

# Elevated CO<sub>2</sub> and O<sub>3</sub> Alter Soil Nitrogen Transformations beneath Trembling Aspen, Paper Birch, and Sugar Maple

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## ABSTRACT

Nitrogen cycling in northern temperate forest ecosystems could change under increasing atmospheric CO<sub>2</sub> and tropospheric O<sub>3</sub> as a result of quantitative and qualitative changes in plant litter production. At the Aspen Free Air CO<sub>2</sub>-O<sub>3</sub> Enrichment (FACE) experiment, we previously found that greater substrate inputs to soil under elevated CO<sub>2</sub> did not alter gross N transformation rates in the first 3 years of the experiment. We hypothesized that greater litter production under elevated CO<sub>2</sub> would eventually cause greater gross N transformation rates and that CO<sub>2</sub> effects would be nullified by elevated O<sub>3</sub>. Following our original study, we continued measurement of gross N transformation rates for an additional four years. From 1999 to 2003, gross N mineralization doubled, N immobilization increased 4-fold, but changes in microbial biomass N and soil total N were not detected. We observed year-to-year variation in N transformation rates, which peaked during a period of foliar insect damage. Elevated

CO<sub>2</sub> caused equivalent increases in gross rates of N mineralization (+34%) and NH<sub>4</sub><sup>+</sup> immobilization (+36%). These results indicate greater rates of N turnover under elevated CO<sub>2</sub>, but do not indicate a negative feedback between elevated CO<sub>2</sub> and soil N availability. Elevated O<sub>3</sub> decreased gross N mineralization (-16%) and had no effect on NH<sub>4</sub><sup>+</sup> immobilization, indicating reduced N availability under elevated O<sub>3</sub>. The effects of CO<sub>2</sub> and O<sub>3</sub> on N mineralization rates were mainly related to changes in litter production, whereas effects on N immobilization were likely influenced by changes in litter chemistry and production. Our findings also indicate that concomitant increases in atmospheric CO<sub>2</sub> and O<sub>3</sub> could lead to a negative feedback on N availability.

**Key words:** *Acer saccharum*; *Betula papyrifera*; Carbon dioxide; FACE; Gross N immobilization; Gross N mineralization; Microbial biomass; Nitrogen cycling; Ozone; *Populus tremuloides*.

## INTRODUCTION

The concentrations of atmospheric carbon dioxide (CO<sub>2</sub>) and tropospheric ozone (O<sub>3</sub>) are increasing at rates that could lead to half of the Earth's forest

ecosystems becoming exposed to both elevated CO<sub>2</sub> and O<sub>3</sub> by the end of this century (Fowler and others 1999). These gases, acting in concert or independently, have the potential to alter carbon (C) and nitrogen (N) cycling in forest ecosystems through their influences on plant growth and litter production. It is difficult to predict ecosystem-level responses because CO<sub>2</sub> responsiveness and O<sub>3</sub>

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sensitivity vary widely both within and among tree species (Karnosky and others 2003). Combined responses could result in positive, negative, or no net effect on growth, as has been observed among clones of aspen (*Populus tremuloides* Michx., McDonald and others 2002). Elevated CO<sub>2</sub> stimulates photosynthesis and plant growth in many temperate tree species (Ceulemans and Mousseau 1994; Curtis and Wang 1998) leading to greater production of leaf and root litter (Cotrufo and Ineson 1996; Cotrufo and others 1998; Allen and others 2000; Wan and others 2004). Elevated O<sub>3</sub> damages photosynthetic tissues and accelerates leaf senescence in O<sub>3</sub>-sensitive species and genotypes, causing reduced plant growth and above- and below ground litter production (Karnosky and others 1996; Bortier and others 2000; Isebrands and others 2001; Noormets and others 2001). Through impacts on plant physiology and growth, elevated CO<sub>2</sub> and O<sub>3</sub> can change plant litter inputs to soil and alter soil microbial processes in a manner that may lead to feedback effects on N availability.

Soil microbial responses to changes in substrate inputs are critical in determining whether N availability will limit plant growth responses to elevated CO<sub>2</sub>. New inputs of organic matter can increase or decrease mineralization of N from the existing pool of soil organic matter and lead to positive or negative long-term feedbacks on N availability to plants (Zak and others 1993; Cheng 1999). The direction of the response depends on the composition of plant and microbial communities, the amount and chemical composition of litter, site conditions (for example, nutrient availability, moisture, and temperature), and duration of CO<sub>2</sub> exposure. Site-to-site variation in these factors could explain why highly variable N cycling responses have been observed (Zak and others 2000a). For example, at the FACTS-I FACE Experiment at the Duke Forest, elevated CO<sub>2</sub> has caused no change in gross or net N mineralization rates (Finzi and Schlesinger 2003), despite significant changes in litter production (Allen and others 2000). By contrast, in a scrub-oak open-top chamber study, elevated CO<sub>2</sub> reduced N mineralization and increased N immobilization, causing a negative feedback on plant growth (Hungate and others 1999).

At the FACTS-II Aspen FACE Experiment in northern Wisconsin, elevated CO<sub>2</sub> has enhanced tree growth and increased the production of leaf and root litter, whereas co-exposure to elevated O<sub>3</sub> has nullified the effect of CO<sub>2</sub> (King and others 2001; Percy and others 2002; R. Lindroth unpub-

lished data). In 1999, three years following tree planting and establishment of the experiment, we observed little effect of elevated CO<sub>2</sub> or O<sub>3</sub> on soil N transformations (Holmes and others 2003). Nonetheless, greater plant litter production under elevated CO<sub>2</sub> has elicited microbial responses, including greater respiration and extracellular enzyme activity (Larson and others 2001; Phillips and others 2001). We hypothesized that (1) with increasing plant growth differential between elevated and ambient CO<sub>2</sub> over time, greater litter production would fuel greater microbial metabolism and increase N turnover in soil; (2) litter produced under elevated CO<sub>2</sub> and CO<sub>2</sub>+O<sub>3</sub> with a relatively lower N concentration (Lindroth and others 2001), would increase N immobilization due to greater microbial N demand; and (3) CO<sub>2</sub> effects on gross N mineralization and immobilization would be counteracted by O<sub>3</sub>. Herein we report gross rates of N mineralization, nitrification, NH<sub>4</sub><sup>+</sup> immobilization, and NO<sub>3</sub><sup>-</sup> immobilization and pools of microbial N and soil total N measured for 4 years (2000–2003) subsequent to our original measurements (1999). Results from all years are combined to facilitate analysis of year-to-year variation in rates, pools, and treatment effects.

## METHODS

### Experimental Design and Field Sampling

We studied soil N transformations over four growing seasons at the Forest-Atmosphere Carbon Transfer and Storage (FACTS-II) FACE experiment near Rhineland, WI (45° 40.5' N, 89° 37.5' E, 490 m elevation; Karnosky and others 1999; Dickson and others 2000). The site was established in 1997 and consists of twelve 30-m diameter plots planted with trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.), and sugar maple (*Acer saccharum* Marsh.). Half of each plot was planted with five aspen genotypes of contrasting O<sub>3</sub> sensitivity and leaf phenology (Coleman and others 1995a,b; Curtis and others 2000). One quarter of each plot was planted with sugar maple and aspen, and the remaining quarter was planted with paper birch and aspen. A planting density of 0.95 stems m<sup>-2</sup> yielded 670 stems per 30-m diameter FACE ring. Soils are Alfic Haplorthods with a sandy loam Ap horizon overlaying a sandy clay loam Bt horizon. The site was formerly under agricultural production and was planted with hybrid poplar and larch beginning in 1972. Prior to planting the FACE rings, tree stumps were removed and the areas were disked. Soil physical and

chemical properties are summarized by Dickson and others (2000).

Three replicates of factorial CO<sub>2</sub> and O<sub>3</sub> treatments were arranged in a randomized complete block design. The split-plot effect consisted of the three sub-plots of differing plant species composition, which we refer to as aspen, aspen-birch, and aspen-maple. Each 30-m diameter plot is surrounded by 32 vertical vent pipes and a control system regulates CO<sub>2</sub> and O<sub>3</sub> concentrations within the plots by adjusting flow rates from each vent pipe. Target concentrations of CO<sub>2</sub> and O<sub>3</sub> were maintained during the daylight hours for the duration of the growing season (mid-May through late October). Elevated CO<sub>2</sub> was maintained at 560 µL L<sup>-1</sup> (200 µL L<sup>-1</sup> above ambient) and elevated O<sub>3</sub> was maintained at an average 50–60 nL L<sup>-1</sup> (approximately 1.5 times ambient 30–40 nL L<sup>-1</sup>). Sampling was constrained to the central area in each plot where gas concentrations are most accurately controlled. We collected 6 soil cores (2.5 cm diameter) to a depth of 10 cm within each split plot (3 per plot) on the following dates: 24 July 1999, 16 Sept 2000, 14 July 2001, 16 July 2002, and 24 June 2003. Samples were combined within each split plot, stored at 4°C, and were processed within 24 h of field collection. All results are expressed on an oven-dry (105°C) mass basis.

### Soil N Transformations

We measured gross rates of N mineralization, nitrification, and N immobilization using the <sup>15</sup>N pool dilution method (Davidson and others 1992; Hart and others 1994). Soils were prepared by removing roots (leaving rhizosphere soil), sieving through a 2-mm mesh, and thoroughly homogenizing by hand. Four 12-g subsamples were placed into 120 mL specimen containers. Two soil samples were enriched with 1 mL <sup>15</sup>N-NH<sub>4</sub>Cl solution and two samples were enriched with 1 mL of <sup>15</sup>N-KNO<sub>3</sub> solution. The <sup>15</sup>N solutions were prepared using a combination of <sup>15</sup>N-enriched (99.5%) and unenriched compounds to yield approximately 2–5 µg N g<sup>-1</sup> soil and 2–4 atom % excess <sup>15</sup>N in either the NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> pool. The volume of labeling solution was adequate to disperse the isotope evenly throughout each 12-g sample, and it brought soil samples to near field capacity. Within 1 h after isotope addition, one <sup>15</sup>NH<sub>4</sub><sup>+</sup>-enriched sample and one <sup>15</sup>NO<sub>3</sub><sup>-</sup>-enriched sample was extracted with 2 M KCl. The remaining <sup>15</sup>NH<sub>4</sub><sup>+</sup>-enriched and <sup>15</sup>NO<sub>3</sub><sup>-</sup>-enriched samples were extracted following a 48 h incubation at 20°C. Ammonium and nitrate in soil extracts were dif-

fused onto acidified disks and analyzed for atom % <sup>15</sup>N on a Delta Plus isotope ratio mass spectrometer with a ConFlo II interface (Thermo Electron, San Jose, CA).

### Microbial N and Soil Organic C & N

We measured microbial biomass using the chloroform fumigation-extraction procedure (Horwath and Paul 1994). Soil samples were fumigated with chloroform 5 d in a vacuum desiccator. Fumigated and unfumigated samples were extracted with 0.5 N K<sub>2</sub>SO<sub>4</sub> (in a ratio of 4:1). Dissolved organic N in the extracts was determined by alkaline persulfate digestion (Cabrera and Beare 1993). Blanks and glycine standards were digested simultaneously with samples. Nitrate-N concentrations in the digestates were measured with a Flow Solution 3000 continuous flow analyzer (OI Analytical, College Station, TX). Microbial biomass was calculated using K<sub>N</sub> = 0.54. Soil samples were prepared for C and N analysis by drying at 60°C and grinding in a Certiprep 8000M mill (Spex Industries, Metuchen, NJ). Soil organic C and N concentrations were measured using a CE Instruments NC2500 elemental analyzer (CE Elantech, Lakewood, NJ).

### Statistical Analyses

The experimental design is a split-plot randomized complete block with two factorial treatments (CO<sub>2</sub>, *n* = 2; O<sub>3</sub>, *n* = 2). Each main plot is split by plant community (*n* = 3; aspen, aspen-birch, and aspen-maple). We tested the influence of elevated CO<sub>2</sub>, O<sub>3</sub>, and plant community composition on all pools and fluxes over a 5-year period using a repeated measures analysis of variance (ANOVA) as described by King and others (2001). We tested CO<sub>2</sub>, O<sub>3</sub>, and community effects within years using a split-plot ANOVA and specified appropriate error terms using type III sums of squares (PROC GLM, SAS, Cary, NC). We compared interaction means using the Tukey-Kramer adjustment for multiple comparisons. Results were accepted as significant at *P* less than 0.05. Pearson correlations were used to assess the relationships between microbial N, soil organic N, and rates of gross N mineralization and gross NH<sub>4</sub><sup>+</sup> immobilization within and among years and treatments.

## RESULTS

### Gross N Transformation Rates

Rates of gross N mineralization and gross NH<sub>4</sub><sup>+</sup> immobilization varied significantly from 1999 to

**Table 1.** Summary of *P*-values for Responses of Soil N Transformation Rates and Belowground N Pools to CO<sub>2</sub>, O<sub>3</sub>, Community, Time and their Interactions

| Source  | Gross N Mineralization | Gross NH <sub>4</sub> <sup>+</sup> Immobilization | Gross Nitrification | Gross NO <sub>3</sub> <sup>-</sup> Immobilization | Microbial Biomass N | Soil Organic N |
|---|------------------------|---|---------------------|---|---------------------|----------------|
| Between Subjects                                    |                        |   |                     |   |                     |                |
| CO <sub>2</sub>                                     | 0.007                  | 0.001   | NS                  | NS  | NS                  | NS             |
| O <sub>3</sub>                                      | 0.042                  | NS  | NS                  | NS  | NS                  | NS             |
| CO <sub>2</sub> × O <sub>3</sub>                    | NS                     | NS  | NS                  | NS  | NS                  | NS             |
| Community   | 0.006                  | NS  | NS                  | NS  | 0.019               | NS             |
| CO <sub>2</sub> × Community                         | NS                     | NS  | NS                  | NS  | NS                  | NS             |
| O <sub>3</sub> × Community                          | NS                     | NS  | NS                  | NS  | NS                  | NS             |
| CO <sub>2</sub> × O <sub>3</sub> × Community        | NS                     | NS  | NS                  | NS  | NS                  | NS             |
| Within Subjects                                     |                        |   |                     |   |                     |                |
| Time  | <0.001                 | <0.001  | NS                  | <0.001  | <0.001              | <0.001         |
| CO <sub>2</sub> × Time                              | 0.004                  | 0.002   | NS                  | NS  | NS                  | NS             |
| O <sub>3</sub> × Time                               | NS                     | 0.015   | NS                  | NS  | NS                  | NS             |
| CO <sub>2</sub> × O <sub>3</sub> × Time             | 0.023                  | 0.013   | NS                  | NS  | NS                  | NS             |
| Community × Time                                    | <0.001                 | <0.001  | NS                  | NS  | 0.005               | 0.017          |
| CO <sub>2</sub> × Community × Time                  | NS                     | NS  | NS                  | NS  | NS                  | NS             |
| O <sub>3</sub> × Community × Time                   | NS                     | NS  | NS                  | NS  | NS                  | NS             |
| CO <sub>2</sub> × O <sub>3</sub> × Community × Time | NS                     | NS  | NS                  | NS  | NS                  | NS             |

2003 and the effects of elevated CO<sub>2</sub> on these rates varied among years (Table 1). Rates of gross N mineralization increased 3-fold from 2000 to 2001, then decreased in 2002 (data not shown). Rates of gross NH<sub>4</sub><sup>+</sup> immobilization increased 4-fold from 1999 to 2000, reached a maximum in 2001, and decreased in 2002. Over all years, elevated CO<sub>2</sub> increased gross N mineralization and gross NH<sub>4</sub><sup>+</sup> immobilization by 34% and 36%, respectively (Tables 2 and 3: main effects). When analyzed by year, elevated CO<sub>2</sub> increased gross N mineralization and NH<sub>4</sub><sup>+</sup> immobilization in most years of the study (Tables 2 and 3: main effects).

Responses of gross N mineralization and gross NH<sub>4</sub><sup>+</sup> immobilization to elevated O<sub>3</sub> were weaker and less consistent than responses to CO<sub>2</sub> (Table 1). Over all years, elevated O<sub>3</sub> decreased gross N mineralization by 16%, but the degree of responses varied among years (Table 2). Elevated O<sub>3</sub> had no effect on gross NH<sub>4</sub><sup>+</sup> immobilization (Table 3). The interactive effects of CO<sub>2</sub> and O<sub>3</sub> varied significantly among years, most likely due to the variable responses of gross N mineralization and gross NH<sub>4</sub><sup>+</sup> immobilization to the elevated CO<sub>2</sub>+O<sub>3</sub> treatment compared to the CO<sub>2</sub> treatment (Tables 2 and 3: interaction means by year). In 1999, gross N mineralization and NH<sub>4</sub><sup>+</sup> immobilization were significantly greater under CO<sub>2</sub> than under CO<sub>2</sub>+O<sub>3</sub>, whereas in subsequent years responses to CO<sub>2</sub> and CO<sub>2</sub>+O<sub>3</sub> did not differ, resulting in significant CO<sub>2</sub> main effects.

Rates of gross N mineralization and gross NH<sub>4</sub><sup>+</sup> immobilization were similar among communities in most years, but differed significantly among communities in 2001. This inconsistency resulted in significant community×time interactions (Table 1). In 2001, rates of gross N mineralization within aspen were over twice that of the aspen-maple community; rates of gross NH<sub>4</sub><sup>+</sup> immobilization beneath aspen were 73% greater (Table 4). Over all years, gross N mineralization was 30% greater beneath aspen than beneath aspen-maple and gross N mineralization beneath aspen-birch was intermediate (Table 4).

Elevated CO<sub>2</sub> and O<sub>3</sub> had no significant effects on gross nitrification (overall mean = 0.7 ± 0.02 mg N kg<sup>-1</sup> d<sup>-1</sup>, *n* = 180). Gross nitrification did not differ among communities or through time. Gross NO<sub>3</sub><sup>-</sup> immobilization tended to be somewhat greater in elevated CO<sub>2</sub> although this difference was not significant (0.8 ± 0.07 vs. 0.6 ± 0.05 mg N kg<sup>-1</sup> d<sup>-1</sup>, *P* = 0.075, *n* = 90). Gross NO<sub>3</sub><sup>-</sup> immobilization was unchanged by O<sub>3</sub> (overall mean = 0.7 ± 0.02 mg N kg<sup>-1</sup> d<sup>-1</sup>, *n* = 180). There were no differences in gross NO<sub>3</sub><sup>-</sup> immobilization among communities, but rates varied significantly through time, due to unusually high rates in 2001 (data not shown).

### Microbial N and Soil Organic N

Microbial N was not affected by elevated CO<sub>2</sub> and O<sub>3</sub>, but varied among years and plant communities

**Table 2.** Gross N Mineralization Rates ( $\text{mg N kg}^{-1} \text{d}^{-1}$ ) beneath Aspen, Aspen-Birch and Aspen-Maple Communities Exposed to Factorial  $\text{CO}_2$  and  $\text{O}_3$  FACE Treatments from 1999 to 2003.

| Year | Interaction Means         |                           |                           |                           |                        |                       | Main Effect Means     |             |            |              |             |            |
|------|---------------------------|---------------------------|---------------------------|---------------------------|------------------------|-----------------------|-----------------------|-------------|------------|--------------|-------------|------------|
|      | Ambient $\text{O}_3$      |                           |                           | Elevated $\text{O}_3$     |                        |                       | $\text{CO}_2$         |             |            | $\text{O}_3$ |             |            |
|      | Ambient $\text{CO}_2$     | Elevated $\text{CO}_2$    | Ambient $\text{CO}_2$     | Ambient $\text{CO}_2$     | Elevated $\text{CO}_2$ | Ambient $\text{CO}_2$ | Ambient $\text{CO}_2$ | Elevated    | Change (%) | Ambient      | Elevated    | Change (%) |
| 1999 | 0.76 <sup>ab</sup> (0.41) | 0.97 <sup>a</sup> (0.18)  | 0.79 <sup>ab</sup> (0.25) | 0.49 <sup>b</sup> (0.09)  | 0.75 (0.11)            | 0.77 (0.08)           | 0.87 (0.11)           | 0.64 (0.07) | -3         | 0.87 (0.11)  | 0.64 (0.07) | -27        |
| 2000 | 0.68 (0.22)               | 0.93 (0.08)               | 0.55 (0.17)               | 0.72 (0.07)               | 0.82 (0.06)            | 0.61 (0.05)           | 0.79 (0.06)           | 0.63 (0.05) | +33*       | 0.79 (0.06)  | 0.63 (0.05) | -20        |
| 2001 | 1.81 <sup>a</sup> (1.09)  | 3.34 <sup>b</sup> (0.51)  | 1.84 <sup>a</sup> (0.88)  | 2.22 <sup>ab</sup> (0.40) | 2.78 (0.34)            | 1.82 (0.23)           | 2.57 (0.36)           | 2.03 (0.25) | +52*       | 2.57 (0.36)  | 2.03 (0.25) | -21        |
| 2002 | 1.23 <sup>ab</sup> (0.17) | 1.59 <sup>b</sup> (0.15)  | 1.14 <sup>a</sup> (0.26)  | 1.66 <sup>b</sup> (0.17)  | 1.63 (0.11)            | 1.19 (0.05)           | 1.41 (0.09)           | 1.40 (0.11) | +37*       | 1.41 (0.09)  | 1.40 (0.11) | -0.9       |
| 2003 | 1.46 <sup>ab</sup> (0.39) | 1.55 <sup>ab</sup> (0.09) | 1.08 <sup>a</sup> (0.37)  | 1.77 <sup>b</sup> (0.17)  | 1.66 (0.09)            | 1.27 (0.10)           | 1.51 (0.08)           | 1.43 (0.12) | +30        | 1.51 (0.08)  | 1.43 (0.12) | -5         |
| All  | 1.20 (0.10)               | 1.69 (0.17)               | 1.09 (0.09)               | 1.36 (0.13)               | 1.53 (0.11)            | 1.14 (0.07)           | 1.45 (0.10)           | 1.22 (0.08) | +34**      | 1.45 (0.10)  | 1.22 (0.08) | -16*       |

Notes: Values are means with standard errors in parentheses. Interaction means followed by the same letter do not differ significantly ( $P > 0.05$ ). Significant differences between main effect means are denoted by symbols (\*\* $P < 0.01$ , \* $P < 0.05$ ).

**Table 3.** Gross  $\text{NH}_4^+$  Immobilization Rates ( $\text{mg N kg}^{-1} \text{d}^{-1}$ ) beneath Aspen, Aspen-Birch and Aspen-Maple Communities Exposed to Factorial  $\text{CO}_2$  and  $\text{O}_3$  FACE Treatments from 1999 to 2003.

| Year | Interaction Means        |                          |                           |                           |                        |                       | Main Effect Means     |             |            |              |             |            |
|------|--------------------------|--------------------------|---------------------------|---------------------------|------------------------|-----------------------|-----------------------|-------------|------------|--------------|-------------|------------|
|      | Ambient $\text{O}_3$     |                          |                           | Elevated $\text{O}_3$     |                        |                       | $\text{CO}_2$         |             |            | $\text{O}_3$ |             |            |
|      | Ambient $\text{CO}_2$    | Elevated $\text{CO}_2$   | Ambient $\text{CO}_2$     | Ambient $\text{CO}_2$     | Elevated $\text{CO}_2$ | Ambient $\text{CO}_2$ | Ambient $\text{CO}_2$ | Elevated    | Change (%) | Ambient      | Elevated    | Change (%) |
| 1999 | 0.50 (0.33)              | 0.82 (0.15)              | 0.64 (0.12)               | 0.40 (0.06)               | 0.62 (0.09)            | 0.57 (0.08)           | 0.64 (0.09)           | 0.53 (0.07) | -10        | 0.64 (0.09)  | 0.53 (0.07) | -17        |
| 2000 | 2.05 <sup>a</sup> (0.55) | 2.45 <sup>a</sup> (0.17) | 2.67 <sup>ab</sup> (0.19) | 3.21 <sup>b</sup> (0.23)  | 2.81 (0.16)            | 2.36 (0.15)           | 2.81 (0.16)           | 2.92 (0.16) | +19*       | 2.25 (0.13)  | 2.92 (0.16) | +30*       |
| 2001 | 3.67 (1.74)              | 5.15 (0.63)              | 3.72 (0.39)               | 4.63 (0.71)               | 4.89 (0.47)            | 3.69 (0.34)           | 4.89 (0.47)           | 4.17 (0.41) | +33**      | 4.41 (0.45)  | 4.17 (0.41) | -5         |
| 2002 | 1.34 <sup>a</sup> (0.39) | 1.95 <sup>b</sup> (0.13) | 1.58 <sup>ac</sup> (0.09) | 1.88 <sup>bc</sup> (0.14) | 1.91 (0.09)            | 1.46 (0.08)           | 1.91 (0.09)           | 1.73 (0.09) | +31*       | 1.63 (0.12)  | 1.73 (0.09) | +7         |
| 2003 | 2.05 <sup>a</sup> (0.55) | 2.53 <sup>a</sup> (0.15) | 1.93 <sup>a</sup> (0.14)  | 3.65 <sup>b</sup> (0.40)  | 3.09 (0.24)            | 1.99 (0.11)           | 3.09 (0.24)           | 2.79 (0.29) | +55**      | 2.29 (0.13)  | 2.79 (0.29) | +22**      |
| All  | 1.92 (0.20)              | 2.68 (0.25)              | 2.18 (0.18)               | 2.90 (0.27)               | 2.79 (0.18)            | 2.05 (0.13)           | 2.79 (0.18)           | 2.53 (0.16) | +36**      | 2.29 (0.17)  | 2.53 (0.16) | +11        |

Notes: Values are means with standard errors in parentheses. Interaction means followed by the same letter do not differ significantly ( $P > 0.05$ ). Significant differences between main effect means are denoted by symbols (\*\* $P < 0.01$ , \* $P < 0.05$ ).

**Table 4.** Gross N Mineralization and NH<sub>4</sub><sup>+</sup> Immobilization Rates (mg N kg<sup>-1</sup> d<sup>-1</sup>) within 3 Plant Communities from 1999 to 2003

| Year | Gross N Mineralization   |                          |                          | Gross NH <sub>4</sub> <sup>+</sup> Immobilization |                          |                          |
|------|--------------------------|--------------------------|--------------------------|---|--------------------------|--------------------------|
|      | Aspen                    | Aspen-Birch              | Aspen-Maple              | Aspen   | Aspen-Birch              | Aspen-Maple              |
| 1999 | 0.80 (0.14)              | 0.67 (0.10)              | 0.80 (0.11)              | 0.61 (0.11)                                       | 0.50 (0.10)              | 0.66 (0.10)              |
| 2000 | 0.70 (0.07)              | 0.79 (0.07)              | 0.65 (0.07)              | 2.43 (0.19)                                       | 2.67 (0.20)              | 2.62 (0.22)              |
| 2001 | 3.36 <sup>a</sup> (0.42) | 2.06 <sup>b</sup> (0.20) | 1.49 <sup>b</sup> (0.27) | 5.98 <sup>a</sup> (0.48)                          | 3.44 <sup>b</sup> (0.26) | 3.45 <sup>b</sup> (0.42) |
| 2002 | 1.46 (0.15)              | 1.36 (0.14)              | 1.40 (0.08)              | 1.61 (0.09)                                       | 1.64 (0.16)              | 1.79 (0.12)              |
| 2003 | 1.29 (0.11)              | 1.50 (0.14)              | 1.48 (0.12)              | 2.20 (0.17)                                       | 2.69 (0.38)              | 2.67 (0.24)              |
| All  | 1.52a (0.16)             | 1.31ab (0.09)            | 1.17b (0.08)             | 2.62 (0.26)                                       | 2.28 (0.16)              | 2.32 (0.16)              |

Notes: Values are means with standard errors in parentheses. Means followed by the same letter do not differ significantly ( $P > 0.05$ ).

**Table 5.** Pools of Microbial Biomass N (mg N kg<sup>-1</sup>) and Soil Organic N (g N kg<sup>-1</sup>) Pools within 3 Communities from 1999 to 2003.

| Year | Microbial Biomass N      |                           |                          | Soil Organic N           |                           |                          |
|------|--------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
|      | Aspen                    | Aspen-Birch               | Aspen-Maple              | Aspen                    | Aspen-Birch               | Aspen-Maple              |
| 1999 | 35.1 (1.56)              | 35.3 (0.15)               | 36.3 (2.21)              | 1.40 (0.05)              | 1.49 (0.07)               | 1.41 (0.05)              |
| 2000 | –                        | –                         | –                        | 1.19 (0.05)              | 1.14 (0.05)               | 1.12 (0.05)              |
| 2001 | 47.4 <sup>a</sup> (2.80) | 41.2 <sup>ab</sup> (2.32) | 34.8 <sup>b</sup> (2.72) | 1.24 <sup>a</sup> (0.05) | 1.23 <sup>ab</sup> (0.05) | 1.15 <sup>b</sup> (0.05) |
| 2002 | 33.2 (1.43)              | 34.1 (2.32)               | 30.3 (2.41)              | 1.47 <sup>a</sup> (0.04) | 1.40 <sup>ab</sup> (0.04) | 1.36 <sup>b</sup> (0.03) |
| 2003 | 33.4 (1.43)              | 32.4 (1.45)               | 32.6 (1.24)              | 1.31 (0.04)              | 1.32 (0.05)               | 1.32 (0.05)              |
| All  | 37.2a (1.16)             | 35.8ab (0.96)             | 33.5b (1.01)             | 1.33 (0.02)              | 1.31 (0.03)               | 1.27 (0.03)              |

Notes: Values are means with standard errors in parentheses. Means followed by the same letter do not differ significantly ( $P > 0.05$ ).

(Table 1). Microbial N was greatest in 2001 and was significantly greater in aspen than in aspen-maple in 2001 (Table 5). In 2001, there were correlations between microbial N and gross N mineralization (Pearson  $r = 0.896$ ,  $n = 36$ ), as well as between microbial N and gross NH<sub>4</sub><sup>+</sup> immobilization (Pearson  $r = 0.828$ ,  $n = 36$ ). These patterns were not observed in other years. Soil organic N was not affected by elevated CO<sub>2</sub> and O<sub>3</sub>, but varied among years and plant communities (Table 1). Soil organic N was greatest in 2002 and was significantly greater beneath aspen than beneath aspen-maple in 2001 and 2002 (Table 5).

## DISCUSSION

We hypothesized that greater plant growth and litter production under elevated CO<sub>2</sub> would accelerate microbial N transformations by providing greater inputs of substrates for microbial metabolism. Rates of gross N mineralization and NH<sub>4</sub><sup>+</sup> immobilization and NO<sub>3</sub><sup>-</sup> immobilization were 40–41% greater in elevated CO<sub>2</sub> plots compared to ambient controls. This is within the upper range of

responses that have been observed in a variety of ecosystems (Zak and others 2000a). Greater rates of soil N transformations did not result from an increase in soil microbial biomass under elevated CO<sub>2</sub>, but likely resulted from increased organic substrate inputs to soil. In 2003, annual above-ground litter production was 35% greater under elevated CO<sub>2</sub> versus control (Liu and others 2005), and root biomass was 52% greater (King and others 2005a). Unless fine root turnover per unit biomass decreased under elevated CO<sub>2</sub>, belowground litter production was likely enhanced by elevated CO<sub>2</sub> to a similar or greater degree than aboveground litter production. Elevated CO<sub>2</sub> caused a small decrease (16% relative to control) in leaf and root litter N concentration, but did not significantly alter the concentrations of cellulose, lignin, soluble phenolics, tannins, or other compounds (Chapman and others 2005; King and others 2005b; Liu and others 2005). The small decrease in litter N concentration was likely less important than litter production in controlling microbial response to elevated CO<sub>2</sub>. The overall effect of greater litter production was to increase both gross N mineralization and immobi-

lization rates. Although rates of gross N immobilization were greater than gross N mineralization in some years, elevated CO<sub>2</sub> had similar positive effects on both processes, suggesting that CO<sub>2</sub> did not favor immobilization over mineralization of N. Thus, we find no evidence of a negative feedback between elevated CO<sub>2</sub> and soil N availability at the Aspen FACE site.

Elevated O<sub>3</sub> decreased gross N mineralization by 16% and this effect was likely driven by decreased organic substrate inputs to soil. In 2003, annual aboveground litter production was 16% lower under elevated O<sub>3</sub> versus control (Liu and others 2005) and root biomass was 18% lower (King and others 2005a). Although elevated O<sub>3</sub> decreased gross N mineralization, it led to no significant decrease in NH<sub>4</sub><sup>+</sup> immobilization (averaged over years). This response was likely controlled by changes in chemical composition of organic substrates in plant litter or changes in the composition of the microbial community or both. Using molecular techniques, Chung and others (2005) found evidence that plant litter produced under elevated O<sub>3</sub> has caused a shift in fungal community composition in this experiment. In addition, elevated O<sub>3</sub> significantly altered leaf litter concentrations of soluble sugars (+94%) and tannins (+87%; Liu and others 2005), as well as fine root litter concentrations of lignin and hemicellulose (Chapman and others 2005). These responses to O<sub>3</sub> probably contributed to changes in microbial metabolism, leading to a reduction in gross N mineralization but not gross N immobilization.

We found that elevated O<sub>3</sub> did not counteract the positive effect of CO<sub>2</sub> on gross N mineralization or NH<sub>4</sub><sup>+</sup> immobilization, as we had hypothesized. From 2000 to 2003, there were no significant differences in gross N mineralization rates between elevated CO<sub>2</sub> and CO<sub>2</sub>+O<sub>3</sub> treatments, whereas gross N immobilization rates in the elevated CO<sub>2</sub>+O<sub>3</sub> treatment were greater than or similar to those in the elevated CO<sub>2</sub> treatment (Tables 2 and 3, interaction means). We believe these responses resulted from greater litter inputs, as well as changes in litter chemistry. In the elevated CO<sub>2</sub>+O<sub>3</sub> treatment, annual aboveground litter production was 18% greater (Liu and others 2005) than in ambient control and root biomass was 21% greater (King and others 2005a). Compared to the CO<sub>2</sub> treatment, less leaf litter was produced in the elevated CO<sub>2</sub>+O<sub>3</sub> treatment, but the litter contained substantially greater amounts of both simple and complex organic substrates for microbial metabolism. Compared to control, the elevated CO<sub>2</sub>+O<sub>3</sub> resulted in greater leaf litter concentrations of sol-

uble sugars (+162%), tannins (+102%), and soluble phenolics (+74%) and lower leaf litter N concentration (-16%; Liu and others 2005). Greater concentrations of soluble sugars may have increased microbial metabolism, whereas greater concentrations of tannins and other soluble phenolics may have promoted microbial N immobilization. As a result, gross N immobilization in the elevated CO<sub>2</sub>+O<sub>3</sub> treatment increased to a greater degree than gross N mineralization. This suggests that, in a scenario of concomitant increases in atmospheric CO<sub>2</sub> and O<sub>3</sub>, greater microbial N demand could lead to a negative feedback on N availability to plants.

There appears to be a strong connection between plant biomass, litter production, and soil microbial activity, which has developed after several years. Although there were no detectable changes in soil microbial biomass in response to elevated CO<sub>2</sub> or O<sub>3</sub>, gross N mineralization increased under elevated CO<sub>2</sub> and decreased under elevated O<sub>3</sub>. These results mirror the response of soil CO<sub>2</sub> efflux, which is driven by both root and microbial respiration. During the period from 1999 to 2001, soil CO<sub>2</sub> efflux was 28% higher in elevated CO<sub>2</sub> and 13% lower in elevated O<sub>3</sub> compared to control (Pregitzer and others 2006). During the same period, gross N mineralization was 27% higher in elevated CO<sub>2</sub> and 23% lower in elevated O<sub>3</sub>. A similar pattern of CO<sub>2</sub> and O<sub>3</sub> responses has been observed in total belowground biomass (King and others 2005a). These results suggest that CO<sub>2</sub>- and O<sub>3</sub>-induced changes in allocation to root production and turnover resulted in an increase in microbial activity in response to elevated CO<sub>2</sub> and a decrease in response to elevated O<sub>3</sub>.

Several studies have investigated the effects of elevated CO<sub>2</sub> or O<sub>3</sub> on litter chemistry and decomposition, and it has been suggested that litter chemistry may not be the primary factor regulating decomposition in all systems (Norby and others 2001) and that litter inputs drive changes in microbial activity in response to elevated CO<sub>2</sub> (Zak and others 2000a). For example, in a range of ecosystems, leaf and root litter produced under elevated CO<sub>2</sub> have higher C:N and lower N concentration, but such litter does not decompose more slowly in all cases (Cotrufo and Ineson 1995; Cotrufo and Ineson 1996; Allen and others 2000; Hoorens and others 2003). Similarly, litter produced under elevated O<sub>3</sub> or combined CO<sub>2</sub> + O<sub>3</sub> may have lower N concentration or higher phenolic concentration, but these changes do not consistently reduce litter decomposition rates (Findlay and others 1996; Scherzer and others

1998; Kainulainen and others 2003). Our results agree with these findings and support the argument that microbial metabolism of substrates produced by plants exposed to elevated CO<sub>2</sub> or O<sub>3</sub> is controlled by treatment effects on litter production to an equal or greater degree than by effects on litter chemistry. However, our results also indicate that litter chemistry may be an important factor governing CO<sub>2</sub> and O<sub>3</sub> effects on microbial N demand and the potential for impacts on long-term N availability to plants.

We observed significant year-to-year differences in CO<sub>2</sub> and O<sub>3</sub> interaction effects on gross N mineralization and NH<sub>4</sub><sup>+</sup> immobilization rates due to a shift in CO<sub>2</sub> and O<sub>3</sub> effects through time. In 1999, rates of gross N mineralization and NH<sub>4</sub><sup>+</sup> immobilization were greatest in elevated CO<sub>2</sub> and least in the elevated CO<sub>2</sub>+O<sub>3</sub> treatment, resulting in no significant CO<sub>2</sub> main effect. In subsequent years, these rates were greater in CO<sub>2</sub> and CO<sub>2</sub>+O<sub>3</sub> treatments than in control and O<sub>3</sub> treatments, resulting in significant CO<sub>2</sub> main effects. Following 1999, elevated CO<sub>2</sub> led to consistently greater rates of gross N mineralization and immobilization. Unusually high rates of gross N mineralization and NH<sub>4</sub><sup>+</sup> immobilization were observed in 2001. Field measured rates of soil respiration were also high during mid-growing season in 2001 compared to prior years (King and others 2004). Most of the increase in soil microbial activity in 2001 occurred in aspen and aspen-birch, and this increase was accompanied by increases in microbial biomass. We suspect these effects resulted from an outbreak of blotch leaf miner, which damaged as much as 50% of leaf area in 2001 (W. Parsons *unpublished data*). Damaged leaf tissue remained on trees through the growing season and insect frass was retained within leaves following damage. It is not known whether this caused an increase in dissolved organic matter leaching from leaves or if it increased root exudation or dieback, but such effects could have contributed to greater microbial biomass and activity via changes in labile substrate inputs to soil. Our observations are consistent with other observations of increased N availability with insect damage to trees (Belovsky and Slade 2000).

Our finding of increased rates of soil N transformations under elevated CO<sub>2</sub> cannot be generalized to other forest types. For example, our results differ from findings at the Duke Forest and Oak Ridge FACE experiments in which elevated CO<sub>2</sub> increased litter production (Finzi and others 2001, 2002; Norby and others 2002), but had no discernable effects on N transformations as of 2001 (Finzi and Schlesinger 2003; Zak and others 2003;

Johnson and others 2004). Stand ages in the Duke and Oak Ridge FACE experiments were 13 and 9 years at the time of initiation of CO<sub>2</sub> exposure, whereas the trees at Aspen FACE were planted the year prior to initiation of CO<sub>2</sub> and O<sub>3</sub> exposure. Although trees at all sites were exposed to CO<sub>2</sub> for 3–5 years as of 2001, those at Aspen FACE were exposed to CO<sub>2</sub> since an early stage of development. This, along with differences in species composition, soil type, climate, and ambient O<sub>3</sub> levels among the three sites could explain differences in CO<sub>2</sub> responses among these forest FACE sites.

The manner in which plant physiological responses to CO<sub>2</sub> and O<sub>3</sub> cascade through ecosystems to influence soil microbial community composition and activity has implications for long-term N availability and soil C storage. Our results show that CO<sub>2</sub> and O<sub>3</sub> are important modifiers of soil N transformations at the Aspen FACE site and that responses are consistent among the plant communities. Plant responses to CO<sub>2</sub> have influenced belowground N dynamics by altering the production of substrates for microbial metabolism. Elevated O<sub>3</sub> modifies the effects of CO<sub>2</sub> by altering the composition of litter inputs, which in turn may lead to a negative feedback on N availability. Therefore, long-term effects of elevated CO<sub>2</sub> on plant production depend on co-exposure to elevated O<sub>3</sub>.

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