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Does deep soil N availability sustain long-term ecosystem responses to elevated CO_2?

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Abstract

A scrub-oak woodland has maintained higher aboveground biomass accumulation after 11 years of atmospheric CO_2 enrichment (ambient $+350\,\mu mol\,CO_2\,mol^{-1}$), despite the expectation of strong nitrogen (N) limitation at the site. We hypothesized that changes in plant available N and exploitation of deep sources of inorganic N in soils have sustained greater growth at elevated CO_2. We employed a suite of assays performed in the sixth and 11th year of a CO_2 enrichment experiment designed to assess soil N dynamics and N availability in the entire soil profile. In the 11th year, we found no differences in gross N flux, but significantly greater microbial respiration ($P \leq 0.01$) at elevated CO_2. Elevated CO_2 lowered extractable inorganic N concentrations ($P = 0.096$) considering the whole soil profile (0–190 cm). Conversely, potential net N mineralization, although not significant in considering the entire profile ($P = 0.460$), tended to be greater at elevated CO_2. Ion-exchange resins placed in the soil profile for approximately 1 year revealed that potential N availability at the water table was almost 3 × greater than found elsewhere in the profile, and we found direct evidence using a ^{15}N tracer study that plants took up N from the water table. Increased microbial respiration and shorter mean residence times of inorganic N at shallower depths suggests that enhanced SOM decomposition may promote a sustained supply of inorganic N at elevated CO_2. Deep soil N availability at the water table is considerable, and provides a readily available source of N for plant uptake. Increased plant growth at elevated CO_2 in this ecosystem may be sustained through greater inorganic N supply from shallow soils and N uptake from deep soil.

Keywords: deep soil nitrogen availability, elevated CO_2, global change, gross N mineralization, nitrogen cycling, progressive nitrogen limitation, rising atmospheric CO_2, water table

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Introduction

Atmospheric carbon dioxide (CO_2) concentrations are predicted to double by the end of this century (IPCC, 2007). Although elevated CO_2 typically increases photosynthesis, plant biomass, net primary productivity (NPP), and net ecosystem production in short-term experiments (Ainsworth & Long, 2005; Norby *et al*., 2005; Reich *et al*., 2006a, b), empirical and modeling studies have suggested that nutrient limitation will constrain productivity responses to elevated atmospheric CO_2 over the long term (Gifford *et al*., 1996; Oren *et al*., 2001; Hungate *et al*., 2003; Luo *et al*., 2004; de Graaff *et al*., 2006; Korner, 2006; Reich *et al*., 2006a; van Groenigen *et al*., 2006; Finzi *et al*., 2007). Long-term field atmospheric CO_2-enrichment experiments are needed in order to predict the direction and magnitude of forest ecosystem responses that may feedback on the rate of atmospheric CO_2 increase (Schimel *et al*., 2001).

It is important to understand the effect of elevated CO_2 on soil nitrogen (N) availability because plant growth and NPP are already N limited across many forest ecosystems (Reich *et al*., 1997). As ecosystem C uptake and storage rise in response to elevated CO_2, ecosystem N demand may also increase resulting in N sequestration into plant biomass and soils (Luo *et al*., 2004, 2006a). The net transfer of labile (i.e. easily mineralizable) or available N pools in soils to longer-lived plant and soil pools over time may reduce plant avail-

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able forms of N (i.e. inorganic N) in the soil (Luo *et al*., 2004). Luo *et al*. (2004) proposed that progressive nitrogen limitation (PNL) would be a result of such a plant–soil nutrient feedback loop and, given enough time, decreased N availability could constrain plant and whole ecosystem responses to increased atmospheric CO_2 concentrations.

Evidence of PNL in long-term elevated field studies is equivocal (Luo *et al*., 2006a), suggesting either that many ecosystems have the ability to avoid greater N constraints or field studies have not run long enough for changes in N availability to fully manifest. In a recent meta-analysis, Luo *et al*. (2006a, b) reported that elevated CO_2 increased the total ecosystem N pool. The mechanisms for net ecosystem N accrual at elevated CO_2 are a matter of much speculation, but they include changes in N inputs, lowered N loss, or greater root exploration and acquisition of N in formerly root-free areas (Luo *et al*., 2006a, b). N-fixation may increase, but a recent review of forest and grasslands has found this effect to be negligible (van Groenigen *et al*., 2006). Greater retention of N from atmospheric deposition has not been found to be a significant source of new N in most CO_2 studies (Reich *et al*., 2006b). Leaching (mainly NO_3^-) or gaseous N losses (NO_x, N_2O, and N_2) in long-term CO_2 enrichment experiments are expected to decrease relative to ambient CO_2 (Barnard *et al*., 2005), but there is not much evidence that lowered N losses are significant. A number of elevated CO_2 studies have reported greater root production (Pregitzer *et al*., 2000; Finzi *et al*., 2007; Stover *et al*., 2007), which could allow greater acquisition of N in portions of the soil that are not typically accessed by plants at ambient CO_2 concentrations. However, few long-term elevated CO_2 studies have empirically examined N cycling or N availability deep in the soil profile as a possible mechanism that allows greater plant N uptake, ecosystem N accrual, and, consequently, avoidance of PNL.

A long-term (11 years) elevated CO_2 experiment in a scrub-oak ecosystem in Florida provided a unique opportunity to study changes in soil N processes and other attributes throughout the entire soil profile ($\sim$ 300 cm) over time in the context of PNL. After 11 years of elevated CO_2 treatment, there was a $\sim$ 50% increase in aboveground plant biomass of the dominant oak, *Quercus myrtifolia*, into the last year of the study (Seiler *et al*., 2009). An initial increase in NPP is necessary for symptoms of PNL to appear, but stimulation of NPP is expected to decline or disappear within a few years in strongly N-limited systems (Luo *et al*., 2004). Thus, sustained CO_2 stimulation was surprising given that this ecosystem was considered to be severely N limited and external inputs are small (Hungate *et al*., 2004). Elevated CO_2 stimulated soil organic matter mineralization in this ecosystem (Carney *et al*., 2007) and increased root production (Stover *et al*., 2007), suggesting at least two mechanisms for alleviating PNL (Johnson, 2006; Luo *et al*., 2006b).

Our objective was to comprehensively evaluate N cycling and availability in the entire soil profile after 11 years of elevated CO_2 exposure in a scrub-oak forest to understand how this ecosystem has avoided the more severe symptoms of N limitation that would be anticipated with PNL. We hypothesized that: (1) rates of gross N mineralization and soil microbial respiration would increase due to increased SOM decomposition; (2) N availability (net N mineralization) would decrease as microbial N demand increased in response to greater plant C input; (3) increased exploitation of soil resources by roots would cause these responses to occur deeper in the soil over time; and (4) plants utilize deep sources of inorganic N.

Materials and methods

Study site

The study site was located on Merritt Island, a barrier island that is home to NASA's Kennedy Space Center on the east coast of central Florida, USA (28°38′N, 80°42′W). The climate is subtropical; temperatures reach an average daily maximum of 33.3 °C in July and a minimum of 9.6 °C in January. Annual precipitation averages 131 cm, with most of the precipitation falling from June through October. Three perennial evergreen oaks, *Q. myrtifolia* Willd., *Quercus geminata* Small, and *Quercus chapmanii* Sarg., comprise up to 90% of the aboveground biomass of the short-statured forest at the site. This is a fire-adapted ecosystem that has an estimated fire-return interval of 8–12 years. The site was burned twice in 1995 just before the establishment of the chambered sites and commencement of CO_2 fumigation in May 1996. The soils under the chambers are Orsino series (hyperthermic uncoated Spodic Quartzipsamments), which are very deep, moderately well-drained, very rapidly permeable soils that form in thick beds of sandy marine or eolian deposits (Huckle *et al*., 1974). Similar soils that occur in the area are the Pomello and Zolfo series; both are sandy, siliceous, hyperthermic Oxyaquic Alorthods.

Experimental design

Sixteen octagonal open-top chambers (OTCs), 2.5 m tall, each enclosing a surface area of 9.42 m^2, were constructed with a PVC frame and covered with rectangular panels of Mylar (Melinez 071, Courtaulds Performance Films, Martinsville, VA, USA). Aboveground biomass

and stem densities were detailed elsewhere (Seiler *et al.*, 2009). A frustum was constructed atop each chamber to reduce wind effects, which reduced the opening to 5.9 m^2. From May 1996 to June 14, 2008, eight of the OTCs were maintained at ambient CO_2 concentrations and eight at elevated CO_2 (ambient + 350 μmol CO_2 mol^{-1}). The experimental setup, chamber design, and operation were detailed elsewhere (Dijkstra *et al.*, 2002).

In July 2007, five soil cores (10 × 10 cm) were taken at least 60 cm from the inner edge of each chamber to a depth of 100 cm in 10 cm increments using an auger designed to prevent soil compaction. Surface litter (or *O* horizon) was collected in 10 × 10 cm quadrates to the mineral soil. In May 2002, the same protocol was used to obtain soil to a depth of 60 cm, and to prepare the soil for subsequent analyses. In 2007, a sand bucket auger (5.5 cm i.d.) was used to obtain soil in 30 cm increments from 100 to 300 cm; but, only a 30 cm portion of the *E*′ horizon (190–277 cm) found directly above the B_h horizon was collected for analysis. The mean starting depth of the B_h horizon was 277 ± 14 cm. This approach minimized environmental differences between the *E*′ (a second *E* or elluvial horizon separated from the first by unlike horizon, i.e., B_1 and B_2) and B_h horizons (h = illuvial accumulation of organic matter) in order to facilitate comparisons (Buol *et al.*, 2003). Soils from each core at each target depth increment were pooled and sieved just after collection with a 2 mm sieve followed by a 1 mm sieve to acquire root-free soil and thoroughly mix the soil. Target depth increments of the mineral soil were: 0–10, 10–30, 30–60, 60–100, 100–130, 130–160, 160–190 cm, *E*′ (190–277 cm, found just above the B_h), and B_h, if able to be sampled. The water table was often found at or above the B_h horizon (~ 235–245 cm). Within 12 h, aliquots of these samples were used for several assays: salt-extractable inorganic N (NH_4^+ and NO_3^-), potential N mineralization, gross N mineralization (ammonification), gross nitrification and microbial respiration. N flux estimates in the B_h horizon may have been overestimated because the handling of the soil introduced aerobic conditions. The remainder of the soil was retained for analyses of total C and N concentration and other chemical properties.

Field extractable N

In 2002 and 2007, 15 g of sieved field moist mineral soil and *O* horizon (surface litter) was extracted with 50 mL 2 M KCl by shaking at 180 r.p.m. on an orbital shaker for 1 h, then filtered (0.45 μm). Inorganic N (NH_4^+-N and NO_3^- + NO_2^--N) concentrations in 2007 were determined colorimetrically with a Lachat QuickChem 8000 Flow Injection Auto-analyzer (Lachat Instruments, Loveland, CO, USA) using the indophenol blue method for NH_4^+-N and cadmium reduction followed by diazotization with sulfanilamide for NO_2^-/NO_3^--N. In 2002, inorganic N concentrations were determined with the same chemistry as in 2007, but were analyzed with an Astoria-Pacific Solution Autoanalyzer (Astoria-Pacific, Clackamas, OR, USA).

Ion-exchange measure of inorganic N availability

The purpose of the ion-exchange resin assay provided an integrated measure of inorganic N availability over time to complement our instantaneous measures. We used mixed-bed cation–anion exchange capsules (Unibest PST-1, Unibest, Bozeman, MT, USA) that were spherically shaped, 2.54 cm – diameter, water-permeable polyester mesh capsules containing 1 g dry weight of resins. They were inserted at depths of 10, 50, 100, 150, 200, and 250 cm (just below the water table at the time) into the soil profile using a WECSA Soil Access System (Warrington Ecological Systems Analysis, Bozeman, MT, USA). Three 2 m^2 plots were selected within 10 m of the experimental CO_2 chambers to assess soil N availability by depth, excluding a CO_2 treatment effect. Each set of access tubes ($n = 3$) (about 2.54 cm i.d.) designed for each of the target depth were placed into the soil at a 60° angle after boring with a sand auger. The resin capsules were inserted to the bottom of the access tubes so that one side (50%) of the surface area was in direct contact with the soil. The exchange resins were deployed starting June 12, 2007 and ended June 3, 2008. The capsules were replaced twice during this time period to avoid saturating the ion-exchange sites on the resins. The first set of resin capsules were in place for approximately 1 month before the elevated CO_2 study ended. Ion-exchange resins were extracted by three consecutive 10 mL rinses of 2 M KCl, each shaken for 30 min. Inorganic N concentrations in the ion-resin extracts were determined colorimetrically (described previously).

Potential net N mineralization

Ten grams aliquots (dry wt. equivalent) of soil (5 g for *O* horizon) were placed in 125 mL I-chem bottles (Amherst, New York, NY, USA) and brought to 11% soil moisture (60% water filled pore space). The headspace for jars containing the B_h horizon was flushed with N_2 gas to simulate anaerobic conditions assumed to occur below the water table. Soil water content was determined gravimetrically using another aliquot that was oven-dried at 105 °C. Each bottle was sealed and incubated in the dark at 23 °C for 30 days. The bottles were opened periodically for 2 h to allow equilibration with ambient O_2 concentrations. At the end of the incubation, soils were extracted with 2 M KCl to determine inorganic N concentrations (described previously). Rates of

net N mineralization were determined by subtracting the final concentration after 30 days from the starting concentration at time 0. Estimates in 2002 were made with comparable methods, except that 1 L mason jars and lids were used to incubate the soils; the soils were incubated for 28 days and soil mass (dry wt. equivalent) in the jars was $\sim$ 100 g. For standardization, soil N flux was expressed as a daily rate by dividing the cumulative flux by the number of days incubated.

Gross N fluxes and microbial respiration

Within 16 h of collection, 15 g of each field-moist, homogenized soil sample was placed in a 20 mL glass scintillation vial for assays of gross ammonification and nitrification. Soil samples were brought up to 11% gravimetric soil moisture, accounting for our addition of 0.25 mL of labeled ^{15}N suspension either as $(NH_4)_2SO_4$ or KNO_3 (highly enriched 99% ^{15}N). A micro-syringe was used to make multiple uniform injections of the^{15}N labeled solutions into the soil. Total ^{15}N additions did not exceed 10% of the ambient inorganic N pools. Duplicate samples were extracted with 2 M KCl after 15 m to serve as initial (T_0) samples, and the remaining (T_1) samples were placed in 125 mL I-chem bottles (Amherst), retrofitted with a rubber septum in the lid, and incubated in the dark at 23 °C for 24 h. The headspace for jars containing the B_h horizon was flushed with N_2 gas to simulate anaerobic conditions assumed to occur below the water table. After 24 h, the headspace was sampled for CO_2 concentration and measured with a LI-COR infrared gas analyzer (model Li-7000, Lincoln, NE, USA) configured for in-line injection with an 8-port injection valve (Valco, Valco Instruments, Houston, TX, USA) and N_2 as the carrier gas. After sampling for headspace CO_2 concentrations, the soils were extracted using standard KCl extraction for NH_4^+ and analyzed colorimetrically (described previously).

Preparations of the soil extracts for ^{15}N isotopic analyses were carried out using a diffusion method described elsewhere (Davidson *et al*., 1992). All samples were analyzed with an isotope-ratio mass spectrometer (NC 2100 Elemental Analyzer interfaced with a Finnagan Delta Plus XL isotope ratio mass spectrometer) at the Colorado Plateau Stable Isotope Laboratory. Gross rates of mineralization (ammonification) and nitrification, as well as consumption, were calculated using standard published formulae (Hart *et al*., 1994).

Plant ^{15}N uptake from deep soil

Clusters of five PVC pipes (5 cm i.d.) were installed outside of the chambers to a depth of $\sim$ 280 cm; one pipe in a center position and the remaining four pipes were installed 2 m from the center pipe at four cardinal directions. Several hundred evenly spaced holes (2 mm) were drilled through a 40 cm section of one end each PVC pipe, which was then sealed with a PVC cap. Each pipe was installed so that the entire perforated end of the pipe was below the surface of the water table at the time. This was repeated with one reference (control) plot. On May 12, 2008 at 1200 hours EST, 0.4 g highly enriched $(^{15}NH_4)_2SO_4$ (99% atom percent ^{15}N) dissolved in 4 L of DI water was injected into each of the five PVC tubes. Five minutes after each injection of the ^{15}N tracer, 8 L of DI water was injected into each pipe creating positive pressure that forced the ^{15}N tracer solution into the water table. Just before the ^{15}N injection, recently budded leaves from discrete plants or stems, *Q. myrtifolia* ($n = 10$), *Q. geminata* ($n = 10$), and *Serenoa repens* ($n = 4$) were collected from within the treatment and reference plot (a 4 m diameter circular plot centered on the middle pipe), and again with leaves on the same stems (marked with survey tape) exactly 24 h later (May 13). May 12 and 13 were clear cloudless days, with a mean daily temperature of 25 and 20 °C, respectively. The leaves were dried (60 °C), finely pulverized, and analyzed with an isotope-ratio mass spectrometer for ^{15}N (described previously).

Soil chemical properties

Bulk mineral soils were analyzed for C and N content using dry combustion/gas chromatography with a LECO CN 2000 C/N analyzer (LECO Corporation, St. Joseph, MI, USA). Soil pH was measured using a 1 : 1 slurry with deionized water. The Bray-1-P test (available P) was used for extractable phosphorus, which utilized a HCl-ammonium fluoride extractant followed by a colorimetric analysis. Cation exchange capacity (CEC) was determined by displacement with ammonium acetate.

Data analyses

A randomized complete block was used as the experimental design in 2002 and 2007; each of the eight chambers was an experimental unit ($n = 8$). However, soil depth increments were not fully replicated in 2007 because the deeper depths could not always be sampled [100–130 ($n = 7$), 130–160 ($n = 7$), 160–190 ($n = 5$)]. Significant differences in treatment means of ambient and elevated CO_2 treatments in 2002 and 2007 (extractable N and mean N residence times, potential N mineralization, gross N fluxes, microbial respiration, and soil chemical properties), were determined using a two-way repeated-measures analysis. The model had a

Table 1 Results of repeated-measures analysis for extractable N, net N mineralization (30 d), and microbial respiration of 2002 and 2007 measurements

	0–60 cm (2002)		0–60 cm (2007)		0–190 cm (2007)	
Source	*F*	*P*	*F*	*P*	*F*	*P*
Extractable N						
Depth	78.06	0.0001***	12.80	0.0003***	5.81	<0.0004***
Treatment	7.32	0.0150**	2.10	0.1694	3.12	0.0960*
Depth × treatment	3.09	0.0730*	0.10	0.9091	0.36	0.9010
Net N mineralization						
Depth	78.06	<0.0001***	23.74	<0.0001***	4.59	<0.0044***
Treatment	1.82	0.1970	0.51	0.4889	0.58	0.4602
Depth × treatment	0.58	0.5711	0.02	0.9767	0.47	0.8249
Microbial respiration						
Depth	146.39	<0.0001***	66.07	<0.0001***	22.22	<0.0001***
Treatment	0.03	0.8682	13.40	0.0030***	10.03	0.0060***
Depth × treatment	0.89	0.4309	3.05	0.0783*	2.65	0.0386**

*,**,***Significance at the 0.10, 0.05, and 0.01 probability levels for repeated-measures analysis, respectively.

first-order covariance autoregressive structure (SAS, version 8.02) ($\alpha = 0.10$) because the variables were autocorrelated by soil depth (Durbin–Watson statistic). A one-way repeated-measures analysis with first-order, autoregressive covariance structure was used to determine significant differences in total N sorption on ion-exchange resins with depth. Pairwise comparison tests using least squares means were used to compare treatment effects at each soil depth increment. Flux estimates in 2007 in the E' and B_h soil horizons were not included in the statistical (repeated measures) analyses because of poor replication ($n = 3$) and their specific depths were not consistent, as required for the covariance structure in the statistical model. Soil depth increments at 0–60 cm in 2007 were also analyzed independently of the rest of the sampled horizons to allow statistical comparisons with 2002 when samples were taken only to a depth of 60 cm. One-way ANOVAs were used to determine significant differences in extractable N and N fluxes of the forest floor (O horizon), E' horizon, and B_h horizon. Repeated-measures analysis of variance was use determine significant differences in ^{15}N values of leaves collected in reference and treatment plots and over time. Critical *P*-values were set ad-hoc at 0.1 due to soil heterogeneity and associated variability in soil flux measurements.

Results

Extractable inorganic N

In 2002, total field extractable inorganic N concentrations were lowered at elevated CO_2 ($P = 0.015$) at depth increments to 60 cm (Table 1, Fig. 1a–c). There was a significant depth × treatment interaction ($P = 0.073$), owing to strong treatment differences at 0–10 cm ($P = 0.038$), which diminished with soil depths from the 30 to 60 cm ($P = 0.971$) (Fig. 1a–c). Considering just the top 60 cm increment in 2007 (Fig. 1a–c), there was no significant depth × treatment interaction ($P = 0.909$) and no CO_2 effects ($P = 0.169$), with the exception of a significant CO_2 effect at 10–30 cm ($P = 0.089$).

Total field extractable inorganic N concentrations were lowered at elevated CO_2 in 2007 when considering the entire profile (0–190 cm) ($P = 0.096$) (Table 1, Fig. 1a–d). Ammonium was the dominant form of inorganic N through the profile, constituting more than 90% of the total inorganic N pool at most depths. There was not a CO_2 effect on nitrate concentrations ($P > 0.10$), and concentrations were relatively uniform with depth, generally ranging between 0.1–0.2 μg N g soil^{-1} (data not shown). Total extractable N decreased significantly with depth with the highest concentrations at 0–10 cm in both treatments ($P < 0.001$) in both 2002 and 2007. The strongest CO_2 effects occurred at depths of 10–30 ($P = 0.089$), 30–60 ($P = 0.014$), and 60–100 cm ($P = 0.015$) (Fig. 1a–d). Mean total extractable N concentrations for the *O*, E', and B_h horizons were 5.04 ± 0.87, 1.40 ± 0.17, and 1.25 ± 0.12 μg N g soil^{-1}, respectively (mean values were pooled because there were no significant differences among treatments).

Ion-exchange measure of inorganic N availability

The total amount of inorganic N ($NH_4 + NO_3$) sorbed from June 2007 to June 2008 was not affected by depth from 10 to 200 cm, but increased significantly ($\sim 3\times$) at 250 cm (Fig. 2). There were sharp contrasts in the

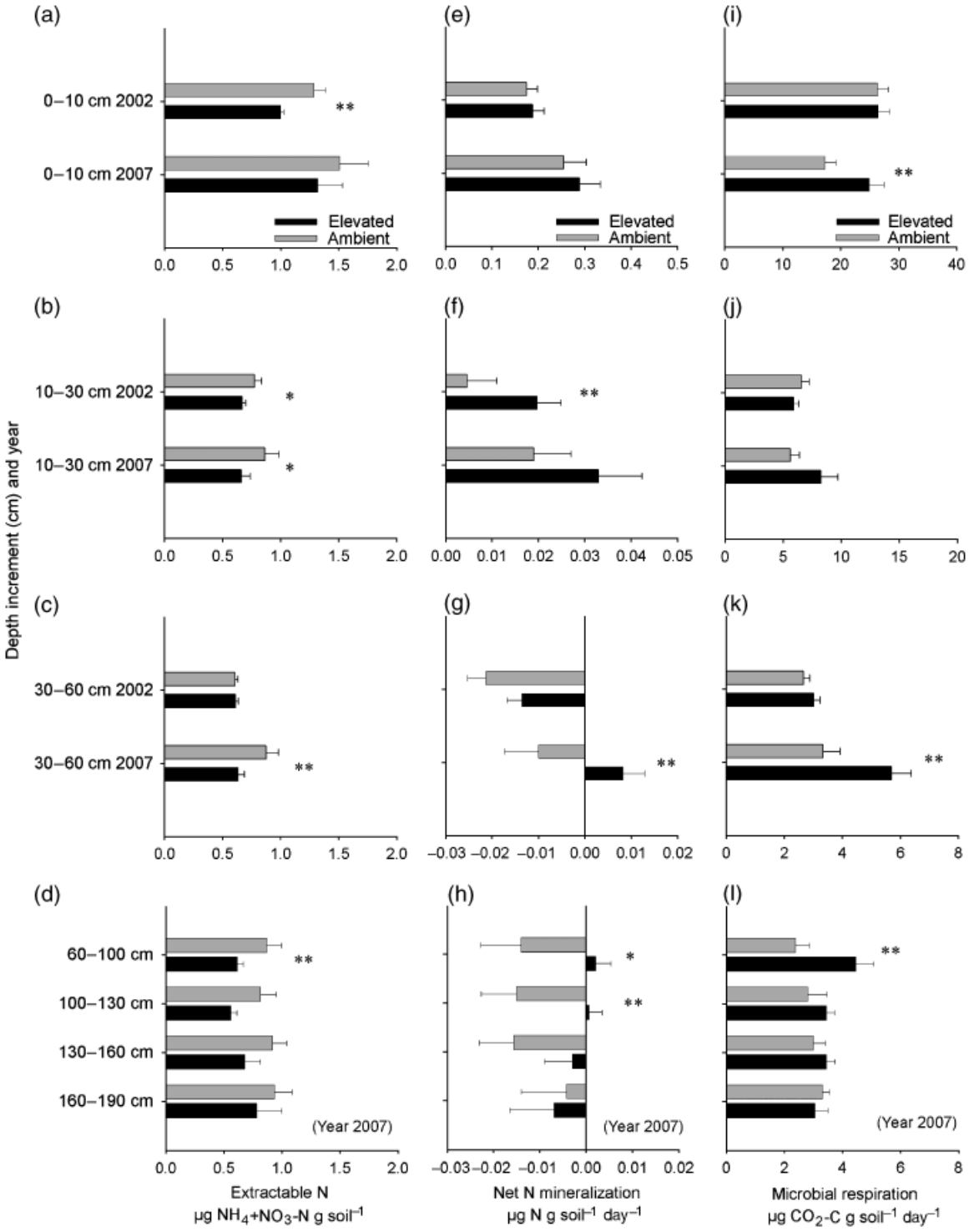

Fig. 1 Field extractable inorganic N (left panel, a–d), potential net N mineralization (middle panel, e–h), and microbial respiration (C mineralization) (right panel, i–l) (± 1 SE) by depth increments (0–190 cm) measured in 2002 and 2007. * and **Significant differences at the 0.10 and 0.05 probability levels for pairwise comparisons, respectively. (Note scale change in middle and right panels.)

composition of inorganic N in the soil profile. Nitrate was the dominant form (~ 95%) of inorganic N sorbed by resins at 10 cm, but the amount of nitrate sorbed fell quickly with increasing soil depth (Fig. 2). By contrast, NH_4^+ sorption was the lowest at the surface, but increased dramatically with soil depth

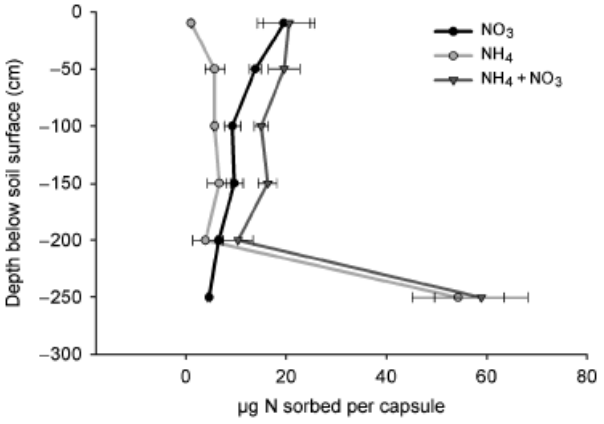

Fig. 2 Total inorganic N, NH_4^+, and NO_3^- ($\pm$ 1 SE) sorbed on ion-exchange resins over approximately 1 year at depth intervals of 10, 50, 100, 150, 200, and 250 cm ($n = 3$). The deepest resin capsules (250 cm) were placed in the B_h horizon, just below the water table.

to a maximum at 250 cm. Ammonium was the dominant form in the very deep soil ($\sim$ 92% at 250 cm) (Fig. 2).

Potential net N mineralization

In 2002, laboratory-based estimates of net N mineralization showed no CO_2 effect at depths of 0–60 cm ($P = 0.197$) (Fig. 1e–g), but there was a CO_2 effect at the 10–30 cm depth increment ($P = 0.049$) (Table 1, Fig. 1f). In 2007, there was also no CO_2 effect on N mineralization in the top 60 cm ($P = 0.489$) (Fig. 1e–g) or in the soil profile to 190 cm ($P = 0.460$) (Table 1, Fig. 1e–h). Also, there were no significant differences in potential N mineralization of the forest floor (O-horizon) in elevated ($0.01 \pm 2.24\,\mu g\,N\,g\,soil^{-1}\,day^{-1}$) and ambient treatments ($-1.62 \pm 1.06\,\mu g\,N\,g\,soil^{-1}\,day^{-1}$) ($P = 0.534$). In both years, net N mineralization decreased significantly with depth with the highest rates at the 0–10 cm increment regardless of the treatment ($P < 0.01$), and there were no depth-by-treatment interactions. Mean net N mineralization for the O, E', and B_h horizons were -0.80 ± 1.25, -0.05 ± 0.16, and -0.31 ± 0.13 µg-N g soil^{-1} day^{-1}, respectively (mean values were pooled because of no significant differences among treatments).

Although the overall model was not significant in 2007 (Table 1), net N mineralization at elevated CO_2 was significantly greater in the 30–60 cm ($P = 0.022$), 60–100 cm ($P = 0.062$), and 100–130 cm ($P = 0.041$) depth increments (Fig. 1g–h). Net N immobilization started to occur at 30 cm in ambient treatments (Fig. 1g–h), but did not occur until 130 cm at elevated CO_2 (Fig. 1 h). There was not a depth-by-treatment interaction ($P = 0.825$).

Net nitrification rates were very low and sometimes negative (10–30 cm had the maximum positive rates, $0.0043\,\mu g\,N\,g\,soil^{-1}\,day^{-1}$), and there was no CO_2 effect when considering the entire profile ($P = 0.389$). There was not a depth $\times$ treatment interaction ($P = 0.160$) for nitrification.

Gross N fluxes and microbial respiration

There was no effect of CO_2 in either gross mineralization (ammonification) (0–190 cm) or nitrification (0–30 cm) (Table 2, Figs 3a and 4a). Gross N mineralization was about 10% greater at 0–10 cm in the elevated CO_2 treatments ($8.21 \pm 1.04\,\mu g\,N\,g\,soil^{-1}\,day^{-1}$) compared with ambient treatments ($7.46 \pm 1.17\,\mu g\,N\,g\,soil^{-1}\,day^{-1}$). Gross N mineralization rates at 0–10 cm greatly exceeded ($\sim 3\times$) those found in the rest of the profile (Fig. 3a). Mean gross N mineralization for the E' and B_h horizons were 1.83 ± 0.12 and $2.03 \pm 0.09\,\mu g\,N\,g\,soil^{-1}\,day^{-1}$, respectively (mean values were pooled because there were no significant differences among treatments).

In 2007, there was a CO_2 effect on mean residence times (MRTs; inorganic N pool divided by gross N mineralization) of NH_4^+ when considering the entire profile (190 cm) (Table 2), but were shorter at elevated CO_2 in the 0–10 cm ($P = 0.031$) and 10–30 cm ($P = 0.021$) depth increments (Fig. 3b). Nitrate had much shorter MRTs (< 4 h) than NH_4^+ (Fig. 4b) (0–30 cm), but there was no CO_2 effect (Table 2).

In 2002, there was no effect of CO_2 on microbial respiration in the entire sampled profile (0–60 cm) ($P = 0.868$) or in any individual soil increment and depth $\times$ treatment interaction (Table 1, Fig. 1i–k). Conversely, in 2007, there was a CO_2 effect at 0–60 cm ($P = 0.003$) where elevated CO_2 stimulated respiration, and a significant depth-by-treatment interaction ($P = 0.078$).

In 2007, elevated CO_2 increased potential soil microbial respiration ($P \leq 0.006$) when considering the whole profile (Table 1, Fig. 1i–l). Microbial respiration decreased significantly with soil depth with the highest rates at the 0–10 cm increment in both treatments ($P < 0.001$) and this trend was significant in both 2002 and 2007. Strong positive effects of CO_2 were found in 0–10 ($P = 0.014$), 30–60 ($P = 0.019$), and 60–100 cm ($P = 0.012$) depth increments (Fig. 1i–l). There was a significant depth-by-treatment interaction for microbial respiration ($P = 0.039$). Mean microbial respiration for the E', and B_h horizons were 3.16 ± 0.29 and $3.43 \pm 0.43\,\mu g\,CO_2\text{-}C\,g\,soil^{-1}\,day^{-1}$, respectively (mean values were pooled because there were no significant differences among treatments).

Table 2 Results of repeated-measures analysis for gross N mineralization N, gross NH_4^+ consumption, gross nitrification, and gross NO_3^- consumption in 2007

	0–190 cm		0–60 cm		0–30 cm	
Source	*F*	*P*	*F*	*P*	*F*	*P*
Gross N mineralization						
Depth	9.08	<0.0001***	25.59	<0.0001***		
Treatment	0.02	0.8867	0.20	0.6628		
Depth × treatment	0.42	0.8610	0.23	0.7943		
Gross NH_4^+ consumption						
Depth	12.42	<0.0001***	34.06	<0.0001***		
Treatment	0.39	0.5411	0.64	0.4353		
Depth × treatment	0.35	0.9054	0.40	0.6752		
MRT NH_4^+						
Depth	6.96	<0.0003***	34.06	<0.0004***		
Treatment	1.32	0.2694	2.40	0.1430		
Depth × treatment	0.89	0.5198	2.52	0.1132		
Gross Nitrification						
Depth					2.11	0.1688
Treatment					0.78	0.3908
Depth × treatment					0.00	0.9756
Gross NO_3^- consumption						
Depth					2.93	0.1091
Treatment					0.46	0.5103
Depth × treatment					0.01	0.9283
MRT NO_3^-						
Depth					3.38	0.0871*
Treatment					0.52	0.4830
Depth × treatment					0.96	0.3446

*,**,***Significance at the 0.10, 0.05, and 0.01 probability levels for repeated-measures analysis, respectively.

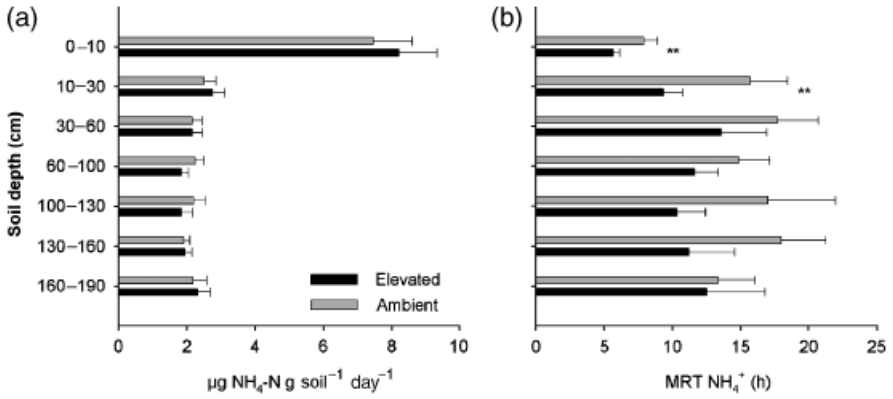

Fig. 3 Gross N mineralization (ammonification) (a), and mean residence times (MRTs) (± 1 SE) (b) of NH_4^+ by depth increments (0–190 cm) measured in 2007. **Significance at the 0.05 probability level for pairwise comparisons.

Plant ^{15}N uptake from deep soil

Leaves from all three sampled species had a significant enrichment of ^{15}N 24 h (T_{24} treated plot) after the water table was injected with ^{15}N, compared with mean values of the treated and reference plot at T_0 and the reference plot at T_{24} (Table 3). There was a significant ($P = 0.010$) time × species interaction, where *S. repens* leaves had the greatest relative ^{15}N enrichment compared with both *Quercus* species. Both main effects of time ($P < 0.001$) and species ($P < 0.001$) were also significant.

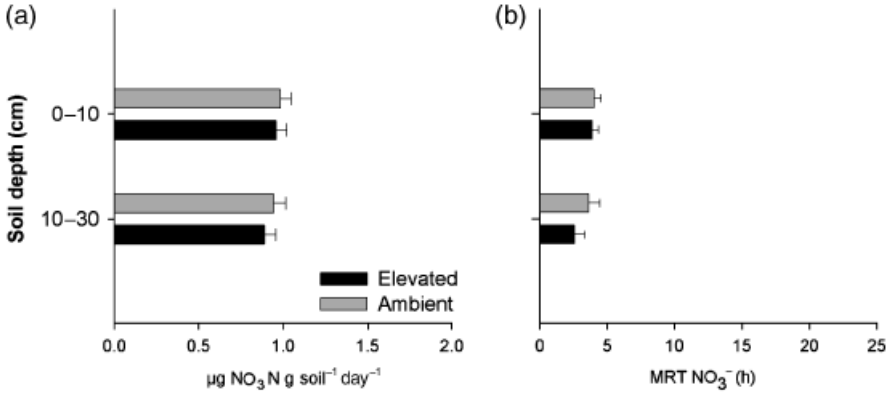

Fig. 4 Gross nitrification (a), and mean residence times (MRTs) (± 1 SE) (b) of NO_3^- by depth increments (0–30 cm) measured in 2007.

Table 3 Delta ^{15}N values (± 1 SE) of leaves collected in reference and treatment plots before (T_0) and 24 h (T_{24}) after ^{15}N tracer in the form of ammonium sulfate was injected into the water table

Plant species	Reference ($\delta^{15}N$)	Treated ($\delta^{15}N$)
T_0		
Q. myrtifolia	−4.29 (0.35)a	−4.25 (0.29)a
Q. geminata	−2.82 (0.26)a	−2.79 (0.20)a
S. repens	−0.99 (0.79)a	−0.69 (0.63)a
T_{24}		
Q. myrtifolia	−4.21 (0.29)a	−2.82 (0.36)b
Q. geminata	−2.95 (0.20)a	−0.93 (0.38)b
S. repens	−0.68 (0.65)a	3.07 (1.38)b

Means with different letters are significantly different, by species.
Q. myrtifolia, *Quercus myrtifolia*; *Q. geminate*, *Quercus geminata*; *S. repens*, *Serenoa repens*.

Soil chemical properties

Soil chemical properties varied greatly with soil depth (Table 4). Soil organic C and N, as well as CEC decreased substantially with increasing soil depth to 190 cm. The carbon to nitrogen ratios (C : N) were wide at 0–10 cm and at the B_h horizon. Soil organic N at ambient ($0.053 \pm 0.03\,mg\,g^{-1}$ soil) and elevated CO_2 ($0.045 \pm 0.05\,mg\,g^{-1}$ soil) were not different ($P > 0.05$) at 0–10 cm. The extremely low values of organic N from 10 to 190 cm were below the detection limit of our analytical instruments, precluding us from obtaining accurate C : N ratios for those portions of the profile. Soil pH increased markedly with soil depth. Plant-available P had markedly high concentrations in the *B* horizon (60–190 cm). The B_h horizon had much greater soil organic C, CEC, and P than much of the soil profile between 10 and 190 cm.

Discussion

Elevated CO_2 stimulation of biomass accumulation in this scrub-oak ecosystem was sustained over the 11 year duration of this study (Dijkstra *et al*., 2002; Hungate *et al*., 2006; Stover *et al*., 2007; Seiler *et al*., 2009), despite nutrient-poor soils that were expected to quickly cause PNL. Nutrient limitations on plant responses to elevated CO_2 in other forests have occurred in less than a decade (Oren *et al*., 2001; Norby *et al*., 2002; Ellsworth *et al*., 2004; Finzi *et al*., 2006, 2007), although they do not always entirely negate the stimulatory effects of CO_2 on NPP (e.g., Duke and ORNL) (Finzi *et al*., 2006, 2007; Norby & Iversen, 2006). Our data indicate that elevated CO_2 and increased plant N demand influenced soil N cycling throughout the soil profile, and suggest that deep soil N sources helped alleviate PNL.

The PNL hypothesis (Luo *et al*., 2004) predicts that soil N availability will decrease in forest ecosystems with strong plant growth responses to elevated CO_2 over time, inducing a negative feedback on the CO_2 fertilization effect. Many of the responses to elevated CO_2 in this scrub-oak ecosystem are consistent with the development of PNL (Johnson *et al*., 2003; Hungate *et al*., 2006; Stover *et al*., 2007), yet there had not been a cessation of the CO_2 stimulation of plant production by the end of the study. Consistent with the PNL hypothesis, plant biomass (above- and belowground) was greater at elevated CO_2 (Hungate *et al*., 2006; Stover *et al*., 2007). Also, elevated CO_2 increased plant N uptake and plant N use efficiency in this ecosystem (Johnson *et al*., 2003; Hungate *et al*., 2006), and there was evidence of greater litter inputs and widening soil C : N ratios (Hungate *et al*., 2006). However, key soil responses to elevated CO_2 did not develop according to PNL predictions.

A requisite for PNL to occur is that labile or mineralizable N soil pools decrease below the level needed to

Table 4 Chemical characteristics (± 1SE) of soil horizons in 2007

Soil properties	SOC (mg g^{-1})	SON (mg g^{-1})	C : N	pH	CEC (mEq 100 g)	P (μg g^{-1})
Soil horizon (depth)						
A (0–10 cm)	17.9 (1.1)	0.49 (0.04)	36.7 (1.5)	4.04 (0.14)	5.93 (0.28)	15 (2)
E (10–60 cm)	3.2 (0.1)	<0.1	na	4.44 (0.14)	2.80 (0.54)	17 (3)
B_1 (60–130 cm)	2.0 (0.5)	<0.1	na	4.97 (0.03)	2.11 (0.07)	154 (12)
B_2 (130–190 cm)	1.5 (0.0)	<0.1	na	5.00 (0.02)	1.90 (0.18)	104 (2)
E′ (190–B_h)	1.1 (0.4)	<0.1	na	5.46 (0.55)	0.94 (0.02)	19 (9)
B_h (mean 277 cm)	11.3 (4.5)	0.17 (0.08)	71.5 (1.7)	4.95 (0.29)	5.68 (1.90)	132 (20)

support the elevated CO_2 stimulation of plant growth (Hu *et al*., 2006). We observed almost an 18% decrease in total soil organic N in the top 10 cm at elevated CO_2, but in contrast with our hypotheses (1 and 2) we found no differences in gross N mineralization (an index of SON decomposition) (Fig. 2a) or potentially mineralizable N (an index of labile N) (Fig. 1e–h). Extractable inorganic N concentrations were significantly lower at elevated CO_2 (Fig. 1a–d, Table 1), indicating greater plant N demand, but the shorter MRT of NH_4^+ (significantly shorter in the top 30 cm) suggests that the NH_4^+ pool in elevated CO_2 soils was replenished at a faster rate than in ambient CO_2 soils (Fig. 3b). Other CO_2 enrichment studies have also reported greater plant N uptake and depletion of SON without changes in soil net N mineralization rates (Johnson *et al*., 1998, 2000, 2003; Finzi *et al*., 2002; Zak *et al*., 2003). In summary, we did not detect a decrease in the potential plant-available N supply in elevated CO_2 soils as predicted by PNL hypothesis (Luo *et al*., 2004).

Increased N uptake appears to be the primary mechanism by which other forests (Rhinelander, Duke, ORNL) exhibiting increased rates of biomass accrual in response to CO_2 enrichment have avoided an increase in plant N limitation (Norby & Iversen, 2006; Finzi *et al*., 2007). Finzi *et al*. (2007) speculated that greater plant N uptake was a result of greater fine root production, increased rates of SOM decomposition, and increased allocation to mycorrhizal fungi (Chalot & Brun, 1998). The mechanisms that are most likely to maintain the supply of plant-available N to sustain greater N uptake in the present ecosystem at elevated CO_2 include: (1) greater N release from existing SOM pools, (2) increased exploitation of inorganic N deep in the soil profile, and (3) N uptake directly from the water table.

Potential N supply from increased SOM mineralization

Our data suggest that soil N cycling has changed over the course of the study to increase N availability, although our temporal sampling was limited to avoid comprising the experiment. Elevated CO_2 caused an initial decline in extractable inorganic N in the top 10 cm for the first 6 years of treatment (2002 data this study; Johnson *et al*., 2001; Hungate *et al*., 2006,Fig. 1a), but by year 11 (2007) the difference between treatments had narrowed ($P = 0.61$). In the first year of the Florida study, elevated CO_2 lowered gross N mineralization by 31% and gross N immobilization by 21% (Hungate *et al*., 1999). By contrast, our 2007 results after 11 years show a slight increase in both gross N mineralization ($\sim$ 10%) and N immobilization (14%) in the top 10 cm compared with ambient CO_2. An increase in the rate of N cycling in the top 10 cm in 2007 caused by elevated CO_2 is also indicated by a decrease in the MRT of SON at 0–10 cm (SON divided by gross N mineralization rates) from 70 ± 17 days at ambient CO_2 ($P = 0.16$) to 55 ± 6 days at elevated CO_2. The short MRT of SON at our site is consistent with the poorly developed soil structure and low silt and clay mineral content of sandy soils (Six *et al*., 2002). Although the increase in gross N mineralization was small ($\sim$ 10%), increases of <10% are often enough to explain differences in plant N uptake at elevated CO_2, owing to the magnitude of the gross N flux (Reich *et al*., 2006b). Enhanced microbial activity as indexed by microbial respiration and gross N mineralization may reflect an increase in SON decomposition resulting from microbial priming and a resulting increase in the supply of plant available N in shallow portions of the soil.

Microbial priming has been strongly implicated as a mechanism that lowered soil C stocks and increased N supply at elevated CO_2 at this site (Carney *et al*., 2007; Langley *et al*., 2009). Many hypotheses have been advanced to explain the priming effect, but the mechanism that would most likely explain an increase in soil organic matter decomposition at the Florida site is *microbial activation* (Kuzyakov, 2002). In this case, elevated CO_2 would cause microbial activation by increasing the input of C-rich organic matter from the growth and death of roots (Iverson *et al*., 2008) to which soil microorganism respond initially by immobilizing available nutrients, then later by mineralizing older, N-rich soil organic matter or humic substances, resulting in a net release of N. Priming has been reported in other ecosystems exposed to elevated CO_2 (Hoosbeek *et al*.,

2004; Lichter *et al*., 2005) and might explain declines or minimal increases in total soil C and N, as well as maintenance of soil N availability (Johnson *et al*., 2000; Pendall *et al*., 2004).

Potential N supply from deep soil and water table

Fine root production at elevated CO_2 increased approximately 180% in the first 21 months of the Florida study (Dilustro *et al*., 2002), but by the third year this difference completely disappeared (Day *et al*., 2006). However, ground-penetrating radar showed that elevated CO_2 dramatically stimulated large root biomass compared with ambient CO_2 (Stover *et al*., 2007). Lower extractable N concentrations at elevated CO_2 (Fig. 1a–d) may reflect increased inorganic N uptake by plants given that net N mineralization rates (or rate of inorganic N supply) at elevated CO_2 (Fig. 1e–h) equaled or exceeded rates at ambient CO_2. Decreased concentrations of inorganic N can be associated with greater plant uptake (Hu *et al*., 2001). Using extractable N as a proxy for plant N uptake, the differences between 2002 and 2007 inorganic N concentrations suggest that plant N demand was satisfied by increased uptake from the top 10 cm in the first 6 years of the experiment, then transitioned to much deeper soil N sources (at least 130 cm) by 2007 (Fig. 1a–d), consistent with our predictions in hypotheses 3 and 4. The amount of coarse and fine root biomass in the last year of study is currently unknown, but fine root density was much greater under elevated CO_2 to a depth of 100 cm in the first 1.5 years of the study (Day *et al*. 2006). Enhanced root production and exploitation of resources in deep soil is likely to be an important mechanism for greater plant N uptake at elevated CO_2.

Our temporally integrated index of N availability using ion-exchange resins indicated that N availability (mostly in the form of NH_4^+) was about three times greater in the B_h horizon than elsewhere in the soil profile (Fig. 2). The dominance of NO_3^- on the ion-exchange resins at 10 cm is likely due to leaching from portions of the soil above 10 cm; NO_3^- is a highly mobile ion in the soil solution and is subject to greater potential leaching than NH_4^+. Organic matter and mineral surfaces in the B_h horizon are not a likely source of N. The B_h horizon is relatively rich in SOC, but it is poor in SON (Table 4). It is also very resistant to decomposition, as shown by low N mineralization rates and very wide C : N ratios. If anaerobic conditions occur in the portion of the B_h horizon below the water table, this would slow decomposition further. The resistance of this relatively large SOC pool in the B_h horizon to decomposition is evident by its age; radiocarbon dating placed the mean age of this horizon at 17 690–18 020 years before present (calendar years) (D.C. McKinley *et al*., unpublished data). The water table that occurs roughly at the B_h horizon is a more likely source of the relatively large amounts of plant-available N found on ion-exchange resins. The source of NH_4^+ in the B_h horizon (Fig. 2) might be leaching from shallower horizons in the soil profile (vertical transport) with low cation-exchange capacity (CEC, Table 4). But, in recent work (D.C. McKinley *et al*., unpublished data) that tracked the fate of ^{15}N applied to the soil surface in May 1998 (Hungate *et al*. 2006), we found very little ^{15}N tracer in microbial biomass below 130 cm by 2007 (most of the tracer was still concentrated at 0–10 and 10–30 cm), suggesting that vertical transport (leaching) of N via the soil to the water table is very slow. Rather, a reservoir of inorganic N from hydrologic sources deep below chambers may already exist without much contemporary N input; however, the origin of this N in the water table remains a matter of much speculation.

We found that plants in the scrub-oak ecosystem took up NH_4^+ (an energetically favorable form of inorganic N) from water table about 2.5 m below the surface of the soil (Table 3), a source much deeper than most elevated CO_2 studies consider. Deep soil pools of inorganic N are not unprecedented; Walvoord *et al*. (2003) found that leached NO_3^- accumulated in the sub-soil of arid-to-semiarid ecosystems over thousands of years. In an earlier study at the site, Hungate *et al*. (2002) found that the two co-occurring oaks at the site were receiving 79–95% of their water from the water table, which provides further evidence of a physical link between plants and deep soil horizons. It is not known how much the dominant oaks depend on groundwater year-round, but we found direct evidence that there is inorganic N uptake from this relatively N-rich source during a period of peak ecosystem C uptake (Table 3). This mechanism may explain avoidance of PNL in the Florida scrub-oak ecosystem.

Plant access to available N at the water table in this study may not reflect realized conditions with rising CO_2 in the case that the major source of inorganic N at the water table is derived from outside the experimental chambers, and this external source could also be influenced by elevated CO_2 without a significant time lag. It is not clear how N availability at the water table and the ability of the ecosystem to avoid PNL would be different if the entire island were treated with elevated CO_2, effectively removing artifacts of scale. In this scenario, plants growing under elevated CO_2 may reduce the amount of inorganic N leached to the water table, owing to greater plant demand for N, thus the development of PNL could occur quickly unless there are

other nutrient subsidies. Alternatively, increased decomposition of SOC and increased release of inorganic N in shallow soil previously observed at elevated CO_2 in this ecosystem (Carney *et al.*, 2007; Langley *et al.*, 2009) might increase the availability of NH_4^+ at the water table, further delaying the onset of PNL. Further studies are needed to determine the openness of the N cycle in deep soils by elucidating the origin of N at the water table and contribution to ecosystem N budgets at elevated CO_2.

Conclusions

Potential feedbacks between the plant and atmospheric systems at elevated CO_2, in particular those that influence ecosystem responses to elevated CO_2, are increasingly recognized as being important predictors of potential terrestrial C uptake and sequestration (Heimann & Reichstein, 2008). After 11 years of CO_2 fumigation, we found few changes in potential plant available N supply at elevated CO_2 that would lead to PNL of plant responses. Greater microbial respiration and slightly enhanced gross N mineralization in the shallower portions of the soil profile where the greatest N flux occur, suggest that SOM decomposition may be enhanced by elevated CO_2, resulting in greater plant available N. Since 2002, we found indirect evidence for greater plant N uptake or exploitation of inorganic N pools at elevated CO_2 in deep portions of the soil profile. Lastly, and perhaps most significant, we found that the water table could be a relatively large source of plant-available N, and direct evidence that plants are taking up inorganic N from the water table. Sustained NPP in the Florida scrub-oak ecosystem is likely a result of increased N availability, resulting from both increased N mineralization in shallow soils and greater exploitation of deep soil N, including N uptake from the water table. These results underscore the value of long-term CO_2 experimentation and the importance in taking a comprehensive approach to fully understand availability of inorganic N in soil that could modulate whole-ecosystem functioning and C uptake on decadal time scales.

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