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ATMOSPHERIC CO_2 AND THE COMPOSITION AND FUNCTION OF SOIL MICROBIAL COMMUNITIES

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Abstract. Elevated atmospheric CO_2 has the potential to increase the production and alter the chemistry of organic substrates entering soil from plant production, the magnitude of which is constrained by soil-N availability. Because microbial growth in soil is limited by substrate inputs from plant production, we reasoned that changes in the amount and chemistry of these organic substrates could affect the composition of soil microbial communities and the cycling of N in soil. We studied microbial community composition and soil-N transformations beneath *Populus tremuloides* Michx. growing under experimental atmospheric CO_2 (35.7 and 70.7 Pa) and soil-N-availability (low N = 61 ng $N \cdot g^{-1} \cdot d^{-1}$ and high N = 319 ng $N \cdot g^{-1} \cdot d^{-1}$) treatments. Atmospheric CO_2 concentration was modified in large, open-top chambers, and we altered soil-N availability in open-bottom root boxes by mixing different proportions of A and C horizon material. We used phospholipid fatty-acid analysis to gain insight into microbial community composition and coupled this analysis to measurements of soil-N transformations using ^{15}N-pool dilution techniques. The information presented here is part of an integrated experiment designed to elucidate the physiological mechanisms controlling the flow of C and N in the plant–soil system. Our objectives were (1) to determine whether changes in plant growth and tissue chemistry alter microbial community composition and soil-N cycling in response to increasing atmospheric CO_2 and soil-N availability and (2) to integrate the results of our experiment into a synthesis of elevated atmospheric CO_2 and the cycling of C and N in terrestrial ecosystems.

After 2.5 growing seasons, microbial biomass, gross N mineralization, microbial immobilization, and nitrification (gross and net) were equivalent at ambient and elevated CO_2, suggesting that increases in fine-root production and declines in fine-root N concentration were insufficient to alter the influence of native soil organic matter on microbial physiology; this was the case in both low- and high-N soil. Similarly, elevated CO_2 did not alter the proportion of bacterial, actinomycetal, or fungal phospholipid fatty acids in low-N or high-N soil, indicating that changes in substrate input from greater plant growth under elevated CO_2 did not alter microbial community composition. Our results differ from a substantial number of studies reporting increases and decreases in soil-N cycling under elevated CO_2. From our analysis, it appears that soil-N cycling responds to elevated atmospheric CO_2 in experimental situations where plant roots have fully colonized the soil and root-associated C inputs are sufficient to modify the influence of native soil organic matter on microbial physiology. In young developing ecosystems where plant roots have not fully exploited the soil, microbial metabolism appears to be regulated by relatively large pools of soil organic matter, rather than by the additional input of organic substrates under elevated CO_2.

Key words: atmospheric CO_2 and soil-N availability; carbon dioxide, elevated atmospheric; feedback, ecosystem; global climate change; microbial community composition; microbial immobilization; mineralization; nitrogen immobilization, gross and net; phospholipid fatty acids (PFLAs); Populus tremuloides; *soil microorganisms; soil-N transformations.*

INTRODUCTION

The rising atmospheric-CO_2 concentration is an anthropogenic environmental change that has the potential to alter the biogeochemical cycling of carbon (C) and nitrogen (N) in terrestrial ecosystems (Vitousek 1994). However, it is presently unclear whether greater plant growth under elevated atmospheric CO_2 will alter the cycling of C and N in terrestrial ecosystems. Some experimental evidence suggests that soil C and N cycling will slow under elevated CO_2 due to changes in litter chemistry that reduce rates of decomposition (Cotrufo et al. 1994, Cotrufo and Ineson 1995) or increase rates of microbial immobilization (Berntson and Bazzaz, 1997, 1998). However, others have observed in-

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creased rates of C and N transformations under elevated CO_2 (Zak et al. 1993*a*, Hungate et al. 1997), suggesting that greater plant growth under elevated CO_2 elicited a positive feedback effect on the flow of C and N in soil. Whether rising atmospheric CO_2 will increase or decrease rates of soil C and N cycling has important implications for C sequestration in terrestrial ecosystems.

At a fundamental level, the response of C and N cycles to rising atmospheric CO_2 will be controlled by changes in plant growth and the extent to which changes in above- and belowground litter production alter the metabolism of heterotrophic microorganisms in soil. Because microbial growth in soil is limited by the amount and chemistry of organic substrate inputs from plants (Babiuk and Paul 1970, Smith and Paul 1990), greater plant production and changes in tissue chemistry under elevated CO_2 could alter substrate availability for microbial biosynthesis. Such a response could lead to changes in the composition of soil microbial communities, the rate at which soil microorganisms assimilate inorganic N to build new biomass, and, in turn, the amount of inorganic N available for plant uptake. However, there are gaps in our knowledge of the mechanisms by which rising atmospheric CO_2 will alter the exchange of C and N between plants and soil microorganisms. For example, we are just beginning to understand how elevated atmospheric CO_2 will modify the production and timing of root-associated C inputs to soil and how such a change in substrate input will influence the composition and function of microbial communities in soil (Coûteaux et al. 1991, Pregitzer et al. 1995, Zak et al. 1996, Hungate et al. 1997, Berntson and Bazzaz 1998). Moreover, it is likely that differences in soil-N availability will modify plant growth responses to atmospheric CO_2 (Curtis 1996, Hungate et al. 1997, Zak et al. 2000), but we have a limited understanding of how soil-N availability will modify the input of belowground litter under elevated CO_2 and its subsequent metabolism by soil microorganisms.

We have hypothesized that plant growth, belowground allocation, and the microbial metabolism of plant-derived substrates are linked processes that regulate ecosystem-level changes in the cycling of C and N under elevated atmospheric CO_2 (Zak et al. 1993*a*, Pregitzer et al. 1995, Curtis et al. 1996). Specifically, we reasoned that greater belowground plant growth and belowground litter production under elevated CO_2 would lead to a more rapid turnover of N through microbial biomass, thus increasing the amount of N available for plant growth (Zak et al. 1993*a*, Curtis et al. 2000). In this paper, we describe how changes in plant growth under elevated atmospheric CO_2 influenced the composition and function of soil microbial communities. Our objectives were to (1) determine whether changes in plant growth, in response to atmospheric CO_2 and soil-N availability, alter microbial community composition and rates of microbial N transformations in soil, and (2) integrate our results with those from the literature to better understand potential changes in soil C and N cycling under elevated CO_2. To accomplish our first objective, we used phospholipid fatty acids (PLFA) to gain insight into the composition of soil microbial communities under experimental atmospheric-CO_2 and soil-N-availability treatments, and coupled this analysis to measurements of gross and net rates of soil-N transformations. We addressed our second objective by linking changes in belowground plant growth under elevated CO_2 to changes in soil-N transformations in our experiment and those from the literature.

Methods

Experimental design and field sampling

We grew *Populus tremuloides* for 2.5 growing seasons under two levels of atmospheric CO_2 and soil-N availability. Our experiment consisted of factorial atmospheric-CO_2 (35.7 and 70.7 Pa) and soil-N-availability (low and high) treatments arranged in a randomized complete-block design. Each atmospheric-CO_2–soil-N availability treatment combination was replicated 5 times, once in each block (i.e., 20 open-top chambers). Our experiment was constructed in a large, open field with level topography at the University of Michigan Biological Station (45°34′ N, 84°40′ W), located in the northern portion of Michigan's lower peninsula (USA). We removed and discarded the Oa, A, and E soil horizons in the open field, which exposed the surface of the underlying Bs soil horizon.

We manipulated soil-N availability by mixing the A and C horizon of native forest soils in open-bottom root boxes (10.9 $m^2 \times 0.4$ m deep) that rested atop the Bs soil horizon. The high-N-availability soil consisted of A horizon from a Typic Haplorthod (Kalkaska series), and the low-N-availability soil was prepared by mixing A horizon (20% volume per volume [v/v]) with the C horizon (80% v/v) of an Entic Haplorthod (Rubicon series). Both experimental soils are very sandy (72–93% sand) and are representative of forest soils covering a large geographic area of the Upper Great Lakes region. Differences in organic matter (low N = 3559 mg C/kg; high N = 12 489 mg C/kg) between our experimental soils resulted in net N mineralization that averaged 61 ng $N \cdot g^{-1} \cdot d^{-1}$ in low-N soil and 319 ng $N \cdot g^{-1} \cdot d^{-1}$ in high-N soil (see *Results: Gross and net N transformations*, below). Mean growing-season rates of net N mineralization in Lake States forests, estimated by isotope dilution and incubation techniques, range from 50 ng $N \cdot g^{-1} \cdot d^{-1}$ in xeric oak-dominated forests on outwash sands to 1000 ng $N \cdot g^{-1} \cdot d^{-1}$ in mesic sugar maple-dominated forests on finer-textured glacial moraines (calculated from Pastor et al. 1984, Zak and Pregitzer 1990, Holmes and Zak 1999). *Populus tre-*

muloides commonly establishes across this range of soil conditions following harvest, fire, or other stand-destroying disturbance. Rates of net N mineralization in our experiment fall within the range of values *P. tremuloides* commonly encounters in the field. A complete summary of the physical, chemical, and biological properties of our experimental soils can be found in Curtis et al. (2000).

In early spring 1994, we placed open-top chambers (2.3-m diameter, 3-m height) over the 20 root boxes in order to control the atmospheric-CO_2 concentration. We then planted two softwood cuttings of six *P. tremuloides* genotypes into each open-bottom root box. Softwood cuttings were propagated from root cuttings of locally occurring genotypes that differed in autumnal leaf senescence; three genotypes senesce in early autumn and three senesce in late autumn. Cuttings of each genotype were graded for size prior to planting; height averaged 17.3 ± 0.45 cm (mean ± SE) and the number of leaves averaged 12.3 ± 0.33 leaves/plant. Leaves produced during the first and second growing season were allowed to abscise and fall onto the soil surface, where they remained for the duration of the experiment. Further details regarding the performance of our CO_2-exposure system, construction of the open-bottom root boxes, and plant propagation can be found in Curtis et al. (2000).

Near the end of the second growing season (9 September 1995) and prior to destructive harvest (6 June 1996), we systematically collected six soil cores (7.6-cm diameter) to a depth of 40 cm in each open-bottom root box. We collected three soil cores in two concentric rings within each chamber and composited them into one soil sample per chamber. The composited samples were placed on ice and transported to the laboratory for analysis, which occurred within 12 h of field collection. All values are expressed on an oven-dry (105°C) mass basis.

Microbial biomass

We determined microbial-biomass N using the $CHCl_3$ fumigation–direct extraction procedure (Brooks et al. 1985). A 25-g subsample of soil collected from each root box (1995 and 1996) was fumigated for 24 h with C_2H_5OH-free $CHCl_3$ in a vacuum dessicator. A second 25-g subsample, which served as a control, was simultaneously incubated in a dessicator without $CHCl_3$. Following fumigation, we extracted each sample with 100 mL of 0.5 mol/L K_2SO_4. Fifty-milliliter portions of each extract were digested with 12 mL K_2SO_4 and a kjeltab for 2 h at 395°C. Concentrations of NH_4^+-N in the diluted digests were determined colorimetrically with an Alpkem RFA 300 (Astoria-Pacific International, Clackmas, Oregon, USA). Microbial-biomass N (in micrograms of N per gram of dry mass [μg N/g]) was estimated by subtracting the amount of inorganic N (NH_4^+-N + NO_3^--N) in control samples from quantities in the fumigated samples; this value was divided by a correction factor (K_N = 0.68) to estimate microbial-biomass N.

Microbial community composition

The cell membranes of soil bacteria, actinomycetes, and fungi contain phospholipid fatty acids (PLFAs), and the fatty-acid portion of these molecules can serve as a general, as well as specific, marker for these different taxonomic groups. We used PLFA analysis to understand whether changes in plant C inputs to soil under elevated CO_2 altered the composition of soil microbial communities. Phospholipid fatty acids were extracted from soil collected on 6 June 1996, prior to destructive harvest. Freeze-dried soil from each root box was extracted with a single-phase $CHCl_3$-CH_3OH solvent that was buffered with PO_4^{3-} (Bligh and Dyer 1959, White et al. 1979). The extracted lipids were then separated into functional classes using silicic-acid column chromotography (Gehron and White 1983). The polar lipid fraction was transesterified into fatty-acid methyl esters (FAMEs) using a mild-alkaline system containing methanol (White et al. 1979). The resulting FAMEs were separated and identified using a coupled gas chromatograph–mass spectrometer as described by Ringelberg et al. (1994). We used the mole fraction (in percentage) of each PLFA to indicate the relative proportion of bacteria, actinomycetes, and fungi in soil. PLFA is degraded within minutes to hours on entering soil, and the total amount extracted from soil can serve to estimate live microbial biomass. We therefore used the total PLFA (in picomoles per gram) extracted from soil as an index of living microbial biomass.

Individual PLFAs are indicated by their shorthand notation, which is described in detail by Vestal and White (1989). PLFAs are designated by the number of C atoms to the number of double bonds, followed by the location of the double bond from the methyl end (ω) of the molecule. For example, 16:1ω6 is a 16-C fatty acid with one double bond located on the sixth C from the methyl end. The letters ''c'' and ''t'' are used to indicate the cis and trans isomers of a particular molecule. Methyl branches in the iso- (first C) or anteiso (second C) positions are indicated by the prefixes ''i'' and ''a,'' respectively. A methyl branch in positions other than the iso or anteiso positions are determined by its location from the carboxyl end of the molecule. For example, the notation 10Me18:0 indicates the presence of a methyl branch on the tenth C from the carboxyl end of an 18-C fatty acid with no double bonds. The prefix ''cy'' denotes the cyclopropane fatty acids. Our PLFA analysis was conducted at the Center for Biotechnology, University of Tennessee–Knoxville.

Gross and net nitrogen transformations

We used ^{15}N isotope dilution to determine gross and net rates of N mineralization, microbial immobiliza-

tion, and nitrification in soil collected during the 1995 and 1996 growing seasons (Davidson et al. 1992, Hart et al. 1994). Two 130-g samples from each root box were placed in 500-mL plastic beakers and covered with Parafilm. To determine initial pool sizes, we extracted 10-g subsamples with 20 mL of 2 mol/L KCl and colorimetrically determined the NH_4^+ and NO_3^- concentrations as described above. The NH_4^+ pool of one sample (120 g) was enriched to ~10% ^{15}N with $(^{15}NH_4^+)_2SO_4$. Similarly, we enriched the NO_3^- pool of the second sample (120 g) to ~10% ^{15}N with $K^{15}NO_3$. Labeled compounds (99% atom excess ^{15}N) were dissolved in deionized water and evenly applied to the surface of each soil sample in 4-mL aliquots; sufficient water was added to bring each sample to field capacity. Immediately following isotope addition, a 20-g sample was removed to determine the initial ^{15}N enrichment of the extractable NH_4^+ or NO_3^- pool. The ^{15}N-labeled soil samples were then incubated at 20°C for 5 d.

Following the 5-d incubation, we determined the ^{15}N enrichment of extractable NH_4^+ and NO_3^- pools. A diffusion procedure was used to separate NH_4^+ and NO_3^- in soil extracts (2 mol/L KCl) for ^{15}N analysis. Twenty-five-milliliter aliquots of each soil extract were placed in plastic specimen containers; the concentration of NH_4^+ and NO_3^- in the extract was determined as described above. When necessary, we added sufficient quantities of $^{14}NH_4SO_4$ or $K^{14}NO_3^-$ to raise the N content of extracts above 50-ug N, which brought the sample into the optimal detection range for mass spectrometry. A cellulose disk (Whatman number 3, Whatman Incorporated, Clifton, New Jersey) was acidified with $KHSO_4$ (10 μL of 2.5 mol/L) and suspended on a wire above the solution inside each specimen container. Following a 6-d diffusion with MgO to trap NH_4^+-N, Devarda's alloy was added to convert NO_3^- to NH_4^+, which was diffused onto the fresh acid traps for 6 d. Cellulose disks containing ^{15}N were placed in Sn capsules and analyzed for ^{15}N abundance by a Europa Scientific Integra CN mass spectrometer (Europa Scientific, Franklin, Ohio, USA).

Air-dried samples from each ^{15}N-enriched soil were ground in a bar mill, placed in Sn capsules, and analyzed for ^{15}N; this information was used to determine the total recovery of ^{15}N in each sample. Gross rates of N mineralization, microbial immobilization ($NH_4^+ + NO_3^-$), nitrification, and NO_3^- immobilization were calculated using the methods of Hart et al. (1994). Microbial immobilization of NH_4^+ was calculated as the difference between NH_4^+ consumption and gross nitrification. We estimated microbial immobilization and the assimilation of both NH_4^+ and NO_3^-. Net N mineralization was calculated as the difference between gross N mineralization and NH_4^+ immobilization. In 1995, recovery of ^{15}N averaged 74% ± 4.4% (mean ± 1 SE) in $^{15}NH_4^+$-enriched samples and 76 ± 4.2% in $^{15}NO_3^-$-enriched samples. Recovery of ^{15}N in 1996 was slightly greater and averaged 81 ± 4.9% in $^{15}NH_4^+$-enriched samples and 84 ± 4.2% in $^{15}NO_3^-$-enriched samples. Recoveries did not significantly differ from 100% (*t* test) in either year. Moreover, none of the $^{15}NO_3^-$ immobilized by soil microorganisms was recovered as $^{15}NH_4^+$, indicating that our 5-d incubation was sufficient for the isotope to move into, but not out of, microbial cells.

Statistical analyses

The influence of atmospheric-CO_2 concentration and soil-N availability on microbial N and rates of soil-N transformations was determined using a repeated-measures analysis of variance (ANOVA) for a completely randomized block design with two factorial treatments. Atmospheric-CO_2 (35.7 and 70.7 Pa) concentration and soil-N availability (low and high) were fixed effects in this ANOVA model. An ANOVA for a completely randomized block design with two factorial treatments (atmospheric CO_2 and soil-N availability) was used to determine differences in total PLFA and the mole fractions of bacterial, actinomycetal, and fungal PLFAs. Main-effect and interaction means were compared using a protected Fisher's least-significant-difference test. We also used linear-regression analysis to explore the relationship between microbial N and living microbial biomass estimated by total PLFA. All analyses were performed using SYSTAT (Wilkinson 1990), and statistical significance was accepted at $\alpha = 0.05$.

RESULTS

Microbial biomass

In 1995 and 1996, atmospheric CO_2 did not influence the nitrogen biomass of soil microorganisms at either level of soil-N availability (Table 1 and Fig. 1). In our analysis, atmospheric CO_2 did not interact with sampling date or soil-N availability in a significant manner, nor was atmospheric CO_2 significant as a main effect. However, soil-N availability was a significant main effect in our analysis; high-N-availability (24.9 ± 0.44 μg N/g; mean ± 1 SE) soil had 5 times more microbial N than the low-N-availability soil (5.5 ± 0.85 μg N/g). Sampling date also was significant (Table 1), and microbial N in 1996 (13.8 ± 1.02 μg N/g) was significantly lower than microbial N in 1995 (16.6 ± 1.20 μg N/g).

Microbial community composition

At the end of our experiment (1996), elevated atmospheric CO_2 did not influence the mole fraction of bacterial, actinomycetal, or fungal phospholipid fatty acids (PLFAs; Fig 2B), indicating that greater belowground plant growth under elevated atmospheric CO_2 did not alter the composition of the soil microbial community. Of the 21 PLFAs we used to characterize microbial community composition, elevated atmospheric CO_2 significantly increased only one, polyenoic PLFA

(18:2ω9c), which was indicative of soil fungi. On the other hand, a substantial number of PLFAs (13 of 21) significantly differed between low-N and high-N soil, suggesting that microbial communities differed between our experimental soils (Fig. 2B). The majority of PLFAs influenced by soil-N availability were indicative of soil bacteria, whereas actinomycetal and fungal PLFAs (tuberculosteric acid and polyenoic fatty acids, respectively) did not differ between low- and high-N soil (Fig. 2A). With the exception of 16:0, Gram-positive bacterial PLFAs increased from low- to high-N-availability soil, while Gram-negative PLFAs (except 18:1ω7c) declined from low- to high-N-availability soil (Fig. 2A). These results indicate a difference in the bacterial community composition between low- and high-N soil, with no difference in actinomycetal or fungal communities.

Living microbial biomass, as estimated by total PLFA, was not influenced by atmospheric CO_2; however, we did observe significantly greater total PLFA in high-N soil vs. low-N soil. In low-N soil, for example, total PLFA was 4551 ± 714.5 pmol/g (all data reported as mean ± 1 SE) at ambient CO_2 and 4165 ± 264.9 pmol/g under elevated CO_2. These values were much greater in high-N soil and averaged 15 410 ± 755.8 pmol/g at ambient CO_2 and 17 020 ± 1583.1 pmol/g under elevated CO_2. At the time of our harvest, living microbial biomass was significantly and linearly related to microbial-biomass N (pmol/g living microbial biomass = 519 (ug N/g microbial N) + 3187; r^2 = 0.72; n = 20 observations; $P < 0.001$). Both the chloroform fumigation–extraction procedure and PLFA analysis of microbial biomass indicate that atmospheric CO_2 did not significantly alter the biomass of soil microorganisms.

Gross and net nitrogen transformations

Atmospheric CO_2 did not alter gross rates of N mineralization at either level of soil-N availability and this was true in 1995 and 1996 (Table 1 and Fig. 3A). Sampling date and soil-N availability were significant main effects in our analysis, but atmospheric CO_2 was not (Table 1). Averaged across CO_2 and soil-N-availability treatments, gross N mineralization significantly increased from 316 ± 74.6 ng $N \cdot g^{-1} \cdot d^{-1}$ in 1995 to 388 ± 71.9 ng $N \cdot g^{-1} \cdot d^{-1}$ in 1996. As one would expect, gross rates of N mineralization were significantly greater in high-N soil (478 ± 55.9 ng $N \cdot g^{-1} \cdot d^{-1}$) than in low-N soil (226 ± 38.4 ng $N \cdot g^{-1} \cdot d^{-1}$).

Greater belowground plant growth under elevated atmospheric CO_2 (Pregitzer et al. 2000; Zak et al. 2000) did not alter the microbial demand for inorganic N. For example, atmospheric CO_2 did not interact with sampling date or soil-N availability to influence rates of microbial immobilization ($NH_4^+ + NO_3^-$), nor was atmospheric CO_2 significant on its own (Table 1 and Fig. 3B). However, sampling date significantly influenced microbial immobilization (Table 1). Rates of microbial immobilization were significantly greater in 1996 (459 ± 47.8 ng $N \cdot g^{-1} \cdot d^{-1}$) compared to rates measured in 1995 (346 ± 46.6 ng $N \cdot g^{-1} \cdot d^{-1}$). Microbial immobilization averaged 430 ± 62.1 ng $N \cdot g^{-1} \cdot d^{-1}$ in high-N soil, which was not significantly greater than the average rate in low-N soil (369 ± 43.5 ng $N \cdot g^{-1} \cdot d^{-1}$).

Atmospheric CO_2 had no effect on net N mineralization (i.e., gross N mineralization minus NH_4^+ immobilization) and, hence, did not influence the amount of N available for plant growth in our experiment (Table 1 and Fig. 4). Again, as expected, net rates of N mineralization differed significantly only with respect to our soil-N-availability treatments (Table 1). Net N mineralization averaged 61 ± 26.5 ng $N \cdot g^{-1} \cdot d^{-1}$ in low-N soil and 319 ± 32.6 ng $N \cdot g^{-1} \cdot d^{-1}$ in high-N soil.

Gross rates of nitrification differed significantly between soil-N-availability treatments, but rates were not influenced by atmospheric CO_2 (Table 1 and Fig. 5A). In low-N soil, gross nitrification declined from 265 ± 20.9 ng $N \cdot g^{-1} \cdot d^{-1}$ in 1995 to 249 ± 23.8 ng $N \cdot g^{-1} \cdot d^{-1}$ in 1996, while gross nitrification in high-N soil increased from 549 ± 22.0 ng $N \cdot g^{-1} \cdot d^{-1}$ in 1995 to 625 ± 46.5 ng $N \cdot g^{-1} \cdot d^{-1}$ in 1996. This response produced a significant year × soil-N-availability interaction. Gross nitrification averaged 589 ± 40.0 ng $N \cdot g^{-1} \cdot d^{-1}$ in high-N soil and 257 ± 22.2 ng $N \cdot g^{-1} \cdot d^{-1}$ in low-N soil; these means were significantly different. Nitrate immobilization was influenced by soil-N availability, but not by atmospheric CO_2 (Table 1, Fig. 5B), further supporting the idea that greater belowground plant growth under elevated CO_2 did not alter the microbial demand for inorganic N. Rates of NO_3^- immobilization were significantly greater in high-N soil (266 ± 28.5 ng $N \cdot g^{-1} \cdot d^{-1}$) compared to low-N soil (196 ± 21.4 ng $N \cdot g^{-1} \cdot d^{-1}$).

Atmospheric CO_2 did not influence net rates of nitrification (Table 1 and Fig. 6) nor did atmospheric CO_2 interact with date or soil-N availability to influence net nitrification. However, the relatively substantial difference in gross nitrification between low- and high-N soil, combined with the relatively small difference in NO_3^- immobilization, gave rise to significantly greater rates of net nitrification in high-N soil (low-N soil = 60 ± 12.8 ng $N \cdot g^{-1} \cdot d^{-1}$; high-N soil = 323 ± 19.2 ng $N \cdot g^{-1} \cdot d^{-1}$). In high-N soil, gross rates of nitrification were 180% greater than net rates, and gross nitrification rates in low-N soil exceeded net rates by 430%. We also observed an interaction between date and soil-N availability in which net nitrification in high-N soil was significantly lower in 1996, compared to rates in 1995.

DISCUSSION

Central to predicting changes in ecosystem C storage due to elevated atmospheric CO_2 is understanding its effect on soil-N cycling. Because elevated atmospheric CO_2 could alter the production and chemistry of or-

TABLE 1. Results of analyses of variance (ANOVA) for microbial biomass N, gross N mineralization, microbial N mineralization, net N mineralization, gross nitrification, NO_3^- immobilization, and net nitrification, using a repeated-measures, randomized block design with two factorial treatments.

		Microbial biomass N		Gross N mineralization		Microbial immobilization	
Source of variation	df	MS	*F*	MS	*F*	MS	*F*
Between subjects							
Block	4	13.8	2.6**	4 873.9	0.30	6 403.8	0.28
CO_2	1	0.8	0.15	591.9	0.04	5 061.4	0.22
Nitrogen (N)	1	3 169.4	596.82***	633 835.9	38.6***	64 083.9	2.85
$CO_2 \times N$	1	3.3	0.62	1 566.3	0.09	131.3	0.01
Error	12	5.3		16 406.9		22 684.9	
Within subjects							
Year	1	76.9	14.12**	51 148.2	6.94**	126 095.9	28.2***
Year × Block	4	10.5	1.94	11 235.2	1.52	12 987.3	2.90
Year × CO_2	1	9.8	1.80	6 736.2	0.91	9 994.5	2.24
Year × N	1	2.1	0.39	9 308.7	1.26	20 387.6	4.56
Year × CO_2 × N	1	0.8	0.16	18 343.1	2.49	5 073.6	1.13
Error	12	5.4		7 374.0		4 470.4	

Notes: Atmospheric CO_2 concentration (35.7 and 70.7 Pa), and soil-N availability (low and high) were fixed effects in the ANOVA model. Microbial immobilization included the assimilation of both NH_4^+ and NO_3^-, and net N mineralization is the difference between gross N mineralization and NH_4^+ immobilization. Similarly, net nitrification is the difference between gross nitrification and NO_3^- immobilization.

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

ganic substrates (i.e., plant litter) entering the soil, it could potentially affect microbial biosynthesis and, thus, the flow of N between soil microorganisms and plant roots. We hypothesized that greater belowground plant growth under elevated CO_2 would increase rates of N turnover through microbial biomass (Zak et al. 1993*a*, Curtis et al. 2000). In our experiment, elevated atmospheric CO_2 produced a substantial increase in net fine-root production in high-N soil, but did not alter net fine-root production in low-N soil (Pregitzer et al. 2000). We also observed consistent declines in the N concentration of fine roots produced under elevated CO_2, with the greatest reduction occurring in high-N-availability soil (Pregitzer et al. 2000; Zak et al. 2000). In combination, these results indicate that elevated CO_2 caused increased turnover and decreased N concentration of fine roots. However, the magnitude of this response was dependent on soil-N availability. Conceptually, these changes have the potential to modify the availability of organic substrates for microbial metabolism, which could, in turn, influence composition and function of soil microbial communities. Nevertheless, over the duration of our experiment, we found that changes in belowground litter under elevated atmospheric CO_2 did not change microbial community composition nor did they change microbial transformations of N in soil. Thus, our observations do not support the idea that greater belowground plant growth under elevated CO_2 will increase rates of soil-N cycling.

There is an accumulating body of information regarding feedback between greater plant growth under elevated atmospheric CO_2 and soil-N cycling; however, a clear pattern has yet to emerge. Regardless of differences in initial soil-N availability, an increase in belowground plant production under elevated CO_2 did not change microbial biomass, gross rates of N mineralization, or microbial immobilization in our present experiment. In a previous experiment with *Populus grandidentata,* we observed that elevated atmospheric CO_2 significantly increased plant C assimilation, fine-root turnover, microbial biomass, and rates of net N mineralization, supporting the idea that rates of soil-N cycling will increase under elevated atmospheric CO_2 (Zak et al. 1993*a*). Díaz et al. (1993) also observed an increase in microbial biomass under elevated CO_2 and, in the absence of measuring microbial-N transformations, speculated that greater inputs of plant-derived C fueled microbial immobilization and reduced the amount of N available for plant uptake. More recently, Hungate et al. (1997) observed that the species-specific growth response of grasses and forbs to elevated atmospheric CO_2 could either increase or decrease soil-N availability, depending on the whether elevated CO_2 increased or decreased plant growth. Additionally, Berntson and Bazzaz (1997, 1998) have argued that elevated atmospheric CO_2 can produce simultaneous positive and negative feedback on soil-N availability. Although seedlings of *Betula alleghaniensis* acquired more soil-N under elevated CO_2, rates of plant N uptake declined and rates of microbial immobilization increased toward the end of their experiment (Berntson and Bazzaz 1997, 1998). Understanding the mechanisms producing these divergent observations requires a careful examination of how organic substrates produced under elevated CO_2 alter the demand for N during microbial biosynthesis.

If soil-N dynamics are altered by elevated atmospheric CO_2, then increases in plant production or

TABLE 1. Extended.

Net N mineralization		Gross nitrification		NO_3^- immobilization		Net nitrification	
MS	*F*	MS	*F*	MS	*F*	MS	*F*
19 035.4	1.63	18 961.3	1.25	5 566.4	2.91	2 205.8	0.48
6 167.6	0.52	21 356.8	1.41	831.9	0.43	4 734.4	1.03
530 232.3	45.36***	777 699.7	51.22***	31 509.9	16.51***	553 052.8	119.9***
4 511.9	0.39	233.6	0.01	5 216.8	2.73	2 488.2	0.54
11 689.5		15 181.2		1 907.8	3.25	4 611.9	
735.1	0.14	32 116.1	7.60	10 770.2	3.25	2 531.0	3.37
15 941.1	2.98	7 618.2	1.80	3 061.7	0.92	2 411.4	3.21
6 706.0	1.25	9 137.5	2.16	197.1	0.06	3 043.4	4.06
19 702.3	3.68	40 078.9	9.49**	1 607.3	0.49	17 573.1	23.4***
19 242.8	3.59	15 119.9	3.58	4 564.1	1.38	988.8	1.32
5 349.7		4 225.2		3 309.3		750.3	

changes in litter chemistry must be of sufficient magnitude to modify the influence of native soil organic matter on microbial physiology. This may, in part, help explain why greater plant growth under elevated CO_2 has produced the array of results described above. Because all of the aforementioned studies occurred over one or two growing seasons, the major plant C input to soil was derived from roots. In our experiment, soil organic C and labile substrates for microbial metabolism present at the start of the experiment were much greater than C inputs from belowground plant growth. For example, soil organic-C content of both low- and high-N soil (0 to 40 cm) was 1000 times greater than the C content of root biomass. Labile C represents a pool of substrates readily available for microbial metabolism in soil. If we assume it was ~10% of soil organic C (Zak et al. 1993*a*, *b*), then labile C pools were 100 times greater than the C content of root biomass, a portion of which entered the soil via fine-root mortality (Pregitzer et al. 2000). Given these large pools of C already in soil, root-associated substrates were apparently not sufficient to alter the physiology of soil microbial communities in our experiment.

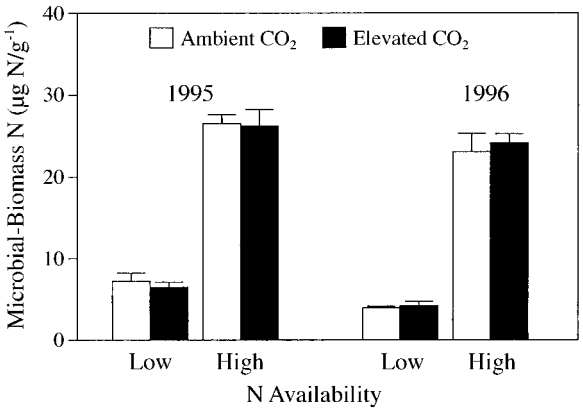

FIG. 1. The influence of atmospheric CO_2 and soil-N availability on microbial biomass N during the 1995 and 1996 field seasons. The illustrated interaction between atmospheric CO_2, soil-N availability, and sampling date was not significant. Atmospheric CO_2 also was not a significant main effect in the ANOVA model, but soil-N availability significantly influenced microbial N as a main effect.

Previously, we reported that elevated CO_2 increased C assimilation and fine-root turnover in *Populus grandidentata*, which led to an increase in soil microbial biomass and rates of net N mineralization (Zak et al. 1993*a*). In our earlier experiment, soil organic C was much lower than in the present experiment, and it represented a much smaller pool of C relative to belowground plant biomass. For example, soil organic C (to 40 cm) in our previous experiment was only 20 times greater than the C content of belowground plant biomass, compared to the 1000-fold difference in the present experiment. Moreover, labile C in our previous experiment was 650 times *less* than belowground plant biomass (calculated from Zak et al. 1993*a*). In the relatively organic-matter-poor soil of our previous experiment, C inputs to soil from belowground plant production likely exerted a much greater influence over substrate availability and microbial physiology, compared to the relatively small root-associated C inputs to low- and high-N soil in the present experiment. Nevertheless, in the present experiment, the amount of C in low- and high-N soil (1.4 and 4.9 kg C/m^2, respectively; 0 to 40 cm; D_b [bulk density of soil] = 0.98 Mg/m^3) was comparable to that of that of most Lake States forests (3.3–5.1 kg C/m^2 in 0–25 cm depth, Zak et al. 1993*b*). In young, regenerating forest stands, like those in our open-top chambers, changes in the production and chemistry of belowground plant litter under elevated atmospheric CO_2 are likely to have a minor influence on substrate availability for microbial me-

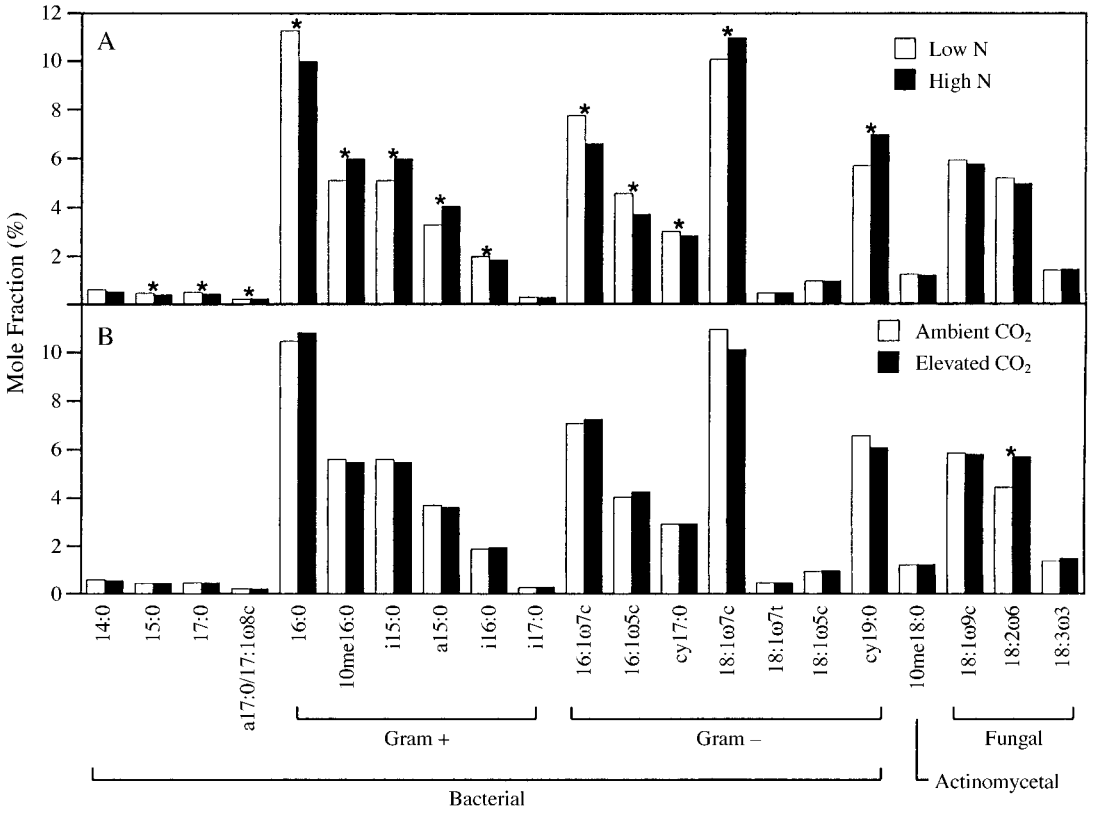

FIG. 2. Phospholipid fatty acids (PLFAs) indicative of soil bacteria, actinomycetes, and fungi beneath *Populus tremuloides* grown (A) in soils of low- and high-N availability and (B) under ambient and elevated atmospheric CO_2. Values presented are the mole fractions (in percentage) of the 21 most abundant PLFAs extracted from soil. An asterisk above a pair of bars indicates a significant difference at $\alpha = 0.05$.

tabolism, given the relatively large pool of organic substrates already stored in most forest soils.

Most studies reporting changes in soil-N transformations under elevated CO_2 are characterized by relatively large belowground inputs of plant C and relatively small soil organic-matter pools. In California (USA) annual grasslands, where plant C inputs under elevated CO_2 can increase or decrease net N mineralization, soil organic matter is 13–15 times greater than plant roots and belowground detritus (ambient CO_2, calculated from Hungate et al. 1997). Plant roots in these grassland soils are equivalent to the biomass of soil microorganisms, in contrast to our experiment in which microbial biomass was 50–90 times greater than plant roots (calculated from Zak et al. 2000). Berntson and Bazzaz (1997) observed that elevated CO_2 decreased gross N mineralization and increased microbial immobilization in soil where the fine roots of *Betula alleghaniensis* were 4.5 times greater than microbial biomass (microbial C estimated using carbon-to-nitrogen ratio (C/N) = 11.5, Berntson and Bazzaz 1998). In a study of deciduous tree regeneration under elevated CO_2, Berntson and Bazzaz (1998) observed that greater belowground plant growth under elevated CO_2 altered soil-N dynamics in an identical manner; fine roots were 5–9 times greater than microbial biomass in that study. Although the soils used by Berntson and Bazazz (1997, 1998) contained organic-matter-rich forest floor (i.e., Oe, Oa), the large difference between root and microbial biomass suggests that plant roots exerted substantial influence on labile C pools in soil. In our previous experiment, where greater belowground plant growth increased rates of soil-N cycling (Zak et al. 1993*a*), root biomass was equivalent to (ambient CO_2) or somewhat greater (1.3 times, elevated CO_2) than microbial biomass. We were unable to make similar comparisons for the experimental system used by Díaz et al. (1993), because they did not report root biomass, microbial biomass, or soil-C pools. Clearly, comparisons of root standing crop to soil-C pools are limited, because they do not include C inputs from root mortality, exudation, and mycorrhizae. Notwithstanding this limitation, our analysis of the literature supports the contention that soil N cycling will respond to elevated atmospheric CO_2 in ecosystems where root-associated C inputs are sufficient to modify the influence of native soil organic matter on microbial physiology.

Microbial growth on organic compounds contained

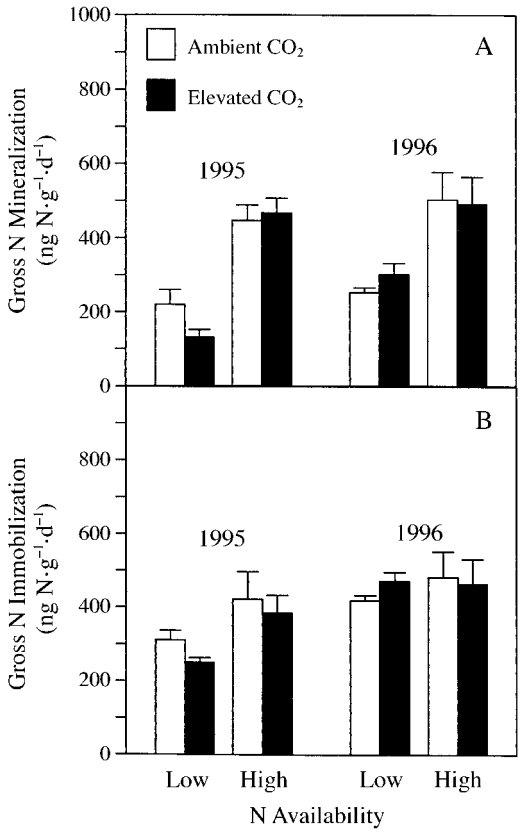

FIG. 3. The influence of atmospheric CO_2 and soil-N availability on (A) gross N mineralization and (B) immobilization rates during the 1995 and 1996 field seasons. Gross N immobilization is the microbial assimilation of NH_4^+ and NO_3^-. Values are treatment means, and the length of each error bar is 1 SE ($n = 5$ observations). The atmospheric-CO_2 × soil-N-availability × sampling-date interactions illustrated in this figure were not significant.

in litter will control whether CO_2-induced changes in tissue chemistry will modify gross rates of N mineralization and microbial immobilization. Most often, changes in tissue chemistry under elevated CO_2 are evaluated using the C to N ratio (C/N) of plant litter, and several studies have observed that elevated CO_2 widens fine-root C/N ratio (Berntson and Bazzaz 1997, 1998, Jackson and Reynolds 1996, Pregitzer et al. 2000). Given that the decomposition of leaf litter slows as the C/N or lignin/N ratio widens, one might predict that the widening of fine-root C/N under elevated CO_2 should slow decomposition and the net release of inorganic N. Although litter C/N has been a useful tool for predicting ecosystem-level patterns of organic-matter decomposition and net N mineralization, litter C/N provides limited insight into substrate availability for microbial biosynthesis. Organic-matter decomposition and net N mineralization are controlled by the biochemical constituents of litter, their use during microbial biosynthesis, and the N required to synthesize proteins, nucleic acids, and other N-containing compounds within microbial cells (Paul and Clark 1996, Barnes et al. 1998). At a physiological level, soil microorganisms do not respond to the relative proportion of C to N in litter, but they do respond to organic compounds that fuel biosynthesis. Clearly, we need to better understand how changes in litter chemistry under elevated atmospheric CO_2 affect microbial biosynthesis and, in turn, microbial demand for N.

Unfortunately, very few studies have investigated biochemical changes in fine roots produced under elevated CO_2 and their effects on microbial biosynthesis and soil-N transformations. It appears that elevated CO_2 does not alter the non-structural carbohydrate concentration (i.e., simple sugars) of live fine roots in some studies (King et al. 1997, Rothstein et al. 1999), and does produce an increase in non-structural carbohydrates in others (BassiriRad et al. 1996). Greater concentrations of non-structural carbohydrates in root litter would promote microbial biosynthesis and stimulate N immobilization. Elevated atmospheric CO_2 has been observed to increase the concentration of some secondary plant metabolites (i.e., condensed tannins and phenolics) in live fine roots (Gebauer et al. 1998), which are poor substrates for heterotrophic metabolism. Thus, greater concentrations of these compounds under elevated CO_2 would slow fine-root decomposition, but would not alter N immobilization to a significant extent. Biochemical changes in plant litter will ultimately determine the response to elevated CO_2 of microbial physiology, soil-N transformations, and C sequestration in soil.

Increased rates of microbial immobilization do not

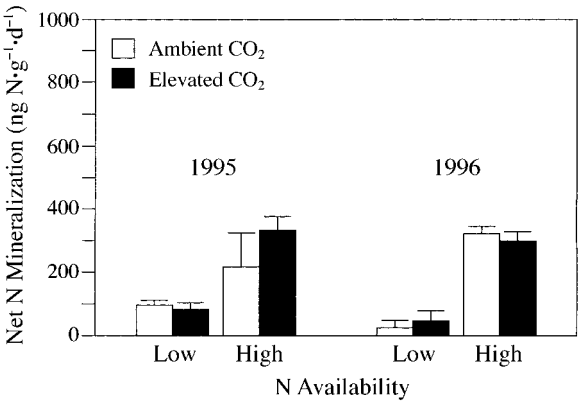

FIG. 4. Net rates of N mineralization in experimental atmospheric-CO_2 and soil-N-availability treatments. Net N mineralization was calculated as the difference between gross rates of N mineralization and NH_4^+ immobilization. (Note that microbial immobilization in Fig. 3 was calculated as the microbial assimilation of both NH_4^+ and NO_3^-.) Values are atmospheric-CO_2–soil-N-availability treatment means during the 1995 and 1996 field seasons. The length of each error bar is 1 SE ($n = 5$). The atmospheric-CO_2 × soil-N-availability × sampling-date interaction illustrated in this figure was not significant.

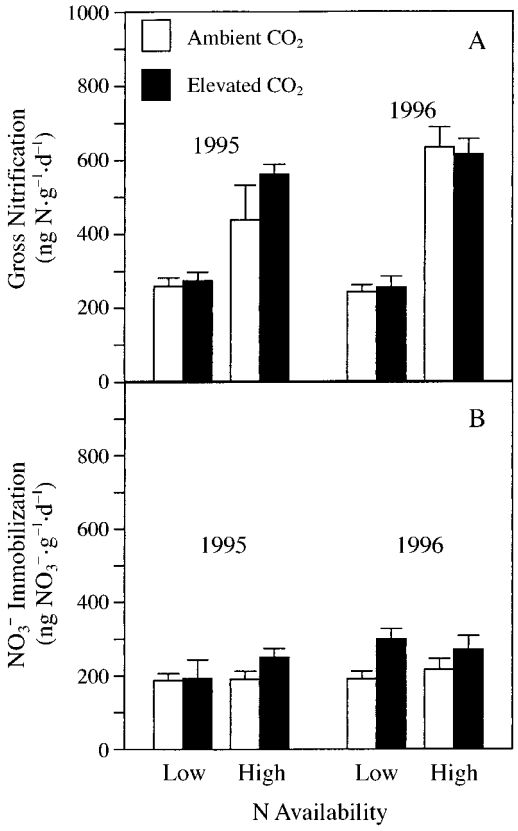

FIG. 5. (A) Gross rates of nitrification and NO_3^- immobilization in experimental atmospheric-CO_2 and (B) soil-N-availability treatments. Values are atmospheric-CO_2–soil-N-availability treatment means during the 1995 and 1996 field seasons. The length of each error bar is 1 SE ($n = 5$). The atmospheric-CO_2 × soil-N-availability × sampling-date interactions illustrated in this figure were not significant.

necessarily mean that elevated CO_2 will alter N availability to plants, especially in developing ecosystems where plant roots have not fully colonized the soil (C. J. Mikan, D. R. Zak, K. S. Pregitzer, and M. E. Kubiske, *unpublished manuscript*). For example, the amount of N plants acquire from soil is controlled by the uptake of N per unit of fine root, the volume of soil exploited by fine roots, and the rate at which N is released from soil organic matter (i.e., the balance between gross mineralization and microbial immobilization). Knowledge of each process is needed to assess whether changes in N flow through soil microbial biomass will indeed alter the amount of N available for plant uptake. In our experiment with *Populus tremuloides*, elevated atmospheric CO_2 and soil-N availability interacted to influence the amount of N plants acquired from soil. In high-N soil, elevated atmospheric CO_2 increased whole-plant N content by 17%, but it did not alter whole-plant N content in low-N soil. This paralleled the response of net fine-root production (Pregitzer et al. 1999), suggesting that elevated CO_2 enabled plants to more effectively forage for soil resources in high-N soil, a response also observed in *Pinus ponderosa* (Johnson et al. 1997). In low-N soil, it appears that greater C acquisition under elevated CO_2 did not facilitate greater root foraging. Furthermore, elevated CO_2 did not significantly influence gross N mineralization and microbial immobilization, suggesting that changes in plant N acquisition under elevated CO_2 resulted from changes in root foraging. Berntson and Bazzaz (1997) observed that greater root foraging under elevated CO_2 increased whole-plant N content, despite greater rates of microbial immobilization and lower rates of gross mineralization. In aggrading systems where plant roots have not fully occupied the soil volume, changes in both root foraging and soil-N transformations will control whether atmospheric CO_2 alters the acquisition of soil resources by plants.

Although elevated CO_2 did not significantly affect microbial immobilization in our study, several studies have observed greater rates of microbial immobilization under elevated CO_2 and concluded that soil N availability will decline (Berntson and Bazzaz 1997, 1998, Hungate et al. 1996). Greater rates of microbial immobilization under elevated CO_2 could decrease soil-N availability only if microbial biomass N or soil organic N increase to the same extent. Alternatively, greater rates of microbial immobilization could occur with greater soil-N availability, if microbial biomass does not change or increases slightly. In this scenario, greater turnover of N through microbial biomass (i.e., microbial biomass N divided by immobilization rate) would increase N availability to plants, assuming a small fraction of immobilized N entered soil organic matter (see Holmes and Zak 1994, 1999). Although many studies report that elevated CO_2 can increase soil microbial biomass (Berntson and Bazzaz 1997, Hungate et al. 1996, Rice et al. 1994, Zak et al. 1993*a*),

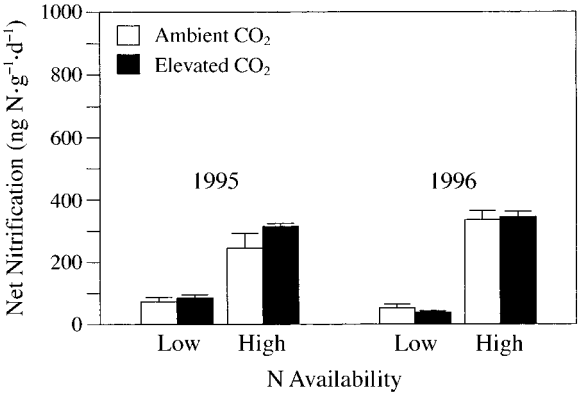

FIG. 6. Net nitrification in experimental atmospheric-CO_2 and soil-N-availability treatments. Values are atmospheric-CO_2–soil-N-availability treatment means during the 1995 and 1996 field seasons. The length of each error bar is 1 SE ($n = 5$). The atmospheric-CO_2 × soil-N-availability × sampling-date interaction illustrated in this figure was not significant.

few have reported concurrent changes in microbial biomass N and soil organic N, making it difficult to assess whether greater microbial immobilization will indeed lead to a decline in soil-N availability. Understanding of the physiologically linked changes in substrate availability, N immobilization, microbial biomass, and the incorporation of N into stable organic matter is needed to predict how changes in microbial activity under elevated CO_2 will alter soil-N availability.

Although elevated CO_2 can alter the nature and availability of organic substrates in soil, we have no evidence that such changes will, in the short term, alter the proportion of bacteria, actinomycetes, and fungi in soil. We found that changes in plant growth under elevated CO_2 did not alter the proportion of bacterial, actinomycetal, or fungal PLFAs (phospholipid fatty acids). In our previous experiment with *Populus grandidentata* (Zak et al. 1993*a*), changes in belowground production and substrate inputs to soil were relatively more important; nevertheless, they also did not alter the composition of soil microbial communities (Zak et al. 1996). In our present experiment, Gram-positive bacterial PLFAs significantly increased and Gram-negative bacterial PLFAs significantly declined from low- to high-N soil; actinomycetal and fungal PLFAs did not differ with soil-N availability. Given the relatively small input of plant substrates during our experiment, it is likely that differences in microbial communities related to our experimental soils resulted from initial differences in their physical and biochemical properties (see Curtis et al. 2000). However, the fact that rates of gross mineralization and microbial immobilization per unit of organic matter were more rapid in low (75 and 104 ng $C \cdot g^{-1} \cdot d^{-1}$, respectively) vs. high-N soil (39 and 35 ng $C \cdot g^{-1} \cdot d^{-1}$), in combination with differences in bacterial community composition, suggests a link between the community of soil bacteria and soil-N transformations.

Summary and implications

In our experiment, elevated CO_2 altered substrate availability for microbial metabolism by increasing net fine-root production and decreasing fine-root N concentration (Pregitzer et al. 2000). Although changes in substrate availability have the potential to alter the composition and function of soil microbial communities, elevated CO_2 did not alter the proportions of soil bacteria, actinomycetes, or fungi, nor did it alter the processes controlling the flow of N from soil microorganisms to plants (i.e., gross and net mineralization, immobilization, and nitrification). From our results, we conclude that changes in substrate availability, resulting from greater root production under elevated CO_2, were not sufficient to alter the influence of native soil organic matter on the composition and function of soil microbial communities. Thus, the results of our experiment do not support our hypothesis that greater belowground plant growth will increase rates of soil-N cycling, nor do they support the idea that rates of soil-N cycling will slow under elevated CO_2. Understanding how changes in the amount and chemistry of belowground C inputs alter substrate availability for microbial biosynthesis, the turnover of N through microbial biomass (biomass N divided by immobilization rate), and the incorporation of N into stable forms of soil organic matter is central to predicting whether elevated CO_2 will increase, decrease, or not alter soil-N availability in forest ecosystems. One cannot conclude that greater rates of microbial immobilization under elevated CO_2 will reduce N availability in terrestrial ecosystems without a better understanding of the biological and physical factors limiting amounts of microbial biomass and rates of organic-matter formation.

Although plants obtained greater amounts of N from high-N soil under elevated CO_2 (Zak et al. 2000), it is likely that greater N acquisition will cease once plant roots have fully exploited soil. The fact that rates of net N mineralization were unaltered by elevated CO_2 suggests that plants growing under elevated CO_2 will likely acquire the same amount of N at root closure as plants growing under ambient CO_2; however, plants under elevated CO_2 will do so at a more rapid rate. Johnson et al (1997) drew the same conclusion from a 3-yr study of Ponderosa pine (*Pinus ponderosa*) in which whole-plant N content increased under elevated CO_2, but rates of net N mineralization did not. Thus, it appears that greater belowground plant growth under elevated CO_2 will not influence soil-N availability in aggrading ecosystems where plant roots have not yet fully colonized the soil, but it will increase the rate at which plants acquire N from soil, at least in the short term. From our analysis of the literature, it appears that soil-N cycling will respond to elevated atmospheric CO_2 in ecosystems where root-associated C inputs are sufficient to modify the influence of native soil organic matter on microbial physiology. In forest ecosystems, this situation likely occurs during, or following, canopy closure aboveground and root closure belowground. Thus, understanding how elevated CO_2 will alter plant–microbe interactions in soil at this point in ecosystem development is of great importance for predicting the long-term response of forest ecosystems to rising atmospheric CO_2.

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