Biogeochemistry (2009) 94:233–242
DOI 10.1007/s10533-009-9321-0

Microbial assimilation of new photosynthate is altered by plant species richness and nitrogen deposition

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Received: 27 November 2008 / Accepted: 31 March 2009 / Published online: 16 April 2009

Abstract To determine how plant species richness impacts microbial assimilation of new photosynthate, and how this may be modified by atmospheric N deposition, we analyzed the microbial assimilation of recent photosynthate in a 6-year-long field experiment in which plant species richness, atmospheric N deposition, and atmospheric CO_2 concentration were manipulated in concert. The depleted $\delta^{13}C$ of fumigation CO_2 enabled us to investigate the effect of plant species richness and atmospheric N deposition on the metabolism of soil microbial communities in the elevated CO_2 treatment. To accomplish this, we determined the $\delta^{13}C$ of bacterial, actinobacterial, and fungal phospholipid fatty acids (PLFAs). In the elevated CO_2 conditions of this study, the $\delta^{13}C$ of bacterial PLFAs (i15:0, i16:0, 16:1ω7c, 16:1ω9c, 10Me16:0, and 10Me18:0) and the fungal PLFA 18:1ω9c was significantly lower in species-rich plant communities than in species-poor plant communities, indicating that microbial incorporation of new C increased with plant species richness. Despite an increase in plant production, total PLFA decreased under N deposition. Moreover, N deposition also decreased fungal relative abundance in species-rich plant communities. In our study, plant species richness directly increased microbial incorporation of new photosynthate, providing a mechanistic link between greater plant detritus production in species-rich plant communities and larger and more active soil microbial community.

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Keywords Atmospheric N deposition · ^{13}C · Grassland ecosystems · Phospholipid fatty acid (PLFA) · Plant species richness · Soil microorganisms

Abbreviations

ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BioCON	Biodiversity, CO_2, and nitrogen
FACE	Free air carbon dioxide enrichment
FAME	Fatty acid methyl ester
PLFA	Phospholipid fatty acid
SOM	Soil organic matter

Introduction

Declines in plant diversity can negatively influence aboveground ecosystem processes, as evidenced by lower plant production and resistance to invasion in species-poor plant communities (Hooper and Vitousek 1998; Hector et al. 1999; Tilman et al. 2001; Kennedy et al. 2002). Human activities have decreased plant diversity at an unprecedented rate and scale, which has the potential to alter ecosystem services provided by plants (Hooper et al. 2005). Concurrent with declining plant diversity, atmospheric N deposition has increased tenfold over the past century in eastern North America (Galloway et al. 1995). Nitrogen deposition can significantly alter ecosystem function because insufficient N availability can limit the productivity of terrestrial ecosystems in temperate regions (Vitousek and Howarth 1991). Increasingly more ecosystems are simultaneously experiencing loss of plant species and higher levels of atmospheric N deposition (Sala et al. 2000; Matson et al. 2002), so it is important to understand the combined as well as the individual impacts of decreasing plant species richness and atmospheric N deposition on soil C and N cycling.

Plant diversity can enhance ecosystem function by increasing belowground detritus production, which can increase microbial activity and rates of soil C and N cycling (Spehn et al. 2000; Zak et al. 2003; Chung et al. 2007). Species-rich plant communities likely elicit such an effect by providing a more consistent supply of organic substrates at a variety of temporal and spatial scales due to interspecific differences in phenology, root structure, and rooting depth (Tilman et al. 2001; Craine et al. 2003a, b). Although greater plant species richness can increase microbial biomass (Spehn et al. 2000; Zak et al. 2003; Chung et al. 2007) and support higher rates of N mineralization (Zak et al. 2003; West et al. 2006), few studies have examined the mechanisms responsible for these responses.

Atmospheric N deposition is a wide-spread agent of global change, and it can alter the rates of soil C cycling by modifying substrate availability for microbial metabolism or by exerting a direct negative effect on microbial activity (Johnson et al. 1998; Carreiro et al. 2000). For example, N deposition can indirectly influence microbial activity by enhancing plant production and detrital inputs to soil (Johnson et al. 1998). Moreover, N deposition can hasten the decomposition of plant litter with low lignin: N ratio and inhibit the decomposition of plant litter with high lignin: N ratio (Fog 1988; Carreiro et al. 2000). In combination, atmospheric N deposition and reductions in plant species richness can alter the flow of C through soil food webs by changing organic substrate availability for heterotrophic soil microorganisms or by inhibiting the metabolic activity of particular microbial guilds (i.e., ligninolytic soil fungi, *sensu* Fog 1988).

The objective of our study was to determine how plant species richness impacts microbial assimilation of new photosynthate (i.e. the photosynthate produced after the initiation of the experiment) and how this may be modified by atmospheric N deposition. The BioCON (Biodiversity, CO_2, and Nitrogen) experiment, located in Minnesota, USA, is designed to study the interactive effects of plant species richness, elevated CO_2, and N deposition on temperate grassland ecosystem function (Reich et al. 2001a). In this experiment, species-rich plant communities composed of physiologically and phenologically different species are more productive than species-poor plant communities due to a greater capacity to capture resources (Reich et al. 2001b, 2004; Craine et al. 2003b). Due to ^{13}C-depleted CO_2 fumigation gas, plants under elevated CO_2 have significantly lower $\delta^{13}C$ ($-37.2 \pm 0.0‰$) than those under ambient CO_2 ($-23.0 \pm 0.1‰$) (Dijkstra et al. 2004). The ^{13}C-depleted feature of the new photosynthate is propagated to the soil food web upon release via aboveground litterfall and rhizodeposition; therefore, heterotrophic microbial communities that metabolize recent photosynthate will attain the depleted isotopic signature (Niklaus et al. 2001). This attribute allowed us to determine the extent to which plant species richness and atmospheric N deposition influence the ability of soil microorganisms to metabolize recent photosynthate versus older forms of soil organic matter (i.e. soil organic matter that existed before the initiation of the experiment) (Burke et al. 2003; Paterson et al. 2008). Our isotopic technique measures 'recent photosynthate' as any carbon fixed by the plants after the start of the experiment; thus the recent photosynthate could be up to 6 years old. Previously, we have demonstrated that plant species richness can directly increase microbial biomass, fungal abundance, and cellulolytic metabolism, and

also demonstrated that plant species richness is a significant determinant of microbial response to N deposition (Chung et al. 2007). Here, we trace the fate of recent photosynthate in the elevated CO_2 treatment into soil bacteria, actinobacteria, and fungi by analyzing the $\delta^{13}C$ of the phospholipid fatty acids (PLFAs) composing their cell membranes, thereby enabling us to further explore the effects of plant species richness and anthropogenic N deposition on microbial metabolism.

Methods

Experimental design and sampling procedures

Our study was implemented at the BioCON experiment located at the Long Term Ecological Research site in the Cedar Creek Ecosystem Science Reserve (formerly Cedar Creek Natural History Area), Minnesota, USA. The experimental site is on a sandy glacial outwash plain and the soil type is Typic Udipsamments (Grigal et al. 1974). The experiment was organized in a split-plot design in which CO_2 treatment was the whole-plot, and plant species richness and N deposition were the split-plot factors (for more details see Reich et al. 2001a, b). There were a total of six 20-m-diameter FACE (free air carbon dioxide enrichment) rings, and ≈sixty-one 2-m × 2-m plots within each ring. Three experimental rings received elevated CO_2 treatment at 560 μl/l, a concentration likely to be reached at the end of this century (Intergovernmental Panel on Climate Change 2001). The other three rings were exposed to ambient levels of CO_2. The $\delta^{13}C$ of the elevated CO_2 gas mixture was −19.5‰, and it was −8.3‰ for the ambient atmosphere (D. Ellsworth, unpublished data).

In 1997, each 2-m × 2-m plot was planted with 1, 4, 9, or 16 plant species randomly selected from a pool of 16 perennial grassland species. Plots in the 1-, 4-, 9-, and 16-species treatments had ≈1, 4, 8, and 14 species per plot in 2003, based on the two aboveground harvests per year (P. Reich, unpublished data). The plant species were native or have naturalized to the Cedar Creek area. There were four C4 grasses (*Andropogon gerardii, Boutela gracilis, Schizachyrium scoparium, Sorghastrum nutans*), four C3 grasses (*Agropyron repens, Bromus inermis, Koeleria cristata, Poa pratensis*), four legumes (*Amorpha canescens, Lespedeza capitata, Lupinus perennis, Petalostemum villosum*), and four forbs (*Achillea millefolium, Anemone cylindrica, Asclepias tuberosa, Solidago rigida*). Plant species richness treatments were maintained by regularly weeding unwanted species. Plots at each plant species richness level were exposed to factorial CO_2 and N treatments. To simulate increased atmospheric N deposition, NH_4NO_3 pellets were applied at the rate of 4 g N m^{-2} y^{-1}, with equal amounts applied on 3 dates during each growing season. Maximum rates of atmospheric N deposition anticipated to occur during this century over portions of North America and Europe (Galloway et al. 2004) are equivalent to the amount of N delivered in our N deposition treatment.

In July 2003, we collected six soil cores that were 2 cm in diameter and 15 cm in depth from each plot. Cores were composited by plot, immediately frozen, and stored at −80°C prior to PLFA analysis. For the work reported here, we used soil samples from the elevated CO_2 treatment to take advantage of the depleted ^{13}C tracer, which are a subset of soil samples used in our previous study of soil microbial communities in BioCON experiment (Chung et al. 2007). In the elevated CO_2 treatment, we collected soil samples under 1- ($n = 32$), 4- ($n = 20$), 9- ($n = 20$), and 16-species ($n = 20$) treatments. Half of the plots from each species richness level received experimental N deposition. Average concentration of soil organic C in the experimental plots is 6.28 g C kg^{-1} soil and that of soil N is 0.52 g N kg^{-1} soil. Although the $\delta^{13}C$ of C3 and C4 plants varies (Wedin et al. 1995), we included plots containing both C3 and C4 plants in our analyses, because the proportion of C3 biomass was not significantly different among the plant species richness treatments (70% in 1-species, 69% and 4-species, 68% in 9-species, and 83% in 16-species treatments; $P = 0.66$). Interpretation of the results would differ marginally even if these differences were statistically adjusted for the C3 fraction, especially given that the C3 fractions of 1-, 4-, and 9-species treatments were almost identical. Moreover, the $\delta^{13}C$ of plants from plots included in our study under 1-species treatment (−36.8 ± 0.8‰) and 16 species treatment (−38.4 ± 0.8‰) did not differ significantly ($P = 0.27$). In addition, plants under ambient N (−37.2 ± 0.9‰) and N deposition (−37.2 ± 0.9‰) showed no difference in their $\delta^{13}C$

signature ($P = 0.94$; Dijkstra et al. 2004). The $\delta^{13}C$ of soil C in the bare-ground plots was $-23.6 \pm 0.1‰$ (Dijkstra et al. 2004). Given these consistencies, differences in the $\delta^{13}C$ of microbial PLFAs among treatments should arise from differences in substrate assimilation.

Phospholipid fatty acid analysis

We freeze-dried 5 g of soil from each composite sample, and extracted lipids from the soil using a solvent system composed of methanol, choloroform, and a phosphate buffer (Guckert et al. 1985). Total extracted lipids were separated into neutral, glyco-, and polar lipids with chloroform, acetone, and methanol. We converted polar lipids to fatty acid methyl esters (FAME) by subjecting them to 0.2 M methanolic KOH. The resulting FAMEs were analyzed for their concentration and $\delta^{13}C$ employing a Finnigan Delta plus mass spectrometer with a GC/C III interface (ThermoElectron, Austin, TX, USA) coupled to a HP 5973 GC (Agilent Technologies, Palo Alto, CA, USA). Stable isotope ratios ($\delta^{13}C$) are expressed as ‰ difference in ^{13}C to ^{12}C ratio of the sample relative to Pee Dee Belemnite (PDB) standard: $\delta^{13}C$ (‰) $= [(^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{standard})/(^{13}C/^{12}C_{standard})] \times 10^3$.

Phospholipid fatty acids i15:0, i16:0, 16:1ω7c, 16:1ω9c, 10Me16:0, a17:0, cy17:0, 18:1ω7c, cy19:0a are of bacterial origin (Asselineau 1966), and 10Me18:0 is specific to actinobacteria (Kroppenstedt 1985). Phospholipid fatty acids 18:1ω9c and 18:2ω6 are biomarkers for saprophytic soil fungi (Erwin 1973). We used total PLFA as an index of living microbial biomass. The relative abundance of each PLFA was calculated as the percent of each PLFA composing living microbial biomass (i.e., total PLFA).

Statistical analyses

We analyzed the $\delta^{13}C$ of each PLFA, total microbial biomass, and relative abundance of microbial groups using an ANOVA (analysis of variance) (SAS, Cary, NC, USA) for a split-plot, randomized complete block design. The fixed effects in our model were FACE ring, plant species richness, and N deposition. The split-plots were plant species richness and N treatments. To account for the effects of plant production on microbial characteristics, we also performed ANCOVA (analysis of covariance) using total plant biomass as a covariate. Treatment effects were considered significant at $\alpha = 0.05$, and we performed Tukey's honestly significant difference (HSD) test to determine which group means differ from other means within the group ($P < 0.05$).

Results

Microbial assimilation of new photosynthate

Plant species richness significantly decreased the $\delta^{13}C$ of individual PLFAs (Fig. 1). The $\delta^{13}C$ of bacterial PLFAs (i15:0, i16:0, 16:1ω7c, 16:1ω9c, 10Me16:0, and 10Me18:0) was significantly lower in species-rich plant communities than in species-poor plant communities (Fig. 1). Fungal PLFA 18:1ω9c also had a lower $\delta^{13}C$ in species-rich plant communities (Table 1; Fig. 1). The $\delta^{13}C$ of total microbial PLFA had the tendency to decrease with greater plant species richness, but the results were not statistically significant (Table 1; Fig 1). Although individual microbial PLFAs had $\delta^{13}C$ values that were 0.4–1.3‰ lower under N deposition than under ambient N, the effect of experimental N deposition was not statistically significant (Table 1). This was also the case for the $\delta^{13}C$ of total microbial PLFA, wherein N deposition had no significant effect ($-30.4 \pm 0.5‰$ under ambient N vs. $-31.1 \pm 0.4‰$ under N deposition; $P = 0.35$). Plant species richness and N deposition did not interact to alter $\delta^{13}C$ of any microbial PLFAs (Table 1).

Total PLFA and relative abundance of microbial PLFAs

Total PLFA increased ($P = 0.06$) with plant species richness (63.0 ± 6.8, 83.1 ± 11.0, 81.1 ± 12.7, and 97.1 ± 8.4 nmol PLFA g^{-1} under 1-, 4-, 9-, and 16-species treatments, respectively), paralleling the response of plant biomass. In contrast, total PLFA decreased by 27% under N deposition compared to the total PLFA under ambient N (88.1 ± 8.4 nmol PLFA g^{-1} under ambient N vs. 69.4 ± 5.4 nmol PLFA g^{-1} under N deposition; $P = 0.04$), despite higher plant biomass under enriched N. There was no significant interaction between plant species richness and N deposition on total PLFA (Table 2).

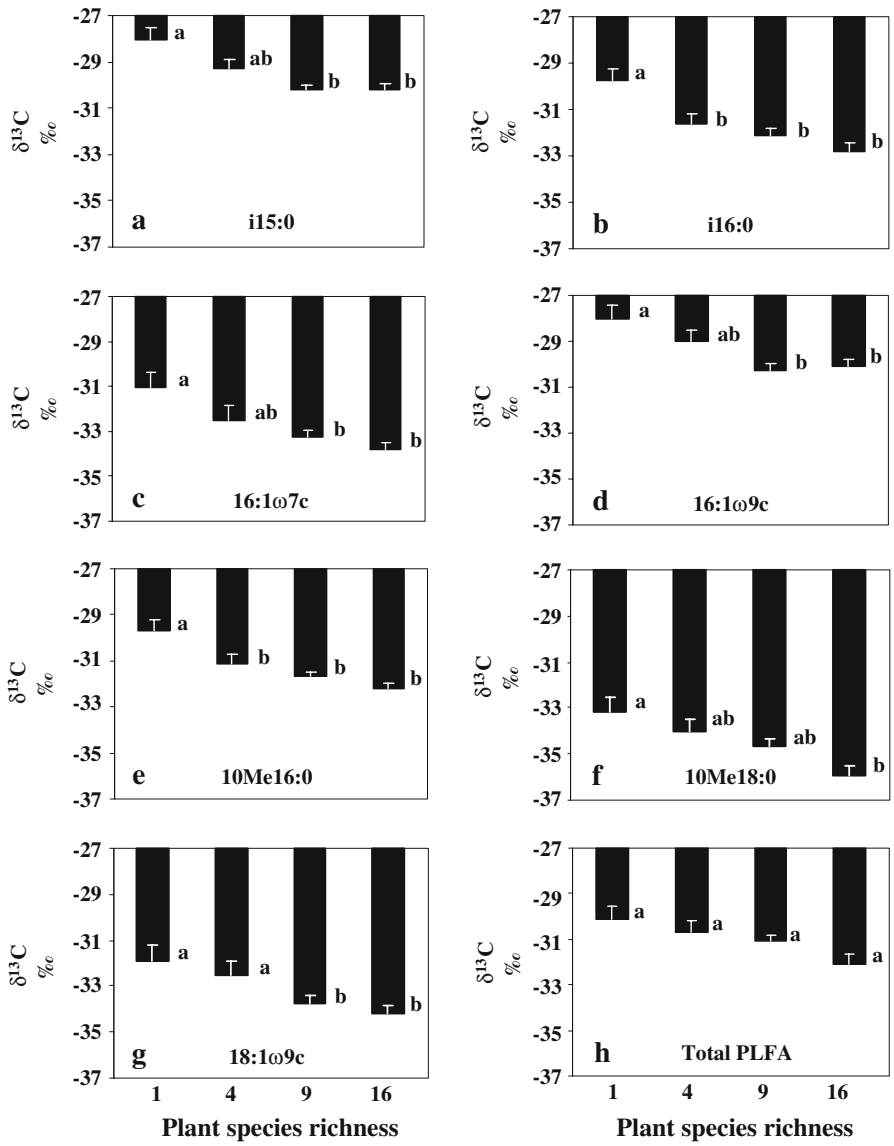

Fig. 1 Main effect of plant species richness treatment on microbial PLFA δ^{13}C. δ^{13}C values were averaged across N treatments. δ^{13}C value of microbial PLFAs **a** i15:0, **b** i16:0, **c** 16:1ω7c, **d** 16:1ω9c, **e** 10Me16:0, **f** 10Me18:0, **g** 18:1ω9c, and **h** total PLFA. *Error bars* indicate one standard error of the mean. Means with the same letter are not significantly different at $\alpha = 0.05$, as determined by Tukey's HSD test

Table 1 The influence of plant species richness and N deposition on microbial PLFA δ^{13}C

	Total PLFA	i15:0	i16:0	16:1 ω7c	16:1 ω9c	10Me 16:0	a17:0	cy 17:0	18:1 ω7c	cy 19:0a	10Me 18:0	18:1 ω9c	18:2 ω6
Species richness	0.16	**<0.01**	**<0.01**	**0.01**	**0.01**	**<0.01**	0.33	0.81	0.13	0.91	**0.02**	**0.05**	0.56
Nitrogen	0.35	0.47	0.32	0.48	0.33	0.33	0.19	0.42	0.37	0.06	0.17	0.34	0.59
Species richness × nitrogen	0.27	0.28	0.47	0.34	0.48	0.27	0.53	0.79	0.23	0.99	0.85	0.24	0.99

P-values for microbial PLFA δ^{13}C were analyzed by analysis of variance (ANOVA)

P-values equal to or lower than 0.05 are in bold face print

Table 2 The influence of plant species richness and N deposition on total PLFA and relative abundance of microbial PLFAs

	Total PLFA	i15:0	i16:0	16:1 ω7c	16:1 ω9c	10Me 16:0	a17:0	cy 17:0	18:1 ω7c	cy 19:0a	10Me 18:0	18:1 ω9c	18:2 ω6
Species richness	0.06	**<0.01**	0.11	**<0.01**	0.19	**<0.01**	0.59	0.06	**<0.01**	0.52	**0.02**	0.09	0.22
Nitrogen	**0.04**	0.94	**<0.01**	0.75	0.80	0.83	0.42	0.57	0.82	0.21	0.21	0.50	0.52
Species richness × nitrogen	0.20	0.41	0.47	0.37	0.50	0.67	0.31	0.98	0.16	0.60	0.53	0.71	**<0.01**

P-values for total PLFA and relative abundance of each microbial PLFA were analyzed by analysis of variance (ANOVA)

P-values equal to or lower than 0.05 are in bold face print

Plant species richness had significant effect on the relative abundance of PLFAs. More specifically, the relative abundance of 16:1ω7c (6.9 ± 0.1, 8.1 ± 0.2, 7.6 ± 0.1, and 8.3 ± 0.2% under 1-, 4-, 9-, and 16-species treatments), 10Me18:0 (1.4 ± 0.0, 1.5 ± 0.0, 1.5 ± 0.1, and 1.6 ± 0.1% under 1-, 4-, 9-, and 16-species treatments), and 18:1ω7c (8.1 ± 0.2, 8.9 ± 0.2, 9.1 ± 0.2, and 9.2 ± 0.2% under 1-, 4-, 9-, and 16-species treatments) increased with plant species richness. On the other hand, the relative abundance of i15:0 (10.4 ± 0.2, 9.4 ± 0.2, 9.5 ± 0.3, and 9.6 ± 0.2% under 1-, 4-, 9-, and 16-species treatments) and 10Me16:0 (6.2 ± 0.2, 5.4 ± 0.1, 5.6 ± 0.2, and 5.4 ± 0.1% under 1-, 4-, 9-, and 16-species treatments) decreased with greater plant species richness.

Although the relative abundance of several PLFAs was generally lowered by N deposition (data not shown), the results were not statistically significant for most of the PLFAs (Table 2); only the relative abundance of i16:0 increased by 6% ($P < 0.01$) under N deposition. Plant species richness and N deposition produced a significant interaction effect on the relative abundance of fungal biomarker 18:2ω6 (Table 2). Under ambient N deposition, the relative abundance of 18:2ω6 increased with greater plant species richness. However, under experimental N deposition, 18:2ω6 relative abundance showed the tendency to decrease in species-rich plant communities (Fig. 2). Total plant biomass was not a significant covariate for total PLFA or relative abundance of any PLFA ($P = 0.17$–0.90).

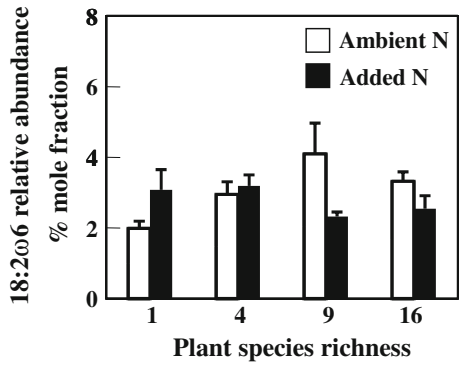

Fig. 2 Interactive effects of plant species richness and N treatments on relative abundance of 18:2ω6. *Error bars* indicate one standard error of the mean

Discussion

The loss of plant species richness and heightened anthropogenic N deposition impact terrestrial ecosystems across broad geographic regions (Sala et al. 2000; Matson et al. 2002; Phoenix et al. 2006). We provide direct evidence for enhanced assimilation of new photosynthate by soil microorganisms in species-rich plant communities. Greater assimilation of recent photosynthate reported here, in combination with complementary observations of higher cellulolytic enzyme activity in species-rich plant communities (Chung et al. 2007), indicate a positive relationship between plant diversity and the rates of C cycling by the soil food web. Lower total PLFA representative of living microbial biomass under experimental N deposition suggests that anthropogenic N deposition could inhibit microbial metabolism of new photosynthate, a contention confirmed here with the use of stable isotope tracing. A reduction in fungal abundance by experimental N deposition in species-rich plant communities indicates that anthropogenic N deposition could eliminate the positive influence of greater plant species richness on soil C cycling.

The decrease in $\delta^{13}C$ of microbial PLFAs in the species-rich plant communities under elevated CO_2 was likely the result of a greater microbial incorporation of new photosynthate that is more available

beneath species-rich plant communities. Plant diversity decreased the $\delta^{13}C$ of PLFAs for a wide range of microbial groups including bacteria, actinobacteria, and fungi, indicating that plant species richness promoted metabolism across different microbial groups. Plant biomass increased with more plant species under elevated CO_2 in this study (Reich et al. 2001a, 2004), which would have led to greater input of ^{13}C-depleted plant detritus to soil. Soil microorganisms under the 16-species treatment tended to have higher proportion of new C than those under the 1-species treatment (data not shown), which also supports our findings. However, this needs to be further confirmed by inclusion of more plots for analysis.

Continuous labeling via ^{13}C-depleted CO_2 results in labeling of the whole plant (de Visser et al. 1997), and this feature allows us to gain a comprehensive view of the flow of C through plants, soil organic matter (SOM), and microorganisms (Paterson et al. 2008). The average $\delta^{13}C$ of total PLFA ($-30.8 \pm 0.5‰$) was similar to that of light fraction ($-31.4 \pm 0.5‰$) (Dijkstra et al. 2004), which is the labile fraction of SOM that mostly consists of partially decomposed plant debris (Gregorich et al. 2006). The plants in plots included in our study had an average $\delta^{13}C$ of $-36.9 \pm 1.0‰$, and $\delta^{13}C$ of the SOM associated with heavy fraction was $-25.3 \pm 0.21‰$ (Dijkstra et al. 2004), which is a recalcitrant SOM pool consisting of organic matter bound to mineral surfaces (Young and Spycher 1979; Strickland and Sollins 1987). Taken together, it is likely that the $\delta^{13}C$ of microbial PLFAs closely reflects the time-integrated average of SOM that existed prior to CO_2 fumigation and C in recent photosynthate.

Higher cellulose-degrading enzyme activity can be a mechanistic link between increased availability of new substrates and a greater microbial metabolism of these substrates in species-rich plant communities under elevated CO_2. In this experiment, species-rich plant communities accumulated more above- and belowground biomass in response to elevated CO_2 than species-poor plant communities (Reich et al. 2001a, b, 2004). Therefore, cellulose availability is undoubtedly higher in species-rich plant communities under elevated CO_2 (Reich et al. 2004). Moreover, cellulose concentration in plant litter increased with plant diversity (Knops et al. 2007), which together could have led to higher cellulolytic enzyme activity in species-rich plant communities (Chung et al. 2007). Increased cellulose-degrading enzyme activity in species-rich plant communities would have made the new photosynthate available for microbial metabolism, a result consistent with the ^{13}C-depletion of microbial PLFAs. In addition, plants under elevated CO_2 can increase root exudation and this can stimulate microbial activity (Pendall et al. 2004; Allard et al. 2006); thus, possible increase in root exudates from greater root biomass in species-rich plant communities under elevated CO_2 could also have promoted microbial metabolism.

Greater total PLFA representative of living biomass in species-rich plant communities under elevated CO_2 is likely due to a higher input of new photosynthate and its subsequent metabolism by soil microorganisms. Microbial biomass has been reported to increase with more plant species in several plant diversity studies (Spehn et al. 2000; Zak et al. 2003), but the mechanism that leads to this observation has not been investigated. This study, in combination with our previous work (Chung et al. 2007), provides evidence that increased cellulolytic enzyme activity, together with higher metabolism of new photosynthate, leads to larger microbial biomass in species-rich plant communities.

The extent to which the greater productivity of species-rich plant communities has increased microbial metabolism was quantified only under elevated CO_2 in this study due to the absence of isotopic tracer in the ambient CO_2 treatment. Nevertheless, we expect that microbial incorporation of recent photosynthate will increase with greater plant species richness under ambient CO_2 as well. In this experiment, species-rich plant communities were more productive than species-poor plant communities and fostered greater microbial cellulolytic potential and biomass regardless of the atmospheric CO_2 concentrations (Reich et al. 2001a, b; Chung et al. 2007), and we reason that a similar mechanism described in our current work could also operate under ambient CO_2. However, this needs to be confirmed with further study.

Reduction of total PLFA under N deposition could be due to the inhibition of microbial activity (Waldrop et al. 2004; Treseder 2008). A meta-analysis on the responses of microbial biomass to N additions in various field studies showed that microbial biomass declined 15% on average under N addition (Treseder

2008), which is consistent with our findings. In our study, total plant biomass and root biomass under elevated CO_2 each increased by 45 and 59% under N deposition in 2003 (Reich et al. unpublished data), which could indicate that experimental N deposition increased the supply of new photosynthate to heterotrophic microbial communities in soil. However, we found no significant effect of N deposition on $\delta^{13}C$ of individual or total microbial PLFAs, which suggests that N deposition could inhibit microbial metabolism of new photosynthate.

Decrease in fungal abundance by experimental N deposition in species-rich plant communities is likely due to differential effect of N deposition on soil fungi associated with a broad range of plant species richness treatments (Chung et al. 2007). Nitrogen deposition can lower soil fungal abundance (Bardgett et al. 1996, 1999; Bossuyt et al. 2001), and also the inhibitory effects of high N availability on specific fungal groups such as white-rot basidiomycetes and mycorrhizae are well documented (Leatham and Kirk 1983; Reddy and D'Souza 1994; Treseder 2004). High inorganic N availability can also lower the activity of ligninolytic enzymes of microbial communities that colonize and metabolize plant litter with high lignin: N ratio (Carreiro et al. 2000; Waldrop et al. 2004). Further analysis of fungal community composition would be required to confirm whether this potential mechanism was operating in our experiment.

In summary, our study provides direct evidence for higher microbial metabolism of recent photosynthate in species-rich plant communities, which likely is mediated by greater cellulolytic activity, which we previously report (Chung et al. 2007). Greater total PLFA in species-rich experimental plant communities appears to result from higher incorporation of new photosynthate by soil microbial communities. The decline in fungal relative abundance elicited by experimental N deposition in species-rich plant communities suggests that N deposition can eliminate the positive effect of greater plant species richness on soil C cycling. In combination, our study demonstrates that plant diversity has the potential to increase soil C cycling by enhancing soil microbial biomass and the metabolism of new photosynthate, and that soil fungi in species-rich plant communities could be negatively affected by future high levels of atmospheric N deposition.

Acknowledgments The BioCON experiment was funded by the Department of Energy (Program for Ecological Research Grant DE-FG02-96ER62291), National Science Foundation (Long Term Ecological Research program: 0080382, Biocomplexity program: 0322057), and by a grant-in-aid from the University of Minnesota. We thank the interns who assisted us with soil sampling at the BioCON site. We also acknowledge Katie Berlin, Jana Gastellum, Michelle Martin, and Dan Rivas for their help with lab analyses at the soils lab in the University of Michigan. Kees-Jan van Groenigen provided valuable comments on the earlier versions of this manuscript.

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