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SOIL NITROGEN CYCLING UNDER ELEVATED CO_2: A SYNTHESIS OF FOREST FACE EXPERIMENTS

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Abstract. The extent to which greater net primary productivity (NPP) will be sustained as the atmospheric CO_2 concentration increases will depend, in part, on the long-term supply of N for plant growth. Over a two-year period, we used common field and laboratory methods to quantify microbial N, gross N mineralization, microbial N immobilization, and specific microbial N immobilization in three free-air CO_2 enrichment experiments (Duke Forest, Oak Ridge, Rhinelander). In these experiments, elevated atmospheric CO_2 has increased the input of above- and belowground litter production, which fuels heterotrophic metabolism in soil. Nonetheless, we found no effect of atmospheric CO_2 concentration on any microbial N cycling pool or process, indicating that greater litter production had not initially altered the microbial supply of N for plant growth. Thus, we have no evidence that changes in plant litter production under elevated CO_2 will initially slow soil N availability and produce a negative feedback on NPP. Understanding the time scale over which greater plant production modifies microbial N demand lies at the heart of our ability to predict long-term changes in soil N availability and hence whether greater NPP will be sustained in a CO_2-enriched atmosphere.

Key words: climate change; elevated CO_2; forest FACE experiments; gross N mineralization; microbial immobilization; soil microorganisms; soil N cycling.

COMMUNICATIONS

INTRODUCTION

A major uncertainty in predicting the response of terrestrial ecosystems to global change is whether the stimulation of plant growth and net primary productivity (NPP) will be sustained as CO_2 accumulates in the Earth's atmosphere. Much of this uncertainty stems from potential interactions between the rising atmospheric CO_2 concentration and other environmental factors, such as soil nitrogen (N) availability, which may constrain greater plant growth in a CO_2-enriched atmosphere (Zak et al. 2000*a*, Oren et al. 2001). Northern temperate forests are globally important sinks for atmospheric CO_2 (Cias et al. 1995), but the degree to which they function as future sinks may depend on the availability of soil N (Oren et al. 2001). Short-term experiments indicate that soil N availability can decrease, increase, or exhibit no significant change beneath temperate trees grown under elevated atmospheric CO_2 (Zak et al. 2000*b*). These observations have divergent implications for C storage in temperate forests and suggest that interactions between C and N cycles will control the long-term response of forests to rising atmospheric CO_2.

Predicting changes in soil N availability under elevated CO_2 requires an understanding of how plant detritus alters heterotrophic microbial metabolism in soil. Constituents of plant litter that fuel microbial growth (i.e., simple carbohydrates and organic acids) stimulate the synthesis of proteins, nucleic acids, and other N-containing biological compounds, thus increasing microbial N uptake and assimilation. In contrast, plant litter containing compounds that provide lower amounts of energy for microbial metabolism (i.e., detritus with high lignin or tannin content) lessen the biosynthetic demand for N and decrease microbial immobilization. Changes in plant litter production under elevated CO_2 could alter microbial N demand and amounts of N available for plant uptake, a response that could potentially feed back to modify plant productivity in a CO_2-enriched atmosphere. We studied the microbial assimilation and release of N in three free-air CO_2 enrichment (FACE) experiments in which forest trees have been growing under ambient and elevated atmospheric CO_2 (Hendrey et al. 1999, Dickson et al. 2000, Norby et al. 2001). In these experiments, elevated atmospheric CO_2 has consistently increased plant growth and litter production (Finzi et al. 2001, King et al. 2001, Norby et al. 2002), and such a response should provide additional substrate for heterotrophic metabolism in soil. Our objectives were to determine (1) how greater substrate availability under elevated CO_2 has altered the biosynthetic N demand of soil microbial communities and (2) whether there is a con-

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TABLE 1. A summary of climate, vegetation, and soil properties in three free-air CO_2 enrichment (FACE) experiments.

Property	Duke	Oak Ridge	Rhinelander
Climate			
Annual temperature (°C)	15.5	14.5	11.5
Annual precipitation (mm)	1140	1150	833
Growing season (d)	200	190	150
Vegetation			
Overstory species	*Pinus taeda*	*Liquidambar styraciflua*	*Populus tremuloides*
Stand establishment†	1983	1988	1997
Initiation of exposure	1996	1998	1997
Soil			
Taxomony	Ustic Hapludalf	Aquic Hapludult	Alfic Haplorthod
Texture	clay loam	silty clay loam	sandy loam
pH	5.0	5.7	5.5
Total organic C (g/kg)	14.4	10.8	15.3
Total N (g/kg)	0.79	1.12	1.20
Soil C:N	18.9	9.0	12.9

Note: Data are summarized from Hendrey et al. (1999), Dickson et al. (2000), and Norby et al. (2001).
† Year plantation was established.

sistent response beneath different tree species. It is plausible that the variable response of soil N cycling to elevated atmospheric CO_2 results, in part, from experiment-to-experiment differences in field sampling, analytical technique, and CO_2 exposure method. Coordinated field sampling, the use of identical laboratory procedures, and a common experimental infrastructure for CO_2 exposure should minimize variability arising from these sources, potentially providing better insight into changes in soil N cycling under elevated CO_2.

METHODS

Study sites

Our research was conducted at FACE experiments located at the Duke Forest, Durham, North Carolina, USA (35°58′ N, 79°5′ W), the Oak Ridge National Environmental Research Park, Oak Ridge, Tennessee, USA (35°54′ N, 84°20′ W), and Rhinelander, Wisconsin, USA (45°40.5′ N, 89°37.5′ W). These experiments were designed using an identical technology to raise the atmospheric CO_2 concentration to that expected during the latter portion of this century (~550 μmol/mol). These experiments differ markedly in climate, vegetation, and soil characteristics (Table 1), but they all contain forest trees ecologically important to each region. Additional details regarding the design and performance of each experiment are reported elsewhere (see Hendrey et al. 1999, Dickson et al. 2000, Norby et al. 2001).

The Duke experiment consists of six 34 m diameter FACE rings that have been placed in an aggrading *Pinus taeda* plantation. Trees in three rings were exposed to the ambient CO_2 concentration, whereas trees in the three remaining rings were exposed to 200 μL/L above ambient CO_2; each treatment occurred in the three blocks. Treatments were initiated in August of 1996, and they have continued to the present. The Oak Ridge experiment was established in 1997 by locating five 25 m diameter FACE rings in a 9-yr-old *Liquidambar styraciflua* plantation and continues to the present. Beginning in April 1998, trees in two rings received elevated CO_2 and those in the remaining rings grew under ambient CO_2. One ambient ring lacked the FACE exposure system and served as an experimental control. For the analysis reported here, the experiment was considered to consist of two blocks each containing an ambient and elevated CO_2 treatment; the ambient ring lacking the FACE system was excluded. Trees in the elevated treatment were exposed to 565 μL/L, identical to the elevated CO_2 treatment at the Duke Forest. The Rhinelander experiment was established in 1997 and continues to the present. It consisted of 12 rings (30-m diameter) in which *Populus tremuloides*, *Betula papyrifera*, and *Acer saccharum* were exposed to factorial CO_2 and O_3 treatments in a randomized block design ($n = 3$). One-half of each FACE ring was planted with *P. tremuloides*, one-quarter contained *P. tremuloides* and *B. papyrifera*, and one-quarter contained *P. tremuloides* and *A. saccharum*. For the current work, we used the half-ring sections containing *P. tremuloides* exposed to control (i.e., ambient CO_2 and O_3) and elevated CO_2 (565 μL/L); no samples were collected in rings receiving O_3.

Microbial N, gross mineralization, and immobilization

Our field sampling took place after plants had been growing for 1–3 yr under the experimental conditions. Midway during the 1999 and 2000 growing seasons, surface soil samples (5 cm diameter, 10 cm deep) were randomly collected from each FACE ring or ring section. Four cores per ring were collected at Duke and eight cores per ring were collected at Oak Ridge and Rhinelander; the four or eight cores collected in each FACE ring were composited in the field. The field-fresh samples were stored on ice and immediately brought

to the laboratory for analysis. Soils from the Oak Ridge and Rhinelander experiments were analyzed at the University of Michigan, Ann Arbor, Michigan, USA, and those collected from the Duke experiment were analyzed at Boston University, Boston, Massachusetts, USA. Fine roots and coarse woody debris were removed, and each composite sample was passed through a 2-mm sieve. Any soil remaining on the roots was returned to the original sample.

We used identical laboratory protocols to ascertain microbial N cycling in the three experiments. Microbial N was determined using a direct extraction procedure (Horwath and Paul 1994). A 15-g subsample from each FACE ring was immediately extracted with 50 mL of 0.5 mol/L K_2SO_4, and a second 15-g subsample was fumigated with $CHCl_3$ for 18 h. Residual $CHCl_3$ was removed by repeated vacuuming (eight times), then 50 mL of 0.5 mol/L K_2SO_4 was added to each fumigated subsample. A 15-mL aliquot of extract from each fumigated and unfumigated soil was pipetted into a glass screwcap vial. Five milliliters of 0.148 mol/L $K_2S_2O_8$ and 0.25 mL 3 mol/L NaOH were added, the vials were sealed with Teflon-lined caps, and placed on a vortex mixer. After autoclaving at 120°C for 1 h and cooling to room temperature, an additional 0.25 mL 3 mol/L NaOH was added, and the solutions were mixed again. Nitrate concentrations in Oak Ridge and Rhinelander samples were analyzed using an OI Analytical Flow Solution 3000 continuous flow analyzer (OI Analytical, College Station, Texas, USA); Duke samples were analyzed using a Lachat QuickChem FIA+ 8000 Series autoanalyzer (Zellweger Analytics, Milwaukee, Wisconsin, USA). We divided the difference in N concentration between fumigated and unfumigated samples by a correction factor ($k_n = 0.54$) to estimate microbial N.

We determined gross rates of soil N transformations using a ^{15}N pool dilution technique (Hart et al. 1994). Four 12-g subsamples were placed in glass vials; two subsamples were enriched with 1 mL of ^{15}N-NH_4Cl solution, and two were enriched with the same volume of ^{15}N-KNO_3 solution. The ^{15}N solutions were prepared using a mixture of ^{15}N-labeled (99.5%) and unlabeled N, which produced target concentrations of N (2–5 μg N/g soil) and ^{15}N (2–4 atom percent excess ^{15}N). Labeling solutions were added by dispensing 1 mL evenly over the soil surface. Within 1 h after isotope addition, one $^{15}NH_4^+$-enriched sample and one $^{15}NO_3^-$-enriched sample from each FACE ring was extracted with 2 mol/L KCl. The remaining two ^{15}N-enriched samples were incubated at 20°C for 2 d, after which we extracted inorganic N with 2 mol/L KCl. Ammonium and NO_3^- concentrations in soil extracts were analyzed as described above.

Ammonium and NO_3^- were sequentially diffused from each KCl extract onto acid traps in preparation for ^{15}N analysis by isotope ratio mass spectrometry. Diffusions were conducted in 120-mL specimen cups, which contained an acidified cellulose disk (10 μL of 2.5 mol/L $KHSO_4$) suspended above the liquid on a stainless steel wire. We added MgO to each solution and collected NH_3 on the acidified cellulose disk. After 6 d, we removed the disk and wire, added Devarda's alloy to convert NO_3^- to NH_4^+; the resulting NH_4^+ was collected on a new acidified cellulose disk. The ^{15}N in the cellulose disks was measured using a Finnigan Delta Plus isotope ratio mass spectrometer with a Conflo II interface (Thermo Finnigan, San Jose, California, USA). Rates of gross N mineralization and gross microbial immobilization were calculated using isotope pool dilution equations (Hart et al. 1994). To gain insight into microbial N demand among experiments and treatments, we calculated the rate of microbial N immobilization per unit of microbial N (i.e., specific microbial immobilization).

Statistical analyses

We used a repeated-measures ANOVA to determine the influence of atmospheric CO_2 concentration on rates of soil N cycling. In our model, experiment ($n = 3$) and CO_2 treatment were fixed effects; blocks were random effects, and they were nested within experiments. This model contained an experiment-by-CO_2 interaction, which allowed us to determine whether microbial N cycling processes responded differently to CO_2 among experiments. All data were assessed for normality and homogeneity of variance prior to ANOVA. We used a Fisher's protected least significant difference (LSD) procedure to compare means among experiments and treatments.

RESULTS

Nitrogen in microbial biomass was not influenced by changes in plant growth under elevated CO_2, and this result was consistent across the three experiments (Table 2). We observed a significant interaction among experiment, CO_2, and time (Table 2); however, across experiments and years, microbial N in the elevated CO_2 treatment varied little relative to the ambient treatment (−3 to 4%). Experiment, atmospheric CO_2, and their interaction had no effect on microbial N (Table 2; Figs. 1A and 2A). Atmospheric CO_2 also had no significant main effect on gross N mineralization, which averaged 1.23 ± 0.267 μg $N \cdot g^{-1} \cdot d^{-1}$ under elevated CO_2 and 1.12 ± 0.220 μg $N \cdot g^{-1} \cdot d^{-1}$ in the ambient treatment. Gross rates of N mineralization differed significantly among experiments, but there was not a significant CO_2 and experiment interaction (Table 2; Fig. 2B). The mean rate at Duke (1.58 ± 0.410 μg $N \cdot g^{-1} \cdot d^{-1}$) was greater than rates measured at Rhinelander (1.11 ± 0.375 μg $N \cdot g^{-1} \cdot d^{-1}$) and Oak Ridge ($0.66 \pm 0.121$ μg $N \cdot g^{-1} \cdot d^{-1}$). Our analysis of microbial immobilization ($NH_4^+ + NO_3^-$) revealed a significant effect of experiment, but we found no evidence that atmospheric CO_2 influenced this process (Table 2; Fig. 1C). We also observed no interaction between experiment and CO_2 on microbial immobilization (Fig. 2C). Averaged across experiment

TABLE 2. A repeated-measures analysis of variance of soil N cycling processes beneath forest trees growing under ambient and elevated atmospheric CO_2.

Source	df	Microbial biomass N		Gross N mineralization		Microbial immobilization		Specific microbial immobilization	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*
Between subjects									
Experiment	2	91.8	1.31	2.05	26.42**	15.35	344.67***	8335.2	86.26**
Block	5	177.1	2.54	1.07	13.83**	0.52	11.71†	667.2	6.90†
CO_2	1	71.1	1.02	0.06	0.75	0.02	0.41	56.0	0.58
Experiment × CO_2	2	18.7	0.27	0.18	2.37	0.02	0.44	117.3	1.21
Error	5	69.8		0.08		0.04		96.6	
Within subjects									
Year	1	38.2	10.12†	0.01	0.01	1.44	18.39	2578.2	11.48†
Year × experiment	2	41.8	11.23†	0.13	0.26	3.17	40.41	1871.8	8.33†
Year × block	5	20.9	5.63†	0.26	0.52	0.28	1.01	443.9	1.96
Year × CO_2	1	0.5	0.14	0.02	0.04	0.03	0.42	3.8	0.02
Year × experiment × CO_2	2	27.8	7.38†	0.01	0.02	0.14	1.81	138.9	0.58
Error	5	3.8		0.51		0.08		224.6	

Notes: Our analyses allowed us to quantify differences among experiments, CO_2 treatment (ambient vs. elevated), and the interaction of experiment and CO_2 treatment; we also accounted for variation associated with blocking in each experiment.
** $P \leq 0.01$; *** $P \leq 0.001$; † $P \leq 0.1$.

and time, rates of gross immobilization were equivalent under ambient (2.00 ± 0.471 μg $N \cdot g^{-1} \cdot d^{-1}$) and elevated CO_2 (2.33 ± 0.468 μg $N \cdot g^{-1} \cdot d^{-1}$; Fig. 1C). However, there were significant differences among experiments (main effect; Table 2), wherein the mean rate of gross immobilization was significantly greater at Duke (3.23 ± 0.301 μg $N \cdot g^{-1} \cdot d^{-1}$) than at Rhinelander (1.89 ± 0.779 μg $N \cdot g^{-1} \cdot d^{-1}$) or Oak Ridge (0.75 ± 0.218 μg $N \cdot g^{-1} \cdot d^{-1}$). Time was not a significant main effect in our analysis of microbial immobilization.

Although we found significant differences among experiments in specific rates of microbial immobilization, we observed no effect of CO_2 on this process nor did we find a significant experiment × CO_2 interaction. Specific microbial immobilization averaged 54.5 ± 13.62 ng $N \cdot (g\ MBN)^{-1} \cdot d^{-1}$ under ambient CO_2 and 55.0

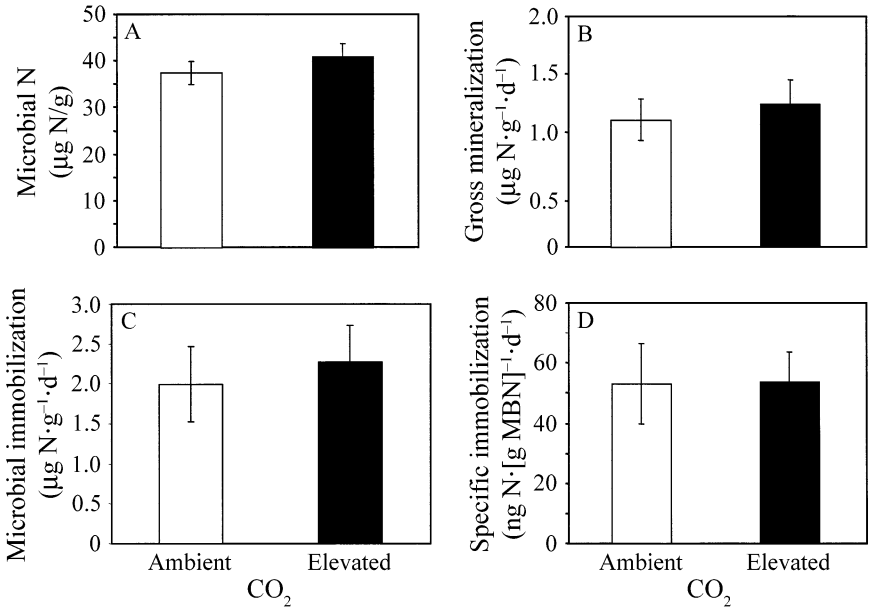

FIG. 1. The influence of atmospheric CO_2 on (A) microbial N, (B) gross N mineralization, (C) microbial immobilization, and (D) specific immobilization. Bars indicate means ± 1 SE averaged across experiment and sampling dates (1999 and 2000). We found no effect of the atmospheric CO_2 concentration on microbial N, gross mineralization, microbial immobilization, or specific immobilization. Research was conducted at three free-air CO_2 enrichment (FACE) experiments located at the Duke Forest, Durham, North Carolina, USA; the Oak Ridge National Environmental Research Park, Oak Ridge, Tennessee, USA; and Rhinelander, Wisconsin, USA.

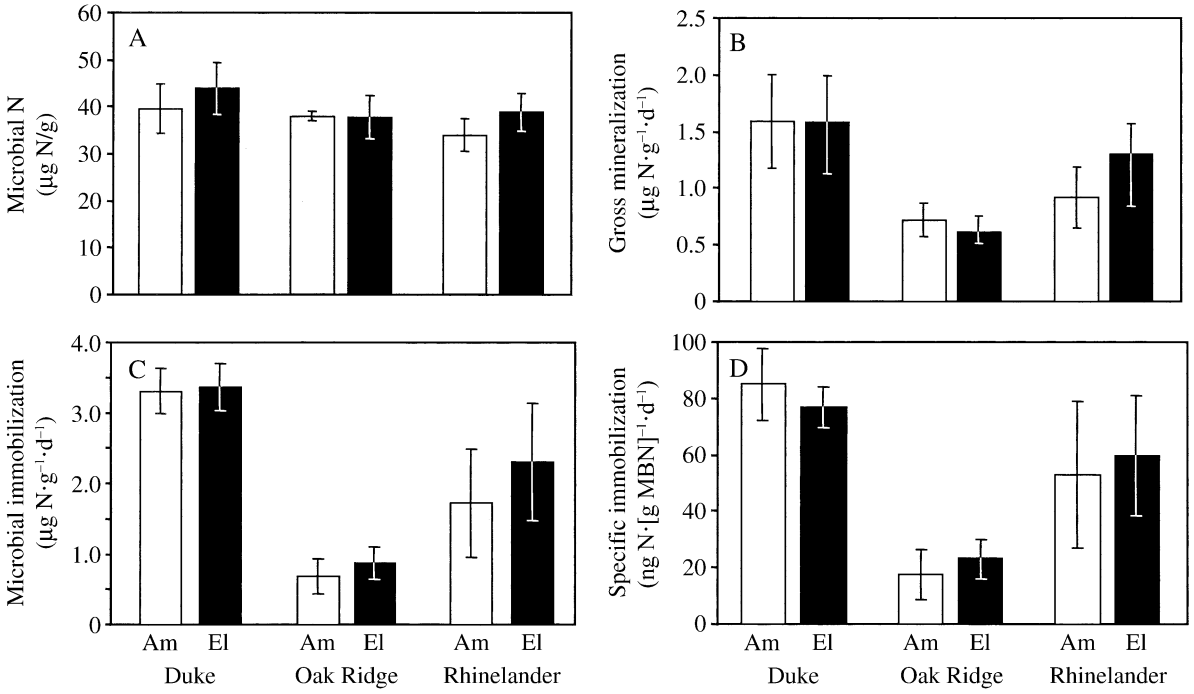

FIG. 2. (A) Mean microbial N, (B) gross N mineralization, (C) microbial immobilization, and (D) specific immobilization under ambient (Am) and elevated (El) CO_2. Bars indicate treatment means ± 1 SE for the Duke, Oak Ridge, and Rhinelander free-air CO_2 enrichment (FACE) experiments averaged across sampling dates (1999 and 2000). The interaction between experiment and CO_2 treatment was not significant for any microbial parameter illustrated in this figure.

± 10.45 ng N·(g MBN)$^{-1}$·d^{-1} in the elevated treatment (Fig. 1D; MBN = microbial biomass nitrogen). Averaged across time and treatment, specific microbial immobilization differed among experiments with significantly greater rates occurring at Duke (78.3 ± 6.09 ng N·g MBN^{-1}·d^{-1}) and Rhinelander (53.8 ± 13.62 ng N·g MBN^{-1}·d^{-1}) than in the Oak Ridge experiment (19.7 ± 3.05 ng N·g MBN^{-1}·d^{-1}). We also observed a significant interaction between experiment and time, in which specific microbial immobilization increased (23.5 ± 4.42 ng N·g MBN^{-1}·d^{-1} in 1999 and 84.0 ± 10.59 ng N·g MBN^{-1}·d^{-1} in 2000) over time at Rhinelander and remained relatively constant at Duke (~80 ng N·g MBN^{-1}·d^{-1}) and Oak Ridge (~20 ng N·g MBN^{-1}·d^{-1}).

DISCUSSION

The rising atmospheric CO_2 concentration has the potential to alter the cycling of C and N in terrestrial ecosystems by enhancing rates of net primary production, altering litter inputs to soil, and modifying the rate at which soil microorganisms release N during decomposition. We used common methods across three forest FACE experiments to better understand how changes in ecosystem C cycling under elevated CO_2 might alter the microbial assimilation and release of N in soil. Although elevated CO_2 consistently increased plant and litter production in our experiments (Finzi et al. 2001, King et al. 2001, Norby et al. 2002), greater litter production did not alter the microbial community biomass or the microbial processes controlling the supply of N to plants (Fig. 1). The consistency among our experiments contrasts with the wide range of results reported in the literature (Zak et al. 2000*b*) and suggests that soil N cycling in young forests may initially show little response as CO_2 increases in the Earth's atmosphere. At this point in our experiments, we have no initial evidence to suggest that plant litter produced under elevated CO_2 will modify microbial activity in a manner that will lessen soil N availability (Berntson and Bazzaz 1996, 1997).

Although our analyses suggest greater plant litter input under elevated CO_2 had no initial effect on microbial N transformations, a wide range of evidence generally indicates that greater substrate availability under elevated CO_2, due to higher levels of litter production, can increase microbial metabolism in soil. Greater microbial respiration (Insam et al. 1999, Andrews and Schlesinger 2001, Phillips et al. 2002), degradative enzyme activity (Mayr et al. 1999, Larson et al. 2002, Sinsabaugh et al. 2003*a*), and metabolism of plant-derived substrates (Phillips et al. 2002) all indicate that soil microbial communities are more metabolically active under elevated CO_2. These observations support the idea that greater plant litter production under elevated CO_2 will enhance microbial metabolism, which, over the long-term, could modify the availability of N for plant growth. Nevertheless, our data

suggest that increases in substrate availability were not sufficient to alter microbial N demand and hence the supply of N to plants. Greater rates of specific microbial immobilization would be clear evidence for increased microbial N demand under elevated CO_2, but that did not occur in our experiments.

This contrast illustrates the need to understand the time steps over which greater litter production under elevated CO_2 could influence microbial metabolism to the point where it alters microbial N demand and the supply of this resource to plants. Given the substantial amount of organic matter (and N) already present in soil, increases in above- and belowground litter production under elevated CO_2 were likely to be insufficient to influence microbial N demand over the duration of our experiments. In the Duke experiment, elevated CO_2 increased leaf litter production by 52 g $C \cdot m^{-2} \cdot yr^{-1}$ and it increased fine root litter by 7 g $C \cdot m^{-2}\ yr^{-1}$ over amounts in the control treatment (Matamala and Schlesinger 2000, Finzi and Schlesinger 2003). Even if these increases were sustained over the experiment (6–7 yr), enhanced litter production under elevated CO_2 only represented 10% of the organic matter already present in forest floor and surface mineral soil (3314 g C/m^2; Schlesinger and Lichter 2001). Similarly, in the Oak Ridge experiment, increases in leaf and fine root litter production under elevated CO_2 totaled 75 g C/m^2 over the 3-yr exposure (Norby et al. 2002; R. Norby, *unpublished data*), which only represented 5% of the organic matter in surface mineral soil (1470 g C/m^2; D. W. Johnson, *unpublished data*). Greater leaf (60 $g \cdot m^{-2} \cdot yr^{-1}$; W. Parsons and R. Lindroth, *unpublished data*) and fine root (58 g $C \cdot m^{-2} \cdot yr^{-1}$; King et al. 2001) litter production are proportional to 12% of the organic matter present in the surface mineral soil of the Rhinelander experiment (2880 g C/m^2; D. R. Zak, *unpublished data*), if this input was constant over the 4-yr duration of the experiment. These calculations overestimate actual litter contributions to soil, because they assume all litter entered mineral soil and that elevated CO_2 stimulated litter production from the initiation of each experiment; this undoubtedly did not occur.

Nonetheless, the aforementioned increases in above- and belowground litter production have been sufficient to generally increase microbial metabolism in the Duke and Rhinelander experiments. For example, rates of microbial respiration in both experiments are greater beneath plants growing under elevated CO_2 (Hamilton et al. 2002, Phillips et al. 2002). Moreover, the activity of extracellular microbial enzymes involved with plant and fungal cell wall degradation are greater under elevated CO_2 (Larson et al. 2002, Sinsabaugh et al. 2003*a*). These extracellular enzymes are induced by the presence of cellulose and chitin; therefore, it appears that microbial communities in these experiments are responding to the greater production of these substrates under elevated CO_2. In the Oak Ridge experiment, microbial communities exhibited no enzymatic response to elevated CO_2 (Sinsabaugh et al. 2003*b*), indicating that greater litter production had not altered substrate availability in soil. These microbial responses parallel differences in the magnitude of plant litter production under elevated CO_2, wherein the additional litter produced under elevated CO_2 represented a larger input of substrate in the Duke and Rhinelander experiments (~10%), relative to the Oak Ridge experiment (5%). In all three cases, we observed no response in the microbial processes controlling N supply to plants, indicating that the magnitude of these inputs has not been sufficient to alter microbial N demand and rates of soil N cycling.

We have studied the microbial processes that control the supply of N for plant growth in three, multiyear field experiments using identical techniques, and we have no evidence that the supply of N to plants will initially change under elevated CO_2. In particular, elevated CO_2 had no effect on microbial N, gross mineralization, or microbial immobilization, suggesting no change in the short-term supply of N to plants. One might argue that short-term laboratory experiments may not provide an accurate assessment of soil N availability at a time scale that would be relevant to long-term trends in forest productivity. Nonetheless, our results are consistent across two successive field seasons and three different experiments, and others have drawn similar conclusions using a wider range of techniques (Finzi et al. 2002, Sinsabaugh et al. 2003*b*). Our results suggest that the ''extra increment'' of plant litter produced under elevated CO_2 has not altered the microbially mediated supply of N to plants. We conclude that understanding the time scale over which greater plant production alters microbial N demand lies at the heart of our ability to predict long-term changes in soil N availability and hence whether greater NPP will be sustained in a CO_2-enriched atmosphere. Extrapolating our results to changes in ecosystem C and N cycling over decades to centuries will require the integration of experimental data with biogeochemical models (Luo and Reynolds 1999, Luo 2001).

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