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PROGRESSIVE NITROGEN LIMITATION OF ECOSYSTEM PROCESSES UNDER ELEVATED CO_2 IN A WARM-TEMPERATE FOREST

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Abstract. A hypothesis for progressive nitrogen limitation (PNL) proposes that net primary production (NPP) will decline through time in ecosystems subjected to a step-function increase in atmospheric CO_2. The primary mechanism driving this response is a rapid rate of N immobilization by plants and microbes under elevated CO_2 that depletes soils of N, causing slower rates of N mineralization. Under this hypothesis, there is little long-term stimulation of NPP by elevated CO_2 in the absence of exogenous inputs of N. We tested this hypothesis using data on the pools and fluxes of C and N in tree biomass, microbes, and soils from 1997 through 2002 collected at the Duke Forest free-air CO_2 enrichment (FACE) experiment. Elevated CO_2 stimulated NPP by 18–24% during the first six years of this experiment. Consistent with the hypothesis for PNL, significantly more N was immobilized in tree biomass and in the O horizon under elevated CO_2. In contrast to the PNL hypothesis, microbial-N immobilization did not increase under elevated CO_2, and although the rate of net N mineralization declined through time, the decline was not significantly more rapid under elevated CO_2. Ecosystem C-to-N ratios widened more rapidly under elevated CO_2 than ambient CO_2 indicating a more rapid rate of C fixation per unit of N, a processes that could delay PNL in this ecosystem. Mass balance calculations demonstrated a large accrual of ecosystem N capital. Is PNL occurring in this ecosystem and will NPP decline to levels under ambient CO_2? The answer depends on the relative strength of tree biomass and O-horizon N immobilization vs. widening C-to-N ratios and ecosystem-N accrual as processes that drive and delay PNL, respectively. Only direct observations through time will definitively answer this question.

Key words: *elevated CO_2; net primary production; nitrogen cycling; temperate forest.*

INTRODUCTION

The community of scientists studying ecosystem responses to elevated CO_2 has faced a conundrum over the last two decades: how is it possible for primary production to remain stimulated by elevated CO_2 despite widespread soil nutrient limitation? Theoretically, single-resource limitation only occurs when the rate of resource supply is very low relative to the rate of uptake (Rastetter and Shaver 1992). An imbalance between supply and demand is much less likely for carbon (C) than it is for nitrogen (N) given that plants are bathed in a vast pool of CO_2 that can diffuse rapidly inside leaves. By contrast, the majority of the fixed N in terrestrial ecosystems is bound to soil organic matter (SOM). This N must be converted to plant available forms prior to plant uptake, and plants have only an indirect effect on the rate at which N is released from SOM to plant-available forms (Schlesinger 1997, Schimel and Bennett 2004). The significant imbalance between nutrient supply and plant demand contributes to widespread N limitation in terrestrial ecosystems (Vitousek and Howarth 1991). Thus it appears improbable for net primary production to respond to elevated CO_2 in the long-term in the absence of an increase in the supply of other limiting resources such as N.

A hypothesis specific to progressive N limitation (PNL) of ecosystem responses to elevated CO_2 has been developing since the early 1980s (Strain and Bazzaz 1983, Commins and McMurtrie 1993, Rastetter et al. 1997, Field 1999, Luo and Reynolds 1999, Luo et al. 2004). This hypothesis postulates that ecosystems exposed to a step-function increase in atmospheric CO_2 have only a transient increase in net primary production (NPP) or net ecosystem production (NEP) because the severity of N limitation increases through time. Luo et al. 2004 proposed two major pathways by which this occurs. First, a rapid rate of NPP under elevated CO_2 immobilizes mineralized N within plant biomass as a

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direct consequence of plant-N uptake from soil. The immobilization of mineralized N depletes soil of relatively labile pools thereby slowing rates of N mineralization, and hence plant-N availability declines (e.g., Gill et al. 2002). Second, an increase in NPP increases microbial-C availability thereby creating a biosynthetic demand for N by microbes (Zak et al. 2000). Nitrogen immobilization within microbial biomass increases and N availability to primary producers declines (Diaz et al. 1993).

The hypothesis for PNL also considers factors that preclude or delay PNL (Luo et al. 2004). Increases in N-use efficiency (NUE) under elevated CO_2 increase the rate of C fixation per unit of N acquired from soils, thus decreasing the quantity of N required to support high rates of NPP (Finzi et al. 2002). Increased inputs of N via atmospheric deposition and N-fixation or decreased N losses can also delay or preclude PNL (Hungate et al. 1999, Lusher et al. 2000, Johnson et al. 2004). Both mechanisms increase ecosystem N capital, which should tend to increase N supply to primary producers (Schimel and Bennett 2004).

Historically, the single largest impediment to testing the hypothesis of PNL was the lack of long-term, ecosystem-scale CO_2 experiments. Among others, the Duke Forest free-air CO_2 enrichment (FACE) experiment now provides an opportunity to examine PNL in an intact ecosystem subject to natural variations in climate and low nutrient availability (Oren et al. 2001, Finzi et al. 2002) without manipulating other ecosystem variables (Hendrey et al. 1999). A behavior consistent with PNL has been observed in the prototype FACE plot at the Duke Forest site (Oren et al. 2001), yet none of the proposed mechanisms were directly investigated. The objectives of this study were (1) to determine whether the initial stimulation in forest productivity under elevated CO_2 declined through time as a consequence of decreasing N availability; (2) to identify the mechanisms responsible for PNL; (3) to determine if processes were delaying PNL; and (4) to predict whether N limitation would eventually preclude any response to elevated CO_2. To meet these objectives we measured the pools and selected fluxes of C and N in biomass, microbes, and soils during the first six years of this experiment.

Materials and Methods

Site description

The FACE experiment in the Duke Forest (Orange County, North Carolina, USA) is composed of six 30 m diameter plots. Three experimental plots are fumigated with CO_2 to maintain the atmospheric CO_2 concentration 200 μL/L above ambient (i.e., 565 μL/L). Three control plots are fumigated with ambient air only (365 μL/L). The experiment began 27 August 1996, about two years after the commencement of CO_2 enrichment at the prototype plot (Oren et al. 2001). CO_2 enrichment was nearly continuous with the exception of time periods when the air temperature was too cold for photosynthesis or when wind speeds exceeded 5 m/s. Additional details on FACE operation can be found in Hendrey et al. (1999).

The current forest is derived from 3-yr-old loblolly pine (*Pinus taeda*) seedlings that were planted in 1983 in a 2.4 × 2.4 m spacing. In 1996, the 13-yr-old pine trees were 14 m tall and accounted for 98% of the basal area of the stand. Since planting, a deciduous understory has recruited from nearby hardwood forests. The most abundant understory tree species is sweet gum (*Liquidambar styraciflua*), with admixtures of winged elm (*Ulmus alata*), red maple (*Acer rubrum*), red bud (*Cercis canadensis*), and dogwood (*Cornus florida*; Naidu and DeLucia 1999). The 32-ha site contains an elevation gradient of 15 m between the highest and lowest points, but topographic relief is ≤1° throughout. Soils are classified as being from the Enon Series (fine, mixed, active, thermic, Ultic Hapludalfs). Enon soils, derived from mafic bedrock, are slightly acidic (0.1 mol/L $CaCl_2$ pH = 5.75), and have well-developed soil horizons with mixed clay mineralogy. Additional site details can be found in Oren et al. (1998), Schlesinger and Lichter (2001), and Finzi et al. (2001).

Plant biomass pools, increments, and turnover

Prior to the initiation of the CO_2 treatment, 30 loblolly pine trees were harvested from nearby stands that varied in age from 10 to 48 years. The trees (dbh: 3.5–35.6 cm) were used to develop allometric regressions between stem diameter and wood, bark, and coarse root mass (Naidu et al. 1998). Martin et al. (1998) and Whittaker and Marks (1975) published similar allometric relationships for several southern Appalachian hardwood species. In each of the six experimental plots all the woody vegetation was surveyed, including stem diameters, prior to the onset of CO_2 fumigation in August of 1996. By using dendrometer bands to monitor diameter growth for 31–34 trees per plot, we used the stem maps and the allometric regressions to calculate loblolly pine and hardwood biomass pools and increments for each year reported in this study. The details of these calculations are presented in Hamilton et al. (2002).

During the second year of CO_2 fumigation, we noted that loblolly pine leaf litter production was greater than that predicted by the pretreatment allometries (Naidu et al. 1998). Therefore, loblolly pine and hardwood foliage pools and increments were based on the data collected from the leaf litter baskets (Finzi et al. 2001). A *t* test indicated that the difference in leaf mass per unit area (LMA, mg/cm^2) between green leaf and litter samples of loblolly pine and several of the hardwood species was not significantly different in any year of this study (Finzi et al. 2002). We therefore assumed that litterfall mass for loblolly pine and the hardwoods was the same as canopy mass.

The longevity of loblolly pine foliage in the Piedmont of NC is 19 mo (Zhang and Allen 1996), so that at any time there are needles of two different ages on a single branch (e.g., two cohorts). Given this longevity, a new cohort of leaves produced in one year does not abscise until the following year. Thus the peak biomass of loblolly pine needles in the canopy in a given year is the sum of litterfall in that year and in the following year. For example, the mass of the loblolly pine needles in the canopy in 1998 was estimated from the sum of litterfall mass in 1998, the needles initially produced in 1997 but present in the canopy during the 1998 growing season, and 1999, the needles produced in 1998 that did not abscise until the end of the growing season in 1999. We used litterfall mass data from 1997 through 2003 to calculate leaf biomass for the period 1997–2002.

Aboveground litterfall mass (turnover) was collected from 5 June 1996 onward by placing 12 replicate 40 $\times$ 40 cm baskets in each plot. Litterfall was collected once per month between January and August and twice per month between September and December to minimize leaching losses of C and nutrients from leaf litter during the period of peak litterfall (Finzi et al. 2001). The samples were brought to the laboratory, dried at 65°C for 4 d, and weighed. The litter was sorted and subdivided into seven categories: pine needles, deciduous leaves, pine branches, deciduous branches, reproductive structures, bark, and ''other.'' The ''other'' category consisted of small, difficult-to-identify fragments of aboveground litterfall and frass. The litterfall data for 1996 through 2000 are from Finzi et al. (2002).

Fine root biomass for the period 1997–1999 was taken from Matamala and Schlesinger (2000) and R. Matamala (*unpublished data*). Fine root biomass for 2003 was taken from R. B. Jackson (*unpublished data*). Fine root increment and turnover data were only available for 1997 and 1998. To estimate fine root increments for each year from 1999 to 2002, we calculated the difference in fine root biomass between 2003 and 1999 and divided that difference by four. Fine root turnover in all years of this study was assumed to be that measured by Matamala and Schlesinger (2000).

Biomass C and N analysis

Wood cores were extracted from a subset of 10 canopy trees sampled in each plot in the autumn of 2002. We separated the tree cores into individual growth increments from 1996 to 2002 and analyzed them for N. Nitrogen concentration of the wood was measured after combustion in an element analyzer (Model NC2500, CE Instruments, Rodano, Italy) and was also assumed to be representative of the concentration of N in coarse woody roots (roots >5 cm diameter). No cores were extracted from sweet gum, the most abundant hardwood species in this forest because of their small size. There is no difference in the concentration of N in the bolewood of sweet gum trees under ambient and elevated CO_2 at the FACE experiment in Oak Ridge, Tennessee (R. J. Norby, *personal communication*). Thus, we assumed that the concentration of N in the bolewood of all hardwood species in this ecosystem was 0.2%, the same as that of the sweet gum trees in the Tennessee FACE experiment.

We collected live foliage of loblolly pine and hardwood species above a randomly selected location on each arm of a cross-shaped boardwalk that extends through each FACE ring to the north, south, east, and west. At each of these locations and heights, we sampled a single branch on each of four trees and collected five to eight fascicles of current and year-old foliage along a primary branch. Foliage samples were collected in September of each year from 1997 through 2002. The September sample represents the peak canopy biomass N (Zhang and Allen 1996).

We measured the N concentration of green leaves and all aboveground litter components in a sulfuric-copper sulfate acid Kjeldahl digestion followed by colorimetric analysis on an automated ion analyzer (Lachat QuickChem FIA+ 8000 Series, Zellweger Analytics, Milwaukee, Wisconsin, USA). The average concentration of N in red bud, red maple, sweet gum, and dogwood leaves was assumed to apply to the N concentration of green leaves for the other deciduous species in the canopy. Fine root N concentration and mass data were taken from Matamala and Schlesinger (2000).

Soil pools of C and N

The content of C and N in the organic (O) and mineral soil horizons was measured in October 1996, 1999, and 2002. Details of the field sampling and chemical analysis can be found in Lichter et al. (2005). In brief, 12 soil samples to a depth of 30 cm were extracted in 4.76 cm diameter cores while 12 forest floor samples were extracted as 10 $\times$ 10 cm monoliths. The forest floor and soil samples were weighed and passed through a 2-mm mesh sieve to remove stones and coarse roots. Samples were dried at 48°C for 5 d and then ground to a fine powder for C and N analysis on an element analyzer.

The concentration of inorganic N (NH_4^+, NO_3^-), the rate of potential net mineralization, and microbial-biomass N were measured in the top 15 cm of mineral soil. Soils were collected two to four times per year in April, June, August, or October. Four replicate soil cores from each plot were collected in 4.76 cm diameter cores to a depth of 15 cm. Soil samples were passed through a 5-mm mesh sieve to remove stones and coarse roots and then composited into a single sample.

The rate of potential net N mineralization was measured in the lab under field-moist conditions at 22°C (Binkley and Hart 1989, Finzi and Schlesinger 2003). Two replicate 20-g subsamples of soil from each core were placed into 250-mL plastic bottles. One bottle (the ''initial'' sample) was extracted immediately in 100 mL of 2 mol/L KCl and the second bottle was incubated

in the dark at 22°C for 28 d after which time it was extracted in 100 mL of 2 mol/L KCl. The rate of potential net N mineralization was calculated as the difference in the amount of NH_4^+ and NO_3^- in the incubated and initial sample. The initial sample estimated the concentration of inorganic N in soil.

Microbial-biomass N was quantified using the fumigation-extraction procedure (Brooks et al. 1985, Gallardo and Schlesinger 1991). In brief, three 10-g subsamples of sieved soil were removed from each of the composite bags and hand picked to remove all fine roots. Each subsample was divided in half, placed into a 50-mL centrifuge tube. The initial sample was immediately extracted with 0.5 mol/L K_2SO_4. The incubated sample was placed under C_2H_5OH-free $CHCl_3$ for 7 d. Following fumigation, we extracted the incubated samples with 0.5 mol/L K_2SO_4. Both the initial and incubated samples underwent persulfate digestion to oxidize all N species to NO_3^-. Microbial biomass N was estimated as the difference in the flush of N following $CHCl_3$ fumigation and that extracted in the initial sample, which was then divided $K_{EN} = 0.54$ (Joergensen and Mueller 1996).

Annual rates of net N mineralization were measured in the top 15 cm of mineral soil using the buried bag technique in 1998 and 2003 (Eno 1960, Finzi et al. 2002). In 1998, we extracted 15 cores per plot per sample period. In 2003, we extracted four cores per plot per sample period. At each sampling date, 4.78 cm diameter × 15 cm deep soil cores were extracted and their contents placed into polyethylene bags. A 20-g subsample of soil was removed from each polyethylene bag for initial determination of NH_4^+ and NO_3^- concentrations in each sample. Samples incubated in the field for one month, after which they were removed and brought back to the lab for analysis of accumulated NH_4^+ and NO_3^-. At the same time a new set of cores was collected for incubation during the following month. Annual rates of net mineralization were calculated as the sum of the difference between the concentration of inorganic N in incubated and initial samples across the twelve months. Concentrations of inorganic N were measured on an autoanalyzer (Lachat QuickChem FIA+ 8000 Series, Zellweger Analytics, Milwaukee, Wisconsin, USA).

Statistical analysis

We used repeated-measures analysis of variance to test for the effects of CO_2 treatment and time on (1) variations in plant C and N pools and fluxes, and (2) N cycling (i.e., extractable pools of inorganic N, potential net N mineralization, and microbial biomass N pools). The data on inorganic N, potential net N mineralization, and microbial-biomass N collected in April, June, August, and October were averaged to provide a single, integrated estimate of these pools and fluxes under ambient and elevated CO_2 within a given year. The test for the main effect of CO_2 was always based on $n = 6$ (two levels of CO_2 × three replicate 30 m diameter plots).

Initial measurements of the pools of C and N in the O and mineral soil horizons in 1996 demonstrated significant pre-treatment, between-plot variation in the pools of C and N at this research site (Schlesinger and Lichter 2001). This underlying between-plot variation often masked the effects of elevated CO_2 on the accumulation of C and N in soils (Schlesinger and Lichter 2001). We therefore used analysis of covariance to analyze the effects of elevated CO_2 on the accumulation of C and N in the O horizon and mineral soil horizon in 1999 and 2002. The 1996 (i.e., pretreatment) data were used as the covariate.

Results

Elevated CO_2 stimulated the production and turnover of biomass during the first six years of this experiment resulting in a consistent stimulation of NPP (Fig. 1a–c). The absolute difference in biomass increments and NPP between ambient and elevated CO_2 declined through time (Fig. 1d, f). However the time × CO_2 interaction term was not statistically significant and the stimulation remained between 20% and 30% (Fig. 1d–e). Biomass turnover under elevated CO_2 increased through time (Fig. 1b, e). The time × CO_2 interaction term was significant ($P < 0.05$), and the percentage of stimulation increased from 7% to 25%.

The content of C in woody biomass increased significantly more rapidly under elevated CO_2 than ambient CO_2 throughout the first six years of this experiment (time × CO_2: $P < 0.01$, Fig. 2a). Similarly, the C content in foliage and fine root pools increased significantly more rapidly under elevated CO_2 through time (time × CO_2: $P < 0.0001$). The content of N stored in woody biomass increased significantly more rapidly under elevated CO_2 through time (time × CO_2: $P < 0.01$, Fig. 2b). There was no statistically significant trend in foliar + fine root N pools through time or with CO_2 treatment (time × CO_2 $P > 0.05$), nor was there a main effect of elevated CO_2 on the N content of these two pools averaged across the first six years of CO_2 treatment. There was no effect of elevated CO_2 on the C-to-N ratio of woody biomass or the C-to-N ratio of foliage + fine roots (Fig. 2c).

The content of C in the top 30 cm of mineral soil was only marginally significantly higher under elevated CO_2 after six years of treatment (Fig. 3a). By contrast, the C content of the O horizon was significantly higher under elevated CO_2 than ambient CO_2 (Fig. 3a). There was no statistically significant difference in the N content of mineral soil between ambient and elevated CO_2 (Fig. 3b). The content of N in the O horizon increased more rapidly under elevated CO_2 than ambient CO_2, but was only significantly higher in the third year of this experiment (Fig. 3b). The C-to-N ratio of mineral soil was significantly lower under elevated CO_2 after

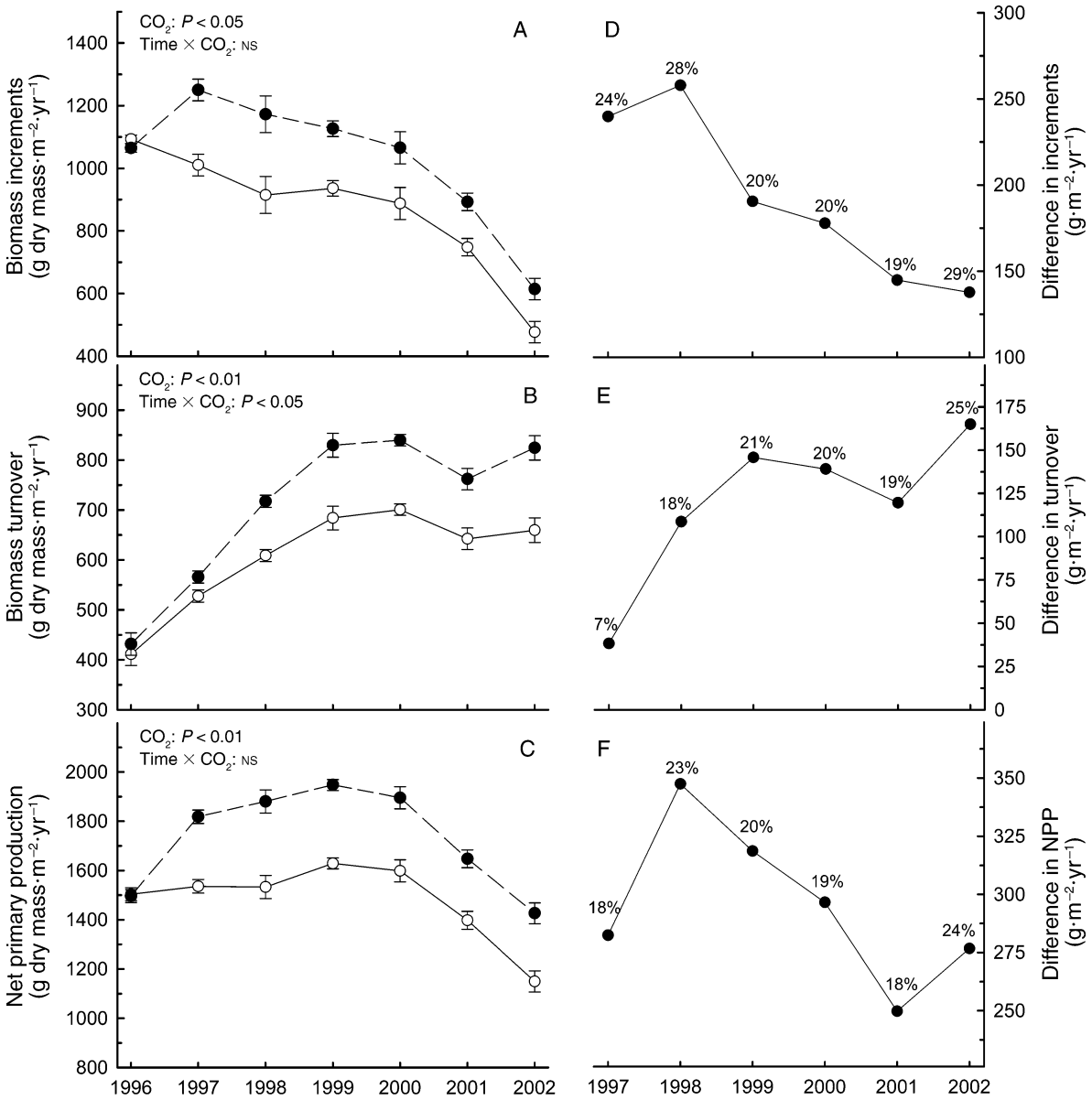

FIG. 1. (A) Increment, (B) turnover of biomass (sum of aboveground litterfall + fine root turnover), and (C) net primary production (increments + turnover) under ambient (open symbols) and elevated CO_2 (solid symbols) during the first six years of this experiment. (D–F) The difference between ambient and elevated CO_2 in (D) biomass increments, (E) biomass turnover, and (F) net primary production, with the percentage of stimulation under elevated CO_2 indicated above each data point. The 1996 data are pretreatment. The 1996–2000 data are recalculated from DeLucia et al. (1999) and Finzi et al. (2002).

six years (Fig. 3c). There was no effect of elevated CO_2 on the C-to-N ratio of the O horizon (Fig. 3c).

The C content of the entire ecosystem (biomass + soils) increased significantly more rapidly under elevated CO_2 than ambient CO_2 (Fig. 4a). The N content of the ecosystem under ambient and elevated CO_2 increased from 1996 through 2002 (208 to 261 g N/m^2 under ambient CO_2, and 227 to 263 g N/m^2 under elevated CO_2), but there was no effect of elevated CO_2 on the rate at which N increased (Fig. 4b). Ecosystem C-to-N ratios increased through time and significantly more rapidly under elevated CO_2 than ambient CO_2 (Fig. 4c).

The average concentration of extractable-inorganic N in mineral soils collected throughout the growing season decreased from the second year (1998) through the sixth year (2002) of this experiment (Fig. 5a). The rate of potential net N mineralization also decreased from the second through the sixth year of this experiment (Fig. 5b). There was no effect of elevated CO_2

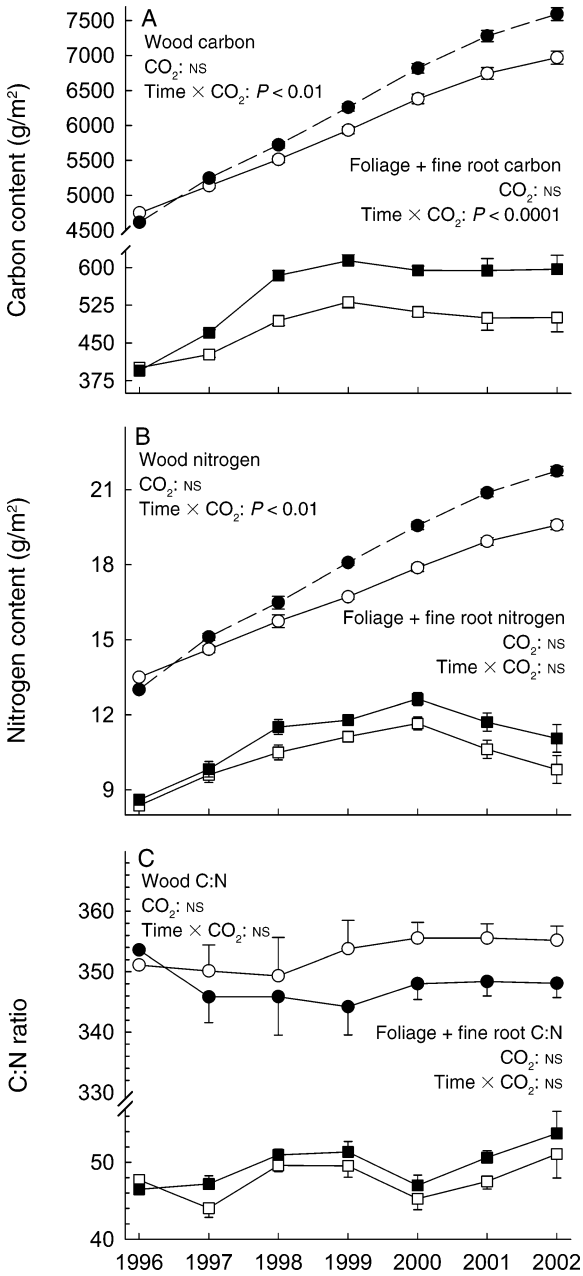

FIG. 2. Content (mean and SE) of (A) carbon, (B) nitrogen, and (C) their ratio in live biomass under ambient (open symbols) and elevated (solid symbols) CO_2 from 1996 through 2002. Circles denote woody biomass; squares denote pine needles + hardwood foliage + fine roots.

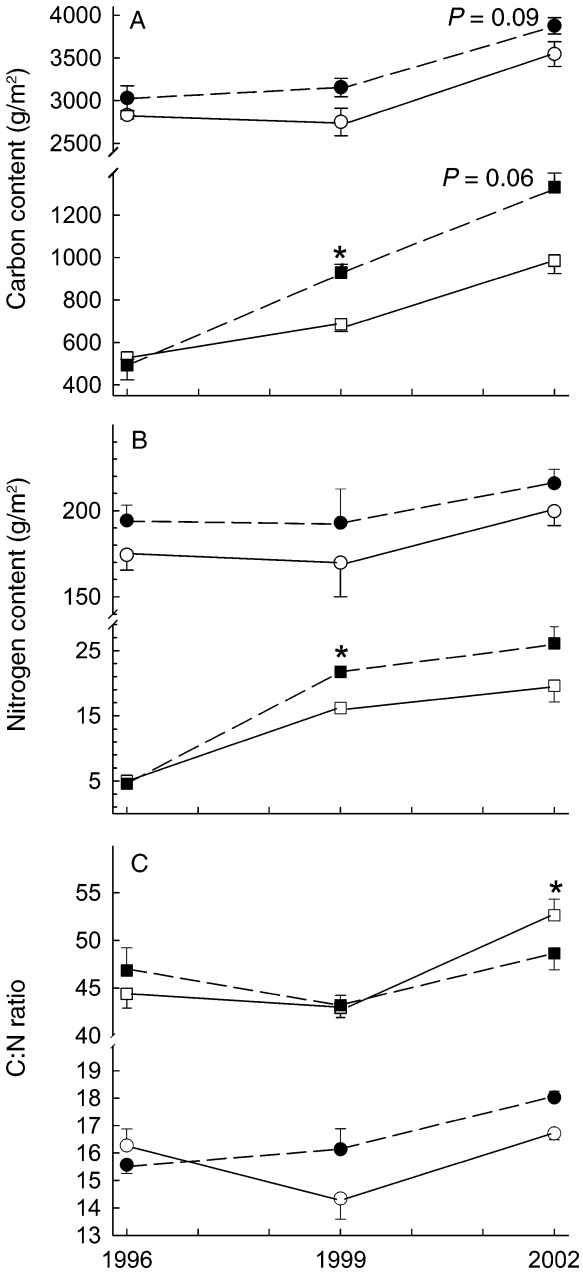

FIG. 3. Content (mean and SE) of (A) carbon, (B) nitrogen, and (C) their ratio in the O horizon and mineral soil to a depth of 30 cm under ambient (open symbols) and elevated (solid symbols) CO_2, initially (1996) and following three (1999) and six years of CO_2 fumigation. Circles denote the top 30 cm of mineral soil; squares denote the organic horizon. Data are taken from Lichter et al. (2005).

on the concentration or mineralization of N in any year of this experiment. The concentration of N in microbial biomass remained constant through time and there was no effect of elevated CO_2 on the concentration of N in microbial biomass (Fig. 5c).

The annual rate of net mineralization in the top 15 cm of mineral soil was significantly ($P < 0.05$) higher in the second year (1998) of this experiment than in the seventh year (2003) of this experiment (Table 1). In neither year was the annual rate of net N mineralization significantly affected by elevated CO_2 (Table 1). From 1998 through 2003, the annual rate of net N mineralization declined by more than half under ambient and elevated CO_2.

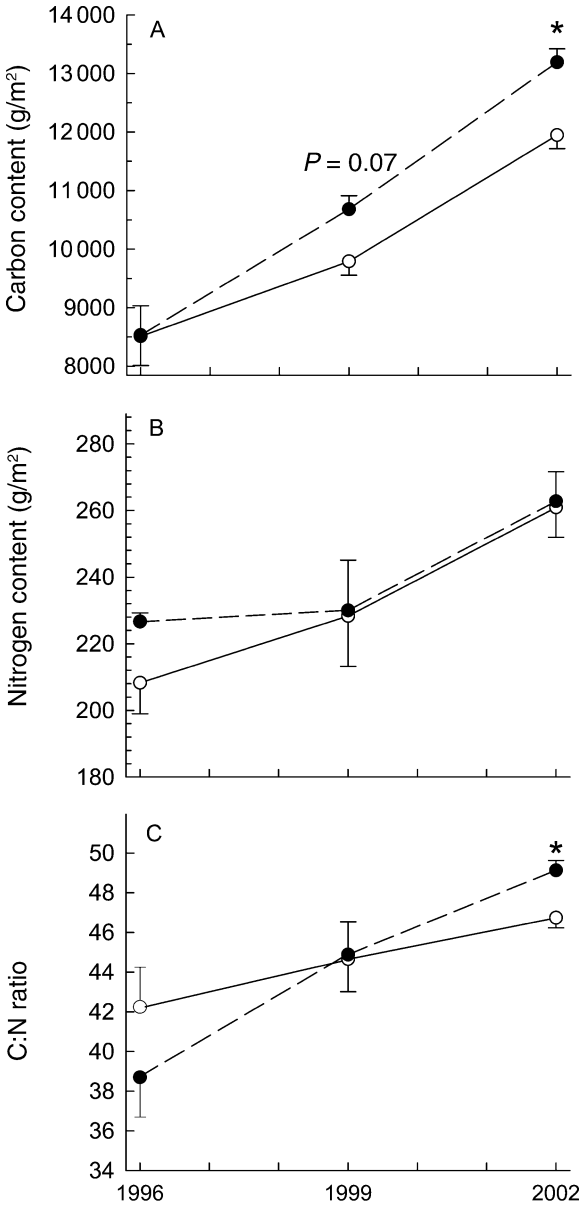

FIG. 4. Content (mean and SE) of (A) carbon, (B) nitrogen, and (C) their ratio in the entire ecosystem (biomass + O horizon + 0–30 cm mineral soil) initially (1996), and after three (1999) and six (2002) years of forest growth under ambient (open symbols) and elevated CO_2 (solid symbols).

DISCUSSION

Mechanisms supporting PNL

A prediction stemming from the hypothesis for progressive N limitation (PNL) is that NPP will decline through time in ecosystems subjected to a step-function increase in atmospheric CO_2 (Luo et al. 2004). One of the primary mechanisms driving this response is a rapid immobilization of N in tree biomass under elevated CO_2 that depletes soils of N causing slower rates of N mineralization (Luo et al. 2004). Consistent with the PNL hypothesis, this study showed that the uptake of N by trees increased significantly under elevated CO_2 as indicated by both the significant increase in the content of N in biomass (Fig. 2b) and the accumulation of N in the O horizon (i.e., an effect of plant uptake and redistribution via litterfall, Fig. 3b). Biomass produced under elevated CO_2 immobilized 41% more N than that under ambient CO_2 (Fig. 2b, Table 2) and N immobilization in the O horizon was 90% greater under elevated CO_2 (Fig. 3b, Table 2).

The accumulation of N in biomass at the expense of mineral soil pools is common during stand development (e.g., Johnson et al. 1988, Hooker and Compton 2003). In this experiment, there was no significant depletion (or accumulation) of N in the top 30 cm of mineral soil through time (Fig. 4b), so the results of this study differ from other chronosequence studies at least during this six-year interval (Richter et al. 2000, Hooker and Compton 2003). Consistent with more rapid uptake of N under elevated CO_2 however, the absolute difference in mineral soil N stocks between elevated and ambient CO_2 narrowed from 20 g/m^2 in 1996 to 16 g/m^2 in 2002 (Fig. 3b) and mineral soil C-to-N ratios increased slightly (Fig. 3c).

The immobilization of N in woody biomass during stand development can exacerbate nutrient limitation to forest productivity (Richter et al. 2000). Several studies have measured declines in N mineralization during the development of *Pinus taeda* stands (Piatek and Allen 1999, Richter et al. 2000, Li et al. 2003). Although we observed a decline in the rate of potential net N mineralization throughout the first six years of this experiment, the rate of decline was not more rapid under elevated CO_2 (Fig. 5b, Table 1). Billings and Ziegler (2005) did, however, observe significantly lower, long-term N mineralization potential under elevated CO_2 at this site. They collected soils in June 2003 to a depth of 10 cm, and incubated them in the lab for 100 d. Beginning on day 51 and continuing through day 100, the rate of potential net N mineralization was significantly lower under elevated CO_2 than ambient CO_2 (Billings and Ziegler 2005). The results of long-term soil incubation must be interpreted with caution because these soils are isolated from inputs of C and N substrates delivered in soil water and root exudates. This artifact of long-term incubation could explain why there was no effect of elevated CO_2 on potential net N mineralization in short-term incubations at this site (i.e., the results of this study and in soils incubated $<$51 d in Billings and Ziegler 2005). Nevertheless, the combination of a reduction in the rate of N mineralization with stand development observed in this study (Fig. 5b), coupled to the lower long-term N mineralization potential of soils under elevated CO_2 (Billings and Ziegler 2005) suggests that the mining of N by trees may be depleting soils of relatively labile N pools more rapidly under elevated CO_2 than ambient CO_2.

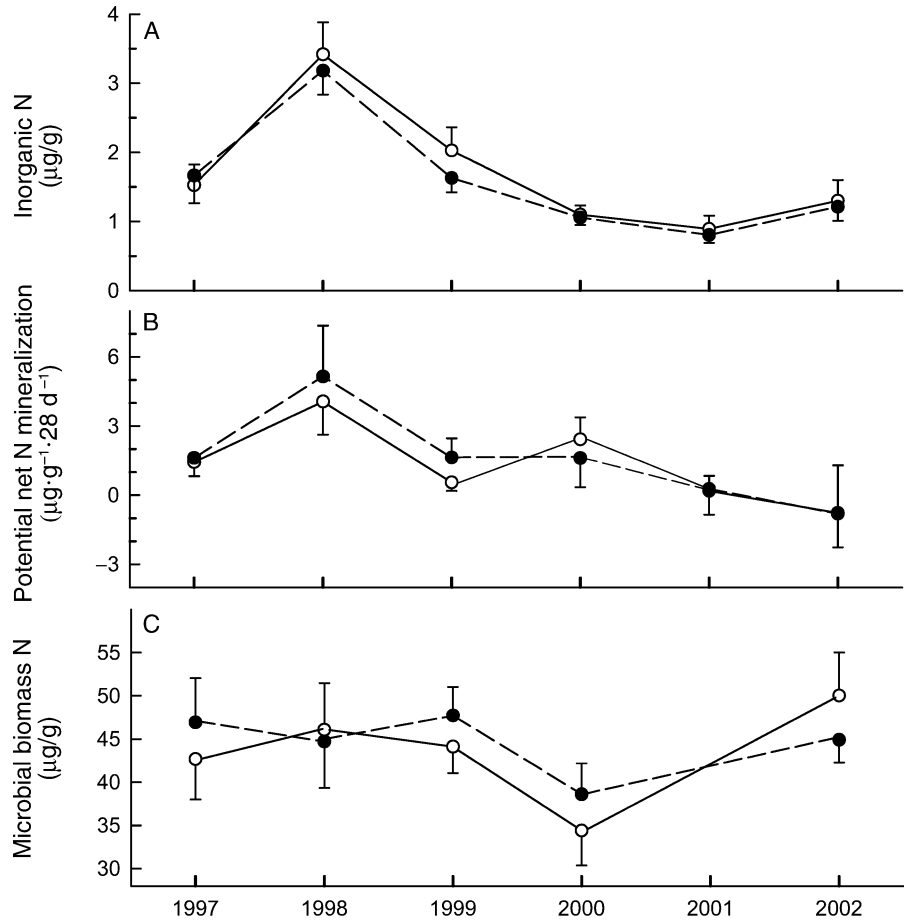

FIG. 5. (A) The concentration of inorganic N, (B) the rate of potential net N mineralization, and (C) the concentration of N in microbial biomass under ambient (open symbols) and elevated (solid symbols) CO_2 during the first six years of this experiment. Data from 1997 to 2001 are from Finzi and Schlesinger (2003).

Mechanisms not important to PNL

Greater microbial-N immobilization under elevated CO_2 is the second major pathway by which PNL should occur. Under elevated CO_2 significant increases in the flux of C to soil should increase microbial-C availability thereby stimulating a biosynthetic demand for N, increasing microbial-N immobilization and decreasing net N mineralization (Zak et al. 2000, Luo et al. 2004). At this site aboveground litterfall, fine root production, and fine root turnover have increased significantly under elevated CO_2 (Fig. 1b; Matamala and Schlesinger 2000, Matamala et al. 2003). Significant increases in the activities of extracellular enzymes associated with the degradation of labile C substrates indicated a stimulation in microbial-C metabolism (Finzi et al., *in press*). However, the pool of N within microbial biomass did not increase under elevated CO_2 through time (Fig. 5c). Nor, has there been a significant increase in the activity of extracellular enzymes that decompose N from SOM (Finzi et al., *in press*). Consequently, microbial-N immobilization driven by increased C inputs to the soil system does not appear to be an important short-term (<6 yr) mechanism driving forest ecosystems towards PNL. The lack of a clear increase in microbial-N pools through time is common to all forest FACE experiments in the US (Zak et al. 2003).

TABLE 1. A comparison of the rate of annual net N mineralization in the plots under ambient and elevated CO_2 in 1998 and 2003 and their difference.

CO_2 treatment	Annual net N mineralization, mean ± SE ($g \cdot m^{-2} \cdot yr^{-1}$)		
	1998	2003	Difference
Ambient	2.85 ± 0.89	0.60 ± 0.18	−2.25 ± 0.83
Elevated	3.18 ± 0.60	1.36 ± 0.43	−1.82 ± 0.47

Mechanisms offsetting progressive N limitation

Increases in N-use efficiency (NUE) can delay or even preclude PNL as a direct consequence of increasing C fixation per unit of N in an ecosystem (Luo et al. 2004). Elevated CO_2 stimulated net ecosystem production (NEP) by an average of 204 g $C \cdot m^{-2} \cdot yr^{-1}$

TABLE 2. Input, output, and accumulation of N in forest plots under ambient and elevated CO_2 during the course of the first six years of this experiment.

Process	Ambient CO_2	Elevated CO_2
Input		
Atmospheric N deposition†	4.2	4.2
Heterotrophic N fixation‡	1.4	1.4
Output§		
N_2O flux	0.042 ± 0.004	0.047 ± 0.004
Leaching (200 cm depth)	<0.001	<0.001
Accumulation		
Biomass	7.80 ± 0.51	11.03 ± 0.46**
O horizon	7.94 ± 1.38	15.06 ± 2.14*
0–30 cm mineral soil	25.26 ± 3.33	21.69 ± 6.62

Note: All units are g $N \cdot m^{-2} \cdot 6\ yr^{-1}$; error terms, where reported, are SE.
* $P < 0.05$; ** $P < 0.01$.
† This flux was calculated by multiplying the deposition measured in 1998 by 6. Original data are from Finzi et al. (2002).
‡ Based on soil collected in 2000, the annual rate of heterotrophic N fixation is measured at 0.23 g $N \cdot m^{-2} \cdot yr^{-1}$ under ambient and elevated CO_2 (K. Hofmockel and W. H. Schlesinger, *unpublished manuscript*). This value was multiplied by 6.
§ Flux was calculated as for atmospheric N deposition, based on annual N_20 emissions reported in Phillips et al. (2001) and leaching losses reported in Finzi et al. (2002).

throughout the first six years of this experiment (Fig. 4a). During this time, the C-to-N ratio of the entire ecosystem widened significantly more rapidly under elevated CO_2 than ambient CO_2 (Fig. 4c). Although on a plant-tissue basis there was no increase in NUE (i.e., a widening of biomass C:N), the reallocation of N from soil with a C:N ≈ 15 (Fig. 3b) into woody biomass and to a lesser extent foliage with C-to-N ratios of ~345 and ~45, respectively (Fig. 2b) significantly increases C fixation per unit of N in the forest plots under elevated CO_2. The widening of ecosystem C-to-N ratios under elevated CO_2 occurred despite increases in N capital through time (Fig. 4b). We do not currently know whether the increase in the C-to-N ratio of this ecosystem will completely preclude PNL from occurring given declining rates of N mineralization (Fig. 5b). At the scale of the entire ecosystem, however increasing C-to-N ratios increases C fixation per unit of N and hence may be an important mechanism delaying PNL.

Increased N inputs or decreased N losses under elevated CO_2 could offset PNL by increasing ecosystem N capital (Hungate et al. 1999, Luo et al. 2004). Previously published estimates show that leaching and gaseous losses of N from this ecosystem are negligible under ambient and elevated CO_2 (Table 2; Phillips et al. 2001, Finzi et al. 2002). Similarly, previously published data indicate that inputs of N through atmospheric deposition and throughfall are not significantly higher under elevated CO_2 (Table 2; Lichter et al. 2000). Based on these observations, we would predict the onset of PNL because inputs of N are too small to account for N immobilization in biomass and the O horizon. Measured rates of atmospheric deposition and N-fixation input ~5.6 g N/m^2 during the first six years of this experiment whereas the immobilization of N in tree biomass and the O horizon consumed ~26 g N/m^2 during this time (Table 2).

In this study, however, we also found a net accrual of ecosystem N capital at an average rate of 12 g $N \cdot m^{-2} \cdot yr^{-1}$ (Fig. 4b, time $P < 0.0001$). This rate of N accrual is much greater than the estimated rate of N input via atmospheric deposition (Finzi et al. 2002) or heterotrophic N fixation (measured by acetylene reduction and estimated at 0.23 $g \cdot m^{-2} \cdot yr^{-1}$; K. Hofmockel and W. H. Schlesinger, *unpublished manuscript*). And, there are no plant species capable of symbiotic N fixation in this ecosystem. Roots at >30 cm depth may be increasing in abundance (e.g., Norby et al. 2004). These roots may be actively taking up N and redistributing N from deeper in the soil profile (Jobbágy and Jackson 2001). Regardless of the mechanism, it is possible that the net accrual of ecosystem N capital is delaying PNL.

Interannual variability in NPP

There were large interannual variations in NPP in this ecosystem (Fig. 1c). D. J. Moore (*unpublished data*) found a significant, positive correlation between the basal area growth of loblolly pine trees and growing season precipitation under ambient and elevated CO_2. We extended their analysis by using our data on NPP and potential net N mineralization to see if the interannual variation in NPP was not only related to precipitation but also to declining rates of net N mineralization. Using multiple regression analysis, we found that the rate of NPP under ambient CO_2 was very tightly regulated by precipitation (partial $r^2 = 0.57$, $P < 0.05$) and net N mineralization (partial $r^2 = 0.36$, $P < 0.05$). NPP under elevated CO_2 was also tightly regulated by precipitation (partial $r^2 = 0.48$, $P < 0.05$) and net N

mineralization (partial $r^2 = 0.46$, $P < 0.05$). Given that NPP in this ecosystem is sensitive to the rate of N mineralization (Finzi et al. 2002) and that growing season precipitation and potential net N mineralization are uncorrelated (Pearson correlation coefficient = 0.04, $P = 0.91$), we hypothesize that continued declines in N mineralization will reduce the stimulation in NPP under elevated CO_2 independent of interannual variation in precipitation. Support for this hypothesis comes from the prototype plot at this site. A recovery of the initial stimulation in tree growth under elevated CO_2 beyond three years of fumigation was only achieved with soil nutrient amendment (Oren et al. 2001).

Conclusions

Results from the first six years of CO_2 fumigation at this site provide mixed support for the hypothesis of PNL. In support of PNL, the initial stimulation in forest productivity under elevated CO_2 immobilized significantly more N in biomass and the O horizon (Figs. 3b, 4b) resulting in a significant redistribution of ecosystem N (Table 2). In the absence of other changes, the uptake and redistribution of N is sufficient to cause PNL via a reduction in soil-N mineralization (Fig. 5; Billings and Ziegler 2005). However, there were mechanisms in operation at this research site that could be delaying the onset of PNL. They were a widening of ecosystem C-to-N ratios under elevated CO_2 (Fig. 4c), and a large accrual of ecosystem N capital (Fig. 4b).

Is PNL occurring in this ecosystem and will NPP under elevated CO_2 decline to levels observed at ambient CO_2? The answer depends on the relative strength of biomass-N immobilization vs. widening C-to-N ratios and N accrual as mechanisms that drive and offset PNL, respectively. The answer also depends on whether processes that are currently unimportant become important (e.g., microbial-N immobilization). During the first six years of this experiment there was no reduction in the average stimulation of NPP by elevated CO_2; NPP was stimulated by 18–24% (Fig. 1). Only direct observations through time will definitively answer this question.

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