

Net mineralization of N at deeper soil depths as a potential mechanism for sustained forest production under elevated [CO₂]

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Abstract

Elevated atmospheric carbon dioxide concentrations [CO₂] is projected to increase forest production, which could increase ecosystem carbon (C) storage. This study contributes to our broad goal of understanding the causes and consequences of increased fine-root production and mortality under elevated [CO₂] by examining potential gross nitrogen (N) cycling rates throughout the soil profile. Our study was conducted in a CO₂-enriched sweetgum (*Liquidambar styraciflua* L.) plantation in Oak Ridge, TN, USA. We used ¹⁵N isotope pool dilution methodology to measure potential gross N cycling rates in laboratory incubations of soil from four depth increments to 60 cm. Our objectives were twofold: (1) to determine whether N is available for root acquisition in deeper soil and (2) to determine whether elevated [CO₂], which has increased inputs of labile C resulting from greater fine-root mortality at depth, has altered N cycling rates. Although gross N fluxes declined with soil depth, we found that N is potentially available for roots to access, especially below 15 cm depth where rates of microbial consumption of mineral N were reduced relative to production. Overall, up to 60% of potential gross N mineralization and 100% of potential net N mineralization occurred below 15 cm depth at this site. This finding was supported by *in situ* measurements from ion-exchange resins, where total inorganic N availability at 55 cm depth was equal to or greater than N availability at 15 cm depth. While it is likely that trees grown under elevated [CO₂] are accessing a larger pool of inorganic N by mining deeper soil, we found no effect of elevated [CO₂] on potential gross or net N cycling rates. Thus, increased root exploration of the soil volume under elevated [CO₂] may be more important than changes in potential gross N cycling rates in sustaining forest responses to rising atmospheric CO₂.

Keywords: elevated [CO₂], fine roots, ¹⁵N isotope pool dilution, potential gross N mineralization, soil depth, sweetgum

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Introduction

Anthropogenic fossil fuel burning is now an accepted cause of atmospheric and climatic change. However, uncertainty lingers over future climatic forcing (Denman *et al.*, 2007). This is due in large part to uncertainties associated with biosphere feedbacks to the climate, such as the fertilization effect of elevated atmospheric carbon dioxide concentrations ([CO₂]) on plant production (Matthews, 2007). Further, feedbacks between limited soil nitrogen (N) availability and plant production are projected to constrain ecosystem carbon (C) uptake under future environmental conditions (Thornton *et al.*, 2007).

Forested ecosystems are an important component of the global C cycle because C taken up from the atmosphere may be stored in aboveground woody biomass

and soil pools over long periods of time (Dixon *et al.*, 1994). While net primary production (NPP) in forests is often limited by the availability of N (Vitousek & Howarth, 1991), elevated [CO₂] has increased the NPP of tree stands in four large-scale forested free-air CO₂ enrichment (FACE) experiments (Norby *et al.*, 2005). Owing to increased primary production and plant N demand, elevated [CO₂] has led to the increased uptake of mineral N from the soil in three of the four forested FACE experiments, even those that were initially N-limited (Finzi *et al.*, 2007). Thus, the long-term sustainability of increased forest production and N uptake under elevated [CO₂] will depend on the rate at which C and N are cycled among plant biomass and soil pools (Luo *et al.*, 2004).

C partitioning among ecosystem components with differing residence times is strongly affected by limiting resources (Litton *et al.*, 2007), and CO₂ enrichment has affected C partitioning in four forested FACE experiments (Norby *et al.*, 2005). At the Oak Ridge National

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Laboratory (ORNL) FACE experiment in a sweetgum (*Liquidambar styraciflua* L.) plantation, CO₂ enrichment nearly doubled the production of fine (i.e., <1 mm diameter) roots that are important in nutrient and water acquisition. Nearly half of the increase in fine-root production at ORNL FACE was below 30 cm soil depth (Norby *et al.*, 2004), and increases in rooting depth distribution under elevated [CO₂] have also been observed in nearly three-quarters of other CO₂ enrichment experiments in woody ecosystems (Iversen, 2010). Given that fine roots often proliferate in patches of nutrient availability (Hodge, 2004), access to nutrients may be one of the main factors driving increased root proliferation at depth in the soil under elevated [CO₂]. However, N cycling in terrestrial forested ecosystems is rarely measured at depths where root proliferation is occurring under elevated [CO₂] (i.e., below the top 10–15 cm of the soil, Booth *et al.*, 2005).

CO₂ enrichment is expected to decrease nutrient availability in terrestrial ecosystems as N is increasingly sequestered in plant biomass and soil organic matter (SOM) (reviewed in Berntson & Bazzaz, 1996; Luo *et al.*, 2004). Although gross N mineralization and immobilization rates have been measured many times at shallower soil depths in CO₂ enrichment experiments, these studies have seen variable results (reviewed in Booth *et al.*, 2005; de Graaff *et al.*, 2006). It has been argued that fine-root dynamics may be the missing link in our understanding of the variation in soil N cycling responses under elevated [CO₂] (Zak *et al.*, 2000). Greater root production under elevated [CO₂] increased C and N inputs to the soil ecosystem over the first 10 years of the ORNL FACE experiment, as the fine-root populations in this forest turn over in approximately 1 year (Iversen *et al.*, 2008). However, it is difficult to predict how the increased input of labile C to the soil from root mortality will affect soil N cycling (i.e., Zak *et al.*, 2000). This is because feedbacks between increased C inputs and soil N availability under elevated CO₂ may either be positive (i.e., increased N mineralization) or negative (i.e., increased N immobilization in microbial biomass) depending on the interplay between microbial community activity and detrital quality (Berntson & Bazzaz, 1996).

Our objectives in this experiment were twofold: (1) to determine a potential explanation for increased fine-root proliferation below 15 cm soil depth under elevated [CO₂] by quantifying the potential amount of mineral N made available for root acquisition throughout the soil profile and (2) to determine how increased inputs of labile C from fine-root mortality under elevated [CO₂] might affect N cycling rates at multiple soil depths. We used isotope pool dilution methodology to determine potential gross rates of NH₄⁺ and NO₃⁻ production and

consumption at four soil depth increments: 0–15, 15–30, 30–45 and 45–60 cm. We hypothesized that relative N availability (i.e., net N mineralization) would increase with soil depth due to decreased microbial competition for mineral N deeper in the soil. We further hypothesized that microbial N consumption would be greater under elevated [CO₂] due to increased C inputs from root mortality, but that consumption would differ with soil depth.

Materials and methods

Site description

Our experiment was conducted in June 2007, at the ORNL FACE experiment located on the National Environmental Research Park in Oak Ridge, TN, USA. The ORNL FACE site has been well-described elsewhere (Norby *et al.*, 2001). Briefly, the experiment consists of five 25 m diameter rings, of which four are encircled by a FACE apparatus. Two of these rings received elevated [CO₂] at a target value of 565 ppm during the daytime (actual daytime concentrations averaged 559 ppm in 2007), while the other two rings received ambient, or current, [CO₂], which averaged 401 ppm during the daytime in 2007 (Riggs *et al.*, 2008). The fifth ring has no FACE apparatus, and is treated as an ambient CO₂ treatment given that there has been no measureable effect of the FACE apparatus on ecosystem function (Norby *et al.*, 2001). The soils in the ORNL FACE experiment consist of Aquic Hapludults, where the soil texture is silty-clay-loam, and soil pH is approximately 5.5–6 (Norby *et al.*, 2001). Soil characteristics are further described in Table 1.

Soil processing

Eight soil cores (2.54 cm inner diameter × 60 cm deep, two cores per quadrant) were collected from each FACE ring on June 18, 2007 (with the exception of the ring with no FACE apparatus in which only six cores were collected due to inclement weather). Before each core was taken, the litter layer was carefully removed (there was no organic horizon at this site). The cores were pooled in 15 cm depth increments by ring in the field: 0–15, 15–30, 30–45 and 45–60 cm. Soils were placed in a cooler on ice directly after sampling, and upon return to the laboratory were refrigerated overnight at 4 °C. The following day, soil was sieved through a 2 mm mesh to remove large roots and pieces of organic matter; smaller roots were removed by hand. Initial soil gravimetric water content (GWC) was determined by oven-drying a 5 g subsample of soil for approximately 48 h at 105 °C. Samples were returned to the refrigerator at 4 °C after sieving.

¹⁵N labeling

Two days after the soil cores were collected, one subset of soil from each depth increment received a highly enriched (99.9 at.%) solution of (¹⁵NH₄)₂SO₄ to determine potential gross N mineralization and NH₄⁺ consumption rates, while

Table 1 Soil characteristics

Treatment	Soil depth (cm)	Soil C (g kg ⁻¹)	Soil N (g kg ⁻¹)	Initial GWC (%)	Initial NH ₄ ⁺ (mg N kg ⁻¹)	Initial NO ₃ ⁻ (mg N kg ⁻¹)
Ambient [CO ₂]	0–15	20.0 ± 1.5	1.55 ± 0.11	17.9 ± 0.7	1.69 ± 0.33	nd
	15–30	8.7 ± 0.5	0.87 ± 0.04	14.4 ± 1.3	0.65 ± 0.07	nd
	30–45	4.1 ± 0.4	0.53 ± 0.04	15.2 ± 0.9	0.36 ± 0.02	nd
	45–60	2.3 ± 0.3	0.46 ± 0.06	15.8 ± 0.5	0.30 ± 0.02	nd
Elevated [CO ₂]	0–15	21.4 ± 0.6	1.61 ± 0.00	17.5 ± 0.1	1.32 ± 0.21	0.12 ± 0.12
	15–30	10.1 ± 0.5	0.97 ± 0.05	15.1 ± 0.4	0.71 ± 0.01	nd
	30–45	4.9 ± 1.6	0.58 ± 0.15	15.9 ± 0.3	0.44 ± 0.10	nd
	45–60	2.6 ± 1.0	0.41 ± 0.12	17.2 ± 0.1	0.38 ± 0.06	nd

Soil C and N content were measured on soil excavated at multiple depth increments from two large soil pits (80 cm × 80 cm) in each treatment ring in June 2009. Initial gravimetric water content (GWC) and extractable NH₄⁺ and NO₃⁻ concentrations were determined from subsamples of soils collected in June 2007 before labeling; GWC after label addition was ~18% higher on average at all soil depths. Initial NO₃⁻ values were below detectable limits in all but five samples (i.e., below 0.005 mg N L⁻¹) and below the lowest standard (0.025 mg N L⁻¹) in all but one sample. nd, not determined.

another subset received a highly enriched solution of K¹⁵NO₃ to determine potential gross nitrification and NO₃⁻ consumption rates. The isotopic label was dissolved in 8 mL of distilled water and added in 1 mL increments to approximately 200 g of field-moist soil in a large polyethylene bag. The labeled soil was homogenized by hand for 30 s. We added variable amounts of labeled ¹⁵N depending on the soil depth (Table 1); label amounts were based on levels of NH₄⁺ and NO₃⁻ determined in a previous soil collection from similar depth increments (data not shown). The goal was to enrich soil NH₄⁺ and NO₃⁻ pools to approximately 60 at.% ¹⁵N (i.e., Stark, 2000). Resulting enrichments after blank correction (Stark & Hart, 1996), were between 46 and 76 at.% for soils labeled with ¹⁵NH₄⁺, and approximately 99 at.% for soils labeled with ¹⁵NO₃⁻ (Table 2). Relatively large atom percent enrichments in soils labeled with ¹⁵NO₃⁻ were due to the very small NO₃⁻ pools in unamended soils (below detection limits); large enrichments were necessary in this case to ensure enough recovery of N for sample diffusion.

Immediately after mixing, five subsamples were taken from each bag of labeled soil: a subsample of approximately 5 g was taken to determine GWC by oven-drying at 105 °C for greater than 48 h; two analytical subsamples of ¹⁵NH₄⁺ -*t*₀ (a and b) or ¹⁵NO₃⁻ -*t*₀ (a and b) of approximately 40 g each were extracted immediately with 150 mL of 2 M KCl to determine the fractional recovery of ¹⁵N in labeled soil as in Hart *et al.* (1994b); and two analytical subsamples (¹⁵NH₄⁺ -*t*₁ a, b or ¹⁵NO₃⁻ -*t*₁ a, b) of approximately 40 g each were placed in 237 mL specimen cups (Fisherbrand, Pittsburgh, PA, USA, catalog number: 02-544-212A) and incubated in 1 L Mason jars for 24 h in the dark at 20 ± 2 °C. Approximately 10 mL of distilled water was placed at the bottom of each Mason jar and the jars were capped to prevent soil drying during the incubation. Incubations were performed at field moist conditions.

The *t*₀ extractions were immediately shaken at low speed on a reciprocating shaker for 1 h and the extractant was filtered through a Whatman #1 filter paper that had been preleached with distilled water. An aliquot was frozen at -20 °C until

analysis for total [NH₄⁺] (Phenate method, 12-107-06-1-A) and [NO₃⁻] (Nitrate method, 12-107-04-1-B) on a Lachat QuikChem 8500 autoanalyzer (Lachat Instruments, Loveland, CO, USA). The remainder of the sample was frozen at -20 °C until diffusion to determine atom percent enrichment. After 24 h, the incubated *t*₁ samples were extracted with 150 mL of 2 M KCl, and shaken, filtered and frozen into aliquots for inorganic N analysis (as described above) and diffusion.

Labeled samples (*t*₀ and *t*₁) were diffused in order to obtain ~50 µg N (from the NH₄⁺ incubations) or ~25 µg N (from the highly enriched NO₃⁻ incubations) for ¹⁵N analysis. Specifically, NH₄⁺ was diffused from *t*₀ and *t*₁ samples by converting NH₄⁺ to NH₃ in a closed Mason jar with an excess of MgO and swirling the solution daily over a period of 6 days. NH₃-N was captured on two acidified filter disks consisting of small punches of Whatman #1 filter paper acidified with 2.5 M KHSO₄ and wrapped in Teflon tape as in Stark & Hart (1996). After airing out the solutions for 4 days to ensure the complete removal of NH₃, the remaining NO₃⁻ in the sample was converted to NH₄⁺ in a closed Mason jar with an excess of Devarda's alloy, and the diffusion process was repeated. Diffusion blanks were determined as in Stark & Hart (1996). After each incubation acidified disks were removed from the solution, dried over concentrated sulfuric acid, and packaged in tin capsules for ¹⁵N analysis at the Stable Isotope Facility at Utah State University (UT, USA). Analytical precision averaged 0.2% and 0.9% compared with the standard deviation for low ¹⁵N enrichment samples (<5 at.% ¹⁵N) and more ¹⁵N-enriched samples (>5 at.% ¹⁵N), respectively. The initial and final atom percent derived from mass spectrometer measurements were corrected for possible N contamination using the blank-correction equations [Eqns (2) and (3)] in Stark & Hart (1996).

Gross N mineralization and consumption calculations

Potential gross N mineralization and nitrification, as well as NH₄⁺ and NO₃⁻ consumption, were determined based on

Table 2 Dynamics of ^{15}N label in isotope pool dilution experiment for each species of N ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$)

Treatment	Soil depth (cm)	$\text{NH}_4\text{-N}$					$\text{NO}_3\text{-N}$				
		^{15}N excess (mg N kg $^{-1}$)	t_0 recovery (F_N)	t_0 APE	NH_4^+ consumption (% stim.)	MRT $_{\text{NH}_4\text{-N}}$ (h)	^{15}N excess (mg N kg $^{-1}$)	t_0 recovery (F_N)	t_0 APE	NO_3^+ consumption (% stim.)	MRT $_{\text{NO}_3\text{-N}}$ (h)
Ambient [CO_2]	0–15	2.65 $\pm$ 0.09	0.99 $\pm$ 0.03	60 $\pm$ 4	20 $\pm$ 4	28 $\pm$ 6	0.87 $\pm$ 0.02	0.98 $\pm$ 0.02	99 $\pm$ 0	9 $\pm$ 13	nd
	15–30	1.14 $\pm$ 0.09	0.87 $\pm$ 0.08	63 $\pm$ 2	22 $\pm$ 12	16 $\pm$ 1	0.71 $\pm$ 0.04	0.83 $\pm$ 0.04	99 $\pm$ 0	2 $\pm$ 2	nd
	30–45	0.50 $\pm$ 0.10	0.58 $\pm$ 0.11	58 $\pm$ 3	6 $\pm$ 31	23 $\pm$ 7	0.53 $\pm$ 0.10	0.61 $\pm$ 0.11	99 $\pm$ 0	nd	nd
	45–60	0.47 $\pm$ 0.05	0.54 $\pm$ 0.05	60 $\pm$ 4	130 $\pm$ 123	22 $\pm$ 2	0.23 $\pm$ 0.02	0.52 $\pm$ 0.03	99 $\pm$ 0	nd	nd
Elevated [CO_2]	0–15	2.54 $\pm$ 0.12	0.95 $\pm$ 0.05	65 $\pm$ 5	26 $\pm$ 11	24 $\pm$ 8	0.77 $\pm$ 0.10	0.88 $\pm$ 0.12	96 $\pm$ 3	1 $\pm$ 1	nd
	15–30	1.30 $\pm$ 0.09	0.99 $\pm$ 0.07	65 $\pm$ 1	35 $\pm$ 4	14 $\pm$ 2	0.68 $\pm$ 0.01	0.79 $\pm$ 0.01	99 $\pm$ 0	2 $\pm$ 1	nd
	30–45	0.48 $\pm$ 0.02	0.56 $\pm$ 0.03	53 $\pm$ 5	32 $\pm$ 34	25 $\pm$ 3	0.59 $\pm$ 0.04	0.68 $\pm$ 0.05	99 $\pm$ 0	nd	nd
	45–60	0.50 $\pm$ 0.01	0.57 $\pm$ 0.01	56 $\pm$ 6	44 $\pm$ 56	28 $\pm$ 8	0.31 $\pm$ 0.01	0.70 $\pm$ 0.01	99 $\pm$ 0	nd	nd

^{15}N excess was the ^{15}N added to soils in excess of background ^{15}N . The t_0 (time 0) recovery was the fractional recovery of N (F_N) in the samples that were labeled with ^{15}N and immediately extracted with 2 M KCl; values <1.0 indicate that ^{15}N was likely adsorbed to soil surfaces, especially in deeper soils with high clay content. APE (atom percent enrichment) was the ^{15}N percentage of t_0 sample minus the natural abundance of ^{15}N (assumed to be 0.366‰); target APE was 60%. The percent stimulation in NH_4^+ and NO_3^- consumption was the percent increase in gross N consumption rates due to the addition of the $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$. The MRT (mean residence time; initial inorganic N pool divided by gross N production) of $\text{NO}_3\text{-N}$ could not be determined due to initial NO_3^- pools that were below detection limits.

calculations developed by Kirkham & Bartholomew (1954), where the atom percent enrichment of the samples was corrected using the fractional recovery of ^{15}N ($F_{15\text{N}}$) in the t_0 samples as in Hart *et al.* (1994b)

$$F_{15\text{N}} = \frac{^{15}\text{N excess (mg kg}^{-1}) \times \text{subsample weight (kg)}}{^{15}\text{N injected (mg)}}, \quad (1)$$

$$\text{APE}_{t_0} = \frac{^{15}\text{N injected (mg)} \times F_{15\text{N}}}{\text{Total N injected (mg)} \times F_{15\text{N}} + \text{initial soil N content (mg)}} \times 100\%, \quad (2)$$

$$\begin{aligned} \text{Gross N mineralization (mg N kg}^{-1} \text{ soil day}^{-1}) \\ = \frac{[\text{NH}_4^+]_{t_0} - [\text{NH}_4^+]_{t_1}}{t} \times \frac{\log(\text{APE}_{t_0}/\text{APE}_{t_1})}{\log([\text{NH}_4^+]_{t_0}/[\text{NH}_4^+]_{t_1})}, \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Gross N consumption (mg N kg}^{-1} \text{ soil day}^{-1}) \\ = \text{Gross N mineralization} - \frac{[\text{NH}_4^+]_{t_1} - [\text{NH}_4^+]_{t_0}}{t}, \end{aligned} \quad (4)$$

where $[\text{NH}_4^+]_{t_0}$ and $[\text{NH}_4^+]_{t_1}$ are the total concentrations (mg kg $^{-1}$) of NH_4^+ in the t_0 and t_1 samples, respectively, APE_{t_0} is the atom percent ^{15}N excess of the NH_4^+ pool at t_0 [corrected for fractional recovery as in Eqn (2)], and APE_{t_1} is the atom percent ^{15}N excess of the NH_4^+ pool at t_1 and t is the incubation time (approximately 1 day). We used the same equations to determine gross nitrification and NO_3^- consumption by substituting $[\text{NO}_3^-]$ for $[\text{NH}_4^+]$ in the equations. The use of fractional recovery to correct the APE_{t_0} is suggested for use in heterogeneous soils (Hart *et al.*, 1994b), and has been used previously in incubations with intact cores (Davidson *et al.*, 1991). We used this method due to extremely low concentrations of NO_3^- in the initial samples that made it difficult to estimate initial APE. Adjusting the APE of the t_0 samples based on the fractional recovery of ^{15}N made the largest difference in calculations of gross nitrification; the APE of NH_4^+ samples was altered (on average) by <3 at.%. Gross N fluxes per unit soil mass (mg g $^{-1}$ day $^{-1}$) were converted to soil area (mg m $^{-2}$ day $^{-1}$) using bulk density values of 1.30, 1.43, 1.50 and 1.55 g cm $^{-3}$ for 0–15, 15–30, 30–45 and 45–60 cm depth increments, respectively (there was no difference in bulk density between ambient and elevated [CO_2] treatments).

Unstimulated consumption

Consumption of NH_4^+ as defined using this methodology includes NH_4^+ assimilation by microbes, as well as NH_4^+ volatilization, clay fixation or nitrification; NO_3^- consumption includes assimilatory and dissimilatory NO_3^- reduction by microbes, as well as denitrification (Hart *et al.*, 1994b). Consumption as calculated from the Kirkham & Bartholomew (1954) equations can be greater than rates derived from unlabeled incubations due to increased NH_4^+ or NO_3^- substrate (Booth *et al.*, 2005). Therefore, we determined

unstimulated NH_4^+ and NO_3^- consumption rates for each ring and depth by incubating approximately 40 g of unlabeled, field-moist soil for 24 h in a manner similar to the labeled t_1 samples to determine potential net N mineralization (e.g. Hart *et al.*, 1994a). Distilled water was added to these soils in the same amount as the labeled soil (i.e., 4 mL of distilled water were added to approximately 100 g field-moist soil) to prevent confounding differences in water content between labeled and unlabeled incubations. After 24 h, the samples were extracted as above, and frozen at -20°C until analysis for total $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ on a Lachat QuikChem 8500. Initial $[\text{NH}_4^+]$ was determined as the $[\text{NH}_4^+]$ of the t_0 $^{15}\text{NO}_3^-$ samples, and initial $[\text{NO}_3^-]$ was determined as the $[\text{NO}_3^-]$ of the t_0 $^{15}\text{NH}_4^+$ samples. Net ammonification was calculated as the difference between initial and final $[\text{NH}_4^+]$, whereas net nitrification was calculated as the difference between initial and final $[\text{NO}_3^-]$. Total N immobilization was calculated as the difference between gross N mineralization and net N ($\text{NH}_4^+ + \text{NO}_3^-$) mineralization. Unstimulated NH_4^+ consumption was calculated as the difference between gross N mineralization and net ammonification as in Hart *et al.* (1994a). Unstimulated NO_3^- consumption was calculated in a similar manner.

Ion-exchange resins

We used ion-exchange resins to examine N availability *in situ* and support the results of laboratory incubations described above. Resin access tubes (Warrington Ecological Systems Analysis Inc., Saint Ignatius, MT, USA) had been previously installed in eight locations in each FACE ring at 15 cm depth in 2001 (Johnson *et al.*, 2004). Additional tubes were installed at 30 and 55 cm depth in four locations in each ring (except the ambient plot without a FACE apparatus) in September 2007. WECSA mixed-bed ion-exchange resin capsules were placed in the tubes at 15 cm beginning in June 2001, and were collected and replaced monthly or every 3 months depending on the year. Resin capsules were incubated in the deeper access tubes on the same schedule as the shallower tubes beginning in May 2008. We present data from the overlapping time periods for both shallow and deep resins beginning in May 2008, and ending in September 2009. At each removal, resin capsules from each depth were rinsed with distilled water to remove soil particles, and allowed to air-dry. Each resin capsule was serially extracted three times with 20 mL of 2 M KCl by shaking for 30 min at low speed on a reciprocating shaker (for a total of 60 mL of KCl extractant). The combined extractant was filtered through a Whatman #1 filter paper that had been preleached with distilled water, and frozen at -20°C until analysis for NH_4^+ and NO_3^- on an autoanalyzer as described above.

Statistics

We used the SAS mixed-model procedure (SAS version 9.1, Cary, NC, USA) to analyze differences in gross and net N cycling rates under ambient and elevated $[\text{CO}_2]$ and with soil depth using a two-factor ANOVA where CO_2 treatment and soil depth were treated as fixed factors. Values were compared

across soil depths using Tukey's multiple comparison tests. Inorganic N adsorbed to resin capsules was analyzed in a similar manner, but including collection date as a repeated measure; ring within each treatment $\times$ depth combination was the subject of the repeated measure ($n = 2$ elevated $[\text{CO}_2]$ plots, $n = 3$ ambient $[\text{CO}_2]$ plots), and the covariance structure was specified as autoregressive. The SAS regression procedure was used to examine the relationship between: (1) stimulated and unstimulated consumption rates and (2) soil C content and total gross N immobilization rates. All nonnormal data were log-transformed before analysis; data presented in the figures and tables are the treatment average ± 1 standard error. Differences were considered statistically significant at $P < 0.05$.

Results

Soil characteristics

Soil C and N content (g kg^{-1}) were measured on soil excavated at multiple depth increments from two large soil pits (80 cm $\times$ 80 cm) in each treatment ring in June 2009. Soil C and N content did not differ between ambient and elevated $[\text{CO}_2]$ ($F_{1,12} = 1.8$, $P = 0.21$ and $F_{1,12} = 0.3$, $P = 0.58$, respectively), but each declined with soil depth ($F_{3,12} = 135.0$, $P < 0.0001$ and $F_{3,12} = 65.1$, $P < 0.0001$, respectively, Table 1). Soil C and N content were greatest at 0–15 cm depth, and least from 30 to 60 cm ($P < 0.01$). GWC (%), and initial extractable NH_4^+ and NO_3^- (mg g^{-1}) were determined on a subsample of the soil cores taken in June 2007, that were used for the isotope pool dilution experiment. Soil GWC did not differ by CO_2 treatment ($F_{1,12} = 1.3$, $P = 0.28$) but differed somewhat with soil depth ($F_{3,12} = 5.1$, $P = 0.02$). The soils averaged approximately 28% water-holding capacity before the addition of ^{15}N label and 33% water-holding capacity after label addition. Initial NH_4^+ did not differ between ambient and elevated $[\text{CO}_2]$ ($F_{1,12} = 0.5$, $P = 0.49$), but declined with soil depth ($F_{3,12} = 43.7$, $P < 0.0001$, Table 1); NH_4^+ was greatest at 0–15 cm depth, and least from 30 to 60 cm ($P < 0.01$). Initial NO_3^- was below detection limits in most cases (Table 1).

Consumption stimulation

The addition of enough highly enriched isotopic label to achieve target labeling of the inorganic N pools in soil from several depths resulted in a $\sim 27\%$ stimulation in NH_4^+ consumption throughout the soil profile (stimulated consumption = $3.17 + 1.27 \times$ unstimulated consumption, $R^2 = 0.95$, $P < 0.0001$), where the slope (1.27 ± 0.05) was significantly different from 1 ($P < 0.0001$). There was no difference in the magnitude of the stimulation in NH_4^+ consumption between ambient and elevated $[\text{CO}_2]$ ($F_{1,12} = 0.03$, $P = 0.83$), or

by soil depth ($F_{3,12} = 0.38$, $P = 0.77$, Table 1). The addition of $^{15}\text{NO}_3\text{-N}$ did not systematically affect NO_3^- consumption ($R^2 = 0.10$, $P = 0.17$). To avoid inflated consumption estimates, we used unstimulated NH_4^+ consumption rates determined by the difference between gross NH_4^+ production and net ammonification rates in our analyses of CO_2 enrichment and depth effects on soil N cycling. To be consistent, we also determined unstimulated NO_3^- consumption rates (i.e., difference between gross nitrification and net nitrification rates) for use in our analyses.

Gross and net N fluxes

CO_2 enrichment had no effect on rates of gross N mineralization (i.e., NH_4^+ production, $F_{1,12} = 0.06$,

$P = 0.81$), gross NH_4^+ consumption ($F_{1,12} = 0.26$, $P = 0.62$) or net ammonification ($F_{1,12} = 2.81$, $P = 0.12$). Combined across treatments, gross NH_4^+ production and consumption decreased with soil depth ($F_{3,12} = 17.5$, $P < 0.001$ and $F_{3,12} = 14.3$, $P < 0.001$, respectively); gross NH_4^+ production and consumption were least at 30–60 cm ($P < 0.05$, Fig. 1a). However, the decline in NH_4^+ consumption with soil depth was steeper than the decline in NH_4^+ production; only 41% of NH_4^+ production occurred in top 15 cm, compared with 54% of NH_4^+ consumption. Therefore, net ammonification increased with soil depth ($F_{3,12} = 27.7$, $P < 0.0001$). Combined across treatments, net ammonification was greatest at soil depths deeper than 15 cm ($P < 0.0001$, Fig. 1b). There were no interactions between CO_2 treatment and soil depth for gross or net NH_4^+ fluxes ($P > 0.46$). The

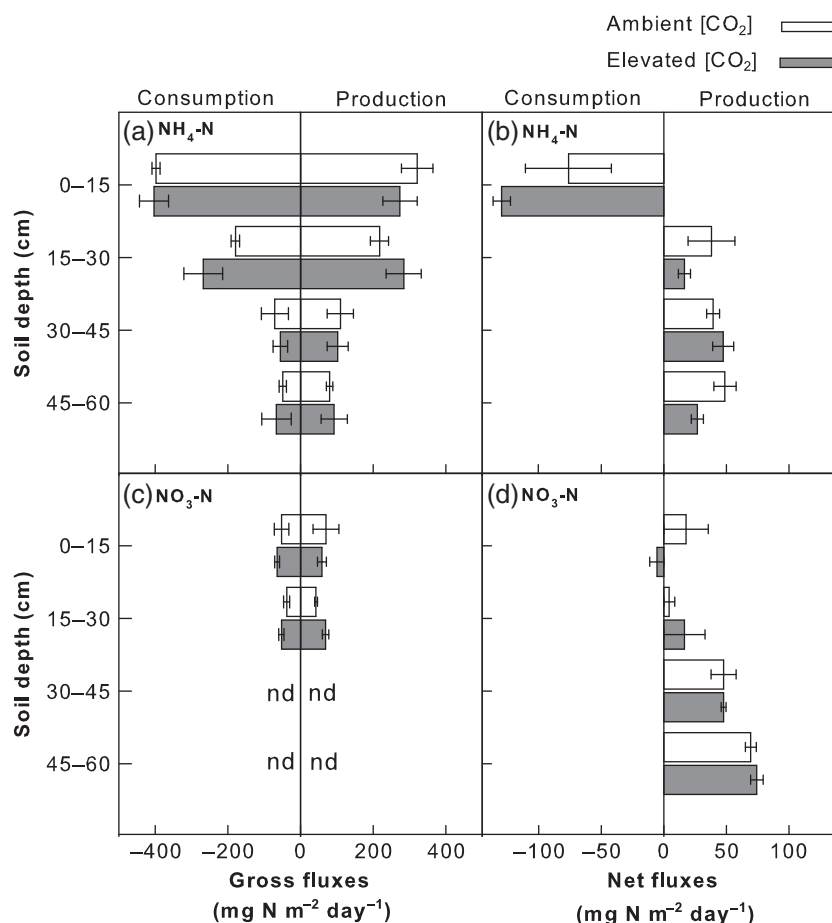


Fig. 1 Potential gross and net rates of N mineralization (a and b) and nitrification (c and d), as well as gross NH_4^+ and NO_3^- consumption under ambient and elevated carbon dioxide concentrations $[\text{CO}_2]$ determined in laboratory incubations of soil sampled from depth increments. Note: Gross production rates are greater than zero, whereas consumption rates are less than zero. Potential gross N fluxes were determined using isotope pool dilution methodology, whereas net N fluxes were measured in unlabeled incubations. Unstimulated gross N consumption was determined as the difference between gross N mineralization (or nitrification) and net ammonification (or nitrification). Owing to low initial NO_3^- pools and high adsorption of label to deeper soils, gross NO_3^- fluxes below 30 cm were unable to be determined using isotope pool dilution methodology (nd, not determined). Note the difference in x-axis scale between panels a and c and b and d, where gross fluxes are many times larger than net fluxes.

mean residence time of NH_4^+ in the soil (i.e., the total initial NH_4^+ pool divided by gross N mineralization rate; Table 2) did not differ between ambient and elevated $[\text{CO}_2]$ ($F_{1,12} = 0.02$, $P = 0.89$) or with soil depth ($F_{3,12} = 1.9$, $P = 0.18$), and there was no interaction between CO_2 treatment and depth ($F_{3,12} = 0.36$, $P = 0.79$).

Gross nitrification rates below 30 cm were difficult to determine with the isotope pool dilution methodology given small initial NO_3^- pool sizes (nearly all were below detectable limits) and greater adsorption of the isotopic label in clay-rich deeper soils ($1 - F_N$, Table 1). This resulted in negative NO_3^- consumption values in the 30–45 and 45–60 cm soil depths after net nitrification rates were subtracted from gross nitrification rates. Thus, we analyzed gross NO_3^- fluxes only in the top 30 cm of the soil. CO_2 enrichment had no effect on gross nitrification (i.e., NO_3^- production, $F_{1,6} = 0.57$, $P = 0.48$), or gross NO_3^- consumption ($F_{1,6} = 0.89$, $P = 0.38$), and no effect on net nitrification rates to 60 cm depth ($F_{1,12} = 0.04$, $P = 0.84$, Fig. 1c and d). Combined across treatments, gross NO_3^- fluxes were small relative to gross NH_4^+ fluxes ($\sim 22\%$), and did not decline from 0–15 to 15–30 cm soil depth ($F_{1,6} = 0.001$, $P = 0.98$ and $F_{1,6} = 0.79$, $P = 0.41$, respectively, for production and consumption). In contrast, net nitrification rates increased with soil depth ($F_{3,12} = 16.6$, $P < 0.001$); combined across treatments, net nitrification was greatest at 30–60 cm depth ($P < 0.05$; Fig. 1d). There were no interactions between CO_2 treatment and soil depth for gross or net NO_3^- fluxes ($P > 0.41$). We were unable to determine the mean residence time of NO_3^- in the soil given that initial NO_3^- values were generally below the detection limit. Assuming that initial NO_3^- values were at the detection limit of $0.005 \text{ mg N L}^{-1}$, NO_3^- -N residence times ranged from 10 min to 8 h, but were generally < 1 h at all soil depths.

CO_2 enrichment had no effect on total gross N immobilization (the difference between gross N mineralization and net NH_4^+ and NO_3^- mineralization; $F_{1,9} = 1.18$, $P = 0.31$). Averaged across treatments, total gross N immobilization declined with depth ($F_{3,9} = 81.61$, $P < 0.0001$); total gross N immobilization was greatest at 0–15 cm soil depth and least from 30 to 60 cm ($P < 0.0001$). There was no interaction between treatment and soil depth ($F_{3,9} = 1.68$, $P = 0.24$). Total gross N immobilization was strongly related to the C content of soils sampled from soil pits in each treatment ring at similar depth increments in June 2009 (total gross N immobilization = $-39.7 + 108.2 \times \text{soil C content}$, Fig. 2). The slope of the relationship between bulk soil C content and total gross N immobilization did not differ between ambient and elevated $[\text{CO}_2]$ ($P = 0.44$).

CO_2 enrichment had no effect on total net N mineralization (net ammonification + net nitrification;

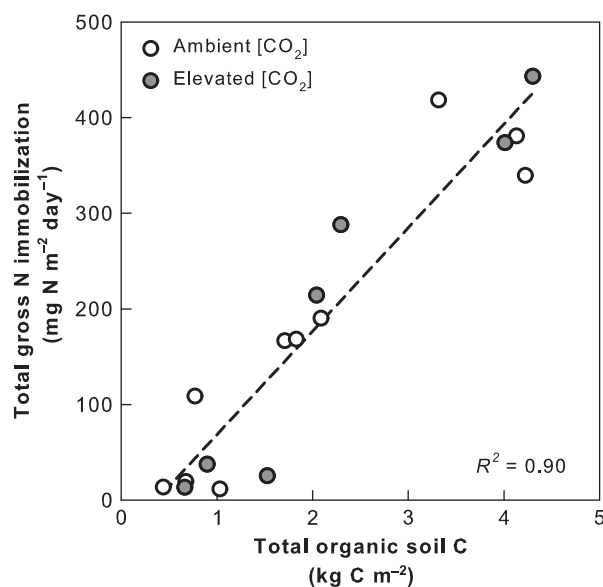


Fig. 2 Positive relationship between soil organic C content and total gross N immobilization [gross N mineralization – net N (NH_4^+ + NO_3^-) mineralization]. Data points are treatment rings at each soil depth; data were omitted if total N immobilization was < 0 (3 points total, 30–60 cm soil depth). While there were greater root C inputs over 10 years under elevated carbon dioxide concentrations $[\text{CO}_2]$ (filled circles), there was no difference in the relationship between soil C content and total gross N immobilization between CO_2 treatments. Total gross N immobilization = $-39.7 + 108.2 \times \text{soil C content}$ ($R^2 = 0.90$, $P < 0.0001$).

$F_{1,12} = 1.87$, $P = 0.20$), but total net N mineralization increased dramatically with soil depth ($F_{3,12} = 28.74$, $P < 0.0001$). Total net mineralization was least at 0–15 cm and greatest from 30 to 60 cm ($P < 0.05$); there was no interaction between CO_2 treatment and soil depth ($F_{3,12} = 1.12$, $P = 0.38$). When combined across treatments, total net N mineralization was well-related to zones where the largest stimulation in cumulative root production under elevated $[\text{CO}_2]$ occurred over the period 1998–2006 (Fig. 3).

Resin N adsorption

Over the period 2008–2009, CO_2 enrichment had no effect on the amount of N adsorbed on the resin capsules ($F_{1,7} = 1.67$, $P = 0.24$), but N adsorption differed by depth and collection date (date $\times$ depth interaction, $F_{10,35} = 2.40$, $P = 0.03$). Inorganic N adsorbed to resin capsules was often greatest at 55 cm soil depth (Fig. 4).

Discussion

The sustainability of greater forest production under rising atmospheric $[\text{CO}_2]$ will be determined by the

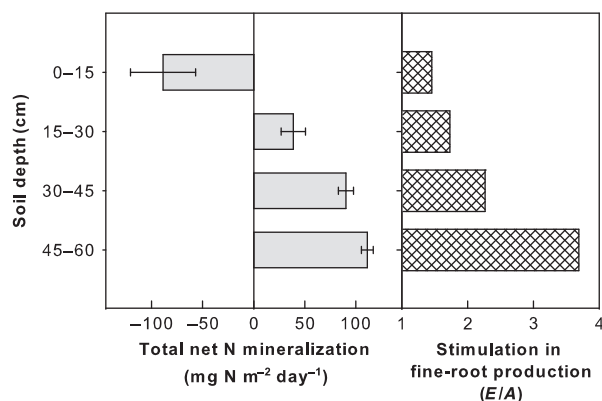


Fig. 3 Total net inorganic N mineralization ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) at depth increments 0–15, 15–30, 30–45 and 45–60 cm measured in laboratory incubations compared with the proportional stimulation (elevated $[\text{CO}_2]/\text{ambient } [\text{CO}_2]$) in cumulative root production measured in minirhizotron tubes at the same depth increments from 1998 to 2006 (fine-root data are from Iversen *et al.*, 2008).

interplay between forest growth, C allocation and soil N mineralization (Luo *et al.*, 2004). We hypothesized that increased root production in response to CO_2 enrichment at ORNL FACE at soil depths >30 cm might be explained by greater relative N availability in deeper soils compared with shallower soils. Further, we hypothesized that a doubling of C inputs from fine-root mortality over a period of 9 years (i.e., Iversen *et al.*, 2008) would increase microbial N consumption (as reviewed in Berntson & Bazzaz, 1996). Our results indicated that deeper rooting distributions in response to CO_2 enrichment were likely associated with relatively greater N availability in deeper soil rather than altered N cycling rates under elevated $[\text{CO}_2]$.

In support of our first hypothesis, our laboratory assays of potential mineralization indicated that significant quantities of inorganic N may be available for tree uptake at soil depths deeper than 15 cm (59% of NH_4^+ production and 45% of NO_3^+ production; Fig. 1). While gross N cycling rates declined with soil depth as expected, rates of microbial N consumption decreased faster than rates of gross inorganic N production, such that 100% of potential net N mineralization occurred at soil depths deeper than 15 cm. We were not able to examine potential gross nitrification or NO_3^- consumption rates in soil deeper than 30 cm, but overall, potential gross nitrification rates were much lower than potential gross N mineralization rates. Gross nitrification accounted for only 22% of gross N mineralization and 23% of NH_4^+ consumption within the top 30 cm of the soil. Low nitrification rates are also found in other undisturbed forested ecosystems (Stark & Hart, 1997).

To corroborate our laboratory data with field measurements, we measured root-available N at three depths in the soil profile using ion-exchange resins. We found the same general pattern of substantial amounts of inorganic N available at deeper soil depths in 2008 and 2009. Inorganic N adsorbed to resins incubated at 55 cm soil depth was equal to or greater than N adsorbed to resins incubated at 15 cm depth at each collection date over a period of 2 years (Fig. 4), indicating that relatively more inorganic N is available for root uptake at depth in the soil. Greater relative N availability at depth under field conditions could be due to several factors, including increased microbial consumption of N in shallower soils as we observed in laboratory incubations (Fig. 1a and c), greater N uptake by roots in shallower soils, or leaching of inorganic N downward through the soil profile (though leaching is

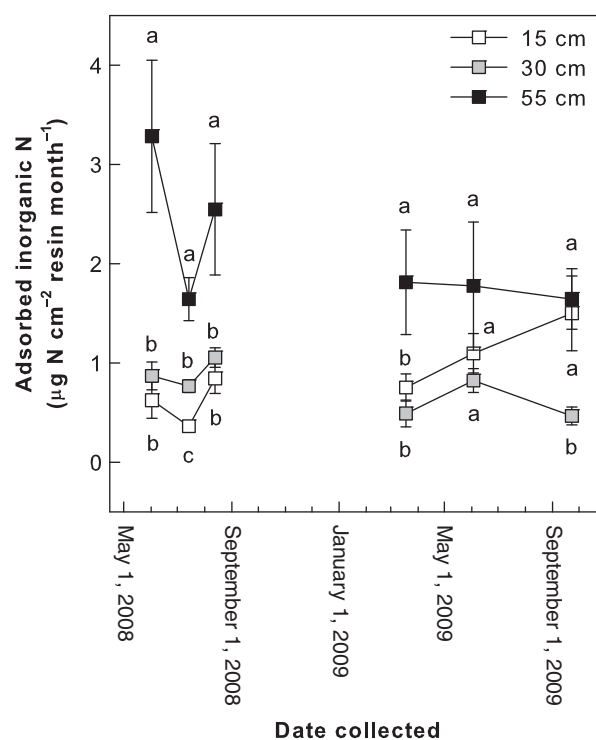


Fig. 4 Total inorganic N ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) adsorbed to ion-exchange resins incubated at three soil depths: 15, 30 and 55 cm. Data are cumulative amounts of inorganic N adsorbed to resin surfaces over the incubation period; values are averaged over all plots ($n = 5$) ± 1 SE, expressed per unit of resin surface area (11.4 cm^2) on a monthly basis (1 month = 30 days). $\text{NO}_3\text{-N}$ averaged from 8% to 75% of total inorganic N depending on the soil depth and time of year. Each incubation period began when the resins from the previous incubation period were removed, except in June 2008 and March 2009, where the incubations began in May 2008 and December 2008, respectively. Lowercase letters indicate a significant difference in inorganic N adsorption between depths within a collection date ($P < 0.05$).

unlikely to be an important factor given that NH_4^+ is the main form of inorganic N at this site). Taken together, our findings indicate that more attention to nutrient cycling at deeper soil depths is merited.

Greater relative N availability at depth in the soil may explain why deeper rooting distributions have been observed in up to three-quarters of CO_2 enrichment experiments in woody ecosystems (Iversen, 2010). Increases in root production in deeper soil observed under elevated $[\text{CO}_2]$ are probably the result of an interaction between alleviated C limitation under elevated $[\text{CO}_2]$ combined with increased forest N demand (Iversen, 2010). Root proliferation tends to occur in zones of greater nutrient availability (Hodge, 2004), and the stimulation in root production under elevated $[\text{CO}_2]$ observed over the first several years of the experiment at ORNL FACE (1998–2006; Iversen *et al.*, 2008) occurred at the soil depths where soil incubations showed the greatest rates of potential net N mineralization (Fig. 3). Relatively greater N availability in deeper soils, combined with increased root proliferation at depth, may have sustained increases in forest N uptake under elevated $[\text{CO}_2]$ in a number of forested CO_2 enrichment experiments (Finzi *et al.*, 2007). This mechanism may be especially important in stands that are limited by N availability, such as the ORNL FACE site (Iversen & Norby, 2008). In addition to internal ecosystem N recycling at depth, external sources of N may also lead to greater root proliferation in deep soil; root access to inorganic N found in groundwater at >2 m depth in CO_2 -enriched chambers may have sustained tree production under elevated $[\text{CO}_2]$ in a scrub-oak system in Florida, USA (McKinley *et al.*, 2009).

While it appears that fine roots in the CO_2 -enriched plots were accessing a larger pool of mineral N by mining deeper soil, we found no effect of elevated $[\text{CO}_2]$ on potential gross N mineralization or consumption in this sweetgum stand. Although similar N cycling rates in the ambient and elevated $[\text{CO}_2]$ treatments is in contrast to our initial hypothesis, this appears to be a common and repeatable result; few differences in gross N mineralization rates have been observed in other CO_2 -enriched ecosystems (de Graaff *et al.*, 2006), and similar N cycling rates were observed in the ambient and elevated $[\text{CO}_2]$ treatments at ORNL FACE after only 2 years of CO_2 fumigation and in the top 10 cm of the soil (Zak *et al.*, 2003). Potential gross N fluxes measured in the top 15 cm of the soil in this experiment were within the same order of magnitude as those found by Zak *et al.* (2003) to 10 cm depth; our measurements of potential gross N mineralization rates in 2007 were ~ 1 to 2 times greater than in those measured by Zak *et al.* (2003) in 1999 and 2000, respectively, while potential gross nitrification rates in 2007 were equal to

or half of rates measured in 1999 and 2000, respectively. While others have observed faster turnover of N in the soil under elevated $[\text{CO}_2]$ even where there were no differences in total mineralization rates (McKinley *et al.*, 2009), we saw no changes in NH_4^+ residence time in the CO_2 -enriched plots (Table 2).

The lack of an effect of elevated $[\text{CO}_2]$ on gross rates of soil N cycling and resin capture after 10 years of CO_2 enrichment was somewhat surprising given the large increases in the input of labile root-derived C and N under elevated CO_2 (Iversen *et al.*, 2008; analyses by Zak *et al.*, 2003 were performed before 2001 when large increases in root production were observed). There are a few possible explanations for similar gross N fluxes in the ambient and elevated $[\text{CO}_2]$ treatments: (1) A study conducted at ORNL FACE in 2006 found no effect of CO_2 enrichment on microbial community composition or enzymatic activity in the top 15 cm of the soil (Austin *et al.*, 2009), indicating that changes in the rate of soil N cycling might not be expected, even after several years of increased C inputs. (2) While microbial N immobilization is often tightly and positively linked with soil C content (Booth *et al.*, 2005), roots are heterogeneously distributed throughout the soil profile and it may take decades for plant inputs to measurably increase the organic matter content of bulk soil (Smith, 2004). Based on the strong linear relationship between bulk soil C content and total gross N immobilization (i.e., the total amount of mineral N immobilized by microbial activity, not including gross nitrification) spanning multiple soil depths in ORNL FACE (Fig. 2), increases in soil C content greater than those currently observed under elevated $[\text{CO}_2]$ would be necessary to lead to increased N immobilization (Fig. 2). (3) The presence of roots has strong effects on soil N cycling (e.g., Dijkstra *et al.*, 2009), and current methodology, in which root inputs and microbial N cycling are often examined using separate experiments, may lead to a disconnect between hypothesized responses and measured results. There have been repeated calls for improved methods to link root C inputs with N cycling at smaller scales (as reviewed in Frank & Groffman, 2009), and at depth in the soil (Iversen, 2010).

Here we provide evidence that N mineralization at depth in the soil, combined with increased root exploration of the soil volume under elevated $[\text{CO}_2]$, may be more important than changes in potential gross N cycling rates in sustaining forest responses to rising atmospheric CO_2 . However, a remaining challenge is to incorporate data on N cycling throughout the soil profile into ecosystem and land surface models projecting forest responses to changing environmental conditions. While there have long been calls for the linkage of root dynamics with soil N cycling throughout the soil

profile (Jackson *et al.*, 2000), most ecosystem and land surface models have no explicit link between root N uptake and soil N cycling (Iversen, 2010). Further, SOM cycling is often simulated in most ecosystem models solely for shallower soils because of the paucity of data on N cycling deeper in the soil (Parton *et al.*, 1988). Our data, combined with observations of rooting distributions and nutrient cycling at depth in other forested ecosystems exposed to elevated $[\text{CO}_2]$ (e.g., McKinley *et al.*, 2009; Iversen, 2010), can help inform the next generation of ecosystem models and reduce the uncertainty in projections of the CO_2 -fertilization effect on forested ecosystems and subsequent climate feedbacks.

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