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Soil nitrogen transformations under *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* following 3 years exposure to elevated CO_2 and O_3

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Abstract

Increases in atmospheric CO_2 and tropospheric O_3 may affect forest N cycling by altering plant litter production and the availability of substrates for microbial metabolism. Three years following the establishment of our free-air CO_2–O_3 enrichment experiment, plant growth has been stimulated by elevated CO_2 resulting in greater substrate input to soil; elevated O_3 has counteracted this effect. We hypothesized that rates of soil N cycling would be enhanced by greater plant productivity under elevated CO_2, and that CO_2 effects would be dampened by O_3. We found that elevated CO_2 did not alter gross N transformation rates. Elevated O_3 significantly reduced gross N mineralization and microbial biomass N, and effects were consistent among species. We also observed significant interactions between CO_2 and O_3: (i) gross N mineralization was greater under elevated CO_2 ($1.0\,mg\,N\,kg^{-1}\,day^{-1}$) than in the presence of both CO_2 and O_3 ($0.5\,mg\,N\,kg^{-1}\,day^{-1}$) and (ii) gross NH_4^+ immobilization was also greater under elevated CO_2 ($0.8\,mg\,N\,kg^{-1}\,day^{-1}$) than under CO_2 plus O_3 ($0.4\,mg\,N\,kg^{-1}\,day^{-1}$). We used a laboratory ^{15}N tracer method to quantify transfer of inorganic N to organic pools. Elevated CO_2 led to greater recovery of NH_4^+-^{15}N in microbial biomass and corresponding lower recovery in the extractable NO_3^- pool. Elevated CO_2 resulted in a substantial increase in NO_3^--^{15}N recovery in soil organic matter. We observed no O_3 main effect and no CO_2 by O_3 interaction effect on ^{15}N recovery in any soil pool. All of the above responses were most pronounced beneath *Betula papyrifera* and *Populus tremuloides*, which have grown more rapidly than *Acer saccharum*. Although elevated CO_2 has increased plant productivity, the resulting increase in plant litter production has yet to overcome the influence of the pre-existing pool of soil organic matter on soil microbial activity and rates of N cycling. Ozone reduces plant litter inputs and also appears to affect the composition of plant litter in a way that reduces microbial biomass and activity.

Keywords: Acer, *Betula*, CO_2, immobilization, mineralization, ^{15}N, N cycling, O_3, *Populus*

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Introduction

The concentrations of carbon dioxide (CO_2) and ozone (O_3) in the Earth's troposphere are expected to increase over the next century due to fossil fuel burning and the conversion of forests to other land uses (Keeling *et al*., 1995; Finlayson-Pitts & Pitts, 1997; Fowler *et al*., 1998; Stevenson *et al*., 1998). The combined effects of greater CO_2 and O_3 on ecosystem carbon (C) and nitrogen (N) cycling are difficult to predict, because CO_2 and O_3 affect plant growth in opposing ways. While greater CO_2 stimulates photosynthesis and the growth of many temperate tree species (Curtis, 1996; Curtis & Wang, 1998), greater O_3 could decrease photosynthesis and the growth of some species (Wang *et al*., 1986; Coleman *et al*., 1995a; Karnosky *et al*., 1998) by inducing foliar damage and accelerating leaf senescence (Karnosky, 1976; Karnosky *et al*., 1996). Further complicating the matter is the fact that tree species differ in their

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responsiveness to CO_2 and in their susceptibility to O_3 damage. Thus, forest net primary productivity may be enhanced by increasing atmospheric CO_2 (DeLucia *et al*., 1999), but it is uncertain how a concomitant increase in tropospheric O_3 will alter plant productivity, litter production and, in turn, ecosystem C and N cycling.

Rates of soil N cycling may be altered by the influence of elevated CO_2 and O_3 on plant litter production, which controls substrate availability for heterotrophic microbial metabolism. There is considerable uncertainty regarding how changes in microbial activity under elevated CO_2 will influence soil N cycling (Zak *et al*., 2000a), and we know little of the relationship between plant response to elevated O_3 and soil microbial activity (Islam *et al*., 2000). We have been studying the influence of elevated CO_2 and O_3 on the growth of ecologically contrasting temperate tree species at the Forest–Atmosphere Carbon Storage and Transfer (FACTS-II) FACE experiment near Rhinelander, WI, USA. Our results to date suggest that changes in plant production resulting from our treatments have altered the input and metabolism of plant litter by soil microbial communities. Elevated CO_2 has increased the production of leaf and fine root litter (King *et al*., 2001; Lindroth *et al*., 2001), which has resulted in greater rates of microbial respiration and increased activities of extracellular enzymes involved with the degradation of plant and fungal cell wall components (i.e. cellobiohydrolase and *N*-acetylglucosamidase; Larson *et al*., 2002; Phillips *et al*., 2002). The magnitude of this response varied between early-successional (i.e. *Populus tremuloides* and *Betula papyrifera*) and late-successional (*Acer saccharum*) tree species, but the presence of elevated O_3 eliminated the influence of elevated CO_2 on microbial activity. A greater metabolic capability to degrade plant and fungal litter under elevated CO_2 suggests that microbial communities have a greater biosynthetic demand for N, which could potentially decrease N availability to plants and increase the movement of N into soil organic matter. However, this may be counteracted by elevated O_3.

Our primary objective was to determine how elevated CO_2 and O_3 alter the cycling of N among soil solution, microbial communities, and soil organic matter. We measured gross N transformation rates and traced inorganic ^{15}N transfer into microbial biomass and organic matter in soil beneath stands of *P. tremuloides*, *B. papyrifera*, and *A. saccharum* growing under free-air CO_2 and O_3 enrichment treatments. Because the growth response of these temperate trees varies under elevated atmospheric CO_2 and O_3, we reasoned that the changes in microbial activity and soil N cycling would be governed by the response of the dominant vegetation to our experimental treatments.

Methods

Experimental design and field sampling

We studied soil ^{15}N transformations at the FACTS-II FACE experiment (45°40.5′N, 89°37.5′E, 490 m elevation; Karnosky *et al*., 1999; Dickson *et al*., 2000). The site consists of 12–30 m diameter plots planted in 1997 with trembling aspen (*P. tremuloides* Michx.), paper birch (*B. papyrifera* Marsh.), and sugar maple (*A. saccharum* Marsh.). One-half of each plot is planted with five aspen genotypes that differ in O_3 sensitivity and leaf phenology (Coleman *et al*., 1995a, b; Curtis *et al*., 2000). One-quarter of each plot is planted with aspen and sugar maple, and the remaining quarter is planted with aspen and paper birch. All sections were planted at a density of 0.95 stems m^{-2}, providing a total of 670 stems per 30 m diameter plot. Our study was confined to the central plot area. Soils are Alfic Haplorthods with a sandy loam Ap horizon overlaying a sandy clay loam Bt horizon. Soil physical and chemical properties have been summarized by Dickson *et al*. (2000).

Factorial CO_2 and O_3 treatments were arranged in a randomized complete block design with three replicates (blocks). The split-plot effect consisted of the three sections differing in species composition (aspen, aspen–birch, and aspen–maple) within each plot. Fumigation gases are delivered to 32 vertical vent pipes spaced uniformly around the perimeter of each 30 m diameter plot. The control system maintains target CO_2 and O_3 concentrations by regulating the release of fumigation gases from the upwind vent pipes. Fumigation occurs during the daylight hours for the duration of the growing season. In 1999, atmospheric CO_2 concentrations were 346.5 L L^{-1} in the ambient CO_2 plots and 547.8 L L^{-1} in the elevated CO_2 plots. Atmospheric O_3 concentrations were 36.9 and 51.7 nL L^{-1} in the ambient and elevated plots, respectively (Dickson *et al*., 2000).

In July 1999, we collected six soil cores (2.5 cm *D*) to a depth of 10 cm from each split plot. Samples were combined by split plot, stored on ice, and were processed within 24 h of collection. All results are expressed on an oven-dry (105 °C) mass basis.

Soil N transformations

We determined gross rates of N mineralization and nitrification (Davidson *et al*., 1992; Hart *et al*., 1994) and traced inorganic ^{15}N from soil solution into microbial biomass and soil organic matter. Soils were prepared by removing fine roots and coarse woody debris by hand and passing the remaining material through a 2 mm

mesh. Soil adhering to roots was removed during sorting and returned to the original sample, which was homogenized. From each soil sample, precisely 12.00 g ($\pm$0.02 g) was weighed into each of five glass vials (29 mm $D \times$ 94 mm H). Two soil samples were enriched with precisely 1.00 mL ($\pm$0.01 mL) ^{15}N-NH_4Cl solution, two were enriched with the same volume of ^{15}N-KNO_3 solution, and the remaining sample received 1.00 mL deionized water. The ^{15}N solutions were prepared using a mixture of ^{15}N-labeled (99.5%) and unlabeled N, which produced similar target concentrations of N (2–5 g N g^{-1} soil) and ^{15}N (2–4 at.% excess ^{15}N) in both $^{15}NH_4^+$- and $^{15}NO_3^-$-labeled soil. Isotope label solutions were added by pipetting evenly over the soil surface. The volume of the solution was adequate to disperse the label evenly throughout each 12 g sample, as indicated by uniform wetting of the entire soil volume, and to bring the soil samples to field capacity. Within 1 h after isotope addition, one of the pair of $^{15}NH_4^+$-enriched samples and one of the pair of $^{15}NO_3^-$-enriched samples were extracted with 2 M KCl and samples amended with deionized water were also extracted. The remaining $^{15}NH_4^+$- and $^{15}NO_3^-$-enriched samples were incubated at 20 °C for 2 days, after which we extracted inorganic N with 2 M KCl.

We devised a sequential extraction procedure to isolate tracer ^{15}N within each soil N pool. In the first step, inorganic N (NH_4^+ and NO_3^-) and dissolved organic nitrogen (DON) were separated from microbial N and soil organic N. Twenty milliliters of 2 M KCl was added to each sample in its original vial. The vials were capped, placed on a shaker for 20 min, and centrifuged for 15 min at 800 rpm. The supernatant was decanted into a 60 mL plastic syringe equipped with a 0.45 m filter attachment. The extraction was repeated with a second 20.0 mL aliquot of 2 M KCl. Particulate organic matter and suspended cells were removed from the solutions by forcing them through the 0.45 m filter. The filter from each syringe was placed back into the vial containing the corresponding soil. Filtrates were stored in 120 mL specimen cups at 4 °C prior to isotopic analysis.

A second extraction step was performed to separate microbial N and soil organic N. Vials containing soils and filters were fumigated with CH_3Cl for 5 days in a vacuum desiccator. Residual CH_3Cl was removed by repeated vacuuming (eight times) and 20.0 mL of 0.5 N K_2SO_4 was added to each vial. Vials were capped, placed on a shaker for 30 min, and centrifuged for 15 min at 800 rpm. The supernatant was decanted into a 120 mL specimen cup. This extraction was repeated with an additional 20.0 mL aliquot of 0.5 N K_2SO_4 and the extracts were frozen until they were digested to determine microbial N. The soil samples remaining in the vials were dried to a constant weight at 60 °C in a forced-air oven. The dried soils were transferred to grinding jars and pulverized with stainless steel pins (0.5 cm $D \times$ 13.8 cm L) in a roller mill (Model 755RMV, US Stoneware, East Palestine, OH, USA), and stored for analysis of organic N.

Ammonium-N and NO_3^--N concentrations in KCl extracts were measured with a Flow Solution 3000 continuous flow analyzer (OI Analytical, College Station, TX, USA). Ammonium-N and NO_3^--N were sequentially diffused from the KCl extracts onto acid traps in preparation for ^{15}N analysis using the method of Brooks *et al*. (1989). The acid traps were analyzed for atom% ^{15}N on a Finnigan Delta Plus isotope ratio mass spectrometer with a Conflo II interface (Thermo Finnigan, San Jose, CA, USA).

Microbial N within the K_2SO_4 extracts was determined by alkaline persulfate digestion (Cabrera & Beare, 1993). Blanks and glycine standards were digested simultaneously with samples. Nitrate-N concentrations in the digestates were measured with a continuous flow analyzer. Nitrate-N in the digested solutions was captured onto acid traps during a 5-day diffusion with MgO and Devarda's alloy. The acid traps were analyzed for atom% ^{15}N by isotope ratio mass spectrometry.

Soil organic N concentration was measured using a CE Instruments NC2500 elemental analyzer (CE Elantech, Lakewood, NJ, USA). Atom% ^{15}N in soil organic N was determined by isotope ratio mass spectrometry. We used the resulting N concentration and atom% ^{15}N data to calculate the recovery of ^{15}N label within inorganic N, microbial N, and soil organic N pools following the 2 day incubation. For each pool, we calculated atom% ^{15}N excess (APE) using atom% ^{15}N of the same pool in the water-amended control as background. We calculated the rates of gross N mineralization and nitrification and rates of gross N immobilization using the isotope pool dilution equations of Hart *et al*. (1994).

Microbial biomass and soil organic C and N

We measured microbial biomass using the chloroform fumigation–extraction procedure (Horwath & Paul, 1994). Soil samples were fumigated with chloroform for 5 days in a vacuum dessicator. Fumigated and control soils were extracted with 0.5 N K_2SO_4 (in a ratio of 4 : 1). Organic N in the extracts was determined by alkaline persulfate digestion followed by analysis of NO_3^--N on a continuous flow analyzer, as described above. Soil organic C and N concentrations were measured using an elemental analyzer, as described above.

Statistical analyses

We tested the influence of elevated CO_2, O_3, and species composition on all pools, fluxes, and ^{15}N recoveries using an analysis of variance (ANOVA) for a randomized complete block, split-plot design with two factorial treatments, as described by King *et al*. (2001). In this design, species composition splits the main plot. When a particular *F*-test was significant, we compared means using Tukey's HSD multiple comparison procedure. The results were accepted as significant at $P < 0.10$.

Results

Gross N transformation rates

We observed a significant interaction between CO_2 and O_3 on rates of gross N mineralization, wherein O_3 significantly reduced rates of N mineralization that occurred under elevated CO_2 (compare elevated CO_2 vs. elevated CO_2 + elevated O_3 means); NH_4^+ immobilization displayed an identical response (Table 1). Gross N mineralization was significantly reduced under elevated O_3 ($P = 0.09$, main effect), but was not influenced by CO_2 (main effect). The effect of O_3 on gross N mineralization was consistent among species and effects were most pronounced in aspen. The gross rates of nitrification and immobilization of NO_3^- were not affected by CO_2, O_3, or their interactions (Table 1). Species composition and the interaction of species composition with CO_2 or O_3 had no significant effects on any of the gross N transformations that we measured.

Distributions of ^{15}N among soil pools

The total recovery of NH_4^+-^{15}N averaged 88.3 ± 8.19% and did not differ among experimental treatments (Table 2). Elevated CO_2 (main effect) significantly increased the recovery of NH_4^+-^{15}N in microbial biomass ($P = 0.05$) and significantly reduced the recovery of NH_4^+-^{15}N in the NO_3^- pool ($P = 0.10$) relative to the ambient CO_2 treatment. Ozone (main effect) did not have a significant effect on the recovery of NH_4^+-^{15}N in any soil pool. The interaction of CO_2 and O_3 also had no effect on NH_4^+-^{15}N recovery.

The recovery of NH_4^+-^{15}N in soil organic N, microbial biomass, and NO_3^- pools differed by species, such that species with greater recovery in the soil organic N and microbial biomass pools had lower recoveries in the NO_3^- pool. Recoveries of NH_4^+-^{15}N in the pools of microbial biomass ($P < 0.01$) and soil organic N ($P = 0.05$) were significantly greater in aspen compared with aspen–maple (Table 2). The recovery of NH_4^+-^{15}N in the NO_3^- pool was significantly lower in aspen and aspen–birch than in aspen–maple ($P < 0.01$, Table 2).

We observed significant species-by-O_3 interactions, indicating that the effect of O_3 on NH_4^+-^{15}N recoveries in the NO_3^- pool and soil organic N pool differed by species (data not shown). In aspen, O_3 led to a higher

Table 1 Gross rates of N mineralization, nitrification, and microbial immobilization of NH_4^+ and NO_3^- measured in a 2-day ^{15}N pool dilution experiment

	Interaction means							
	Ambient O_3		Elevated O_3		Main effect means			
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient O_3	Elevated O_3
	$mg\,N\,kg^{-1}\,day^{-1}$				$mg\,N\,kg^{-1}\,day^{-1}$			
Gross N mineralization	0.8ab (0.41)	1.0a (0.53)	0.8ab (0.25)	0.5b (0.27)	0.8 (0.33)	0.8 (0.48)	0.9 (0.47)	0.6* (0.29)
Gross nitrification	0.8 (0.29)	1.0 (0.38)	1.0 (0.39)	0.6 (0.22)	0.9 (0.35)	0.8 (0.36)	0.9 (0.34)	0.8 (0.37)
NH_4^+ immobilization	0.5ab (0.33)	0.8a (0.44)	0.7ab (0.35)	0.4b (0.17)	0.6 (0.34)	0.6 (0.40)	0.6 (0.40)	0.5 (0.30)
NO_3^- immobilization	0.1 (0.23)	0.2 (0.30)	0.1 (0.13)	0.2 (0.26)	0.1 (0.18)	0.2 (0.27)	0.2 (0.26)	0.2 (0.20)

Values are means with standard deviations in parentheses. Significant differences among interaction means are indicated by letters. Means followed by the same letter do not differ significantly ($P < 0.10$). Significant CO_2 or O_3 main effects are denoted by asterisks (*$P < 0.10$; **$P < 0.05$).

Table 2 Main effects of CO_2, O_3, and species composition on recoveries of ^{15}N following a 2-day laboratory incubation of soils amended with either ^{15}N-labeled NH_4^+ or ^{15}N-labeled NO_3^-

	Ambient CO_2	Elevated CO_2	Ambient O_3	Elevated O_3	Aspen only	Aspen–birch	Aspen–maple
	%		%		%		
$^{15}NH_4^+$ amended soils							
Extractable NH_4^+	0.9	0.6	0.7	0.7	0.4	0.7	1.0
	(0.98)	(1.28)	(1.21)	(1.08)	(0.65)	(1.15)	(1.46)
Extractable NO_3-	35.9	27.8*	34.2	29.6	26.7a	29.29a	39.6b
	(9.04)	(11.73)	(9.60)	(12.30)	(11.09)	(11.14)	(6.71)
Microbial biomass N	5.0	7.0**	5.7	6.4	6.8a	6.1ab	5.3b
	(0.88)	(1.92)	(1.05)	(2.28)	(1.84)	(1.92)	(1.35)
Soil organic N	49.0	51.7	47.7	53.0	53.2a	52.1ab	45.6b
	(7.49)	(10.38)	(9.10)	(8.37)	(10.76)	(6.80)	(7.78)
Total ^{15}N recovery	90.3	86.3	87.9	88.6	87.0	87.8	90.1
	(8.01)	(8.08)	(9.19)	(7.31)	(8.75)	(8.47)	(7.72)
$^{15}NO_3$ amended soils							
Extractable NH_4^+	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	(0.07)	(0.02)	(0.04)	(0.06)	(0.02)	(0.05)	(0.07)
Extractable NO_3^-	78.7	68.9	71.8	76.6	71.3ab	67.7a	82.5b
	(14.33)	(14.98)	(14.55)	(16.06)	(14.69)	(17.21)	(10.26)
Microbial biomass N	3.6	3.9	3.8	3.7	3.6	4.1	3.6
	(1.65)	(2.15)	(2.36)	(1.21)	(1.71)	(2.24)	(1.78)
Soil organic N	7.8	14.2*	10.3	11.3	11.4ab	14.2a	7.1b
	(6.23)	(9.60)	(10.12)	(6.57)	(7.22)	(11.47)	(4.96)
Total ^{15}N recovery	90.1	87.5	86.0	91.7	86.5a	86.6a	93.3b
	(11.00)	(8.25)	(8.25)	(10.36)	(9.38)	(9.33)	(9.45)

Values are mean recoveries (with standard deviations) of ^{15}N in pools of extractable NH_4^+ and NO_3^-, microbial biomass N, and soil organic N. Significant CO_2 or O_3 main effects are denoted by asterisks (*$P<0.10$; **$P<0.05$). Species main effect means not followed by the same letter are significantly different ($P<0.10$).

recovery of NH_4^+-^{15}N in soil organic N and a lower recovery in the NO_3^- pool. In contrast, no such trends were observed in aspen–birch. Although these species-by-O_3 interactions were significant, multiple comparisons did not indicate significant O_3 effects *within* any given species composition (i.e. the only significant contrasts were across species).

The total recovery of NO_3^--^{15}N averaged $88.8 \pm 9.67\%$ and differed by species composition only ($P = 0.02$, Table 2). Elevated CO_2 (main effect) significantly increased the recovery of NO_3^--^{15}N in soil organic N ($P = 0.08$). There was a corresponding decrease in the recovery of NO_3^--^{15}N in the NO_3^- pool, but it was not significant ($P = 0.16$). Ozone (main effect) and the interaction of CO_2 and O_3 had no effect on the recovery of NO_3^--^{15}N in any soil pool.

The distribution of NO_3^--^{15}N among soil pools differed by species. The recovery of NO_3^--^{15}N in the pool of soil organic N was greater in aspen–birch compared with aspen–maple ($P = 0.10$, Table 2). The recovery of NO_3^--^{15}N in the NO_3^- pool was lower in aspen–birch than in aspen–maple ($P<0.02$, Table 2).

We observed significant species-by-O_3 interaction effects on NO_3^--^{15}N recoveries in the pools of NO_3^-, microbial biomass, and soil organic N. In aspen, elevated O_3 led to a greater recovery of NO_3^--^{15}N in microbial biomass and soil organic N and a lower recovery in NO_3^-. In aspen–birch, the effect of O_3 was the reverse – there was greater recovery of NO_3^--^{15}N in NO_3^- and lower recovery in microbial biomass and soil organic N. However, within-species contrasts were not significant.

Microbial biomass and soil C and N

Microbial biomass averaged $35.9 \pm 9.43\,mg\,N\,kg^{-1}$ over all plots and was not affected by the interaction of CO_2 and O_3 or the main effect of CO_2. However, the main effect of O_3 was significant ($P = 0.10$). Microbial biomass was 15% lower under elevated O_3 ($32.9 \pm 7.12\,mg\,N\,kg^{-1}$) compared with ambient O_3 ($38.9 \pm 10.64\,mg\,N\,kg^{-1}$); this effect was consistent among species. Soil organic C and N were not significantly influenced by CO_2, O_3, species composition, or

their interactions. Soil organic C averaged 18.0 ± 3.39 g C kg^{-1} and soil organic N averaged 1.45 ± 0.231 g N kg^{-1} across all treatments.

Discussion

Plant growth at the FACTS II FACE site has been strongly influenced by elevated atmospheric CO_2 and O_3 (Isebrands *et al*., 2001; Karnosky *et al*., 2003; Sober *et al*., 2003). In 1999, fine root production and mortality were significantly greater under elevated CO_2 compared with ambient CO_2, and the effects of CO_2 were counteracted by elevated O_3 (King *et al*., 2001). Others have observed similar CO_2 and O_3 effects on cellulolytic and chitinolytic enzyme activity by soil microbial communities (Larson *et al*., 2002; Phillips *et al*., 2002), suggesting that changes in fine root turnover have altered belowground substrate availability for microbial metabolism.

Nevertheless, we found no significant main effect of elevated CO_2 on microbial biomass N or gross rates of N transformations. In cases where we observed significant CO_2-by-O_3 interaction effects, there were no differences between the control and elevated CO_2 treatments. This suggests that the effects of elevated CO_2 alone (no O_3) on plant biomass production and substrate inputs to soil were not sufficient to overcome the influence of the native pool of soil organic matter on microbial activity. This result is consistent with previous studies of gross N transformation rates beneath aspen exposed to elevated CO_2 in open-top chambers for a similar length of time (Zak *et al*., 2000b). Our experiment consists of aggrading stands of young trees, which still display a substantial growth response to CO_2. The effect of CO_2 on soil N transformations could become significant if this growth response continues. Our results differ from observations at the Duke FACE site, which consists of loblolly pine stands at more advanced stages of development. There, the response plant growth to CO_2 diminished 3 years following initiation of exposure to elevated CO_2 (Oren *et al*., 2001). In our study, we have no evidence to suggest that N availability will initially limit the CO_2-induced growth response in our experiment.

Ozone had significant negative effects on microbial biomass and N transformation rates. The influence of O_3 was most distinct in the elevated CO_2 treatments, wherein O_3 significantly reduced gross N mineralization (−47%), gross NH_4^+ immobilization (−54%), and microbial biomass (−23%). This suggests that O_3 substantially modified the effect of CO_2 on plant litter production. There is some evidence that O_3 reduced aspen dead root production under elevated CO_2 (King *et al*., 2001), but we have yet to determine whether O_3 altered the chemical composition of belowground litter inputs in a way that would affect N transformation rates. Gross nitrification rates were unaffected by CO_2 or O_3 and were approximately equal to gross N mineralization rates across treatments and species. Thus, any N mineralized from organic matter and not taken up by plants is rapidly nitrified.

The tree species that we studied differ in life-history traits, litter production and biochemistry, and responsiveness to elevated CO_2 and O_3. We hypothesized that the response of microbial activity would parallel species-specific changes in plant growth under elevated CO_2 and O_3. In general, the response of plant production to CO_2 and O_3 was least in aspen–maple (Karnosky *et al*., 2003); the N cycling responses that we observed display a similar pattern. The responses of gross N mineralization, NH_4^+ immobilization, and microbial biomass to elevated O_3 and the interaction of CO_2 and O_3 were similar in aspen, aspen–birch, and aspen–maple, but the magnitudes of responses were greatest in aspen and aspen–birch. Thus, at this point, we have no evidence that soil N cycling beneath the tree species in our experiment will respond differently to elevated CO_2 and O_3.

In our ^{15}N tracer experiment, the majority of ^{15}N added as NH_4^+ was incorporated into soil organic matter (50%) or converted to NO_3^- (32%) during the 2-day laboratory incubation. A small portion (6%) was recovered in soil microbial biomass. Microbial biomass is a dynamic pool and its ^{15}N enrichment may not reflect the net amount of ^{15}N that has passed through it during the incubation. Consequently, we cannot determine what proportion of the ^{15}N recovered in soil organic N is attributable to microbial immobilization or whether the high ^{15}N recoveries in soil organic matter resulted from abiotic N immobilization (Nömmik, 1965; Nömmik & Vahtras, 1982; Fog, 1988; Johnson *et al*., 2000).

Elevated CO_2 led to a 40% increase in recovery of NH_4^+-^{15}N in microbial biomass and a corresponding decrease in NH_4^+-^{15}N recovery in the NO_3^- pool. This likely resulted from greater substrate input under elevated CO_2. Greater heterotrophic N demand may have led to reduced NH_4^+ availability to autotrophic nitrifying bacteria and, thus, reduced ^{15}N recovery in the NO_3^- pool. Greater heterotrophic N demand reflects an increase in gross NH_4^+ immobilization under elevated CO_2, although the increase we observed in this rate was not significant.

Most of the ^{15}N added to soil in the form of NO_3^- was unmodified (74%), and the remainder was divided between microbial biomass (4%) and soil organic matter (11%). The recovery of NO_3^--^{15}N in soil organic N

increased by 100% in the aspen and aspen–birch, and by 50% in aspen–maple, in both elevated CO_2 and elevated $CO_2 + O_3$ treatments. Greater transfer of NO_3^--^{15}N to soil organic N under elevated CO_2 (no O_3) could result from greater plant litter inputs to soil (King *et al.*, 2001). However, greater transfer of NO_3^--^{15}N to soil organic N under elevated $CO_2 + O_3$ cannot be explained by greater substrate inputs to soil and, thus, is more likely due to a change in the chemical composition of belowground litter inputs.

We found that elevated CO_2, both alone and in combination with elevated O_3, caused greater transfer of N from inorganic pools to organic pools. This could occur if the turnover of organic pools increased or if organic pools accumulated N. We found no significant increases in microbial N or soil organic N under elevated CO_2, but it could take several years for such changes to become detectable. Our gross N mineralization and immobilization results indicate that microbial N turnover is greater under elevated CO_2 alone than under elevated $CO_2 + O_3$. Thus, greater transfer of inorganic N to organic pools under elevated $CO_2 + O_3$ is not likely due to greater microbial N turnover, but may have resulted from changes in plant litter biochemistry or microbial community composition. Greater N accumulation in organic pools could be a sign of reduced N availability under elevated $CO_2 + O_3$, but this may or may not further limit plant productivity under O_3.

From our analyses, we conclude that tropospheric O_3 is an important factor modifying the response of both plants and soil microorganisms to elevated atmospheric CO_2. We observed similar N cycling responses beneath ecologically distinct tree species, which were consistent with their individual growth responses to elevated CO_2 and O_3. The changes that we observed in microbial biomass, gross N mineralization, and the transfer of N into soil pools appear to be driven by CO_2 and O_3 effects on plant production and the production and chemical composition of plant litter. These results indicate that changes in plant growth due to climate change cascade rapidly through the ecosystem to influence microbial activity in soil. Understanding how changes in microbial activity will alter the long-term availability of N, which will feed back on plant growth responses to atmospheric CO_2 and O_3, remains elusive. To accomplish this, we need to understand the factors controlling the formation and degradation of soil organic matter over time scales longer than the duration of most current experiments (i.e. 3–6 years). Many of our most critical questions can only be addressed through continued long-term field research in conjunction with mechanistic models of ecosystem C and N dynamics.

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