water use efficiency by 50% (Lee *et al.*, 2001) and percentage soil water in C_3 grass monocultures by 9% (Reich *et al.*, 2001b), both the most of any functional group, which may indicate they experienced the greatest reduction in water stress. Decreased water stress can promote sporulation by foliar fungi (Woolacott & Ayres, 1984). This mechanism is perhaps most plausible, because it can potentially explain why the effect was restricted to C_3 grasses. Second, elevated CO_2 increased leaf longevity of C_3, but not C_4, grasses by 7% (Craine & Reich, 2001). Increased leaf longevity under elevated CO_2 can result in increased disease severity by increasing exposure time to the pathogen (Tiedemann & Firsching, 1998). However, the increase in C_3 grass leaf longevity was less than for nonleguminous C_3 forbs (Craine & Reich, 2001), so if this were a general mechanism, elevated CO_2 should have also increased forb pathogen load. Third, elevated CO_2 increased net photosynthetic rate per leaf area by 15% in C_3 grasses, the most of any functional group (Lee *et al.*, 2001). Increased photosynthetic rate can increase the growth rate of successful infections, but also can decrease infection success per fungal spore (Hibberd *et al.*, 1996b). For increased photosynthetic capacity to explain the increased pathogen load, its effect on pathogen growth rate would have had to outweigh its effect on pathogen infection success, but we were unable to test this hypothesis.

Nitrogen and disease

Pathogen load of C_4 grasses was more sensitive to nitrogen addition than were pathogen loads of the other plant functional groups. These results partially support the nitrogen—disease hypothesis, although the hypothesis predicted that nitrogen addition would also increase disease severity of C_3 plants, as has been observed elsewhere (Snaydon & Davies, 1972; Nordin *et al.*, 1998). Nitrogen addition can cause grasslands to shift from C_4—dominated to C_3—dominated (Tilman, 1987); our results suggest that increased foliar fungal disease could contribute to this shift. However, this contribution is probably small relative to the shift from nitrogen to light competition (Wedin & Tilman, 1993) because foliar fungal disease inhibits root growth more than shoot growth (Mitchell, 2003). Foliar fungal disease can decrease leaf longevity (Mitchell, 2003), and therefore also likely contributed to the decrease in C_4 grass leaf longevity under nitrogen addition observed in our experiment, although similar decreases in C_3 species suggest that the primary mechanism is more general (Craine & Reich, 2001).

Our results suggest that increased foliar nitrogen concentration was the major cause of the increased C_4 grass pathogen load under nitrogen addition. Increased foliar nitrogen concentration can increase disease severity by increasing pathogen infection success, lesion growth, and spore production (Hartleb & Heitefuss, 1997; Jensen & Munk, 1997; Sander & Heitefuss, 1998). In monocultures

of our experiment, foliar nitrogen concentration in C_4 grasses was lower than in all other functional groups at ambient nitrogen supply, and increased the most both proportionally and absolutely in response to nitrogen addition (Reich *et al*., 2001b). Thus, pathogens of C_4 grasses received the greatest benefit of nitrogen addition in terms of foliar nitrogen concentration.

Interactive effects

Elevated CO_2 and plant diversity interacted to determine pathogen load of C_3 grasses. Specifically, higher diversity prevented elevated CO_2 from increasing C_3 grass pathogen load. Higher diversity also enhanced the positive effect of elevated CO_2 on primary production (Reich *et al*., 2001a). Foliar fungal pathogens can decrease grassland production and biomass (Mitchell, 2003), suggesting that the interactive effects of diversity and elevated CO_2 on disease contributed to their interactive effects on production. Joint effects of elevated CO_2 and disease on plant production have been observed in potted crop plants (Hibberd *et al*., 1996a; Malmstrom & Field, 1997; Chakraborty *et al*., 2000; Tiedemann & Firsching, 2000). However, leguminous and nonleguminous forbs responded more strongly to elevated CO_2 than did C_3 grasses (Reich *et al*., 2001a, b), so the contribution of disease to the interactive effects of diversity and elevated CO_2 on production was likely minor. Furthermore, the interactive effects of elevated CO_2 and decreased diversity on C_3 grass pathogen load comprised the strongest interaction detected in this study, suggesting that the interactive effects of elevated CO_2, nitrogen deposition, and decreased plant diversity will be less important than their separate effects in determining grassland pathogen loads.

Conclusions

This study demonstrates that decreased plant diversity, elevated CO_2, and nitrogen addition can all increase foliar fungal pathogen loads, either of key functional groups or across the plant community. It also shows that some of these components of environmental change can amplify each other's effects on pathogen loads, but suggests that such interactive effects will be less frequent and weaker than these drivers' independent effects. Pathogen loads will likely also be functions of other components of global change, such as climate change (Harvell *et al*., 2002), and perhaps of other interactions among environmental drivers. For example, if the increased pathogen loads of C_3 grasses under elevated CO_2 observed here resulted from decreased water stress, climate change could enhance or reduce this effect depending on local changes in precipitation regime. Additionally, the sensitivity of pathogens to climate suggests that the effects of environmental change on pathogen load will vary temporally and spatially. As our study was restricted to one location and one year, further studies to examine the generality of our results are needed. Nonetheless, together with recent results showing that altering foliar fungal pathogen load can influence grassland ecosystem processes (Mitchell, 2003), our study suggests that increased foliar fungal pathogen load is one mechanism by which global change can impact grassland ecosystems.

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