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Plant species richness, elevated CO_2, and atmospheric nitrogen deposition alter soil microbial community composition and function

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Abstract

We determined soil microbial community composition and function in a field experiment in which plant communities of increasing species richness were exposed to factorial elevated CO_2 and nitrogen (N) deposition treatments. Because elevated CO_2 and N deposition increased plant productivity to a greater extent in more diverse plant assemblages, it is plausible that heterotrophic microbial communities would experience greater substrate availability, potentially increasing microbial activity, and accelerating soil carbon (C) and N cycling. We, therefore, hypothesized that the response of microbial communities to elevated CO_2 and N deposition is contingent on the species richness of plant communities. Microbial community composition was determined by phospholipid fatty acid analysis, and function was measured using the activity of key extracellular enzymes involved in litter decomposition. Higher plant species richness, as a main effect, fostered greater microbial biomass, cellulolytic and chitinolytic capacity, as well as the abundance of saprophytic and arbuscular mycorrhizal (AM) fungi. Moreover, the effect of plant species richness on microbial communities was significantly modified by elevated CO_2 and N deposition. For instance, microbial biomass and fungal abundance increased with greater species richness, but only under combinations of elevated CO_2 and ambient N, or ambient CO_2 and N deposition. Cellobiohydrolase activity increased with higher plant species richness, and this trend was amplified by elevated CO_2. In most cases, the effect of plant species richness remained significant even after accounting for the influence of plant biomass. Taken together, our results demonstrate that plant species richness can directly regulate microbial activity and community composition, and that plant species richness is a significant determinant of microbial response to elevated CO_2 and N deposition. The strong positive effect of plant species richness on cellulolytic capacity and microbial biomass indicate that the rates of soil C cycling may decline with decreasing plant species richness.

Keywords: complementary resource use, extracellular enzymes, FACE (free-air carbon dioxide enrichment), global change, grassland ecosystem, microbial biomass, phospholipid fatty acid (PLFA), plant diversity, soil C cycling, soil fungi

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Introduction

Human activity has altered the global biogeochemical cycling of carbon (C) and nitrogen (N) by increasing atmospheric CO_2, accelerating rates of atmospheric N deposition, and decreasing plant diversity (Vitousek, 1994; Chapin *et al*., 2000). Owing to fossil fuel combustion, atmospheric CO_2 has risen $100\,\mu L\,L^{-1}$ since the mid 1800s (Neftel *et al*., 1985), and atmospheric N deposition has increased 10-fold in eastern North America (Galloway *et al*., 1995). In addition, human activity has increased the rate of plant extinction by 100–1000 times relative to the rates before the appear-

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ance of *Homo sapiens* (Pimm *et al*., 1995). Although individual effects of elevated CO_2, increased N deposition, and declining plant diversity on ecosystem function have received substantial attention (Norby, 1998; Körner, 2000; Naeem, 2002), we have an incomplete understanding of the combined impacts of these global change factors on ecosystem function (Reich *et al*., 2001a). Moreover, the main focus of many studies has been on primary producers (Navas *et al*., 1999; He *et al*., 2002; Shaw *et al*., 2002; Zavaleta *et al*., 2003), and we are only beginning to understand the response of higher trophic levels, such as soil microbial communities, to co-occurring human-induced changes (Horz *et al*., 2004, 2005; Henry *et al*., 2005).

Reductions in biological diversity may negatively affect ecosystem function (Pimm *et al*., 1995; Matson *et al*., 1997; Chapin *et al*., 2000). Grassland studies in North America and Europe have demonstrated that plant productivity increases with greater plant species richness, implying that plant species richness may be positively related to ecosystem function (Tilman *et al*., 1996, 2001; Hector *et al*., 1999). However, little is known about the relationship between plant species richness and soil microbial communities that mediate nutrient cycling (Loreau *et al*., 2001; Catovsky *et al*., 2002; Zak *et al*., 2003).

In combination, increased atmospheric CO_2 and N deposition also could alter ecosystem function (Melillo *et al*., 1993; Vitousek, 1994). Plants grown under elevated CO_2 have higher productivity, greater root exudation, and lower tissue N concentration (Norby *et al*., 2001; Nowak *et al*., 2004; Philips *et al*., 2006). On the other hand, plants exposed to high levels of atmospheric N deposition increase both production and tissue N concentration (Falkengren-Grerup, 1998; Fenn *et al*., 1998). N deposition can also directly change soil C-cycling rates by inhibiting the microbial production of ligninolytic enzymes and enhancing cellulolytic enzyme activity (Carreiro *et al*., 2000). Altogether, CO_2 enrichment and N deposition can alter the rates of heterotrophic microbial metabolism in soil, and consequently the flow of C and N through soil food webs.

Evidence suggests that the response of plant productivity to atmospheric CO_2 and soil N availability depend on plant species richness (Niklaus *et al*., 2001a; Reich *et al*., 2001a, 2004; He *et al*., 2002), and this effect may be propagated to heterotrophic soil microorganisms via plant litter production. For example, Niklaus *et al*. (2001a) found that plant production increased as plant diversity increased, and elevated CO_2 enhanced this response. Others have observed a similar response to elevated CO_2 and high nutrient (N, P, and K) availability (He *et al*., 2002). These observations suggest that the relationship between plant production and plant species richness can be modified by CO_2 and soil N availability, potentially altering the availability of organic substrates in plant litter which structure soil microbial communities and control the rates of soil C and N cycling.

We investigated the response of microbial community composition and function to decreasing plant species richness, elevated CO_2, and increased N deposition at the BioCON (Biodiversity, CO_2, and N) experiment in east-central Minnesota, USA. In this experiment, plant production increased with plant species richness, an effect that was amplified by CO_2 enrichment and N deposition (Reich *et al*., 2001a and unpublished data). Because plant productivity responded more to elevated CO_2 and N deposition in species-rich than species-poor plant communities (Reich *et al*., 2001a), we hypothesized that more diverse plant assemblages will provide heterotrophic soil microbial communities with an enhanced supply of organic substrates, especially under the elevated CO_2 and N treatments. It is plausible that such a response could alter the composition and increase the biomass and activity of soil microbial communities. We also hypothesized that microbial biomass and extracellular enzyme activity, a measure of metabolic capability, will increase with plant species richness due to greater substrate availability induced by higher rates of plant litter production. Our predictions are different from those of Loreau (2001), who suggested that higher diversity of plant organic compounds will reduce nutrient-cycling efficiency, and will have negative, or no effect on ecosystem processes mediated by soil microorganisms.

Material and methods

Experimental design and soil sampling

Our study was conducted at the BioCON experiment in Cedar Creek Natural History Area in east-central Minnesota, USA (Reich *et al*., 2001a, b). A split-plot design was employed with CO_2 treatment as the whole-plot, and plant species richness and N treatments as the split plots. The experiment was established in previously mid-secondary successional grassland on sandy soil. To destroy the seedbank, plots were tilled and methyl bromide was applied. The soils were then inoculated with unfumigated soil from the surrounding old field. The experiment consists of six 20-m diameter free-air carbon dioxide enrichment (FACE) rings. In each ambient and elevated FACE ring, there are sixty-one 2-m × 2-m plots. Elevated CO_2 was applied at $560\,\mu L\,L^{-1}$, which is $\sim 200\,\mu L\,L^{-1}$ above the ambient concentration. Plants are exposed to elevated CO_2 during daylight hours from spring (early April) to fall (late October to mid-November), and 1-min

averages were within 10% of the target concentration 94% of the time in 1998 and 95% of the time in 1999 (Reich *et al.*, 2001b). There was little variation in the mean concentration of elevated CO_2 among plant species richness treatments (one species = $560 \pm 10\,\mu L\,L^{-1}$; four species = $561 \pm 10\,\mu L\,L^{-1}$; nine species = $561 \pm 10\,\mu L\,L^{-1}$; sixteen species = $559 \pm 10\,\mu L\,L^{-1}$) or between ambient N and N-amended plots (560 vs. $561\,\mu L\,L^{-1}$, respectively; Reich *et al.*, 2001a).

The main BioCON experiment ($n = 296$ plots) was a random-assembly, full factorial of species richness, CO_2 and N, including ample replication of the one ($n = 32$), four ($n = 15$), nine ($n = 15$), and 16 ($n = 12$) species treatments at each unique combination of CO_2 and N. In 1997, the $2\,m \times 2\,m$ plots were planted with 1, 4, 9, or 16 perennial grassland species with species for each plot randomly chosen from a pool of 16 locally occurring plant species. In 1999, the average number of plant species in the treatments was one, four, eight, and fourteen (Reich *et al.*, 2001a) with comparable number through 2003. The 16 species used in this experiment were native or have naturalized in the Cedar Creek Natural History Area; they include four C4 grasses (*Andropogon gerardii*, *Boutela gracilis*, *Schizachyrium scoparium*, and *Sorghastrum nutans*), four C3 grasses (*Agropyron repens*, *Bromus inermis*, *Koeleria cristata*, and *Poa pratensis*), four legumes (*Amorpha canescens*, *Lespedeza capitata*, *Lupinus perennis*, and *Petalostemum villosum*), and four forbs (*Achillea millefolium*, *Anemone cylindrica*, *Asclepias tuberosa*, and *Solidago rigida*). Plots were regularly weeded to remove unwanted species. Half of the randomly selected plots received $4\,g\,N\,m^{-2}\,yr^{-1}$, applied as NH_4NO_3 in equal amounts on three dates during each growing season. All plots were burned in the spring of 2000, 2002, and 2003.

On a single day in July 2003, we collected soil samples from 184 of the 296 plots. There were 64 replicates for the one-species treatment, and 40 replicates each of the four-, nine-, and sixteen-species treatments. For the one-species treatment, we collected soil samples from 16 monocultures that were exposed to each CO_2-N treatment combination. For the four-, nine-, and sixteen-species treatments, plots were randomly chosen from a pool of four-, nine-, and sixteen-species plots that were exposed to all CO_2 by N treatment combinations, and there were 10 replicates for each. Six soil cores, 2 cm in diameter and 0–15 cm in soil depth, were randomly collected from each 2-m × 2-m plot. Cores were composited by plot, immediately frozen, and stored at $-80\,^{\circ}C$. Soil subsamples were thawed, ground, and analyzed for C and N content using a Flash EA 1112 (ThermoQuest, Austin, TX, USA). Averaged across all samples, soil C was $6.37 \pm 0.50\,mg\,C\,g^{-1}$ and N was $0.53 \pm 0.01\,mg\,N\,g^{-1}$ ($n = 161$).

Microbial community composition

Phospholipid fatty acid (PLFA) analysis was used to gain insight into microbial community composition. Microbial lipids were extracted from 5 g of freeze-dried soil with a solvent system that included methanol, choloroform, and a phosphate buffer (Guckert *et al.*, 1985). Total extracted lipids collected in the organic phase were fractionated into neutral, glyco-, and polar lipids with chloroform, acetone, and methanol using silicic acid chromatography (Gehron & White, 1983). Polar lipids were methylated to form fatty acid methyl esters (FAME) by subjecting them to 0.2 M methanolic KOH (White *et al.*, 1979). The resulting FAMEs were analyzed by a Finnigan Delta plus isotope ratio mass spectrometer with a GC/C III interface (ThermoElectron, Austin, TX, USA) connected to a HP 5973 GC (Agilent Technologies, Palo Alto, CA, USA). The recovery of FAMEs was calculated based on the amount of an internal standard (21 : 0) added before the analysis and present at the end of the analysis. FAME were identified and quantified based on the retention time and peak area of FAME standards.

Bacterial-specific PLFAs were i15 : 0, a15 : 0, i16 : 0, 16 : 1ω7c, 16 : 1ω9c, 10Me16 : 0, i17 : 0, a17 : 0, cy17 : 0, 17 : 0, 18 : 1ω7c, 18 : 1ω7t, and cy19 : 0a (Frostegård *et al.*, 1993; Pennanen *et al.*, 1998; Grayston *et al.*, 2001). Bacterial biomass and relative abundance were calculated using the sum of all the bacterial PLFAs. Relative abundance of bacteria in the microbial communities was calculated as the percentage of bacterial biomass comprising total microbial biomass. The biomarkers for saprophytic fungi were 18 : 1ω9c and 18 : 2ω6 (Bardgett *et al.*, 1996; Stahl & Klug, 1996). The sum of these two PLFAs was used to estimate fungal biomass and relative abundance. The amount of 16 : 1ω5c can be used to estimate the biomass and abundance of arbuscular mycorrhizal (AM) fungi (Olsson, 1999; Madan *et al.*, 2002; Olsson *et al.*, 2003), but this interpretation requires some caution because this PLFA can also exist in some bacteria (Olsson, 1999). Nevertheless, bacterial 16 : 1ω5c concentration is considerably lower than AM fungi, particularly if the soil organic matter content is low (Olsson, 1999). Because soil in this experiment has low organic matter content, we used 16 : 1ω5c to infer the biomass and relative abundance of AM fungi. PLFAs i14 : 0, 14 : 0, 15 : 0, 16 : 0, 20H-16 : 0, 18 : 3ω3, 18 : 1ω5c, 18 : 0, 22 : 0, and 24 : 0 are common to both bacteria and fungi. Total PLFA was used as an index of living microbial biomass.

Microbial community function

To gain insight into microbial community function, we performed fluorometric assays using methylumbelliferone (MUB)-linked substrates to determine the activities

of 1,4-β-glucosidase, cellobiohydrolase, 1,4,-β-*N*-acetylglucosaminidase, and phosphatase, which are enzymes that mediate key functions during the microbial degradation of litter (Saiya-Cork *et al*., 2002). The enzymes 1,4-β-glucosidase and cellobiohydrolase degrade cellulose. 1,4,-β-*N*-acetylglucosaminidase decomposes chitin; acid phosphatase cleaves phosphoester bonds. Using a colorimetric assay, we also measured the activity of peroxidase and phenol oxidase, enzymes that depolymerize lignin.

Two grams of each composite soil sample were combined with 125 mL of sodium acetate buffer (pH 5.0). The soil slurry was loaded on a 96-well microplate and there were eight analytical replicates of each enzyme assay. For each enzyme assay, we combined 200 μL of soil slurry and 50 μL of substrate specific for each enzyme. All enzyme assays were incubated at 21 °C. The incubation time was 0.5 h for phosphatase and 1,4,-β-*N*-acetylglucosaminidase assays, and 2 h for the 1,4-β-glucosidase and cellobiohydrolase assays. We measured fluorescence using an *f*-Max fluorometer (Molecular Devices Corp., Sunnydale, CA, USA); excitation energy was 355 nm and emission was measured at 460 nm. Enzyme activity was expressed as nmol 4 MUB $g^{-1}\,h^{-1}$.

The activity of phenol oxidase and peroxidase was measured using 25 mM L-3,4-dihydroxy-phenylalanine (L-DOPA) as the substrate (Saiya-Cork *et al*., 2002). Procedures for these colorimetric assays were similar to those of the fluorometric assays described above. Phenol oxidase and peroxidase assays had 16 analytical replicates for each soil sample. The 96-well microplates were incubated for 24 h at 21 °C, and absorbance was measured at 450 nm on EL-800 plate reader (Biotek Instruments Inc., Winooski, VT, USA). Activity was expressed in nmol L-DOPA oxidized $g^{-1}\,h^{-1}$.

Statistical analyses

We analyzed PLFA and enzyme activities using an analysis of variance (ANOVA) for a split-plot design. The fixed effects in this model were ring, plant species richness, CO_2, and N. Carbon dioxide treatment was the whole-plot factor, and the subplot factors were the plant species richness and N treatments. The effect of CO_2 was tested against the random effect of ring nested within CO_2. The main effects of plant species richness and N, as well as interactions among plant species richness, CO_2, and N, were tested against the residual error. After testing the main effects using ANOVA, we performed analysis of covariance (ANCOVA) using total plant biomass to account for the effects of plant production on microbial community composition and function. Significant effects of plant species richness, CO_2, and N treatments and their interactions were accepted at $\alpha = 0.05$. Tukey's honestly significant difference (HSD) test was performed to assess which group means differ from other means within the group ($\alpha < 0.05$).

Results

Microbial biomass and microbial community composition

Total microbial biomass significantly increased with plant species richness (Table 1). For example, microbial biomass in the sixteen-species treatment (89.6 ± 6.3 nmol PLFA g^{-1}) was significantly greater than in the one-species treatment (65.5 ± 4.4 nmol PLFA g^{-1}). Microbial biomass increased with plant species richness in treatments with elevated levels of either CO_2 or N, but not in the treatments with ambient levels of both or elevated levels of both (three-way interaction, $P = 0.05$; Fig. 1a).

Fungal ($P < 0.01$) and AM ($P < 0.01$) relative abundance increased significantly with plant species richness (Figs 1b and 2a, Table 1). Fungal relative abundance generally increased with higher plant species richness in treatments with elevated levels of either CO_2 or N deposition, but not in the treatments with ambient levels of both or elevated levels of both (three-way interaction, $P < 0.01$; Fig. 1b). The relative abundance of

Table 1 Influence of elevated CO_2, N deposition, and plant species richness on microbial biomass and community composition

	Total microbial biomass	Fungal relative abundance	Bacterial relative abundance	AM relative abundance
CO_2	0.89	0.27	0.08	0.46
Nitrogen	0.31	0.59	0.26	0.09
Species richness	**0.01**	**<0.01**	0.63	**<0.01**
CO_2 × species richness	0.63	0.96	0.88	0.91
Nitrogen × species richness	0.99	0.76	0.91	0.83
CO_2 × nitrogen	0.08	0.68	0.58	0.07
CO_2 × nitrogen × species richness	**0.05**	**<0.01**	**0.05**	0.51

P-values for total microbial biomass and community composition analyzed by analysis of variance (ANOVA) are shown. *P*-values equal to or lower than 0.05 are in bold face print.
AM, arbuscular mycorrhizal.

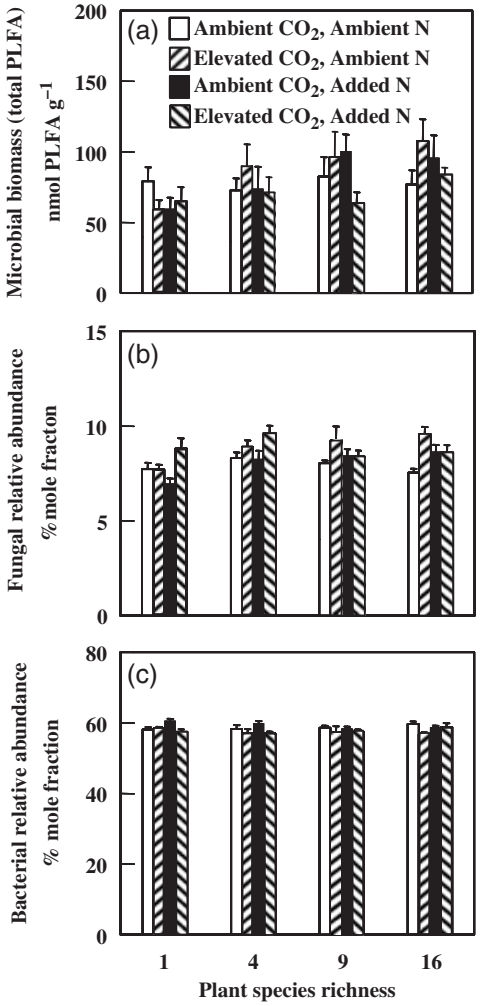

Fig. 1 Influence of plant species richness, elevated CO_2, and N deposition on microbial biomass and community composition. (a) Microbial biomass, (b) fungal abundance, and (c) bacterial abundance. Error bars indicate 1 SE of the mean.

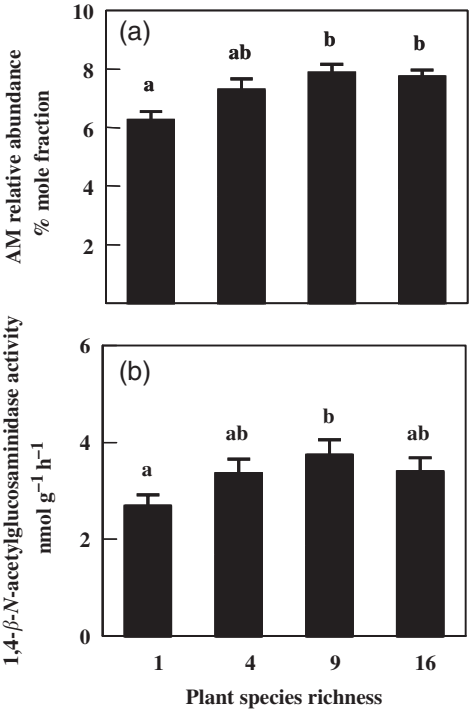

Fig. 2 Main effect of plant species richness on microbial community composition and function. (a) Arbuscular mycorrhizal relative abundance and (b) 1,4-*N*-acetylglucosaminidase activity. For both (a) and (b), values were averaged across CO_2 and N treatments. Error bars indicate 1 SE of the mean. Means with the same letter are not significantly different at $\alpha = 0.05$, as determined by Tukey's honestly significant difference test.

fungal biomarkers 18:1ω9c ($P<0.01$) and 18:2ω6 ($P=0.01$) significantly increased with greater plant species richness (data not shown). For instance, relative abundance of 18:1ω9c was $5.5 \pm 0.1\%$ in the one-species treatment, whereas it was $6.0 \pm 0.1\%$ under the sixteen-species treatment. Similarly, the relative abundance of 18:2ω6 was $2.1 \pm 0.1\%$ in the one-species treatment, and it was $2.5 \pm 0.2\%$ in the sixteen-species treatment.

Plant species richness had significant interactive effects with both CO_2 and N on bacterial relative abundance ($P=0.05$), but there was no clearly interpretable trend related to this three-way interaction (Fig. 1c). At the individual PLFA level, the relative abundance of bacterial biomarkers 16:1ω7c ($P<0.01$), cy17:0 ($P<0.01$), and 18:1ω7c ($P<0.01$) increased with plant species richness, whereas the relative abundance of i15:0 ($P<0.01$), a15:0 ($P=0.02$), i16:0 ($P<0.01$), and 10Me16:0 ($P<0.01$) decreased with increasing plant species richness (data not shown).

To determine the influence of plant production on microbial biomass and community composition, as well as to separate it from treatment effects, we performed analysis of covariance (ANCOVA) using total plant biomass. Total plant biomass was not a significant covariate for total microbial biomass, bacterial, or fungal relative abundance ($P=0.15$–0.98). However, it was a significant covariate for AM relative abundance ($P=0.02$). Accounting for variability attributable to plant biomass, plant species richness no longer had a significant effect ($P=0.20$) on AM relative abundance, whereas N addition significantly decreased AM relative abundance by 9% ($P=0.02$). This indicates that the increase in AM relative abundance with plant species richness was due to higher plant biomass, and that N addition had a direct inhibitory effect on AM fungal abundance.

Microbial degradative potential

Averaged across CO_2 and N deposition treatments, 1,4-*N*-acetylglucosaminidase activity increased with high

species richness (Fig. 2b). Cellobiohydrolase activity also increased with higher plant species richness, and this response was enhanced by CO_2 enrichment (Fig. 3, Table 2). Activity of phenol oxidase was not significantly influenced by any of the treatments or their interaction (Table 2).

β-glucosidase activity was enhanced under higher plant species richness (main effect). N deposition increased β-glucosidase activity by 25% (main effect), but this was only the case for the four- and nine-species treatments (Fig. 4a, Table 2). N deposition increased peroxidase activity under the four- and nine-species treatments, but decreased it under the sixteen-species treatment (Fig. 4b). Phosphatase activity was enhanced by 26% by N deposition ($12.4 \pm 0.9\,nmol\,g^{-1}\,h^{-1}$ under ambient N vs. $15.6 \pm 1.0\,nmol\,g^{-1}\,h^{-1}$ under added N).

We carried out analyses of covariance (ANCOVA) employing total plant biomass as a covariate to account for the influence of plant production on microbial degradative potential. Total plant biomass was a significant covariate for phosphatase ($P = 0.01$), but not for any other extracellular enzyme ($P = 0.16-1.00$). The effect of N addition on phosphatase remained significant even after adjusting for the effect of plant biomass ($P = 0.04$), which indicates that N addition directly enhanced phosphatase activity.

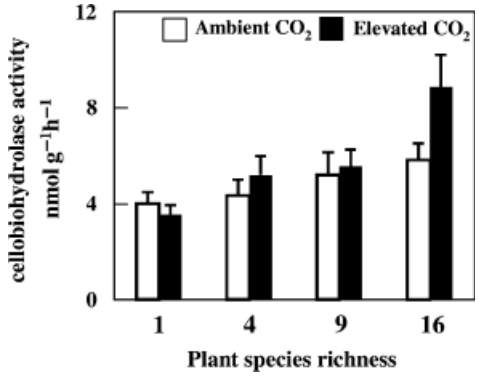

Fig. 3 Interactions between plant species richness and CO_2 enrichment on cellobiohydrolase activity. Enzyme activity was averaged across N treatment. Error bars indicate 1 SE of the mean.

Discussion

Concurrent changes in plant diversity and atmospheric chemistry driven by human activity have the potential to interact and alter nutrient cycling in terrestrial eco-

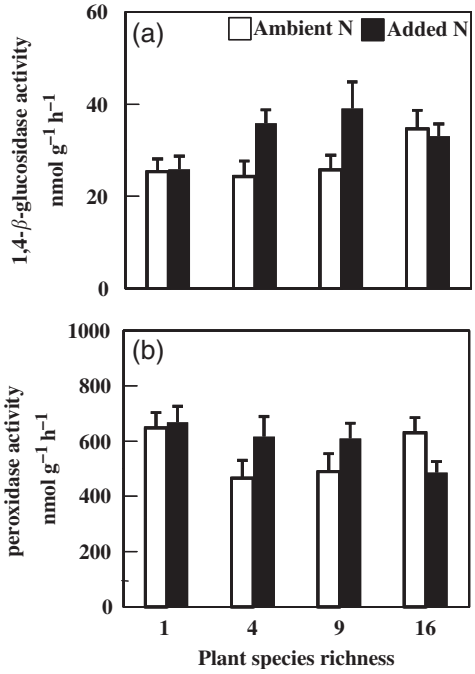

Fig. 4 Interactions between plant species richness and N deposition on β-glucosidase and peroxidase activity. (a) β-glucosidase activity and (b) peroxidase activity. For both (a) and (b), enzyme activity was averaged across CO_2 treatment. Error bars indicate 1 SE of the mean.

Table 2 Influence of elevated CO_2, N addition, and plant species richness on microbial enzyme activity

	1,4-β-glucosidase	Cellobiohydrolase	1,4,-β-*N*-acetyl-glucosaminidase	Phosphatase	Peroxidase	Phenol oxidase
CO_2	0.56	0.35	0.33	0.19	0.62	0.71
Nitrogen	**0.01**	0.89	0.57	**0.01**	0.34	0.30
Species richness	**0.03**	< **0.01**	**0.02**	0.07	0.15	0.98
CO_2 × species richness	0.73	**0.04**	0.65	0.94	0.55	0.26
Nitrogen × species richness	**0.02**	0.47	0.36	0.38	**0.04**	0.68
CO_2 × nitrogen	0.36	0.22	0.12	0.64	0.95	0.87
CO_2 × nitrogen × species richness	0.44	0.31	0.35	0.49	0.67	0.19

P-values for extracellular enzyme activities analyzed by analysis of variance (ANOVA) are shown. *P*-values equal to or lower than 0.05 are in bold face print.

systems (Niklaus *et al.*, 2001b; Shaw *et al.*, 2002). Our results indicate that higher plant species richness can increase microbial biomass, saprophytic and AM fungal abundance, as well as cellulolytic activity in soil microbial communities. Moreover, microbial responses to elevated CO_2 and N deposition were dependent on the species richness of plant communities. Our study demonstrates that increasing plant species richness can exert a strong positive influence on microbial communities and can regulate changes in microbial community composition and function under CO_2 enrichment and N deposition.

Plant species richness had a significant influence on microbial biomass even after accounting for variation in total plant biomass, indicating that higher plant species richness *per se* can increase microbial biomass. Spehn *et al.* (2000) also found greater microbial biomass in species-rich plant communities, even when accounting for variation in plant production among species-richness treatments. Plant species in the BioCON experiment each have unique phenology, physiology, and morphology (Lee *et al.*, 2001; Reich *et al.*, 2001b; Craine *et al.*, 2003a, b), and plant communities of higher diversity can utilize space more completely due to interspecific differences in resource acquisition than plant communities of lower diversity (Tilman, 1999). For instance, there was up to a 40-fold difference in fine root biomass among individual plant species in this experiment, and plants differed significantly in their vertical distribution of fine roots as well (Craine *et al.*, 2003b). Owing to the complementary occupation of soil by roots in diverse plant communities, substrate can be supplied to soil microorganisms more homogeneously in space in species-rich plant communities. Moreover, differences in phenology among plant species in this experiment led to lower variation in plant production over time in the species-rich experimental communities (Craine *et al.*, 2003a). Relative to species-poor plant communities, this implies that microbial communities can derive energy from plants more consistently in time and space in diverse plant communities.

Greater plant species richness increased fungal relative abundance, and these results are consistent with other studies (Smith *et al.*, 2003; Zak *et al.*, 2003). Microbial C : N significantly increased from one to four species treatment in this experiment (Dijkstra *et al.*, 2005), and this could also indicate greater fungal relative abundance with higher plant species richness, because fungi have higher C : N ratio than bacteria (Paul & Clark, 1996). In a neighboring grassland diversity study, an increase in fungal relative abundance with plant species richness could not be explained by differences in soil pH or water potential among plant species richness treatments, which indicates that plant species richness can enhance fungal relative abundance (Zak *et al.*, 2003). Additionally, Smith *et al.* (2003) have found that cessation of NPK fertilizer use and seed addition increased plant species richness in a grassland ecosystem, a response driven by an increase in legumes and stress-tolerant plants. In that experiment, fungal relative abundance also increased with greater plant species richness (Smith *et al.*, 2003). Other studies have found that a shift from high fertilizer input to unfertilized management can increase plant diversity, and this change in vegetation was accompanied by higher fungal relative abundance (Bardgett *et al.*, 1996, 1999).

Elevated CO_2 and N deposition had differential effects on microbial biomass and fungal abundance depending on plant species richness, and this can be due to distinctive microbial community composition under each plant species-richness treatments. If plant communities of different species-richness level harbor microbial communities that are distinct in species composition (Kowalchuk *et al.*, 2002; Garbeva *et al.*, 2006), differential response of microorganisms to elevated CO_2 and N deposition can produce the results we observed. To ascertain this, further studies using molecular tools that can detect shifts in composition of microbial species will be required.

An increase in cellulolytic potential with greater plant species richness could result from a greater availability of cellulose, the result of high rates of plant productivity. In a neighboring plant diversity study, cellulose content increased in species-rich plant communities (M. P. Waldrop, unpublished data). Because the plant species studied in the BioCON experiment and the neighboring diversity experiment were similar, we expect the cellulose availability in our study to increase with plant species richness as well. The production of cellulose-degrading enzymes is induced by the amount of substrate available (Lynd *et al.*, 2002), so greater cellulose availability in species-rich plant communities likely elicited the observed increases in 1,4-β-glucosidase and cellobiohydrolase activity. Amplification of the effect of plant species richness on cellobiohydrolase by CO_2 enrichment may also result from higher cellulose availability due to the further enhancement of plant productivity by elevated CO_2. Greater fungal relative abundance in species-rich plant communities may account for greater cellulolytic potential because heterotrophic fungi can be dominant cellulase producers in soil (Lynd *et al.*, 2002).

Higher *N*-acetylglucosaminidase activity in species-rich experimental plant communities is likely due to an increase in fungal abundance. Miller *et al.* (1998) have documented a significant positive relationship between *N*-acetylglucosaminidase activity and fungal biomass. In their study, *N*-acetylglucosaminidase was constitu-

tively produced by a diverse group of fungi, but not by any of the bacterial species. Therefore, greater *N*-acetylglucosaminidase activity is indicative of a larger active fungal population in species-rich plant communities, a response that can lead to greater rates of chitin degradation.

AM fungi are the dominant plant symbionts in this experiment (Wolf *et al*., 2003), and higher root biomass in species-rich plant communities can account for the increase in AM abundance we observed. Wolf *et al*. (2003) also found higher AM spore abundance and spore biovolume under the 16-species treatment than under the one-species treatment. The effect of plant species richness on AM relative abundance was no longer significant when the effect of total plant biomass was taken into account, which supports our argument that greater plant biomass leads to higher AM abundance. Zak *et al*. (2003) also found a greater relative abundance of 16 : 1ω5c with higher plant species richness; total plant N content ($g\,N\,m^{-2}$) also increased with plant species richness. In combination, these observations suggest that higher AM abundance may facilitate N acquisition in species-rich plant communities.

The lack of a CO_2 main effect on soil microbial communities can be attributed to a small increase in plant production under elevated CO_2. Elevated CO_2 did not alter other belowground properties in this experiment either; microbial C or N, organic matter decomposition rates, or N mineralization were unaffected by CO_2 enrichment (Reich *et al*., 2001a; Dijkstra *et al*., 2005, 2006). Photosynthesis of plants in the BioCON experiment was stimulated to a smaller degree than plants of other elevated CO_2 studies (Lee *et al*., 2001; Reich *et al*., 2001b), and photosynthesis significantly acclimated to CO_2 enrichment in all of the plant functional groups (Lee *et al*., 2001). Therefore, a limited increase in plant production by CO_2 enrichment can explain the lack of response by soil microbial communities in some regards.

Interactive effects of plant species richness and N deposition on β-glucosidase and peroxidase activity may result from a change in litter biochemistry with increasing plant species richness, especially due to the greater inclusion of different plant functional groups. Ammonium nitrate addition can enhance cellulose degradation of labile plant litter, whereas it can repress lignin decomposition of plant litter with high lignin : N ratio (Carreiro *et al*., 2000). This results from high inorganic N concentrations repressing lignin-degrading enzyme synthesis by white-rot fungi, a few bacteria, and actinomycetes (Keyser *et al*., 1978; Kirk & Farrell, 1987). Whole plant N concentration ($mg\,N\,g^{-1}$) decreased by 26% when plant species richness increased from one to sixteen species in this experiment (Reich *et al*., 2001a), and this suggests that N deposition differentially affected cellulose and lignin degradation as plant litter biochemistry changed with greater plant species richness. Whether the repression of peroxidase activity was due to decrease in the abundance of lignin-degrading microorganisms remains to be determined by employing molecular methods that can detect changes in this functional group.

Greater phosphatase activity is likely due to a higher microbial demand for P in N-amended soil. Johnson *et al*. (1998) found that phosphomonoesterase activity and utilization of organic P increased in response to N addition; similar observations have been made in N-limited soils in Hawaii (Olander & Vitousek, 2000). When the influence of total plant biomass was accounted for, a negative effect of N addition on AM abundance was apparent, which indicates that experimental N deposition affects microbial communities directly rather than by an indirect effect mediated via plant litter.

In summary, we have found that plant species richness has a strong positive influence on microbial biomass and potential rates of litter degradation. Higher plant species richness enhanced microbial biomass, fungal abundance, and AM abundance, and this change in microbial community composition was accompanied by a greater cellulolytic and chitinolytic activity. Except for the increase in AM abundance, changes in microbial communities with higher plant species richness could not be explained by increase in plant production. Therefore, it is likely that greater plant species richness influenced microbial communities through resource provision at a more temporally and spatially consistent level, or by alteration of plant litter quality. Although exact mechanism needs to be further elucidated, microbial responses to elevated CO_2 and N deposition were dependent on plant species richness. Taken together, our results demonstrate that plant species richness is a significant determinant of microbial community composition and function. The strong positive effect of plant species richness on cellulolytic capacity and microbial biomass indicate that soil C-cycling rate may decrease with declining plant species richness.

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