Global Change Biology

Global Change Biology (2011) **17**, 3338–3350, doi: 10.1111/j.1365-2486.2011.02465.x

Sources of increased N uptake in forest trees growing under elevated CO_2: results of a large-scale ^{15}N study

KIRSTEN S. HOFMOCKEL*†, ANNE GALLET-BUDYNEK‡§, HEATHER R. McCARTHY†¶, WILLIAM S. CURRIE**, ROBERT B. JACKSON† and ADRIEN FINZI‡

*Department of Ecology, Evolution, & Organismal Biology, Iowa State University, Ames, IA, 50011, USA, †Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC, 27708, USA, ‡Department of Biology, Boston University, 5 Cummington Street, Boston, MA, 02215, USA, §UMR 1220 TCEM, INRA, F-33883, Villenave d'Ornon, France, ¶Department of Earth System Science, University of California, Irvine, CA, 92697, USA, **School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI, 48109, USA

Abstract

Nitrogen availability in terrestrial ecosystems strongly influences plant productivity and nutrient cycling in response to increasing atmospheric carbon dioxide (CO_2). Elevated CO_2 has consistently stimulated forest productivity at the Duke Forest free-air CO_2 enrichment experiment throughout the decade-long experiment. It remains unclear how the N cycle has changed with elevated CO_2 to support this increased productivity. Using natural-abundance measures of N isotopes together with an ecosystem-scale ^{15}N tracer experiment, we quantified the cycling of ^{15}N in plant and soil pools under ambient and elevated CO_2 over three growing seasons to determine how elevated CO_2 changed N cycling between plants, soil, and microorganisms. After measuring natural-abundance ^{15}N differences in ambient and CO_2-fumigated plots, we applied inorganic ^{15}N tracers and quantified the redistribution of ^{15}N for three subsequent growing seasons. The natural abundance of leaf litter was enriched under elevated compared to ambient CO_2, consistent with deeper rooting and enhanced N mineralization. After tracer application, ^{15}N was initially retained in the organic and mineral soil horizons. Recovery of ^{15}N in plant biomass was $3.5 \pm 0.5\%$ in the canopy, $1.7 \pm 0.2\%$ in roots and $1.7 \pm 0.2\%$ in branches. After two growing seasons, ^{15}N recoveries in biomass and soil pools were not significantly different between CO_2 treatments, despite greater total N uptake under elevated CO_2. After the third growing season, ^{15}N recovery in trees was significantly higher in elevated compared to ambient CO_2. Natural-abundance ^{15}N and tracer results, taken together, suggest that trees growing under elevated CO_2 acquired additional soil N resources to support increased plant growth. Our study provides an integrated understanding of elevated CO_2 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 CO_2 beyond the decadal time scale.

Keywords: ^{15}N, atmospheric CO_2, 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 CO_2 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 CO_2-mediated growth enhancement (Mcguire *et al*., 1995; Luo & Reynolds, 1999). However, several free-air CO_2 enrichment (FACE) experiments in North America have shown a continual stimulation in forest productivity under elevated CO_2 over time scales nearly reaching a decade (Finzi *et al*., 2006a; Norby & Iversen, 2006; Zak *et al*., 2007; McCarthy *et al*., 2010); although reduced CO_2-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

Correspondence: Kirsten S. Hofmockel, tel. 515 294 2589, fax 515 294 1337, e-mail: khof@iastate.edu

would expect the CO_2-mediated growth enhancement to diminish. By contrast, after more than a decade of CO_2 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 CO_2 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 CO_2 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 CO_2 at the Duke FACE experiment (Hofmockel *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 CO_2 raises the question of what sources of N support higher rates forest productivity under elevated CO_2.

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 CO_2, 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 ^{15}N to entire forest plots, followed by an analysis of the redistribution of the ^{15}N 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 ^{15}N tracer experiments in conjunction with natural-abundance measures offers opportunities to assess how elevated CO_2 has influenced N cycling. Specifically, we can first identify altered patterns of N cycling with elevated CO_2 using natural-abundance measures, and then measure two different N pools: the quantity of ^{15}N recovered in each ecosystem N pool (percent recovery of the tracer) and the level of ^{15}N 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 ^{15}N tracer (through its absolute mass) and unlabeled N (through tracer dilution) to be compared. We hypothesized that elevated CO_2 will be associated with higher APE and percent recovery of ^{15}N in tree biomass, as an indication of greater N uptake from surface soils. By contrast, lower APE and ^{15}N recovery of biomass under elevated CO_2 compared to ambient CO_2 would be indicative of greater N uptake from unlabeled sources, including older SON or soil below 15 cm, where little of the applied ^{15}N 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 $\mu L\ L^{-1}$) and three receive elevated concentrations of atmospheric CO_2 (ambient +200 $\mu L\ L^{-1}$). Fumigation with elevated CO_2 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 ^{15}N was measured in pine foliage samples collected from the bottom, middle, and top of the pine canopy in 2002 (see

^{15}N methods below). Natural abundance in wood was measured in the last wood increment in April 2003, before the tracer application. In pine branches, natural abundance of ^{15}N was estimated in branches taken outside the plots in September 2005. Natural abundance of ^{15}N 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 ^{15}N were applied to one ambient and one CO_2-fumigated plot per day. Enough water was added to the ^{15}N to simulate a 0.2 mm rain event, using backpack sprayers. Paralleling the proportions of NH_4^+ and NO_3^- in the soil (Finzi & Schlesinger, 2003), the tracer (98 atom% ^{15}N) was added as 75% $^{15}NH_4Cl$ and 25% $K^{15}NO_3$ at a rate of 0.015 g ^{15}N m^{-2} in 0.25 L H_2O 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, $\delta^{15}N$ values averaged 484 (±29)‰, representing 44 (±3)% ^{15}N recovery, with no significant differences between CO_2 treatments.

The redistribution of the ^{15}N label was followed for three growing seasons, corresponding to the seventh–ninth growing seasons after the initiation of CO_2 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 ^{15}N in a total of 144 foliage samples (e.g., 2 CO_2 treatments × 3 replicate plots × 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 1 Natural ^{15}N abundances in the ecosystem pools under ambient and elevated CO_2

Ecosystem pool	Ambient CO_2 $\delta^{15}N$ (‰)	Elevated CO_2 $\delta^{15}N$ (‰)	*P*-value
Pine needles	−3.3 (0.1)	−2.3 (0.7)	0.20
Bottom	−3.6 (0.1)	−2.5 (0.4)	0.07
Middle	−3.4 (0.1)	−2.5 (0.8)	0.31
Top	−2.9 (0.2)	−2.0 (1.0)	0.87
Hardwood foliage	−1.9 (0.3)	−2.5 (0.6)	0.39
Wood			
Pine stem	−4.4 (0.3)	−2.9 (0.2)	0.01
Pine branches	−4.9 (–)	−4.9 (–)	–
Hardwood stem and branches	4.1 (0.6)	3.9 (1.1)	0.84
Roots			
O_{ea}	−5.6 (0.2)	−5.7 (0.3)	0.86
0–15 cm mineral	−1.0 (0.1)	−0.6 (0.3)	0.21
15–30 cm mineral	−2.1 (0.5)	−0.9 (0.5)	0.13
Forest litter	−5.0 (0.3)	−4.2 (0.2)	0.00
SOM			
O_{ea}	−4.7 (0.2)	−4.7 (0.1)	0.90
0–15 cm mineral	1.8 (0.1)	1.1 (0.2)	0.00
15–30 cm mineral	4.5 (0.1)	3.5 (0.2)	0.00
NH_4^+–N			
O_{ea}	−4.2 (0.6)	−5.0 (1.4)	0.59
0–15 cm mineral	3.9 (1.6)	−1.0 (0.8)	0.02
15–30 cm mineral	21.6 (2.4)	23.7 (4.1)	0.66
TDN			
O_{ea}	−6.0 (0.4)	−7.8 (0.2)	0.00
0–15 cm mineral	−1.5 (1.2)	−2.1 (0.3)	0.68
15–30 cm mineral	−0.1 (0.8)	−1.3 (0.3)	0.15

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 homogenous 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 concentration 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 mM $CaCl_2$ 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_2SO_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 chloroform ($CHCl_3$) 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_2SO_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 cadium-reduction method. The quantity of N in DON and microbial biomass pools was measured in the 0.5 M K_2SO_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^+–N plus NO_3^-–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^-–N were below the detection limit (12 ppb NO_3^-–N) and were therefore not diffused. Soil NH_4^+, DON, and microbial biomass extracts were diffused onto acidified disks and analyzed for %N and δ^{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 O_{ea} 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 2 The percent recovery (±1 SE) of the ^{15}N tracer in plant (hardwood + pine) biomass and whole soil under ambient and elevated CO_2 in September of 2003, 2004, and 2005, corresponding to the seventh–ninth growing seasons following the initiation of CO_2 fumigation. Within a row, significant differences ($P < 0.05$) in percent recovery are indicated by different superscript letters

	Year					
	2003		2004		2005	
Ecosystem component	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Tree biomass	3.8^{a} (0.8)	3.5^{a} (0.8)	8.5^{b} (0.8)	9.5^{b} (1.3)	10.6^{b} (0.3)	13.3^{c} (0.5)
Total roots	1.1^{a} (0.2)	0.8^{a} (0.2)	1.6^{ab} (0.4)	1.6^{ab} (0.3)	2.4^{b} (0.2)	2.7^{b} (0.3)
Total canopy	1.2^{a} (0.3)	1.1^{a} (0.3)	3.8^{b} (0.3)	3.7^{b} (0.4)	4.9^{bc} (0.2)	6.3^{c} (0.6)
Bark	0.9^{a} (0.3)	0.9^{a} (0.2)	0.6^{a} (0.1)	0.7^{a} (0.2)	0.3^{a} (0.1)	0.4^{a} (0.1)
Bole	0.1^{a} (0.0)	0.1^{a} (0.0)	0.9^{b} (0.2)	1.2^{b} (0.2)	1.0^{b} (0.0)	1.2^{b} (0.0)
Branches	0.5^{a} (0.1)	0.6^{a} (0.2)	1.7^{b} (0.3)	2.3^{b} (0.4)	2.0^{b} (0.2)	2.7^{b} (0.3)
Forest litter	40.2^{a} (2.5)	42.4^{a} (7.5)	6.7^{bc} (2.2)	7.1^{b} (2.2)	1.5^{bc} (0.4)	0.9^{c} (0.1)
O_{ea} horizon						
SOM	22.0^{ab} (0.3)	16.5^{a} (2.5)	44.1^{ab} (5.8)	42.2^{ab} (5.8)	45.1^{ab} (11.7)	49.2^{b} (11.3)
MB	2.8^{a} (0.2)	2.1^{a} (0.3)	3.0^{a} (0.4)	3.0^{a} (0.3)	5.3^{a} (2.5)	1.3^{a} (0.3)
DON	0.3^{a} (0.1)	0.2^{a} (0.0)	0.8^{b} (0.1)	0.7^{ab} (0.1)	0.8^{ab} (0.1)	0.6^{ab} $(0.3)^{b}$
NH_4^+	0.1^{a} (0.0)	0.0^{a} (0.0)	0.0^{a} (0.0)	0.0^{a} (0.0)	0.1^{a} (0.1)	0.0^{a} (0.0)
Mineral soil 0–15 cm						
SOM	18.0^{a} (1.5)	21.2^{a} (1.1)	16.4^{a} (1.7)	15.2^{a} (0.3)	22.2^{a} (5.1)	20.8^{a} (2.4)
MB	1.5^{a} (0.2)	1.8^{a} (0.4)	0.5^{b} (0.1)	0.4^{b} (0.0)	1.4^{ab} (0.6)	1.0^{ab} (0.2)
DON	0.2^{a} (0.1)	0.3^{a} (0.1)	0.7^{a} (0.1)	0.5^{a} (0.0)	0.5^{a} (0.3)	0.5^{a} (0.1)
NH_4^+	0.2^{ab} (0.1)	0.2^{a} (0.1)	0.0^{b} (0.0)	0.0^{b} (0.0)	0.0^{ab} (0.0)	0.1^{ab} (0.0)
Mineral soil 15–30 cm						
SOM	8.4^{a} (1.7)	6.5^{a} (0.9)	0.0^{b} (0.0)	0.0^{b} (0.0)	0.0^{b} (0.0)	0.0^{b} (0.0)
MB	0.6^{a} (0.2)	0.4^{a} (0.2)	2.7^{b} (0.1)	1.8^{ab} (0.9)	0.3^{a} (0.1)	0.3^{a} (0.2)
DON	0.2^{a} (0.1)	0.3^{a} (0.1)	0.2^{a} (0.0)	0.4^{a} (0.1)	0.2^{a} (0.0)	0.3^{a} (0.1)
NH_4^+	0.0^{a} (0.0)	0.0^{a} (0.0)	0.0^{a} (0.0)	0.0^{a} (0.0)	0.0^{a} (0.0)	0.0^{a} (0.0)
Total recovery	98.3^{a} (12.0)	95.5^{a} (11.1)	83.8^{a} (6.4)	80.8^{a} (8.0)	88.0^{a} (9.2)	88.3^{a} (10.5)

integrated measure of the entire canopy. Pinewood in the elevated CO_2 plots was also more enriched in ^{15}N ($P < 0.01$; Table 1). SON showed the opposite with lower natural ^{15}N abundance under elevated compared to ambient CO_2 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 ^{15}N tracer added to forest plots under ambient CO_2 and 13.3% under elevated CO_2 ($P = 0.007$; Table 2, Fig. 1l). Following application of enriched ^{15}N tracers, APE ^{15}N in the canopies of both treatments increased significantly through time ($P < 0.0001$; Fig. 1b). Although over the course of this experiment the APE ^{15}N in the canopy was lower under elevated CO_2 compared to ambient CO_2, the larger canopy mass and N content under elevated CO_2 resulted in a progressive increase in ^{15}N recovery in the canopy through time ($P < 0.0001$; Fig. 1a, c). Fine root APE ^{15}N was significantly lower under elevated compared to ambient CO_2 ($P = 0.02$), but there were no significant main effects of CO_2 on N content ($P = 0.48$) or ^{15}N percent recovery of fine roots ($P = 0.92$). By the end of the third growing season following tracer application, percent recovery of the ^{15}N tracer in the canopy ($P = 0.10$) and woody biomass ($P = 0.06$) was greater under elevated CO_2 compared to ambient CO_2 (Fig. 1c, f, Table 2).

Despite elevated CO_2 effects on ^{15}N recovery in tree biomass, we detected no significant CO_2 main or interaction effects on the percent ^{15}N recovery in the forest floor, SOM, DON, NH_4^+, or microbial pools (Table 2). Over the course of the experiment, ^{15}N recovery in the forest litter (O_i) significantly decreased over time ($P < 0.00001$; Table 2) and $SO^{15}N$ recovery in the O_{ea}

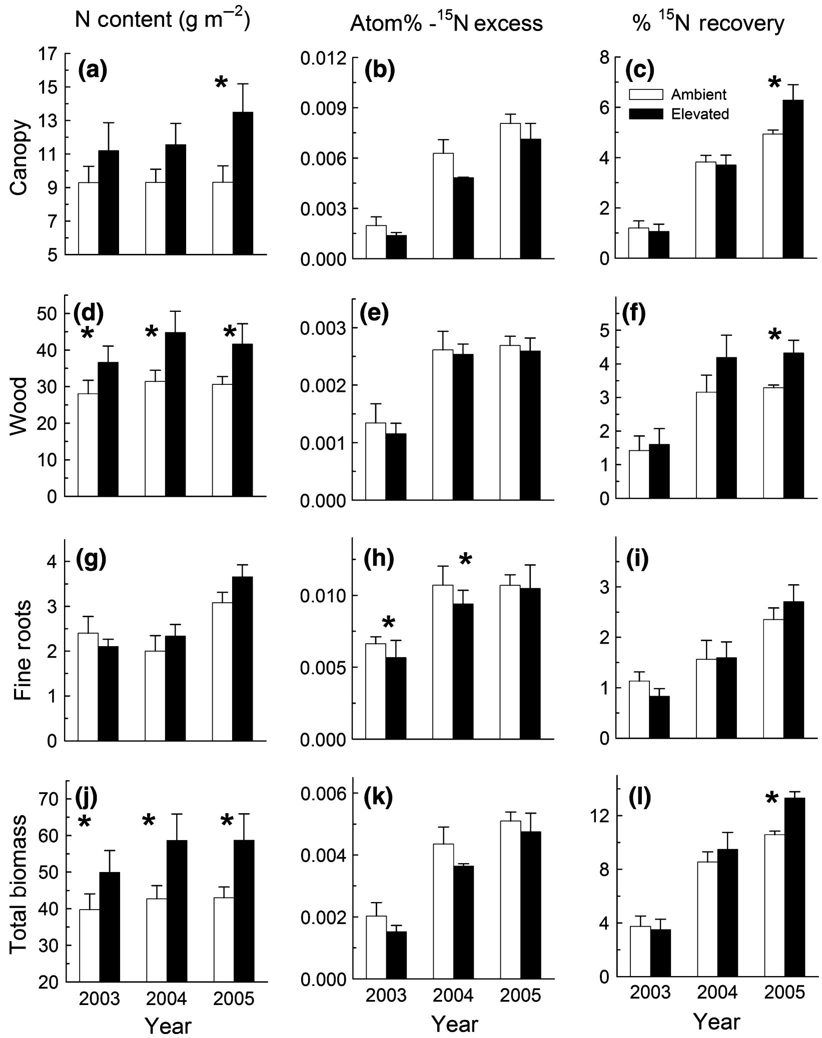

Fig. 1 Effects of atmospheric CO_2 (ambient in white, elevated in black) on the N content (g m^{-2}), atom% ^{15}N excess and %^{15}N recovery of plant pools (hardwood and pine) measured in this experiment. Error bars represent SEM using treatment plots as experimental units ($n = 3$ ambient CO_2 plots and $n = 3$ elevated CO_2 plots). Asterisks represent significant CO_2 effects within year ($P \leq 0.05$).

horizon increased over time ($P = 0.01$). In the 0–15 cm mineral soil, ^{15}N recovery did not differ significantly among years and averaged 19.0 ± 1.1% recovery. Only small quantities of ^{15}N were recovered from the 15–30 cm mineral soil in the first year; tracer recovery rapidly declined in subsequent years ($P < 0.0001$; Table 2). Recovery of $SO^{15}N$ significantly decreased with depth, and average recovery for the 3 years was 37.5% ± 4.1 in the O_{ea}, 15.4% ± 2.0 in the 0–15 cm soil and 2.5% ± 0.9 in the 15–30 cm soil. $DO^{15}N$ recovery increased over time in the O horizon ($P = 0.01$) and the 0–15 cm mineral soil ($P = 0.03$; Table 2). Recovery of ^{15}N in microbial biomass was variable over time with lower recovery in 2004 compared to 2003 and 2005 for both mineral soil horizons ($P < 0.01$; Table 2). Over the 3 years of the experiment, the total recovery of the applied ^{15}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 ($P = 0.31$; Table 2).

Biomass and N concentration

Elevated atmospheric CO_2 significantly increased plant biomass (g m^{-2}) but had no effect on the concentration of N in foliage, wood or fine roots. Loblolly pine canopy mass was higher (ca. 29%) under elevated compared to ambient 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 N in the plant canopy (loblolly pine + hardwoods) was significantly greater under elevated CO_2 only in 2005 ($P = 0.007$; Fig. 1a). Similarly, the ecosystem pool size of N in woody biomass was significantly higher under elevated CO_2 ($P = 0.01$; Fig. 1d).

Elevated atmospheric CO_2 consistently increased the standing crop of fine root biomass in the O_{ea} (58% on average). Contrary to more detailed studies of root biomass (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 g ± 12.8) was four times that of the biomass in the 15–30 cm (50.9 ± 5.5 g) and O_{ea} (37 ± 5.2) horizons ($P < 0.0001$). We did not detect a CO_2 treatment effect on the N concentration of fine roots or the total quantity of N in fine root biomass ($P = 0.48$; Fig. 1g).

We failed to detect an effect of elevated CO_2 on the mass of the surface organic (O) horizon or on its N concentration or content (ecosystem N 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 (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 (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}N recovery in plant biomass 3 years following tracer application (i.e., the 2005 calendar year; 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}N tracers were initially retained. At the same time, the consistently lower atom% ^{15}N excess in plant N pools under elevated CO_2 (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 $\delta^{15}N$ under elevated CO_2, temporal variations in isotope recovery, ^{15}N cycling in the Duke Forest compared to other FACE sites, and additional uptake of unlabeled N sources.

Changes in natural-abundance $\delta^{15}N$ under elevated CO_2

Changes in the N cycle were evident prior to ^{15}N tracer addition (Table 1). Our natural-abundance data are consistent with increased mineralization of SON and/or deeper rooting (15–30 cm) supporting increased NPP under elevated CO_2. Although foliar $\delta^{15}N$ can be positively correlated with net N mineralization in soil due to losses of ^{15}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 $\delta^{15}N$ values with elevated CO_2. For example, our findings are similar to result from the Mojave FACE site, where $\delta^{15}N$ increased in the dominant vegetation (*Larrea tridentate*) grown under elevated CO_2 (Billings *et al.*, 2002, 2004), as well as open-top chamber research on ponderosa pines (*Pinus ponderosa* Dougl.), which revealed significantly enriched $\delta^{15}N$ with elevated CO_2 in both live 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 δ^{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 δ^{15}N signature of leaf litter is also consistent with deeper rooting. Soil δ^{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. twofold) 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 δ^{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. 1l), 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 CO_2 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 CO_2 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 CO_2 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 CO_2.

Within the CO_2 literature, evidence suggests that in low N environments mineralization can decrease soil C accumulation under elevated CO_2 (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 CO_2 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 CO_2 canopy initially showing greater ^{14}N assimilation (depleted APE signature under elevated CO_2; Fig. 1k), but progressively increased ^{15}N uptake (Fig. 1l) 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 CO_2 may be supported by the decomposition of SOM. It is possible, therefore, that under elevated CO_2 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 CO_2 3 years following tracer application (Fig. 1l).

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 CO_2 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 CO_2 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 loblolly pine roots has increased 14% under elevated CO_2 (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 CO_2 may be enhanced by increases in C allocation to mycorrhizal fungi. If this is true, however, depletion in $\delta^{15}N$ of foliage compared to soil would be greater under elevated CO_2, because the discrimination against the heavy isotope during the transfer of N compounds by mycorrhizal fungi causes a decrease in the $\delta^{15}N$ of plants and increases the $\delta^{15}N$ 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 $\delta^{15}N$ is significantly more depleted under elevated relative to ambient CO_2 (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 $\delta^{15}N$ 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 CO_2 (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 δ^{15}N values of SOM normally increase with depth (Högberg, 1997; Billings & Richter, 2006). After the ^{15}N tracer was added, δ^{15}N in the surface soil horizons was artificially elevated well-above natural-abundance levels, resulting in a decline in δ^{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^-

concentration in throughfall is not significantly different between CO_2 treatments (Lichter *et al.*, 2000; Oh *et al.*, 2007). In some seasons, NO_3^-–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 ± 1.6 g N m^{-2} 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 CO_2 plots. An alternative explanation for lower APE in the elevated CO_2 trees could be dilution of the ^{15}N labeling by a larger initial N pool (prior to ^{15}N labeling) in trees grown under elevated CO_2. APE depletion was greatest in the canopy (Fig. 1); yet, we detected no CO_2 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 CO_2 effect on the canopy N pool.

Conclusions

There has been much speculation about the sustainability of high NPP in response to elevated CO_2 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}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-CO_2 conditions, but that this is greater under elevated CO_2 (Fig. 1j). The results from natural-abundance data and this ^{15}N tracer experiment suggest that in pine forests of the southeastern United States, rising CO_2 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 CO_2 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).

References

Asmar F, Eiland F, Nielsen NE (1994) Effect of extracellular-enzyme activities on solubilization rate of soil organic nitrogen. *Biology and Fertility of Soils*, **17**, 32–38.

BassiriRad H, Constable JVH, Lussenhop J *et al.* (2003) Widespread foliage delta N-15 depletion under elevated CO_2: inferences for the nitrogen cycle. *Global Change Biology*, **9**, 1582–1590.

Billings SA, Richter DD (2006) Changes in stable isotopic signatures of soil nitrogen and carbon during 40 years of forest development. *Oecologia*, **148**, 325–333.

Billings SA, Ziegler SE (2008) Altered patterns of soil carbon substrate usage and heterotrophic respiration in a pine forest with elevated CO_2 and N fertilization. *Global Change Biology*, **14**, 1025–1036.

Billings SA, Schaeffer SM, Evans RD (2002) Trace N gas losses and N mineralization in Mojave desert soils exposed to elevated CO_2. *Soil Biology & Biochemistry*, **34**, 1777–1784.

Billings SA, Schaeffer SM, Evans RD (2004) Soil microbial activity and N availability with elevated CO_2 in Mojave desert soils. *Global Biogeochemical Cycles*, **18**, GB1011.

Billings SA, Lichter J, Ziegler SE, Hungate BA, Richter DD (2010) A call to investigate drivers of soil organic matter retention vs. mineralization in a high CO_2 world. *Soil Biology & Biochemistry*, **42**, 665–668.

Brumme R, Leimcke U, Matzner E (1992) Interception and uptake of NH_4 and NO_3 from wet deposition by aboveground parts of young beech (*Fagus silvatica* L) trees. *Plant and Soil*, **142**, 273–279.

Buchmann N, Gebauer G, Schulze ED (1996) Partitioning of N-15-labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understory in a 15-year-old Picea abies plantation. *Biogeochemistry*, **33**, 1–23.

Cabrera ML, Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of America Journal*, **57**, 1007–1012.

Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 4990–4995.

Clarholm M (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil-nitrogen. *Soil Biology & Biochemistry*, **17**, 181–187.

Clark AC, Phillips DR, Fredrick DJ (1986) *Weight, Volume, and Physical Properties of Major Hardwood Species in the Piedmont*. RP-SE-255.USDA Forest Service, Southeastern Forest Experiment Station, Asheville, North Carolina, USA.

Compton JE, Hooker TD, Perakis SS (2007) Ecosystem N distribution and delta N-15 during a century of forest regrowth after agricultural abandonment. *Ecosystems*, **10**, 1197–1208.

Craine JM, Elmore AJ, Aidar MPM *et al.* (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist*, **183**, 980–992.

Currie WS, Aber JD, McDowell WH, Boone RD, Magill AH (1996) Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry*, **35**, 471–505.

Currie WS, Nadelhoffer KJ, Aber JD (1999) Soil detrital processes controlling the movement of N-15 tracers to forest vegetation. *Ecological Applications*, **9**, 87–102.

De Graaff MA, Van Kessel C, Six J (2009) Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated CO_2. *Soil Biology & Biochemistry*, **41**, 1094–1103.

Dijkstra P, Hymus G, Colavito D *et al.* (2002) Elevated atmospheric CO_2 stimulates aboveground biomass in a fire-regenerated scrub-oak ecosystem. *Global Change Biology*, **8**, 90–103.

Drake JE, Gallet-Budynek A, Hofmockel KS *et al.* (2011) Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters*, **14**, 349–357.

Eilers G, Brumme R, Matzner E (1992) Aboveground N-uptake from wet deposition by Norway spruce (*Picea abies* Karst). *Forest Ecology and Management*, **51**, 239–249.

Emmerton KS, Callaghan TV, Jones HE, Leake JR, Michelsen A, Read DJ (2001) Assimilation and isotopic fractionation of nitrogen by mycorrhizal fungi. *New Phytologist*, **151**, 503–511.

Fang ZX, Borders BE, Bailey RL (2000) Compatible volume-taper models for loblolly and slash pine based on a system with segmented-stem form factors. *Forest Science*, **46**, 1–12.

Field CB (1999) *Diverse Controls on Carbon Storage Under Elevarted CO_2: Toward a Synthesis*. Academic Press, San Diego, CA.

Finzi AC, Schlesinger WH (2003) Soil-nitrogen cycling in a pine forest exposed to 5 years of elevated carbon dioxide. *Ecosystems*, **6**, 444–456.

Finzi AC, Delucia EH, Hamilton JG, Richter DD, Schlesinger WH (2002) The nitrogen budget of a pine forest under free air CO_2 enrichment. *Oecologia*, **132**, 567–578.

Finzi AC, Delucia EH, Schlesinger WH (2004) Canopy N and P dynamics of a southeastern US pine forest under elevated CO_2. *Biogeochemistry*, **69**, 363–378.

Finzi AC, Moore DJP, DeLucia EH *et al*. (2006) Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. *Ecology*, **87**, 15–25.

Finzi AC, Sinsabaugh RL, Long TM, Osgood MP (2006) Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. *Ecosystems*, **9**, 215–226.

Finzi AC, Norby RJ, Calfapietra C *et al*. (2007) Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO_2. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 14014–14019.

Garcia MO, Ovasapyan T, Greas M, Treseder KK (2008) Mycorrhizal dynamics under elevated CO_2 and nitrogen fertilization in a warm temperate forest. *Plant and Soil*, **303**, 301–310.

Garten CT (1993) Variation in foliar N-15 abundance and the availability of soil-nitrogen on Walker Branch watershed. *Ecology*, **74**, 2098–2113.

Garten CT, Van Miegroet H (1994) Relationships between soil-nitrogen dynamics and natural N-15 abundance in plant foliage from Great Smoky Mountains National-Park. *Canadian Journal of Forest Research – Revue Canadienne De Recherche Forestiere*, **24**, 1636–1645.

Garten CT, Iversen CM, Norby RJ (2011) Litterfall N-15 abundance indicates declining soil nitrogen availability in a free-air CO_2 enrichment experiment. *Ecology*, **92**, 133–139.

Guggenberger G, Christensen B, Zech W (1994) Land-use effects on the composition of organic-matter in particle-size separates of soil. I. Lignin and carbohydrate signature. *European Journal of Soil Science*, **45**, 449–458.

Hagedorn F, Maurer S, Bucher J, Siegwolf R (2005) Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated CO_2: a N-15 and C-13 tracer study. *Global Change Biology*, **11**, 1816–1827.

Hardy RWF, Holsten RD, Jackson EK, Burns RC (1968) The acetylene-ethylene assay for N2 fixation: laboratory and field evaluation. *Plant Physiology*, **43**, 1185–1207.

Hendrey GR, Ellsworth DS, Lewin KF, Nagy J (1999) a free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology*, **5**, 293–309.

Hobbie EA (2006) Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*, **87**, 563–569.

Hobbie EA, Jumpponen A, Trappe J (2005) Foliar and fungal (15) N:(14) N ratios reflect development of mycorrhizae and nitrogen supply during primary succession: testing analytical models. *Oecologia*, **146**, 258–268.

Hofmockel KS, Schlesinger WH (2007) Carbon dioxide effects on heterotrophic dinitrogen fixation in a temperate pine forest. *Soil Science Society of America Journal*, **71**, 140–144.

Hofmockel KS, Schlesinger WH, Jackson RB (2007) Effects of elevated atmospheric carbon dioxide on amino acid and NH4+-N cycling in a temperate pine ecosystem. *Global Change Biology*, **13**, 1950–1959.

Hofmockel K, Zak D, Moran K, Jastrow J (2011) Changes in forest soil organic matter pools after a decade of elevated CO_2 and O_3. *Soil Biology & Biochemistry*, **43**, 1518–1527.

Högberg P (1997) Tansley review No 95 – N-15 natural abundance in soil-plant systems. *New Phytologist*, **137**, 179–203.

Holmes WE, Zak DR, Pregitzer KS, King JS (2003) Soil nitrogen transformations under *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* following 3 years exposure to elevated CO_2 and O-3. *Global Change Biology*, **9**, 1743–1750.

Holmes WE, Zak DR, Pregitzer KS, King JS (2006) Elevated CO_2 and O-3 alter soil nitrogen transformations beneath trembling aspen, paper birch, and sugar maple. *Ecosystems*, **9**, 1354–1363.

Hoosbeek MR, Scarascia-Mugnozza GE (2009) Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO_2 enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments. *Ecosystems*, **12**, 220–239.

Hungate BA, Dukes JS, Shaw MR, Luo YQ, Field CB (2003) Nitrogen and climate change. *Science*, **302**, 1512–1513.

Hungate BA, Johnson DW, Dijkstra P *et al*. (2006) Nitrogen cycling during seven years of atmospheric CO_2 enrichment in a scrub oak woodland. *Ecology*, **87**, 26–40.

Hungate BA, van Groenigen KJ, Six J *et al*. (2009) Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. *Global Change Biology*, **15**, 2020–2034.

Iversen CM (2010) Digging deeper: fine-root responses to rising atmospheric CO_2 concentration in forested ecosystems. *New Phytologist*, **186**, 346–357.

Jackson RB, Cook CW, Pippen JS, Palmer SM (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, **90**, 3352–3366.

Joergensen RG (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the k(EC) value. *Soil Biology & Biochemistry*, **28**, 25–31.

Johnson DW, Cheng W, Ball JT (2000) Effects of CO_2 and N fertilization on decomposition and N immobilization in ponderosa pine litter. *Plant and Soil*, **224**, 115–122.

Kahmen A, Wanek W, Buchmann N (2008) Foliar delta N-15 values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia*, **156**, 861–870.

Kenward M, Roger J (1997) Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, **53**, 983–997.

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry*, **32**, 1485–1498.

Langley JA, McKinley DC, Wolf AA, Hungate BA, Drake BG, Megonigal JP (2009) Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. *Soil Biology & Biochemistry*, **41**, 54–60.

Létolle R (1980) *Nitrogen-15 in the Natural Environment*. Elsevier, Amsterdam.

Lichter J, Lavine M, Mace KA, Richter DD, Schlesinger WH (2000) Throughfall chemistry in a loblolly pine plantation under elevated atmospheric CO_2 concentrations. *Biogeochemistry*, **50**, 73–93.

Lichter J, Billings SA, Ziegler SE *et al*. (2008) Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. *Global Change Biology*, **14**, 2910–2922.

Littell R (2002) Analysis of unbalanced mixed model data: a case study comparison of ANOVA versus REML/GLS. *Journal of Agricultural Biological and Environmental Statistics*, **7**, 472–490.

Luo YQ, Reynolds JF (1999) Validity of extrapolating field CO_2 experiments to predict carbon sequestration in natural ecosystems. *Ecology*, **80**, 1568–1583.

Luo Y, Su B, Currie WS *et al*. (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience*, **54**, 731–739.

Matamala R, Schlesinger WH (2000) Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology*, **6**, 967–979.

Matthews HD (2007) Implications of CO_2 fertilization for future climate change in a coupled climate-carbon model. *Global Change Biology*, **13**, 1068–1078.

McCarthy HR, Oren R, Kim HS, Johnsen KH, Maier C, Pritchard SG, Davis MA (2006) Interaction of ice storms and management practices on current carbon sequestration in forests with potential mitigation under future CO_2 atmosphere. *Journal of Geophysical Research-Atmospheres*, **111**, doi: 10.1029/2005JD006428 D15103.

McCarthy HR, Oren R, Finzi AC, Ellsworth DS, Kim HS, Johnsen KH, Millar B (2007) Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO_2. *Global Change Biology*, **13**, 2479–2497.

McCarthy H, Oren R, Johnsen K *et al*. (2010) Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric [CO_2] with nitrogen and water availability over stand development. *New Phytologist*, **185**, 514–528.

Mcguire AD, Melillo JM, Joyce LA (1995) The role of nitrogen in the response of forest net primary production to elevated atmospheric carbon-dioxide. *Annual Review of Ecology and Systematics*, **26**, 473–503.

Nadelhoffer K, Fry B (1988) Controls on natural nitrogen-15 and carbon-13 abundance in forest soil organic matter. *Soil Science Society of America Journal*, **52**, 1633–1640.

Nadelhoffer KJ, Emmett BA, Gundersen P *et al*. (1999) Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature*, **398**, 145–148.

Naidu SL, DeLucia EH, Thomas RB (1998) Contrasting patterns of biomass allocation in dominant and suppressed loblolly pine. *Canadian Journal of Forest Research – Revue Canadienne De Recherche Forestiere*, **28**, 1116–1124.

Norby RJ, Iversen CM (2006) Nitrogen uptake, distribution, turnover, and efficiency of use in a CO_2-enriched sweetgum forest. *Ecology*, **87**, 5–14.

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 9689–9693.

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtriec RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences of the United States of America*, **45**, 19368–19373.

Oh NH, Hofmockel M, Lavine ML, Richter DD (2007) Did elevated atmospheric CO_2 alter soil mineral weathering? An analysis of 5-year soil water chemistry data at Duke FACE study. *Global Change Biology*, **13**, 2626–2641.

Oren R, Ellsworth DS, Johnsen KH, *et al*. (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. *Nature*, **411**, 469–472.

Pritchard SG, Rogers HH, Davis MA, Van Santen E, Prior SA, Schlesinger WH (2001) The influence of elevated atmospheric CO_2 on fine root dynamics in an intact temperate forest. *Global Change Biology*, **7**, 829–837.

Pritchard SG, Strand AE, McCormack ML *et al*. (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air-CO_2-enrichment: a six-year-minirhizotron study. *Global Change Biology*, **14**, 588–602.

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist*, **157**, 475–492.

Reich PB, Hungate BA, Luo Y (2006) Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annual Review of Ecology Evolution and Systematics*, **37**, 611–636.

Rumpel C, Eusterhues K, Kogel-Knabner I (2010) Non-cellulosic neutral sugar contribution to mineral associated organic matter in top- and subsoil horizons of two acid forest soils. *Soil Biology & Biochemistry*, **42**, 379.

Schlesinger WH, Lichter J (2001) Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2. *Nature*, **411**, 466–469.

Sievering H, Tomaszewski T, Torizzo J (2007) Canopy uptake of atmospheric N deposition at a conifer forest: part I – canopy N budget, photosynthetic efficiency and net ecosystem exchange. *Tellus Series B – Chemical and Physical Meteorology*, **59**, 483–492.

Simpson AJ, Simpson MJ, Smith E, Kelleher BP (2007) Microbially derived inputs to soil organic matter: are current estimates too low? *Environmental Science & Technology*, **41**, 8070–8076.

Sparks JP, Walker J, Turnipseed A, Guenther A (2008) Dry nitrogen deposition estimates over a forest experiencing free air CO_2 enrichment. *Global Change Biology*, **14**, 768–781.

Stark JM, Hart SC (1996) Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal*, **60**, 1846–1855.

Thornton PE, Lamarque JF, Rosenbloom NA, Mahowald NM (2007) Influence of carbon-nitrogen cycle coupling on land model response to CO_2 fertilization and climate variability. *Global Biogeochemical Cycles*, **21**, GB4018, doi: 10.1029/2006GB002868.

Trueman RJ, Gonzalez-Meler MA (2005) Accelerated belowground C cycling in a managed agriforest ecosystem exposed to elevated carbon dioxide concentrations. *Global Change Biology*, **11**, 1258–1271.

Van Groenigen KJ, de Graaff MA, Six J, Harris D, Kuikman P, van Kessel C (2006) The impact of elevated atmospheric [CO_2] on soil C and N dynamics: a meta-analysis. In: *Managed Ecosystems and CO_2: Case Studies Processes and Perspectives*, (eds Noesberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H), pp. 373–391. Springer-Verlag, Berlin.

Wallander H, Nylund JE (1991) Effects of excess nitrogen on carbohydrate concentration and mycorrhizal development of *Pinus sylvestris* L seedlings. *New Phytologist*, **119**, 405–411.

Zaehle S, Friedlingstein P, Friend AD (2010) Terrestrial nitrogen feedbacks may accelerate future climate change. *Geophysical Research Letters*, **37**, L01401, doi: 10.1029/2009GL041345.

Zak DR, Holmes WE, Pregitzer KS (2007) Atmospheric CO_2 and O-3 alter the flow of N-15 in developing forest ecosystems. *Ecology*, **88**, 2630–2639.

Zhang SS, Allen HL (1996) Foliar nutrient dynamics of 11-year-old loblolly pine (*Pinus taeda*) following nitrogen fertilization. *Canadian Journal of Forest Research – Revue Canadienne De Recherche Forestiere*, **26**, 1426–1439.