Sources of increased N uptake in forest trees growing under elevated CO2: results of a large-scale 15N study

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Abstract

Nitrogen availability in terrestrial ecosystems strongly influences plant productivity and nutrient cycling in response to increasing atmospheric carbon dioxide (CO2). Elevated CO2 has consistently stimulated forest productivity at the Duke Forest free-air CO2 enrichment experiment throughout the decade-long experiment. It remains unclear how the N cycle has changed with elevated CO2 to support this increased productivity. Using natural-abundance measures of N isotopes together with an ecosystem-scale 15N tracer experiment, we quantified the cycling of 15N in plant and soil pools under ambient and elevated CO2 over three growing seasons to determine how elevated CO2 changed N cycling between plants, soil, and microorganisms. After measuring natural-abundance 15N differences in ambient and CO2-fumigated plots, we applied inorganic 15N tracers and quantified the redistribution of 15N for three subsequent growing seasons. The natural abundance of leaf litter was enriched under elevated compared to ambient CO2, consistent with deeper rooting and enhanced N mineralization. After tracer application, 15N was initially retained in the organic and mineral soil horizons. Recovery of 15N in plant biomass was 3.5 ± 0.5% in the canopy, 1.7 ± 0.2% in roots and 1.7 ± 0.2% in branches. After two growing seasons, 15N recoveries in biomass and soil pools were not significantly different between CO2 treatments, despite greater total N uptake under elevated CO2. After the third growing season, 15N recovery in trees was significantly higher in elevated compared to ambient CO2. Natural-abundance 15N and tracer results, taken together, suggest that trees growing under elevated CO2 acquired additional soil N resources to support increased plant growth. Our study provides an integrated understanding of elevated CO2 effects on N cycling in the Duke Forest and provides a basis for inferring how C and N cycling in this forest may respond to elevated CO2 beyond the decadal time scale.

Keywords: 15N, atmospheric CO2, carbon sequestration, FACE experiment, N cycling, Pinus taeda

Received 7 December 2010 and accepted 28 April 2011

Introduction

A major source of uncertainty in calculating the potential for long-term biological carbon sequestration is the demand and availability of soil nitrogen (N; Field, 1999; Hungate et al., 2003; Matthews, 2007). For example, it has been theorized that an initial increase in plant N uptake and subsequent decrease in soil N availability under elevated CO2 could reduce the enhanced plant growth response over the longer term, thereby decreasing net primary productivity (NPP) and the potential for C sequestration in terrestrial ecosystems (Luo & Reynolds, 1999; Thornton et al., 2007; Zaehle et al., 2010). Immobilization of N in plant biomass and soil organic matter (SOM) can feedback to affect negatively plant growth, and may ultimately lead to progressive N limitation (PNL) of CO2-mediated growth enhancement (Mguire et al., 1995; Luo & Reynolds, 1999). However, several free-air CO2 enrichment (FACE) experiments in North America have shown a continual stimulation in forest productivity under elevated CO2 over time scales nearly reaching a decade (Finzi et al., 2006a; Norby & Iversen, 2006; Zak et al., 2007; McCarthy et al., 2010); although reduced CO2-mediated growth enhancement has recently been documented at the Oak Ridge, TN experiment (Norby et al., 2010). It is unclear if, and under what conditions, this stimulation will persist for decades to centuries, including whether N cycling in the plant-soil system will be able to support continued high rates of NPP (Norby et al., 2010). If PNL were occurring at the Duke FACE experiment, we
would expect the CO2-mediated growth enhancement to diminish. By contrast, after more than a decade of CO2 treatment, there is little evidence that PNL is occurring in the replicated Duke experiment based on evidence from aboveground or total NPP (Finzi et al., 2007).

A recent synthesis of studies of N uptake and N-use efficiency at four forest FACE sites highlighted the discrepancy between plant N accumulation without observable changes in soil N and concluded that although the specific mechanism remained unidentified, increased C allocation belowground (root biomass, exudates, mycorrhizae) resulted in greater soil N uptake (Finzi et al., 2007). In a separate synthesis of soil N cycling responses in the Duke FACE experiment, no statistically significant change in gross rates of mineralization or immobilization were detected in response to elevated CO2 treatment (Finzi & Schlesinger, 2003). Similarly, studies from Rhinelander FACE demonstrated that the gross rates of mineralization and immobilization were stimulated to a similar extent, resulting in no significant CO2 treatment effect on net rates of soil N cycling (Holmes et al., 2006). In addition, no significant stimulation of dissolved organic N uptake has been detected in trees grown in elevated relative to ambient CO2 at the Duke FACE experiment (Hofmokel et al., 2007). The discrepancy between the observation of increasing N uptake but no detectable change in N pools of surface and mineral horizons under elevated CO2 raises the question of what sources of N support higher rates forest productivity under elevated CO2.

Measuring increases in N cycling rates in forest soils requires detecting small changes in heterogeneous pools with variable fluxes, which is notoriously difficult, especially in long-term experiments in which alterations in N cycling may be subtle. It is therefore not surprising that short-term analyses of soil N transformations fail to identify the small, but critically important, increases in the rate of soil N cycling that may be occurring under elevated CO2, especially when those measurements include significant soil disturbance (e.g., gross or net rates of N mineralization). Another approach to studying soil N cycling processes is the application of tracer quantities of 15N to entire forest plots, followed by an analysis of the redistribution of the 15N tracer throughout the plant-microbe-soil system (e.g., Buchmann et al., 1996; Nadelhoffer et al., 1999). An advantage to this approach is that the isotopic label can be applied to the surface of the soil without disturbing plant-fungal-bacterial interactions that are known to affect SOM decomposition and N cycling (Kuzyakov et al., 2000, Read & Perez-Moreno, 2003).

The use of ecosystem-scale 15N tracer experiments in conjunction with natural-abundance measures offers opportunities to assess how elevated CO2 has influenced N cycling. Specifically, we can first identify altered patterns of N cycling with elevated CO2 using natural-abundance measures, and then measure two different N pools: the quantity of 15N recovered in each ecosystem N pool (percent recovery of the tracer) and the level of 15N incorporation above the measured natural-abundance value of each plant and soil pool (atom percent excess or APE). When these quantities are used together in a time series of field samples, this allows the cycling of both the 15N tracer (through its absolute mass) and unlabeled N (through tracer dilution) to be compared. We hypothesized that elevated CO2 will be associated with higher APE and percent recovery of 15N in tree biomass, as an indication of greater N uptake from surface soils. By contrast, lower APE and 15N recovery of biomass under elevated CO2 compared to ambient CO2 would be indicative of greater N uptake from unlabelled sources, including older SON or soil below 15 cm, where little of the applied 15N tracer resides.

Materials and methods

Site description

The Duke Forest FACE site is located in a 25-year-old loblolly pine (Pinus taeda) forest in Orange County, North Carolina. In 1983, the site was planted with 3-year-old pine seedlings in 2.4 × 2.4 m spacing. Although initiated as a plantation, there has been no subsequent management of the forest. A diverse assemblage of understory, hardwood tree species has self-recruited from hardwood forest adjacent to the pine plantation.

The experimental design consists of six circular plots, each 30 m in diameter, widely spaced within the homogeneous, now closed-canopy pine stand. Each plot is subdivided into eight alternating sectors that are designated for either belowground or aboveground sampling. Three plots receive ambient air (386 μL L⁻¹) and three receive elevated concentrations of atmospheric CO2 (ambient +200 μL L⁻¹). Fumigation with elevated CO2 began on August 27, 1996. As in all FACE experiments, the experimental plots are open to ambient sunlight, rainfall, winds, deposition, and other environmental variables. Additional details on the FACE technology can be found in Hendrey et al. (1999). The soils of the site are clay loams of igneous origin, classified as Ultic Hapludalfs of the Enon series, and are relatively homogeneous across the experimental area. The soil is highly weathered and moderately acidic (pH = 5.75).

Isotope experiment

As a baseline for the tracer experiment, the natural abundance of 15N was measured in pine foliage samples collected from the bottom, middle, and top of the pine canopy in 2002 (see

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Natural abundance in wood was measured in the last wood increment in April 2003, before the tracer application. In pine branches, natural abundance of 15N was estimated in branches taken outside the plots in September 2005. Natural abundance of 15N was measured in O horizon and mineral soil samples collected in March 2003 (Table 1).

Over three consecutive days in May 2003, trace amounts of 15N were applied to one ambient and one CO2-fumigated plot per day. Enough water was added to the 15N to simulate a 0.2 mm rain event, using backpack sprayers. Paralleling the proportions of NH4+ and NO3− in the soil (Finzi & Schlesinger, 2003), the tracer (98 atom% 15N) was added as 75% 15NH4Cl and 25% K15NO3 at a rate of 0.015 g 15N m−2 in 0.25 L H2O m−2, which represents ca. 3% of the inorganic N pool (0–15 cm depth). To quantify the extent of initial labeling and to test whether the tracers were evenly applied, we sampled the forest floor of all six plots 2 weeks after the tracers were applied. In the forest litter, δ15N values averaged 48 (±29)‰, representing 44 (±3)% 15N recovery, with no significant differences between CO2 treatments.

The redistribution of the 15N label was followed for three growing seasons, corresponding to the seventh–ninth growing seasons after the initiation of CO2 fumigation. In September of 2003, 2004, and 2005, when the canopy reaches its seasonal maximum N content (Zhang & Allen, 1996; Finzi et al., 2004), we sampled all components of the ecosystem from the canopy through 30 cm depth in the mineral soil horizon.

Aboveground sampling

The mean longevity of loblolly pine foliage in the Piedmont of NC is 18 months (Zhang & Allen, 1996). As a result, during the growing season, there are two cohorts of needles in the canopy, one produced in the current year and the other in the previous year. In each experimental plot, 10–15 fascicles from each cohort of needles were sampled from eight trees in 2003 and 2004 and from four trees in 2005. Previous research with this tree species had shown that the concentration of N in needles varies from the bottom to the top of the crown (Zhang & Allen, 1996; Finzi et al., 2004). Consequently, needles from the bottom 25%, middle 50%, and top 25% of the crown were collected from an upright lift and analyzed separately. In 2003 (natural abundance and postlabel) and 2004 (postlabel), we collected and analyzed the concentration of N and 15N in a total of 144 foliage samples (e.g., 2 CO2 treatments × 3 replicates × 8 trees per plot × 3 canopy positions per tree). In 2005, we collected and analyzed samples from 4 trees per plot generating 72 foliage samples.

<table>
<thead>
<tr>
<th>Ecosystem pool</th>
<th>Ambient CO2 δ15N (%)</th>
<th>Elevated CO2 δ15N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine needles</td>
<td>−3.3 (0.1)</td>
<td>−2.3 (0.7)</td>
<td>0.20</td>
</tr>
<tr>
<td>Bottom</td>
<td>−3.6 (0.1)</td>
<td>−2.5 (0.4)</td>
<td>0.07</td>
</tr>
<tr>
<td>Middle</td>
<td>−3.4 (0.1)</td>
<td>−2.5 (0.8)</td>
<td>0.31</td>
</tr>
<tr>
<td>Top</td>
<td>−2.9 (0.2)</td>
<td>−2.0 (1.0)</td>
<td>0.87</td>
</tr>
<tr>
<td>Hardwood foliage</td>
<td>−1.9 (0.3)</td>
<td>−2.5 (0.6)</td>
<td>0.39</td>
</tr>
<tr>
<td>Wood</td>
<td>−4.4 (0.3)</td>
<td>−2.9 (0.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Pine stem</td>
<td>−4.9 (−)</td>
<td>−4.9 (−)</td>
<td>−</td>
</tr>
<tr>
<td>Pine branches</td>
<td>4.1 (0.6)</td>
<td>3.9 (1.1)</td>
<td>0.84</td>
</tr>
<tr>
<td>Hardwood stem and branches</td>
<td>4.1 (0.6)</td>
<td>3.9 (1.1)</td>
<td>0.84</td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oa</td>
<td>−5.6 (0.2)</td>
<td>−5.7 (0.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>0–15 cm mineral</td>
<td>−1.0 (0.1)</td>
<td>−0.6 (0.3)</td>
<td>0.21</td>
</tr>
<tr>
<td>15–30 cm mineral</td>
<td>−2.1 (0.5)</td>
<td>−0.9 (0.5)</td>
<td>0.13</td>
</tr>
<tr>
<td>Forest litter</td>
<td>−5.0 (0.3)</td>
<td>−4.2 (0.2)</td>
<td>0.00</td>
</tr>
<tr>
<td>SOM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oa</td>
<td>−4.7 (0.2)</td>
<td>−4.7 (0.1)</td>
<td>0.90</td>
</tr>
<tr>
<td>0–15 cm mineral</td>
<td>1.8 (0.1)</td>
<td>1.1 (0.2)</td>
<td>0.00</td>
</tr>
<tr>
<td>15–30 cm mineral</td>
<td>4.5 (0.1)</td>
<td>3.5 (0.2)</td>
<td>0.00</td>
</tr>
<tr>
<td>NH4+–N</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Oa</td>
<td>−4.2 (0.6)</td>
<td>−5.0 (1.4)</td>
<td>0.59</td>
</tr>
<tr>
<td>0–15 cm mineral</td>
<td>3.9 (1.6)</td>
<td>−1.0 (0.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>15–30 cm mineral</td>
<td>21.6 (2.4)</td>
<td>23.7 (4.1)</td>
<td>0.66</td>
</tr>
<tr>
<td>TDN</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Oa</td>
<td>−6.0 (0.4)</td>
<td>−7.8 (0.2)</td>
<td>0.00</td>
</tr>
<tr>
<td>0–15 cm mineral</td>
<td>−1.5 (1.2)</td>
<td>−2.1 (0.3)</td>
<td>0.68</td>
</tr>
<tr>
<td>15–30 cm mineral</td>
<td>−0.1 (0.8)</td>
<td>−1.3 (0.3)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Each value is the mean of the three ambient or elevated plots (±SEM), except for pine branches (natural abundance measured outside the plots in 2005)
The concentration of N and $^{15}$N in wood was determined separately for bolewood and branches. For bolewood, five randomly selected pine trees in each plot were cored ca. 1.3 m above the soil surface using a 5 mm diameter increment borer. Bark and each yearly growth increment in each core were separated by year using a razor blade. In 2003, we measured the concentration of N and $^{15}$N in the bark and in the current year of growth. To account for lateral redistribution of the $^{15}$N tracer to growth rings from earlier years, in 2004 and 2005 we analyzed growth increments dating back to the 2000 growing season in each core.

Analysis of N and $^{15}$N in foliated pine branches was conducted in September 2004 and 2005. Three foliated secondary branches from the bottom, middle, and top of the canopy were harvested from each plot. Primary branches were not sampled to avoid leader damage. The branches were stripped of their foliage, dried and subsequently ground in a Wiley mill to create a single homogenized sample for analysis.

Ten to 15 samples of foliage of the four dominant hardwood species were collected from each plot sector in which each species was present. The four hardwood species were: red maple (Acer rubrum L.), sweet gum (Liquidambar styraciflua L.), winged elm (Ulmus alata Michx.), and red bud (Cercis canadensis L.). Because the understory trees were too small to core, we clipped a lateral branch from four individuals within each FACE plot and assumed that the concentration of N and $^{15}$N in the branch was the same as that of the stem.

Belowground sampling

Soil fractions and fine root biomass were sampled from a randomly selected position within each soil sector of each plot. At each sampling location, a square section 100 cm$^2$ in area was cut from both the undecomposed litter ($O_i$) and the underlying partially decomposed organic material ($O_{ea}$). Mineral horizons (0–15, 15–30 cm) were collected directly under the organic horizon sample, using a 5 cm diameter slide hammer bulk-density soil corer. Four vertical sets of organic and mineral-horizon samples per plot were collected and kept separate for chemical, physical, and microbiological analysis.

Immediately after sampling, the soils were brought back to the laboratory where visible roots and rocks were removed from each sample, and a 30 g subsample of the root-free organic and mineral soil horizons was made available for sequential extraction (see below). The remaining mineral soil samples were first sieved through a 2 mm mesh and then quantitatively root picked. The roots from each horizon were separated into live fine roots (<2 mm) and live coarse roots (>2 mm). Because root species were not identified, our results represent a community level measurement. Live fine roots were identified by tensile strength and white or yellow color of the vascular tissue, rinsed three times in 0.5 m m$^{2}$CaCl$_2$ to remove any adsorbed tracer, rinsed in deionized water and placed in an oven at 60 °C for 3 days. The dried fine roots were then ground to a powder and analyzed for N and $^{15}$N.

To separate the different fractions of N within the soil, we used the sequential extraction procedure described in Holmes et al. (2003). In brief, a 30 g subsample of root-free, field-moist soil was placed in a 125 mL plastic bottle, extracted with 90 mL of 0.5 m K$_2$SO$_4$, shaken for 1 h, centrifuged, and filtered through a 0.5 μm glass fiber filter. The filtrate was collected for NH$_4^+$, NO$_3^-$, and DON measurements (see below). Next, the filter was removed from the filtration apparatus and placed in the sample bottle containing the extracted soil, and fumigated with chlorofom (CHCl$_3$) for 10 days in the dark. After incubation, the CHCl$_3$ was removed and the sample was extracted with 90 mL of 0.5 m K$_2$SO$_4$ shaken for 1 h, centrifuged, and filtered through a 0.5 μm glass fiber filter. The flush of N after fumigation corresponded to microbial biomass N (Joergensen, 1996). The remaining soil pellet, representing SON in the soil, was oven-dried at 60 °C, ground with a ball mill, and prepared for mass spectrometry.

The concentration of NH$_4^+$ and NO$_3^-$ in each sample was measured on an autoanalyzer (Lachat QuickChem FIA+ 8000 Series; Zellweger Analytics, Milwaukee, WI, USA). Ammonium concentrations were measured with the phenolate method and NO$_3^-$ concentrations by the cadmium-reduction method. The quantity of N in DON and microbial biomass pools was measured in the 0.5 m K$_2$SO$_4$ extracts following persulfate digestion (Cabrera & Beare, 1993). The quantity of N in DON was measured as the difference in the concentration of N released after persulfate digestion and the concentrations of NH$_4^+\cdot$N plus NO$_3^-\cdot$N initially present in the sample (Currie et al., 1996). Similarly, the concentration of N in microbial biomass was calculated as the difference in the N concentration of the CHCl$_3$-fumigated sample and the DON sample. We used a diffusion procedure to determine the $^{15}$N content of the NH$_4^+$, DON, and microbial biomass pools (Stark & Hart, 1996). Concentrations of NO$_3^-\cdot$N were below the detection limit (12 ppb NO$_3^-\cdot$N) and were therefore not diffused. Soil NH$_4^+$, DON, and microbial biomass extracts were diffused onto acidified disks and analyzed for %N and $\Delta^{15}$N at the University of California, Davis on an Europa Integra mass spectrometer.

Calculations and statistical analysis

The production of woody biomass, branches, and coarse roots was estimated from measurements of tree heights and diameters and allometric equations (Clark et al., 1986; Naidu et al., 1998; Fang et al., 2000) as presented in McCarthy et al. (2010). Because foliar biomass deviated from that predicted by the allometric equations under elevated CO$_2$, the canopy biomass for each experimental plot was estimated according to McCarthy et al. (2007), based on the mass of foliage collected in litter baskets. Fine root biomass was estimated directly from the quantitative root picking.

Each year, for each sample we used the N concentration and $^{15}$N contents of tree tissues, organic horizon and mineral soil fractions to calculate the distribution the $^{15}$N tracer in the three plots under ambient and elevated CO$_2$. We first calculated the atom% excess $^{15}$N (APE $^{15}$N) of each sample as the difference in atom% $^{15}$N of the component collected after labeling of plots with enriched $^{15}$N tracers minus the atom% $^{15}$N in natural abundance of the component, measured prior to the application of $^{15}$N tracers. Because the tracer was 98% $^{15}$N enriched, APE approximates $^{15}$N derived from the applied tracer.
(NDFT), a stock independent measure of tracer recovery. The total quantity of $^{15}$N in each pool (g $^{15}$N m$^{-2}$) was then estimated as the atom% $^{15}$N excess of that pool multiplied by the N content, or pool size (g N m$^{-2}$) of that pool divided by 100. Finally, the recovery of the added label in each ecosystem pool was calculated as the $^{15}$N mass in that pool (g $^{15}$N m$^{-2}$) divided by the mass of $^{15}$N label added at the time of tracer application (i.e., 0.015 g $^{15}$N m$^{-2}$; Currie et al., 1999). Although not statistically so ($P = 0.16$), total ecosystem recovery in 2004 (74.8 ± 6.1%) was lower than recovery in 2003 (96.9 ± 4.3%) and 2005 (88.2 ± 6.3%), likely due to low $^{15}$N recovery in SOM from the 0–15 mineral soil in 2004 (5.3 ± 0.7%) compared to 2003 (19.6 ± 1.0%) and 2005 (21.5 ± 2.5%). We suspect that low recovery in the upper mineral horizon in 2004 was due to variation in the delineation between the Oea and mineral horizons among years. We therefore used the 2003 and 2005 data to interpolate linearly APE values for each 0–15 cm mineral soil sample ($n = 6$). The average measured APE was 0.36759 (±0.00017) in 2004. The average interpolated value was 0.36920 (±0.00024). For each sample, the interpolated APE value was used in subsequent calculations of SO$^{15}$N recovery and ecosystem $^{15}$N recovery (Table 2).

For statistical analysis, each 30 m diameter FACE plot is a replicate experimental unit ($n = 3$ for the ambient and elevated CO$_2$ treatments). All samples collected within a plot were averaged prior to statistical analysis. Forest floor and tree components represent the sum of hardwood and pine trees, unless otherwise stated. We used repeated measures analysis of variance (ANOVA) to test for the effects of CO$_2$ treatment (386 and 586 µL L$^{-1}$) and time (2003, 2004, and 2005) on the biomass (g dry matter m$^{-2}$), N concentration (%), N content or pool size (g N m$^{-2}$), atom% $^{15}$N excess and percent recovery of the isotope. Because initial measurements in 1996 demonstrated significant between-plot variation in plant biomass, NPP and pools of N, the effect of elevated CO$_2$ on N content and biomass were tested using repeated measures analysis (Kenward & Roger, 1997; Littell, 2002) with the 1996 pretreatment data as covariates, using Proc Mixed in SAS (Schlesinger & Lichter, 2001; Finzi et al., 2002). Treatment and interaction means were compared using Tukey’s HSD test, using a significance definition of $\alpha = 0.05$.

### Results

Prior to tracer application, elevated CO$_2$ caused a significant enrichment of the natural abundance of the forest litter (O$_i$; $P = 0.0006$), which provides an

<table>
<thead>
<tr>
<th>Ecosystem component</th>
<th>Year</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ambient</td>
<td>Elevated</td>
<td></td>
</tr>
<tr>
<td>Tree biomass</td>
<td></td>
<td>3.8$^a$ (0.8)</td>
<td>3.5$^a$ (0.8)</td>
<td>8.5$^b$ (0.8)</td>
</tr>
<tr>
<td>Total roots</td>
<td></td>
<td>1.1$^a$ (0.2)</td>
<td>0.8$^a$ (0.2)</td>
<td>1.6$^{ab}$ (0.4)</td>
</tr>
<tr>
<td>Total canopy</td>
<td></td>
<td>1.2$^a$ (0.3)</td>
<td>1.1$^a$ (0.3)</td>
<td>3.8$^b$ (0.3)</td>
</tr>
<tr>
<td>Bark</td>
<td></td>
<td>0.9$^a$ (0.3)</td>
<td>0.9$^a$ (0.2)</td>
<td>0.6$^a$ (0.1)</td>
</tr>
<tr>
<td>Bole</td>
<td></td>
<td>0.1$^a$ (0.0)</td>
<td>0.1$^a$ (0.0)</td>
<td>0.9$^a$ (0.2)</td>
</tr>
<tr>
<td>Branches</td>
<td></td>
<td>0.5$^a$ (0.1)</td>
<td>0.6$^a$ (0.2)</td>
<td>1.7$^a$ (0.3)</td>
</tr>
<tr>
<td>Forest litter</td>
<td></td>
<td>40.2$^a$ (2.5)</td>
<td>42.4$^a$ (7.5)</td>
<td>6.7$^{bc}$ (2.2)</td>
</tr>
<tr>
<td>O$_i$ horizon</td>
<td></td>
<td>22.0$^{ab}$ (0.3)</td>
<td>16.5$^a$ (2.5)</td>
<td>44.1$^{ab}$ (5.8)</td>
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<tr>
<td>SOM</td>
<td></td>
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<td>2.1$^a$ (0.3)</td>
<td>3.0$^a$ (0.4)</td>
</tr>
<tr>
<td>MB</td>
<td></td>
<td>0.3$^a$ (0.1)</td>
<td>0.2$^a$ (0.0)</td>
<td>0.8$^a$ (0.1)</td>
</tr>
<tr>
<td>DON</td>
<td></td>
<td>0.1$^a$ (0.0)</td>
<td>0.0$^a$ (0.0)</td>
<td>0.0$^a$ (0.0)</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td></td>
<td>0.2$^a$ (0.1)</td>
<td>0.2$^a$ (0.1)</td>
<td>0.0$^a$ (0.0)</td>
</tr>
<tr>
<td>Mineral soil 0–15 cm</td>
<td></td>
<td>18.0$^a$ (1.5)</td>
<td>21.2$^a$ (1.1)</td>
<td>16.4$^a$ (1.7)</td>
</tr>
<tr>
<td>SOM</td>
<td></td>
<td>1.5$^a$ (0.2)</td>
<td>1.8$^a$ (0.4)</td>
<td>0.5$^a$ (0.1)</td>
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<td>MB</td>
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<td>0.3$^a$ (0.1)</td>
<td>0.7$^a$ (0.1)</td>
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<tr>
<td>DON</td>
<td></td>
<td>0.2$^{ab}$ (0.1)</td>
<td>0.2$^{ab}$ (0.1)</td>
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<td>NH$_4^+$</td>
<td></td>
<td>8.4$^a$ (1.7)</td>
<td>6.5$^a$ (0.9)</td>
<td>0.0$^a$ (0.0)</td>
</tr>
<tr>
<td>MB</td>
<td></td>
<td>0.6$^a$ (0.2)</td>
<td>0.4$^a$ (0.2)</td>
<td>2.7$^a$ (0.1)</td>
</tr>
<tr>
<td>DON</td>
<td></td>
<td>0.2$^a$ (0.1)</td>
<td>0.3$^a$ (0.1)</td>
<td>0.2$^a$ (0.0)</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td></td>
<td>0.0$^a$ (0.0)</td>
<td>0.0$^a$ (0.0)</td>
<td>0.0$^a$ (0.0)</td>
</tr>
<tr>
<td>Total recovery</td>
<td></td>
<td>98.3$^a$ (12.0)</td>
<td>95.5$^a$ (11.1)</td>
<td>83.8$^a$ (6.4)</td>
</tr>
</tbody>
</table>
integrated measure of the entire canopy. Pinewood in the elevated CO2 plots was also more enriched in 15N ($P < 0.01$; Table 1). SON showed the opposite with lower natural 15N abundance under elevated compared to ambient CO2 for mineral soil at 0–15 cm ($P < 0.0001$) and 15–30 cm depth ($P < 0.0001$; Table 1).

After 3 years of tracer addition, tree biomass (hardwood + pine) accounted for 10.6% of the 15N tracer added to forest plots under ambient CO2 and 13.3% under elevated CO2 ($P = 0.007$; Table 2, Fig. 1). Following application of enriched 15N tracers, APE 15N in the canopies of both treatments increased significantly through time ($P < 0.0001$; Fig. 1b). Although over the course of this experiment the APE 15N in the canopy was lower under elevated CO2 compared to ambient CO2, the larger canopy mass and N content under elevated CO2 resulted in a progressive increase in 15N recovery in the canopy through time ($P < 0.0001$; Fig. 1a, c). Fine root APE 15N was significantly lower under elevated compared to ambient CO2 ($P = 0.02$), but there were no significant main effects of CO2 on N content ($P = 0.48$) or 15N percent recovery of fine roots ($P = 0.92$). By the end of the third growing season following tracer application, percent recovery of the 15N tracer in the canopy ($P = 0.10$) and woody biomass ($P = 0.06$) was greater under elevated CO2 compared to ambient CO2 (Fig. 1c, f, Table 2).

Despite elevated CO2 effects on 15N recovery in tree biomass, we detected no significant CO2 main or interaction effects on the percent 15N recovery in the forest floor, SOM, DON, NH4+, or microbial pools (Table 2). Over the course of the experiment, 15N recovery in the forest litter (Oi) significantly decreased over time ($P < 0.00001$; Table 2) and SO15N recovery in the Oe

![Fig. 1](https://example.com/fig1.jpg) Effects of atmospheric CO2 (ambient in white, elevated in black) on the N content (g m$^{-2}$), atom% 15N excess and %15N recovery of plant pools (hardwood and pine) measured in this experiment. Error bars represent SEM using treatment plots as experimental units ($n = 3$ ambient CO2 plots and $n = 3$ elevated CO2 plots). Asterisks represent significant CO2 effects within year ($P \leq 0.05$).
horizon increased over time \((P = 0.01)\). In the 0–15 cm mineral soil, \(^{15}\text{N}\) recovery did not differ significantly among years and averaged 19.0 \(\pm\) 1.1% recovery. Only small quantities of \(^{15}\text{N}\) were recovered from the 15–30 cm mineral soil in the first year; tracer recovery rapidly declined in subsequent years \((P < 0.0001); \text{Table 2}\). Recovery of \(^{15}\text{O}\)N significantly decreased with depth, and average recovery for the 3 years was 37.5\% \(\pm\) 4.1 in the O\text{er}, 15.4\% \(\pm\) 2.0 in the 0–15 cm soil and 2.5\% \(\pm\) 0.9 in the 15–30 cm soil. DO\(^{15}\text{N}\) recovery increased over time in the O horizon \((P = 0.01)\) and the 0–15 cm mineral soil \((P = 0.03); \text{Table 2}\). Recovery of \(^{15}\text{N}\) in microbial biomass was variable over time with lower recovery in 2004 compared to 2003 and 2005 for both mineral soil horizons \((P < 0.01); \text{Table 2}\). Over the 3 years of the experiment, the total recovery of the applied \(^{15}\text{N}\) isotope ranged from 80\% to 98\%. Percent recovery in the entire ecosystem was not significantly different between CO\(_2\) treatments \((P = 0.74)\) or among years \((P = 0.31);\text{Table 2}\).

**Biomass and \(^{15}\text{N}\) concentration**

Elevated atmospheric CO\(_2\) significantly increased plant biomass \((\text{g} \text{ m}^{-2})\) but had no effect on the concentration of \(^{15}\text{N}\) in foliage, wood or fine roots. Loblolly pine canopy mass was higher \((\text{ca.} 29\%\) under elevated CO\(_2\) \((P = 0.10)\), and increased in mass through time \((P = 0.0001)\). A similar pattern was observed in the understory hardwoods with greater canopy mass under elevated compared to ambient CO\(_2\) \((P = 0.08)\). The content or ecosystem pool size of \(^{15}\text{N}\) in the plant canopy \((\text{loblolly pine} + \text{hardwoods})\) was significantly greater under elevated CO\(_2\) only in 2005 \((P = 0.007); \text{Fig. 1a}\). Similarly, the ecosystem pool size of \(^{15}\text{N}\) in woody biomass was significantly higher under elevated CO\(_2\) \((P = 0.01); \text{Fig. 1d}\).

Elevated atmospheric CO\(_2\) consistently increased the standing crop of fine root biomass in the O\text{er} \((58\% \text{ on average})\). Contrary to more detailed studies of root biomass, \text{(Pritchard et al., 2008; Jackson et al., 2009)}, we detected no significant CO\(_2\) effects on fine root biomass in the mineral soil. The pool of live fine roots in the 0–15 cm horizon \((234.1 \text{ g} \pm 12.8)\) was four times that of the biomass in the 15–30 cm \((50.9 \pm 5.5 \text{ g})\) and O\text{er} \((37 \pm 5.2)\) horizons \((P < 0.0001)\). We did not detect a CO\(_2\) treatment effect on the \(^{15}\text{N}\) concentration of fine roots or the total quantity of \(^{15}\text{N}\) in fine root biomass \((P = 0.48); \text{Fig. 1g}\).

We failed to detect an effect of elevated CO\(_2\) on the mass of the surface organic (O) horizon or on its \(^{15}\text{N}\) concentration or content \((\text{ecosystem pool size})\), but Lichter et al. \((2008)\) report that N accumulated faster under elevated compared to ambient CO\(_2\) during the first 6 years of the experiment, then leveled off between Years 6 and 9 \((2003–2005)\). Similarly, elevated CO\(_2\) had no effect on the content or pool size of N in the top 30 cm of mineral soil or on the concentration of N in DON, NH\(_4^+\), or microbial biomass \((\text{Table 2})\).

**Discussion**

After 9 years of CO\(_2\) fumigation, elevated CO\(_2\) continued to stimulate forest productivity above levels observed under ambient CO\(_2\) at the Duke FACE site \((McCarthy et al., 2010)\). In the final year of this study, the additional N in biomass was 15.7 g N m\(^{-2}\) higher in elevated relative to ambient CO\(_2\) treatments, or about a 4\% average annual increase in N uptake in each of the 9 years of CO\(_2\) fumigation \((\text{Fig. 1j})\). This means that an additional ca. 1.6 g N m\(^{-2}\) yr\(^{-1}\), was acquired by trees under elevated CO\(_2\). The significantly greater \(^{15}\text{N}\) recovery in plant biomass 3 years following tracer application \((i.e.,\ the\ 2005\ calendar\ year); \text{Fig. 1l})\ suggests that some of the additional N taken up under elevated CO\(_2\) was acquired from labeled forest floor and 0–15 cm soil horizons, where the majority of \(^{15}\text{N}\) tracers were initially retained. At the same time, the consistently lower atom\% \(^{15}\text{N}\) excess in plant N pools under elevated CO\(_2\) \((\text{Fig. 1k})\) indicates that nonlabeled pools of N in the ecosystem also supplied the additional N taken up under elevated compared to ambient CO\(_2\).

Below, we discuss processes likely controlling four factors: changes in natural-abundance \(\delta^{15}\text{N}\) under elevated CO\(_2\), temporal variations in isotope recovery, \(^{15}\text{N}\) cycling in the Duke Forest compared to other FACE sites, and additional uptake of unlabeled N sources.

**Changes in natural-abundance \(\delta^{15}\text{N}\) under elevated CO\(_2\)**

Changes in the N cycle were evident prior to \(^{15}\text{N}\) tracer addition \((\text{Table 1})\). Our natural-abundance data are consistent with increased mineralization of SON and/or deeper rooting \((15–30 \text{ cm})\) supporting increased NPP under elevated CO\(_2\). Although foliar \(\delta^{15}\text{N}\) can be positively correlated with net N mineralization in soil due to losses of \(^{15}\text{N}\)-deplete N species throughout the N cycle \((Garten & Van Miegroet, 1994; BassiriRad et al., 2003; Kahmen et al., 2008; Garten et al., 2011), several studies suggest that enhanced rates of SOM mineralization have prompted relatively higher vegetation \(\delta^{15}\text{N}\) values with elevated CO\(_2\). For example, our findings are similar to result from the Mojave FACE site, where \(\delta^{15}\text{N}\) increased in the dominant vegetation \((\text{Larrea tridentata})\) grown under elevated CO\(_2\) \((\text{Billings et al., 2002, 2004})\), as well as open-top chamber research on ponderosa pines \((\text{Pinus ponderosa} \text{Dougl})\), which revealed significantly enriched \(\delta^{15}\text{N}\) with elevated CO\(_2\) in both live

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and senesced needles (Johnson et al., 2000). Mining of N from recalcitrant SOM is the mechanism proposed for inducing $^{15}$N enrichment in these studies (Johnson et al., 2000; Billings et al., 2004). This is because, with some exceptions, recalcitrant SOM is typically enriched in $^{15}$N, while N in more labile fractions is relatively $^{15}$N-depleted. Although our methods cannot distinguish between mineralization of labile and recalcitrant ($^{15}$N enriched) SOM, other studies suggest that enhanced mineralization of relatively slow-turnover SOM can occur with elevated CO$_2$ (Billings & Ziegler, 2008; Langley et al., 2009; Hofmockel et al., 2011). Values of $\delta^{15}$N for ammonium are consistent with increased mineralization, but minimal N loss (Garten, 1993; Compton et al., 2007; Kahmen et al., 2008; Craine et al., 2009) in the Duke FACE experiment elevated CO$_2$ produced isotopically lighter soil extractable NH$_4^+$ (0–15 cm soil; Table 1).

The natural-abundance $\delta^{15}$N signature of leaf litter is also consistent with deeper rooting. Soil $\delta^{15}$N values generally increase with depth (Nadelhoffer & Fry, 1988a; Högberg, 1997), a phenomenon generally attributed to greater age of SON with depth and the fact that mineralization favors the lighter $^{14}$N, thus leaving enriched $^{15}$N in older SON (Létolle, 1980; Nadelhoffer & Fry 1988b). Although annual destructive soil sampling (via soil cores) at the Duke FACE experiment did not reveal a significant CO$_2$ effect on the 15–30 cm fine root biomass, coarse roots sampled from soil pits dug to 32 cm depth revealed significantly greater (ca. two-fold) coarse root biomass under elevated CO$_2$ (Jackson et al., 2009). Fine root minirhizotron data from 1998 to 2004 are consistent with deeper rooting; elevated CO$_2$ significantly increased fine root production in the 15–30 cm soil increment (+25%; Pritchard et al., 2008). Studies from the Oak Ridge National Lab (ORNL) FACE experiment suggest that N may be more available in deep compared to shallow soil, due to decreased microbial and root uptake of mineralized N with depth (Iversen, 2010). In our results, increased fine root production at depth (15–30 cm) combined with higher natural-abundance $\delta^{15}$N in plant litter of elevated relative to ambient CO$_2$ treatments suggests that trees may have acquired some additional N from deeper soil pools under elevated CO$_2$.

Temporal variations in isotope recovery

In the first two growing seasons following tracer application (i.e., 2003, 2004), significantly more N was taken up by trees under elevated CO$_2$ (on average 10.2 and 15.9 g N m$^{-2}$, respectively; Fig. 1j), but not more $^{15}$N (Fig. 1I), suggesting that some of the additional N was acquired from an unlabeled source. It is important to remember that the cycling of unlabeled material in this forest influences temporal trends. For example, the time-lags of wood and needle production relative to plant N uptake and OM synthesis could be important, especially in the first year, when new material is largely built from carbohydrate produced the previous year (i.e., prior to the labeling). In addition, the longevity of loblolly pine foliage in the Piedmont of NC is 18 months (Zhang & Allen, 1996). Therefore during the first year, old natural-abundance needles fell to the forest floor and new needles were generated from unlabeled carbohydrates. During the second growing season, the old unlabeled needles began decomposing on the forest floor, while the first experimental (largely unlabeled) cohort of needles remained on the tree. Thus, the cycling of unlabeled N in the system delays the return of assimilated $^{15}$N to the forest floor, and contributes to the temporal dynamics of $^{15}$N recovery in tree biomass.

A second source of interannual variability in CO$_2$ treatment effects on $^{15}$N recovery is probably related to extreme weather events. In December 2002 (sixth year of the experiment, the winter prior to tracer application), a severe ice storm substantially reduced living tree biomass and increased detrital inputs to the forest floor (McCarthy et al., 2006). No CO$_2$ stimulation of annual litterfall inputs was detected the following 2 years. It was not until the ninth year of the experiment (2005) that a significant increase in net annual C increment returned (ca. 17%; Lichter et al., 2008). Thus, it is feasible that the influence of CO$_2$ fertilization on $^{15}$N assimilation by trees was strongly diluted by the natural-abundance inputs of decomposing branches and leaves as a consequence of the ice storm.

Some of the temporal variation in $^{15}$N recovery in plant biomass may also be the result of the $^{15}$N label being retained in the mineral-bound SOM (De Graaff et al., 2009; Langley et al., 2009). Previous studies have shown that the $^{15}$N tracer may initially be immobilized by microorganisms and retained in relatively stable mineral associated organic pools (Currie et al., 1999; Hagedorn et al., 2005). Similarly, recent NMR data suggest that microbial residues can account for up to 80% of SON (Simpson et al., 2007), supporting the idea that mineral-bound organic matter tends to be dominated by microbial products (Guggenberger et al., 1994; Rumpel et al., 2010). This microbial-derived SO$^{15}$N can take several years to be remobilized as plant available $^{15}$N, consistent with an increase in the APE $^{15}$N signature over time (Fig. 1k).

The progressive increase in percent recovery of $^{15}$N in tree biomass under elevated CO$_2$ compared to ambient CO$_2$ (Fig. 1l) is consistent with uptake from the 0–15 cm soil (O and A horizons), where the majority of
the tracer was retained (Table 2), and may be due to increased turnover of SOM. Calculations of SOM turnover in the Duke Forest have been made based on C mineralization rates under elevated CO2 combined with C : N ratios of the O horizon (45) and the 0–15 cm mineral soil (20; Lichter et al., 2008). The calculated additional N mineralized under elevated CO2 is on average 0.9 g N m\(^{-2}\) yr\(^{-1}\) from the O horizon and 3 g N m\(^{-2}\) yr\(^{-1}\) from the mineral soil (Drake et al., 2011). These small changes in soil N pools are difficult to detect even with the added sensitivity of the \(^{15}\)N tracer (Table 2). Nonetheless, it is reasonable to suggest that increased mineralization of organic and mineral substrates in the upper 15 cm may be contributing to CO2 enhanced growth based on evidence of increased C mineralization (Drake et al., 2011) and widening C : N ratios (Lichter et al., 2008), combined with higher \(^{15}\)N recovery in plants under elevated CO2.

Within the CO2 literature, evidence suggests that in low N environments mineralization can decrease soil C accumulation under elevated CO2 (Carney et al., 2007; Hungate et al., 2009; Langley et al., 2009; Hofmockel et al., 2011). Reduced gains in SOC have been attributed to priming of slow-turnover SOM (Hoosbeek & Scarascia-Mugnozza, 2009), which may be driven by changes in the microbial community, including increased fungal : bacterial ratio (Carney et al., 2007), or increased extracellular enzyme activity of microbes adept at accessing recalcitrant SOM (Billings & Ziegler, 2008; Billings et al., 2010). At the Duke FACE experiment, increases in NPP under elevated CO2 have increased the quantity of C entering the belowground system through fine root production, exudation, and C allocation to ectomycorrhizal fungi (Matamala & Schlesinger, 2000; Pritchard et al., 2001; Norby et al., 2004; Garcia et al., 2008). These processes, alone or in combination, can increase the metabolism of organic substrates by soil microbial communities and the release of N from SOM (Clarholm, 1985; Asmar et al., 1994; Trueman & Gonzalez-Meler, 2005). This is consistent with the elevated CO2 canopy initially showing greater \(^{14}\)N assimilation (depleted APE signature under elevated CO2; Fig. 1k), but progressively increased \(^{15}\)N uptake (Fig. 11) as \(^{15}\)N was slowly remineralized into the available pool (Hagedorn et al., 2005). Our data are also consistent with the idea that enhanced plant N uptake under elevated CO2 may be supported by the decomposition of SOM. It is possible, therefore, that under elevated CO2 soil microbial communities in the Duke Forest are responding to increased plant N demand by increasing the mineralization of SOM, resulting in significantly greater \(^{15}\)N recovery in biomass at elevated compared to ambient CO2 3 years following tracer application (Fig. 11).

Increased root and fungal biomass in the 0–15 cm mineral soil is possibly contributing to the transition from an unlabeled to a labeled N source over the duration of this experiment. Root exploration has been the dominant hypothesis for enhanced N uptake at other forest FACE sites (Norby & Iversen, 2006; Zak et al., 2007). Results from our annual sampling indicate that fine root biomass is greater under elevated CO2 only in the organic horizon, but not the mineral soil. Previous studies that focused explicitly on fine root dynamics indicate that fine root production and biomass are greater under elevated CO2 at the Duke FACE site (average across years, 25–30%, O and 0–15 cm mineral horizons; Pritchard et al., 2001, 2008; Jackson et al., 2009), although not stimulated to the same degree as observed at the Rhinelander (+57%) and ORNL FACE sites (+92%; Finzi et al., 2007). Increases in fine root production augment the volume of soil explored by roots for available N. Furthermore, in the surface mineral soil (O and 0–10 cm mineral horizons) ectomycorrhizal colonization of lobolly pine roots has increased 14% under elevated CO2 (Garcia et al., 2008). Field and laboratory studies show that carbon allocation to ectomycorrhizal fungi increases as the concentration of available N in the soil decreases (Wallander & Nylund, 1991), so the ability of trees to take up additional N from the soil under elevated CO2 may be enhanced by increases in C allocation to mycorrhizal fungi. If this is true, however, depletion in \(^{15}\)N of foliage compared to soil would be greater under elevated CO2, because the discrimination against the heavy isotope during the transfer of N compounds by mycorrhizal fungi causes a decrease in the \(^{15}\)N of plants and increases the \(^{15}\)N of fungi (Emmerton et al., 2001; Hobbie et al., 2005; Hobbie, 2006). The opposite was observed in our natural-abundance data, in which the difference in \(^{15}\)N between litter and mineral soil is greater under ambient conditions, and soil \(^{15}\)N is significantly more depleted under elevated relative to ambient CO2 (Table 1). This suggests that increased mycorrhizal assimilation of N is not the source of greater N uptake. Given the integrative nature of natural-abundance values, it is alternatively possible that the natural-abundance data reflect the net result of increased rates of microbial SOM turnover and the mitigative effect of foliar \(^{15}\)N of mycorrhizal N acquisition (Garcia et al., 2008).

Comparison with other FACE studies
The results of this \(^{15}\)N tracer experiment are consistent with those of Zak et al. (2007) who found significantly higher \(^{15}\)N recovery in aspen and aspen-birch communities growing under elevated CO2 (10.0 ± 2.1%) at the Rhinelander FACE site compared to those growing
under ambient CO$_2$ (7.4 ± 1.3%). An interesting difference between experiments, however, is that greater $^{15}$N recovery under elevated CO$_2$ was observed within 12 months (approximately one growing season) of tracer application at the Rhinelander FACE site, whereas at the Duke FACE site it took 28 months (approximately three growing seasons) for significantly higher $^{15}$N recovery in biomass under elevated CO$_2$.

Differences in plant community composition between the Rhinelander and Duke FACE sites may have contributed to the difference in time elapsed until significantly greater $^{15}$N recovery was observed under elevated CO$_2$. The longevity of loblolly pine foliage in the Duke Forest delays the return of assimilated $^{15}$N to the forest floor relative to the deciduous forests of the Rhinelander FACE experiment. Unlike the deciduous forest, where the $^{15}$N returns to the forest floor in the first fall, the pine forest has a pulse of $^{14}$N entering the soil system when the unlabeled needles fall to the forest floor. Furthermore, the northern hardwood species produce leaf litter that typically decomposes faster than pine needles when compared in similar climate and soil; the warmer climate in NC should have mitigated this difference to some extent. As a result, after 12 months, the O horizon at the Rhinelander FACE experiment retained on average 39% of the applied $^{15}$N (Zak et al., 2007), compared to 79% retention in the O horizon at Duke FACE following 16 months of tracer application. Because the bulk of the $^{15}$N label was retained in the forest litter during the current study (Table 2), root biomass in the surface mineral soil had limited access to $^{15}$N during the first year of our experiment.

Support for PNL was garnered by the $^{15}$N experiment in the Florida scrub oak ecosystem, where initial CO$_2$ enhancements in aboveground mass of N and $^{15}$N declined over time (Hungate et al., 2006). After 4 years, the accumulation of N in oak tissues and the O horizon exceeded the CO$_2$ stimulation of N uptake during the first year of the tracer experiment. Reduced soil N availability diminished aboveground NPP as evidenced by the declining aboveground litter production in Years 2–4 of the Florida scrub oak experiment (Hungate et al., 2006). The results from this study differ from the Duke Forest for several reasons, including well-drained sandy soils, deciduous plant community composition, and the much greater CO$_2$ response, which elicited nearly 80% stimulation of aboveground biomass (Dijkstra et al., 2002). Although other studies have demonstrated the need for additional N to elicit a CO$_2$ response (Reich et al., 2006), including prototype results from the Duke Forest (Oren et al., 2001), our long-term experiment has not yet revealed evidence for reduced N cycling or PNL (Drake et al., 2011).

Additional uptake of unlabelled N sources

In addition to the $^{15}$N tracer, unlabeled sources of N may be supporting increased NPP as suggested by lower APE $^{15}$N of plant biomass under elevated CO$_2$. Sources of unlabeled N include N$_2$ fixation, atmospheric deposition, and soil >15 cm below the soil surface. Of these sources, N below 15 cm is the most likely source of unlabelled N that may have been taken up by trees under elevated CO$_2$. The natural-abundance $\delta^{15}$N values of SOM normally increase with depth (Högberg, 1997; Billings & Richter, 2006). After the $^{15}$N tracer was added, $\delta^{15}$N in the surface soil horizons was artificially elevated well-above natural-abundance levels, resulting in a decline in $\delta^{15}$N with depth. By extending fine roots deeper into the mineral soil, loblolly pine trees may have acquired additional, unlabeled N. Although the Duke FACE CO$_2$ stimulation of fine root production is small, relative to the doubling of fine root production that occurred at the ORNL FACE experiment (Norby et al., 2004), deeper soil N probably contributes to the additional N uptake by trees grown under elevated CO$_2$ at the Duke FACE site.

Previous studies at the Duke FACE experiment indicated that heterotrophic N$_2$ fixation provided an additional source of exogenous N, but elevated CO$_2$ did not enhance N$_2$ fixation in the forest floor or mineral soil (0–10 cm; Hofmockel & Schlesinger, 2007). Although acetylene reduction assays (Hardy et al., 1968) did not reveal a CO$_2$ stimulation of diazotrophs during the 2000 growing season, N$_2$ fixation may have been stimulated in subsequent years of the experiment, as suggested by the natural-abundance results. N$_2$ fixation may be contributing to the natural $^{15}$N abundance in the mineral soil (N$_2$ is 0‰ by definition; Table 1) as well as a fraction of the additional $^{14}$N assimilated by plants under elevated CO$_2$. Previous analyses suggest, however, that the effect of increased N$_2$ fixation by elevated CO$_2$ is too small to account for the additional N demand (Van Groenigen et al., 2006).

Foliar N uptake can be an important component of N inputs in forests, especially under conditions of N deficiency (Brumme et al., 1992; Eilers et al., 1992; Sievering et al., 2007). Although N concentration in throughfall is not different between CO$_2$ treatments (Lichter et al., 2000; Oh et al., 2007), greater canopy biomass under elevated CO$_2$ could increase foliar uptake of atmospheric N deposition, even if leaf-specific rates of uptake are the same under ambient and elevated CO$_2$. Total atmospheric N deposition at the Duke FACE site is 1.37 g N m$^{-2}$ yr$^{-1}$ (Sparks et al., 2008). Previous studies indicate that 0.12 g N m$^{-2}$ yr$^{-1}$ of NH$_4^+$ was absorbed by the canopy, with no significant CO$_2$ treatment effects (Lichter et al., 2000). Similarly NO$_3^-$

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concentration in throughfall is not significantly different between CO₂ treatments (Lichter et al., 2000; Oh et al., 2007). In some seasons, NO₃⁻–N concentrations in throughfall exceed precipitation due to foliar leaching (Lichter et al., 2000) or additional inputs of dry deposition, resulting in average throughfall inorganic N fluxes of \(2.53 \pm 1.6 \text{ g N m}^{-2} \text{ yr}^{-1}\) (1998–2001 from Oh et al., 2007). Therefore, foliar uptake of atmospheric N deposition is minimal and cannot explain the difference in unlabeled N uptake between the ambient and elevated CO₂ plots. An alternative explanation for lower APE in the elevated CO₂ trees could be dilution of the \(^{15}\text{N}\) labeling by a larger initial N pool (prior to \(^{15}\text{N}\) labeling) in trees grown under elevated CO₂. APE depletion was greatest in the canopy (Fig. 1); yet, we detected no CO₂ main effect on the canopy N pool prior to labeling (\(P = 0.45\), or over the course of the experiment (\(P = 0.27\)). Only in 2005 did we detect a significant CO₂ effect on the canopy N pool.

Conclusions

There has been much speculation about the sustainability of high NPP in response to elevated CO₂ in N limited ecosystems (Field, 1999; Luo et al., 2004; Finzi et al., 2006b; Hungate et al., 2006; Norby & Iversen, 2006; Zak et al., 2007). Labeling forests with \(^{15}\text{N}\) has provided information about the short- and long-term fate of N and has led to insights regarding global C cycling. At the Duke FACE site, the rate at which N is being sequestered in plant biomass is greater than the rate of atmospheric deposition and heterotrophic N fixation (Finzi et al., 2002; Hofmockel & Schlesinger, 2007; Sparks et al., 2008), suggesting that SOM decomposition supplies a significant fraction of plant N in both ambient and elevated CO₂ conditions, but that this is greater under elevated CO₂ (Fig. 1). The results from natural-abundance data and this \(^{15}\text{N}\) tracer experiment suggest that in pine forests of the southeastern United States, rising CO₂ may elicit shifts in the mechanisms by which plants acquire N, allowing a sustained increase in NPP for decades. Our study suggests that increased mineralization of N in the organic and 0–15 cm mineral horizon and deeper rooting are likely sustaining the elevated CO₂ enhancement of NPP.

Acknowledgements

We thank Jeff Pippen, Will Cook, Heather Hemric, Isabel Heine, Eddie Brzostek, Vikki Rodgers, and Jennifer Talbot for help in the laboratory and field. We also thank Sharon Billings, John Lichter, and three anonymous referees for useful comments. This research was supported by the US Department of Energy, Office of Science, Biological and Environmental Research (BER) program and by the National Science Foundation (DEB0236356 to ACF and DEB0235425 to RBJ).

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