Microbial Ecology

Elevated Atmospheric CO_2 Alters Soil Microbial Communities Associated with Trembling Aspen (*Populus tremuloides*) Roots

Lori R. Janus[1], Nicholas L. Angeloni[1], John McCormack[1], Steven T. Rier[1,2], Nancy C. Tuchman[1,2] and John J. Kelly[1]

(1) Department of Biology, Loyola University Chicago, Chicago, Illinois 60626, USA
(2) The University of Michigan Biological Station, Pellston, Michigan 49769, USA

Received: 3 June 2004 / Accepted: 18 August 2004 / Online publication: 29 July 2005

Abstract

Global atmospheric CO_2 levels are expected to double within the next 50 years. To assess the effects of increased atmospheric CO_2 on soil ecosystems, cloned trembling aspen (*Populus tremuloides*) seedlings were grown individually in 1 m^3 open bottom root boxes under either elevated (720 ppm, ELEV) or ambient CO_2 (360 ppm, AMB). After 5 years, soil cores (40 cm depth) were collected from the root boxes and divided into 0–20 cm and 20–40 cm fractions. ELEV treatment resulted in significant decreases in both soil nitrate and total soil nitrogen in both the 0–20 cm and 20–40 cm soil fractions, with a 47% decrease in soil nitrate and a 50% decrease in total soil nitrogen occurring in the 0–20 cm fraction. ELEV treatment did not result in a significant change in the amount of soil microbial biomass. However, analysis of indicator phospholipid fatty acids (PLFA) indicated that ELEV treatment did result in significant increases in PLFA indicators for fungi and Gram-negative bacteria in the 0–20 cm fraction. Terminal restriction fragment length polymorphism (T-RFLP) analysis was used to analyze the composition of the soil bacterial communities (using primers targeting the 16SrRNA gene) and the soil fungal communities (using primers targeting the intergenic transcribed spacer region). T-RFLP analysis revealed shifts in both bacterial and fungal community structure, as well as increases in both bacterial and fungal species richness with ELEV treatment. These results indicated that increased atmospheric CO_2 had significant effects on both soil nutrient availability and the community composition of soil microbes associated with aspen roots.

Correspondence to: John J. Kelly; E-mail: jkelly7@luc.edu

Introduction

Global atmospheric CO_2 levels are presently accumulating at their highest rate [20] and are expected to double within the next 50 years [50] owing to the continued burning of fossil fuels and changes in land use patterns [1]. To predict the global and ecosystem-level effects of increased atmospheric CO_2, an understanding of the biological effects of CO_2 enrichment is needed. The impact of CO_2 enrichment on plants has been studied extensively for over 20 years. Researchers have demonstrated that elevated CO_2 can substantially affect the leaf chemistry of plants through increased carbon fixation [6, 41, 51], which can result in increased carbon allocation to structural and nonstructural carbohydrates, lignin, and secondary phenolic defense compounds like tannins, as well as an increase in the carbon to nitrogen ratio of leaves [e.g., 46].

Microbes are critical to the cycling of carbon and inorganic nutrients in soil ecosystems, and so insight into the soil microbial responses to elevated atmospheric CO_2 is critical for improving our understanding of global carbon and nutrient cycling [39]. Because the concentration of CO_2 in soil is 10 to 15 times higher than in the atmosphere, elevated atmospheric CO_2 would not be expected to have a direct effect on soil microorganisms [22]. Indeed, Koizumi et al. [27] observed no change in soil microbial activity with atmospheric CO_2 levels ranging from 100 ppm to 1000 ppm. However, elevated atmospheric CO_2 may affect the soil ecosystem indirectly through CO_2-induced plant responses that influence below-ground processes. For example, CO_2-induced biochemical changes in plant leaves slowed the decomposition of leaf litter by terrestrial microbes [1, 5, 8] and by microbes in stream ecosystems [38], suggesting that alterations in leaf litter

DOI: 10.1007/s00248-004-0120-9 • Volume 50, 102–109 (2005)

quality could have significant effects on nutrient cycling.

The physiological changes in plants caused by elevated CO_2 might also affect the interactions between plant roots and soil. It has been suggested that CO_2-stimulated plant growth might result in an increased uptake of mineral nutrients such as nitrogen from the soil, possibly leading to decreases in soil nitrogen [20], and data from several researchers has supported this theory [19, 21, 33]. CO_2 enrichment can also increase the input of organic carbon to soils by increasing plant root biomass [3, 32, 47, 56] as well as root exudates [35, 39]. Because plant roots are often the single largest source of organic carbon for soil microbial metabolism [4], increases in carbon inputs could have significant effects on the soil microbial communities. For example, several studies have reported significant increases in microbial biomass [37, 56] and microbial activity [37] in soils under elevated CO_2.

Most of the work on microbial responses to elevated CO_2 has focused on total microbial biomass or gross measures of microbial activity [26]. These quantitative measures do not provide insight into population dynamics within soil microbial communities. It has been demonstrated that changes in specific microbial populations can occur even when total microbial community biomass remains unchanged [36], suggesting that measures of microbial community composition may be more sensitive to disturbance than assays that quantify biomass or cumulative microbial activity [24]. Despite the ecological significance of microbial community composition, community assessment techniques have received limited application to the analysis of elevated CO_2 impacts [26].

This study examined the effects of atmospheric CO_2 concentrations predicted for the year 2050 on the chemical and biological properties of soil in the vicinity of roots of trembling aspen (*Populus tremuloides*). The hypothesis that growth of trembling aspen under elevated atmospheric CO_2 would result in a decrease in the concentration of inorganic soil nutrients, an increase in microbial biomass, and a shift in the composition of soil microbial communities, was tested. Two methods were used to analyze the composition of microbial communities; phospholipid fatty acid analysis (PLFA) and terminal restriction fragment length polymorphism analysis (T-RFLP). Both of these methods avoid the limitations of culture-based techniques by using signature molecules as indicators of microbial community composition, and both methods have been widely applied to the analysis of microbial community composition in complex habitats. In the current study T-RFLP was used to examine both the soil bacterial communities (using primers targeting the 16SrRNA gene) and the soil fungal communities (using primers targeting the intergenic transcribed spacer region).

Methods

Aspen Growth under Elevated Atmospheric CO_2. In June 1996, 16 cloned trembling aspen (*Populus tremuloides*) seedlings were individually planted in 1 m^3 open-bottom root boxes at the University of Michigan Biological Station (UMBS) in northern lower Michigan (45°34′N, 84°4034′W). Trembling aspen was chosen because it is the most abundant tree species in Michigan [40] and is easy to grow. The root boxes contained a soil homogenate of 80% native rubicon sand and 20% topsoil. Topsoil was added to augment the low nutrient levels of the native granitic sand. The soils were homogenized in a cement mixing truck and backfilled into the soil boxes. Each tree was enclosed within an above-ground clear plastic open-top chamber (1 m × 1 m × 2 m) and half of the trees were fumigated with 2× ambient CO_2 (ELEV = 720 ppm) while the other 8 trees were treated with unenriched air (AMB = 360 ppm) as per Tuchman et al. [46]. ELEV or AMB air was delivered to the trees between May and leaf senescence in November from 1996 to 2002, and leaves were collected and removed from the chambers after leaf senescence each year. During the treatment periods, all chambers were watered twice weekly with equal volumes provided to each tree. Well water from UMBS, which contained non-detectable levels of total inorganic nitrogen and orthophosphorus (R. VandeKopple, UMBS Resident Biologist, unpublished data), was used for watering.

Soil Sampling. Soils were sampled during leaf senescence in November 2001 after leaf and plant debris was removed from the soil surfaces. Three replicate soil samples were collected from each of three ELEV chambers and three AMB chambers. Soil samples were collected to a depth of 40 cm with a soil corer and immediately separated into 0–20 cm and 20–40 cm fractions. Root and plant debris was removed and each soil sample was well mixed. Subsamples of approximately 1 g were taken from each soil sample, placed on dry ice, and shipped to Loyola University where they were stored at −80°C. Bulk soil samples for soil nutrient analysis were shipped on wet ice and stored at 4°C.

Soil Analysis. In the laboratory all bulk soil samples were sieved through a 2-mm sieve and stored at 4°C. Soil moisture content was determined gravimetrically by drying at 100°C [15], organic matter content was determined by loss on ignition at 500°C [2], and pH was determined in a 1:1 soil:distilled water slurry [43]. Total nitrogen was extracted by acid digestion (EPA Method 3050) [11] and measured by the Kjeldahl method (EPA Method 351.2)[10]. Soil nitrate was determined by extraction with deionized water and measurement with a nitrate electrode [16]. Total phosphorous was extracted

by acid digestion (EPA Method 3050) [11], available phosphorous was extracted by the Mehlich 3 method [12], and phosphorous levels in both extracts were determined by inductively coupled plasma-atomicemission spectrometry (ICP-AES) (EPA Method 6010) [11]. Total microbial biomass was determined by measurement of total phospholipids using the Lipid Phosphate Assay [13]. Soil data were analyzed by analysis of variance (ANOVA) with the SPSS software package (SPSS Inc., Chicago, IL).

PLFA Analysis. Fatty acids were extracted from all soil samples using a modification of the method of White et al. [52] as previously described [25]. Fatty acids were prepared according to the MIDI protocol [31] and analyzed using the MIDI Sherlock Microbial 19 Identification System (MIDI Inc., Newark, DE). Fatty acids with carbon chain lengths between 9 and 20 carbons were identified. Individual phospholipid fatty acid (PLFA) values were expressed as a percentage of the total PLFAs in the sample. The total PLFA data set was analyzed by principal component analysis (PCA) with the SAS software package (SAS Institute Inc., Cary, NC). Signature PLFAs were analyzed by ANOVA with the SPSS software package (SPSS Inc).

T-RFLP Analysis

PCR for Bacterial Community Analysis. Soil genomic DNA and DNA of a bacterial reference culture (*Pseudomonas aeruginosa* PAO1) were isolated with the UltraClean Soil DNA Kit (MoBio Laboratories, Salana Beach, CA). DNA was amplified with polymerase chain reaction (PCR) using primers 8F and 926R [29] targeting the 16S rRNA gene. Primer 926R was synthesized by Operon, Inc. (Alameda, CA) and 8F was synthesized and labeled at the 5′ end with dye IRD-800 by LI-COR, Inc. (Lincoln, NE). Each 25 μL PCR reaction contained 0.4 μM forward primer, 4.0 μM reverse primer, 200 μM deoxynucleoside triphosphates (Amersham Biosciences, Piscataway, NJ), 0.2 $mgmL^{-1}$ bovine serum albumin (Amersham Biosciences), 1× PCR buffer (Promega, Madison, WI), 2.5 mM $MgCl_2$ (Promega), 1.5 units of Taq DNA polymerase (Promega), and 1.0 μL of template DNA. For these samples and this primer pair, a 1:10 dilution of DNA extract was found to be optimal for amplification. The PCR reactions were run in a DNA thermal cycler (PTC-100, MJ Research, Waltham, MA) with the following cycling parameters; initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 15 s, annealing at 56.4°C for 20 s, and extension at 72°C for 30 s, followed by a final extension step for 3 min at 72°C. Four replicate reactions were run for each soil sample and the products were pooled.

PCR for Fungal Community Analysis. Soil genomic DNA and DNA of a fungal reference culture (*Cortinarius multiformis*) were isolated with the UltraClean Soil DNA Kit (MoBio Laboratories). DNA was amplified with PCR primers targeting the intergenic transcribed spacer region, ITS1-F and ITS4 [14]. ITS4 was synthesized by Operon and ITS1-F was synthesized and labeled at the 5′ end with dye IRD-800 by LI-COR. The PCR reactions were set up as described above for bacterial community analysis. Cycling parameters were as descibed previously [14]. Four replicate reactions were run for each sample and the products were pooled.

PCR Product Cleanup and Digestion. The PCR products were purified with the UltraClean PCR Cleanup DNA Purification Kit (MoBio Laboratories) and quantified based on analysis of agarose gel band intensities with Quantity One Software (BioRad, Hercules, CA). The PCR products (25 ng) were digested individually with *Msp*I, *Alu*I, and *Hae*III (New England BioLabs, Beverly, MA) according to the manufacturer's instructions. To verify complete digestion, DNA isolated from pure culture controls was amplified and digested in parallel with soil samples.

T-RFLP Analysis. After denaturation at 94°C for 2 min, digested DNA samples were electrophoresed on a 5.5% acrylamide gel in DNA Sequencer Model 4000L (LI-COR). The size and intensity of the TRFs were determined with Quantity One Software (BioRad). Fungal and bacterial T-RFLP data were analyzed separately by PCA with the SAS software package (SAS Institute, Inc.). For PCA analysis, each individual, terminal restriction fragment (TRF) was scored as present or absent and analyzed as binary data [26], and only TRFs that were present in at least four samples were included. For both the bacterial and fungal data sets, the scores for each sample on PC1 and PC2 were analyzed by ANOVA with the SPSS software package (SPSS Inc).

Results

CO_2 enrichment resulted in no significant changes in soil moisture content, pH, or levels of organic matter as compared to AMB in either the 0–20 cm or the 20–40 cm fractions (Table 1). Soil pH decreased significantly with depth in both treatments ($P < 0.0001$), whereas moisture content and organic matter content showed no change with depth (Table 1). Total soil phosphorous showed no CO_2 treatment effect, and no change with depth (Table 1). Extractable phosphorous showed no change with CO_2 treatment but did increase significantly with depth ($P < 0.0001$; Table 1). Total soil nitrogen decreased significantly with ELEV treatment by 50% in the 0–20 cm fraction ($P < 0.05$) and 34% in the 20–40 cm soil fractions ($P < 0.05$; Fig. 1A). Soil nitrate also decreased significantly with ELEV treatment by 47% in the 0–20 cm

Table 1. Soil physical and chemical parameters

CO_2 treatment	*Depth (cm)*	*Moisture content (%)*	*pH*	*Organic carbon (%)*	*Total P (mg kg^{-1})*	*Extractable P (mg kg^{-1})*
AMB	0–20	14.7 ± 0.9	7.5 ± 0.1[a]	2.9 ± 0.1	642 ± 108	15.3 ± 63[a]
ELEV	0–20	14.9 ± 2.0	7.5 ± 0.1[a]	2.9 ± 0.1	579 ± 137	22.8 ± 5.6[a]
AMB	20–40	13.9 ± 0.7	6.4 ± 0.1[b]	2.9 ± 0.1	582 ± 103	34.1 ± 1.3[b]
ELEV	20–40	14.3 ± 0.8	6.3 ± 0.1[b]	3.1 ± 0.0	783 ± 216	36.9 ± 1.3[b]

Different letters indicate significant differences ($P < 0.0001$) based on ANOVA ($n = 3$).

fraction ($P < 0.05$) and 20% in the 20–40 cm soil fraction ($P < 0.05$; Fig. 1B).

Microbial biomass was not significantly altered by CO_2 enrichment in either the 0–20 cm or the 20–40 cm fractions (Fig, 2). However, within the ELEV soils, there was a significant ($P < 0.01$) decrease in microbial biomass with depth, which was not seen in the AMB soils (Fig. 2).

Phospholipid fatty acid data was used to look for shifts in major microbial groups within the soils based on depth and/or CO_2 treatment. Principle component analysis of PLFA data did not reveal shifts in PLFA profiles with treatment or depth (data not shown). However, analysis of several indicator PLFAs did reveal shifts in specific microbial populations. Based on previously published data, four PLFAs were used as indicators for major microbial groups: 18:2 ω6,9c [18] and 18:1ω9c [54] for fungi, 15:0 iso for Gram-positive bacteria [28], and 18:1ω7c for Gram-negative bacteria [28]. The data for these indicator PLFAs revealed that, as a percentage of the total microbial community, fungal and Gram-negative bacterial PLFAs decreased significantly with depth ($P < 0.001$; Fig. 3), and Gram-positive bacterial PLFAs increased significantly with depth ($P \leq 0.01$) (Fig. 3). Indicator PLFAs also revealed shifts in microbial community composition with CO_2 treatment in the 0–20 cm fraction, where both fungal PLFAs (18:2ω6,9c and 18:1ω9c) and the Gram-negative bacterial PLFA (18:1ω7c) increased significantly ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively) with CO_2 enrichment (Fig. 3).

The T-RFLP analyses revealed some significant differences in the number of TRFs observed for both fungi and bacteria. The total number of fungal TRFs increased significantly with ELEV treatment by 51 % in the 20–40 cm fraction ($P < 0.05$), but showed no treatment effect in the 0–20 cm fraction (Fig. 4A). The total number of bacterial TRFs increased significantly with ELEV treatment by 37% in the 0–20 cm fraction ($P < 0.002$) and 5% in the 20–40 cm fraction ($P > 0.05$; Fig. 4B).

Principal component analysis of fungal T-RFLP data revealed a shift in fungal community composition with CO_2 treatment (Fig. 5A). ANOVA indicated a significant effect of the elevated CO_2 treatment on PC1 ($P < 0.01$), but no significant effect of depth on PC1 or PC2. Principal component analysis of bacterial T-RFLP data also revealed strong shifts in bacterial community composition with CO_2 treatment and with depth (Fig. 5B). ANOVA indicated significant effects of elevated CO_2 treatment on PC_2 ($P < 0.01$) and significant effects of depth on both PC1 ($P < 0.05$) and PC2 ($P < 0.05$). The fact that the elevated CO_2 treatment did not have a significant impact on PC1 indicates that depth had a more significant effect on bacterial community composition than the CO_2 treatment.

Discussion

Previous phytochemical analyses of the *Populus tremuloides* seedlings used in this study demonstrated that CO_2 enrichment resulted in significant increases in C:N ratio and an increase in the levels of lignin and secondary phenolic defense compounds like tannins [38, 44–46]. The results of the present study indicated that the 5-year seasonal CO_2 treatment also had a significant impact on the soil ecosystems in which these *Populus tremuloides* seedlings were grown. The increase in atmospheric CO_2 resulted in large decreases in soil nitrate

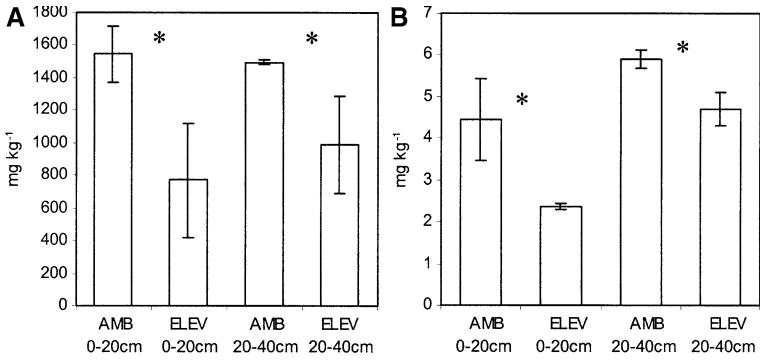

Figure 1. Total Kjeldahl nitrogen (A) and extractable nitrate (B) in soil samples. Each data point represents mean of three replicate chambers ± 1 S.E. Asterisks designate significant differences ($P < 0.05$) between AMB and ELEV treatments within soil depths.

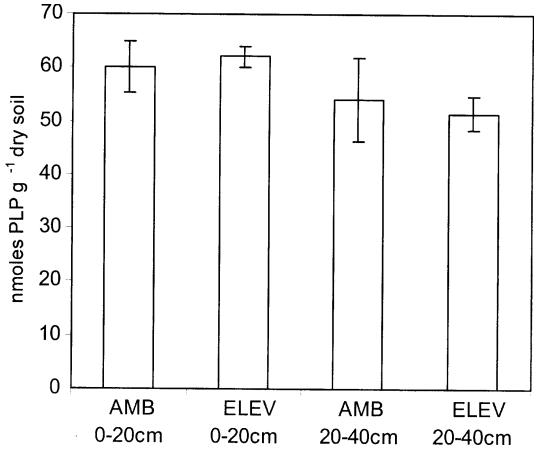

Figure 2. Total microbial biomass as measured by the total lipid phosphate method. Biomass indicated as nmoles phospholipid phosphate per gram of dry soil. Each data point represents the mean of three replicate chambers ± 1 S.E.

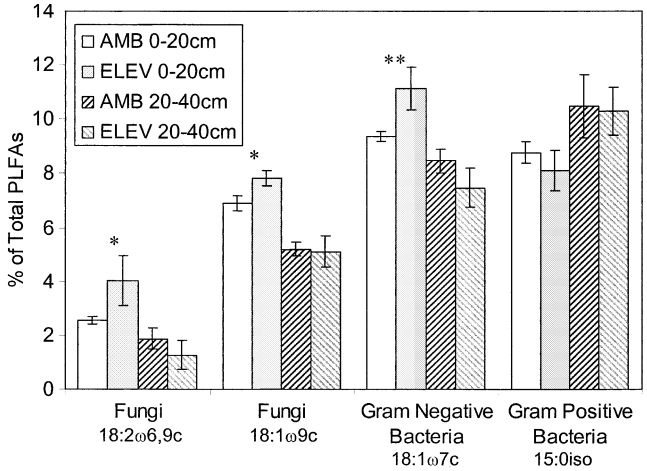

Figure 3. Levels of indicator phospholipid fatty acids. Each data point represents the mean of three replicate chambers ± 1 S.E. Asterisks designate significant differences between AMB and ELEV treatments ($^*P < 0.05$; $^{**}P < 0.01$).

and total soil nitrogen that were most pronounced in the soil fraction closest to the soil surface (0–20 cm). These decreases in soil nitrate and total nitrogen are extremely important because net primary productivity in most soil ecosystems is nitrogen limited [48]. Decreases in soil nitrogen could thus have negative impacts on biological productivity in both natural and agricultural ecosystems and could necessitate the increased use of nitrogen fertilizer in agricultural soils. These decreases in soil nitrogen could also have effects on the global carbon cycle. It has been suggested that as atmospheric CO_2 levels continue to rise, carbon sequestration in soils might increase [5]. Thus soils could serve as a valuable sink for carbon, helping to moderate the impacts of increased CO_2 emissions. However, if soil nitrogen is decreased by elevated CO_2, as was observed in this study, this could severely limit biological productivity which could limit the ability of soils to sequester carbon.

The elevated CO_2 treatment also had significant impacts on the soil microbial community. Although the ELEV treatment did not have a significant effect on the biomass of the soil microbial community (Fig. 2), the PLFA data indicated that the ELEV treatment resulted in a shift in the composition of the communities. The PLFA data indicated a significant increase in fungi as a percentage of the overall microbial community in the 0–20 cm soil fraction (Fig. 3). Other researchers have also reported increases in fungi with elevated CO_2 based on ergosterol content [26] and plate counts [17]. Zak et al. [55] did not observe an increase in fungal PLFAs when a tree species from the same genus, *Populus grandidentata*, was grown under elevated CO_2 for one growing season. However, when *Populus tremuloides* were grown under elevated CO_2 for two growing seasons, Zak et al. [54] did report an increase in fungal PLFA 18:2ω6,9c. These results suggest that the CO_2-induced increase in fungal PLFAs observed in our study and by Zak et al. [54] may have been slow to develop.

The increase in fungi with elevated CO_2 documented in the present study may be connected to the observed decreases in total and available soil nitrogen. Nitrogen limitation tends to favor fungi over bacteria because fungal biomass has a higher C:N ratio and because fungal hyphae are efficient at nitrogen uptake [19]. The increase

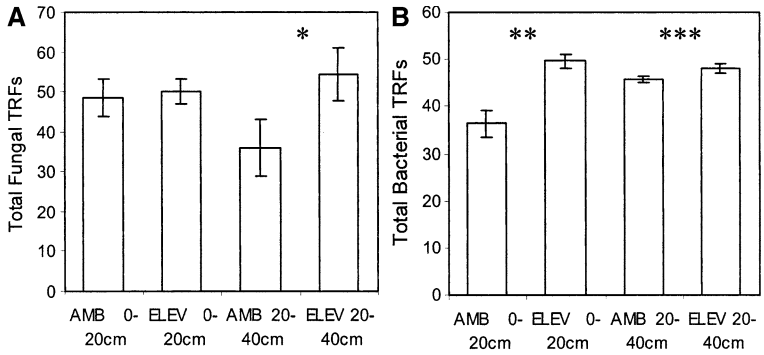

Figure 4. Total number of terminal restriction fragments (TRFs) observed using fungal ITS primers (A) and bacterial 16S rRNA primers (B). Each data point represents the mean of three replicate chambers ± 1 S.E. Asterisks designate significant differences between AMB and ELEV treatments within soil depths ($^*P < 0.05$; $^{**}P < 0.002$; $^{***}P < 0.05$).

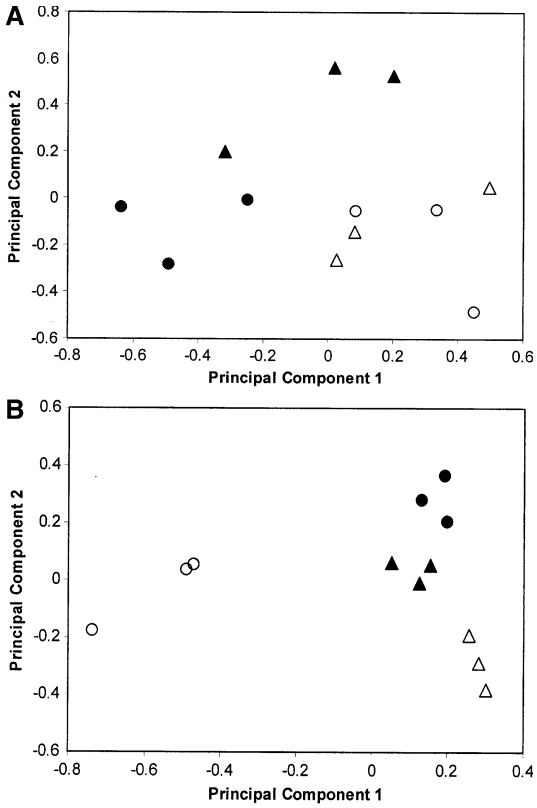

Figure 5. Principal component analysis of T-RFLP data, open circle: AMB 0–20 cm; solid circle: ELEV 0–20 cm; open triangle: AMB 20–40 cm; solid triangle: ELEV 20–40 cm. Fungal data (A): principal component 1 accounted for 19.9% of the variability in the data. Principal component 2 accounted for 14.7% of the variability in the data. Bacterial data (B): principal component 1 accounted for 41.7% of the variability in the data. Principal component 2 accounted for 17.0% of the variability in the data.

in fungi may also be related to an increase in plant root biomass. Several previous studies have demonstrated increased plant root biomass [3, 7, 32] and increased fine root production [7, 53] under elevated CO_2 conditions. These increases in root biomass and fine root production would be expected to favor the growth of mycorrhizal fungi, which form symbiotic relationships with plant roots, and indeed several researchers have observed increased mycorrhizal infection of plant roots [9, 34] and increased arbuscular mycorrhizal root mass [30] under elevated CO_2. The increase in fungi may also be related to lignin production by the trees. Lignin, an integral component of plant cell walls, is a complex aromatic polymer, and fungi are the major microbial group involved in lignin degradation [49]. Lignin is a significant component of root biomass, so an increase in root biomass would result in more lignin in the soil, which would favor the growth of fungi. An increase in fungi as a percentage of the microbial community could be significant to ecosystem function, as fungi play important roles in organic matter degradation, nutrient cycling, and the formation of soil aggregates. Mycorrhizal fungi in particular contribute to nutrient and water uptake by plant roots [42], so increases in these fungi could increase the uptake of these resources from the soil.

Although the PLFA data did not indicate any change in fungal community size in the 20–40 cm fraction (Fig. 3), the fungal T-RFLP data indicated that there was a significant increase in fungal species richness in the 20–40 cm soil fraction, as reflected by the increased number of TRFs observed (Fig. 4A). Although the T-RFLP assay generally provides an underestimate of biodiversity, as it is biased toward the numerically dominant organisms, the number of TRFs produced for a set of samples is a useful indicator of changes in species richness [26]. Principle component analysis of the fungal T-RFLP data also indicated that the ELEV treatment caused a shift in the composition of the fungal communities in both the 0–20 cm and the 20–40 cm soil fractions (Fig. 5A). These shifts in the size, species richness, and composition of the fungal communities may be related to the decrease in soil nitrogen levels or to possible changes in soil root biomass. These shifts suggest that the altered soil conditions caused by ELEV treatment resulted in the stimulation and/or inhibition of certain subsets of the fungal community. Klamer et al. [26] recently used the T-RFLP technique to examine the impact of elevated CO_2 on soil fungal communities in a scrub oak ecosystem, and they did not see an effect of the CO_2 treatment on fungal community composition after 3 years. The difference in results between our study and the Klamer et al. study [26] may be related to the difference in the duration of CO_2 treatment (5 years and 3 years, respectively) or to differences in the ecosystems studied.

The PLFA data also indicated that the ELEV treatment resulted in an increase in Gram-negative bacteria as a percentage of the overall microbial community in the 0–20 cm soil fraction (Fig. 3). An increase in Gram-negative bacterial PLFAs was not observed in previous work by Zak et al. [54], who used the same tree species in very similar soils, but as discussed above, their samples were collected after two growing seasons whereas our samples were collected after five growing seasons, suggesting that this shift in bacterial populations may have been slow to develop. The observed increase in Gram-negative bacteria may be related to an increase in root biomass, as the growth of Gram-negative bacteria is favored in the rhizosphere [42] and the Gram-negative bacterial PLFA (18:1ω7c) has been shown previously to be higher in rhizosphere soils than in non-rhizopshere soils [55].

The bacterial T-RFLP data showed an increase in bacterial species richness in both the 0–20 cm and 20–40 cm soil fractions, as reflected by the increased number of TRFs observed (Fig. 4B). This increased diversity may be related to the increase in Gram-negative bacteria, as an increase in root biomass may have favored the prolifer-

ation of a variety of different Gram-negative bacteria. Principle component analysis of the bacterial T-RFLP data also revealed shifts in bacterial community composition with CO_2 treatment In both the 0–20 cm and 20–40 cm soil fractions (Fig. 5B). These community shifts revealed by PCA may be related to the observed differences in richness, as the lowest richness samples (AMB 0–20 cm) are clearly separated from the other samples on PC1. However, the separation of samples observed in the PCA cannot be completely explained by richness differences, because when all of the samples are examined there is no significant correlation between richness and PC scores (data not shown). In addition, ANOVA indicated significant effects of both elevated CO_2 treatment and depth on the PC scores.

Conclusions

The results of this study demonstrated that a long-term increase in atmospheric CO_2 had significant effects on both soil nitrogen levels and soil microbial community composition. These findings suggest that rising levels of atmospheric CO_2 could have significant impacts on soil ecosystems as well as on global carbon and nutrient cycling.

Acknowledgments

Soil analyzed in this project were collected at the Elevated CO_2 Research Facility of the University of Michigan Biological Station, where infrastructural support was provided by the U.S. DOE National Institute of Global Environmental Change (NIGEC). This research was supported, in part, by grants awarded to N.C.T. from the National Science Foundation (DEB-9903888 and DEB-0108847) and by a supplement to grant DEB-0108847 awarded to N.C.T. and J.J.K. from the National Science Foundation. The authors thank M. J. Kirisits for assistance with the T-RFLP technique, M. Haggblom for use of the MIDI system, M. Parsek for generously providing the *Pseudomonas aeruginosa* culture, and L. Edgerton-Warburton for generously providing the *Cortinarius multiformis* tissue.

Reference

1. Ball, AS, Drake, BG (1997) Short-term decomposition of litter produced by plants grown in ambient and elevated atmospheric CO2 concentrations. Glob Change Biol 3: 29–35
2. Bear, FE (1955) Chemistry of the Soil. Reinhold Publishing Co., New York
3. Cardon, ZG, Hungate, BA, Cambardella, CA (2001) Contrasting effects of elevated CO_2 on old and new soil carbon pools. Soil Biol Biochem 33: 365–373
4. Coleman, DC, Andrews, R, Ellis, JE, Singh, JS (1976) Energy-flow and partitioning in selected man-managed and natural ecosystems. Agro-Ecosystems 3: 45–54
5. Cotrufo, MF, Ineson, P (1996) Elevated CO_2 reduces field decomposition rates of *Betula pendula* (Roth) leaf litter. Oecologia 106: 525–530
6. Curtis, PS, Teeri, JA (1992) Seasonal responses of leaf gas-exchange to elevated carbon-dioxide in *Populus grandidentata*. Can J For Res 22: 1320–1325
7. Curtis, PS, Zak, DR, Pregitzer, KS, Lussenhop, J, Teeri, JA (1996) Linking above-ground and below-ground responses to rising CO_2 in northern deciduous forest species. In: Koch, GW, Mooney, HA (Eds.) Carbon Dioxide and Terrestrial Ecosystems. Academic Press, San Diego, CA, pp 41–51
8. De Angelis, P, Chigwerewe, KS, Mugnozza, GES (2000) Litter quality and decomposition in an CO_2-enriched Mediterranean forest ecosystem. Plant Soil 224: 31–41
9. Dhillion, SS, Roy, J, Abrams, M (1996) Assessing the impact of elevated CO_2 on soil microbial activity in a Mediterranean model ecosystem. Plant Soil 187: 333–342
10. Environmental Protection Agency (1993) Methods for the Determination of Inorganic Substances in Environmental Samples. EPA/600/R-93/100
11. Environmental Protection Agency (1996) Test Methods for Evaluating Solid Waste: Physical/Chemical Methods. EPA SW-846, Chapter 3
12. Frank K, Beegle D, Denning, J (1998) Phosphorus. In: Brown, JR (Ed.) Recommended Chemical Soil Test Procedures for the North Central Region. University of Missouri-Columbia, Columbia, MO, pp 21–30
13. Frostegard, A, Tunlid, A, Baath, E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. J Microbiol Meth 14: 151–163
14. Gardes, M, Bruns, TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2: 113–118
15. Gardner, WH (1986) Water Content. In: Klute, A (Ed.) Methods of Soil Analysis, Part I. Soil Science Society of America, Madison, WI, pp 493–544
16. Gelderman RH, Beegle, D (1998) Nitrate-nitrogen. In: Brown, JR (Ed.) Recommended Chemical Soil Test Procedures for the North Central Region. University of Missouri-Columbia, Columbia, MO, pp 17–20
17. Grayston, SJ, Campbell, CD, Lutze, JL, Gifford, RM (1998) Impact of elevated CO_2 on the metabolic diversity of microbial communities in N-limited grass swards. Plant Soil 203: 289–300
18. Guckert, JB, Antworth, CP, Nichols, PD, White, DC (1985) Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol Ecol 31: 147–158
19. Hu, S, Chapin, FS, Firestone, MK, Field, CB, Chiarello, NR (2001) Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. Nature 409: 188–191
20. Hungate, BA, Holland, EA, Jackson, RB, Chapin, FS, Mooneyk, HA, Field, CB (1997) The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388: 576–579
21. Insam, H, Baath, E, Berreck, M, Frostegard, A, Gerzabek, MH, Kraft, A, Schinner, F, Schweiger, P, Tschuggnall, G (1999) Responses of the soil microbiota to elevated CO_2 in an artificial tropical ecosystem. J Microbiol Meth 36: 45–54
22. Kandeler, E, Tscherko, D, Bardgett, RD, Hobbs, PJ, Kampichler, C, Jones, TH (1998) The response of soil microorganisms and roots to elevated CO_2 and temperature in a terrestrial model ecosystem. Plant Soil 202: 251–262
23. Keeling, CD, Whort, TP (1999) Atmospheric CO_2 records from sites in the SIO air sampling network. In: Trends: Compendium of Data on Global Change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN
24. Kennedy, AC, Smith, KL (1995) Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170: 77–86

25. Kelly, JJ, Haggblom, M, Tate, RL (1999) Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. Soil Biol Biochem 31: 1455–1465
26. Klamer, M, Roberts, MS, Levine, LH, Drake, BG, Garland, JL (2002) Influence of elevated CO_2 on the fungal community in a coastal scrub oak forest soil investigated with terminal-restriction fragment length polymorphism analysis. Appl Environ Microbiol 68: 4370–4376
27. Koizumi, H, Nakadai, T, Usami, Y, Satoh, M, Shiyomi, M, Oikawa, T (1991) Effect of carbon dioxide concentration on microbial respiration in soil. Ecol Res 6: 227–232
28. Konopka, A, Zakharova, T, Bischoff, M, Oliver, L, Nakatsu, C, Turco, RF (1999) Microbial biomass and activity in lead-contaminated soil. Appl Environ Microbiol 65: 2256–2259
29. Liu, W, Marsh, TL, Cheng, H, Forney, LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63: 4516–4522
30. Lussenhop, J, Treonis, A, Curtis, PS, Teeri, JA, Vogel, CS (1998) Response of soil biota to elevated atmospheric CO_2 in poplar model systems. Oecologia 113: 247–251
31. MIDI (1995) Sherlock Microbial Identification System Operating Manual: Version 5. MIDI Inc., Newark, DE
32. Mikan, CJ, Zak, DR, Kubisker, ME, Pregitzer, KS (2000) Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. Oecologia 124: 432–445
33. Niklaus, PA, Kandeler, E, Leadley, PW, Schmid, B, Tscherko, D, Korner, C (2001) A link between plant diversity, elevated CO_2, and soil nitrate. Oecologia 127: 540–548
34. O'Neill, EG, Luxmoore, RJ, Norby, RJ (1987) Increases in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO_2 atmosphere. Can J For Res 17: 878–883
35. Norby, RJ, O'Neill, EG, Hood, WG, Luxmoore, RJ (1987) Carbon allocation, root exudation, and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO_2 enrichment. Tree Physiol 3: 203–210
36. Pennanen, T (2001) Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH — a summary of the use of phospholipid fatty acids. Biolog and H-3-thymidine incorporation methods in field studies. Geoderma 100: 91–126
37. Rice, CW, Garcia, FO, Hampton, CO, Owensby, CE (1994) Soil microbial response in tallgrass prairie to elevated CO_2. Plant Soil 165: 67–74
38. Rier, ST, Tuchman, NC, Wetzel, RG, Teeri, JA (2002) Elevated-CO_2-induced changes in the chemistry of quaking aspen (*Populus tremuloides* Michaux) leaf litter: subsequent mass loss and microbial response in a stream ecosystem. J North Am Benthol Soc 21: 16–27
39. Sadowsky, MJ, Shortemeyer, M (1997) Soil microbial responses to increased concentrations of atmospheric CO_2. Glob Change Biol 3: 217–224
40. Schmidt, TL, Spencer, JS, Bertsch, R (1993) Michigan's forests 1993: an analysis. U.S. Department of Agriculture, Forest Service, North Central Experiment Station. Resource Bulletin NC-179
41. Strain, BR, Bazaaz, FA (1983) Terrestrial plant communities. In: Lemon, EH (Ed.) The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide (AAAS Selected Symposium 8), Washington, DC, pp 117–222
42. Tate, RL (1995) Soil Microbiology. John Wiley and Sons, Inc. New York
43. Thomas, GW (1996) Soil ph and soil acidity. In: Sparks, D (Ed.) Methods of Soil Analysis. Part II. Soil Science Society of America, Madison, WI
44. Tuchman, NC, Wahtera, KA, Wetzel, RG, Russo, NM, Kilbane, GM, Sasso, LM, Teeri, JA (2003) Nutritional quality of leaf detritus altered by elevated atmospheric CO_2: effects on development of mosquito larvae. Freshwater Biol 48: 1432–1439
45. Tuchman, NC, Wahtera, KA, Wetzel, RG, Teeri, JA (2003) Elevated atmospheric CO_2 alters leaf litter quality for stream ecosystems: an *in-situ* leaf decomposition study. Hydrobiology 495: 203–211
46. Tuchman, NC, Wetzel, RG, Rier, ST, Wahtera, KA, Teeri, JA (2002) Elevated atmospheric CO_2 lowers leaf litter nutritional quality for stream ecosystem food webs. Global Change Biol 8: 145–153
47. van Ginkel, JH, Gorissen, A, Polci, D (2000) Elevated atmospheric carbon dioxide concentration: effects of increased carbon input in a Lolium perenne soil on microorganisms and decomposition. Soil Biol Biochem 32: 449–456
48. Vitousek, PM, Howarth, RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13: 87–115
49. Wagner, GH, Wolf, DC (1999) Carbon transformation and soil organic matter formation. In: Sylvia, DM, Fuhrmann, JJ, Hartel, PG, Zuberer, DA (Eds.) Principles and Applications of Soil Microbiology. Prentice-Hall, Upper Saddle River, NJ
50. Watson, RT, Miera-Filho, LG, Sanjueza, E, Janetos, A (1992) Greenhouse gases: sources and sinks. In: Houghton, JT, Callander, BA, Varney, SK (Eds.) Climate Change 1992: The Supplementary Report to the IPCC Scientific Assessment. University Press, Cambridge, UK, pp 25–46
51. Wetzel, RG, Grace, JB (1983) Atmospheric CO_2 enrichment effects on aquatic plants. In: Lemon, EH (Eds.) The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide. American Association for the Advancement of Science. DC, Washington, pp 223–280
52. White, DC, Davis, WM, Nickels, JS, King, JD, Bobbie, RJ (1979) Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40: 51–62
53. Wiemken, V, Laczko, E, Ineichen, K, Boller, T (2001) Effects of elevated carbon dioxide and nitrogen fertilization on mycorrhizal fine roots and the soil microbial community in beech-spruce ecosystems on siliceous and calcareous soil. Microb Ecol 42: 126–135
54. Zak, DR, Pregitzer, KS, Curtis, PS, Holmes, WE (2000) Atmospheric CO_2 and the composition and function of soil microbial communities. Ecol Appl 10: 47–59
55. Zak, DR, Ringelberg, DB, Pregitzer, KS, Randlett, DL, White, DC, Curtis, PS (1996) Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecol Appl 6: 257–262
56. Zak, DR, Pregitzer, KS, Curtis, PS, Teeri, JA, Fogel, R, Randlett, DL (1993) Elevated atmospheric CO_2 and feedbacks between carbon and nitrogen cycles. Plant Soil 151: 105–117