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# The nitrogen budget of a pine forest under free air $CO_2$ enrichment

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**Abstract** Elevated concentrations of atmospheric $CO_2$ increase plant biomass, net primary production (NPP) and plant demand for nitrogen (N). The demand for N set by rapid plant growth under elevated $CO_2$ could be met by increasing soil N availability or by greater efficiency of N uptake. Alternatively, plants could increase their nitrogen-use efficiency (NUE), thereby maintaining high rates of growth and NPP in the face of nutrient limitation. We quantified dry matter and N budgets for a young pine forest exposed to 4 years of elevated $CO_2$ using free-air $CO_2$ enrichment technology. We addressed three questions: Does elevated $CO_2$ increase forest NPP and the demand for N by vegetation? Is demand for N met by greater uptake from soils, a shift in the distribution of N between plants, microbes, and soils, or increases in NUE under elevated $CO_2$? Will soil N availability constrain the NPP response of this forest as $CO_2$ fumigation continues? A step-function increase in atmospheric $CO_2$ significantly increased NPP during the first 4 years of this study. Significant increases in NUE under elevated $CO_2$ modulated the average annual requirement for N by vegetation in the first and third growing seasons under elevated $CO_2$; the average stimulation of NPP in these years was 21% whereas the average annual stimulation of the N requirement was only 6%. In the second and fourth growing seasons, increases in NPP increased the annual requirement for N by 27–33%. Increases in the annual requirement for N were largely met by increases in N uptake from soils. Retranslocation of nutrients prior to senescence played only a minor role in supplying the additional N required by trees growing under elevated $CO_2$. NPP was highly correlated with between-plot variation in the annual rate of net N mineralization and $CO_2$ treatment. This demonstrates that NPP is co-limited by C availability, as $CO_2$ from the atmosphere, and N availability from soils. There is no evidence that soil N mineralization rates have increased under elevated $CO_2$. The correlation between NPP and N mineralization rates and the increase in the annual requirement for N in certain years imply that soil N availability may control the long-term productivity response of this ecosystem to elevated $CO_2$. Although we have no evidence suggesting that NPP is declining in response to >4 years of $CO_2$ fumigation, if the annual requirement of N continues to be stimulated by elevated $CO_2$, we predict that the productivity response of this forest ecosystem will decline over time.

**Keywords** Elevated $CO_2$ · Nitrogen · Net primary production · N limitation · Nutrient-use efficiency

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## Introduction

The atmospheric concentration of $CO_2$ has increased from ~280 µl/l to ~360 µl/l during the past 150 years and may double during this century (IPCC 2001). Tree biomass increases under elevated atmospheric $CO_2$ (Bazzaz and Miao 1993; Curtis and Wang 1998; Zak et al 2000a; Hamilton et al. 2002), and forest productivity may be enhanced by the projected increase in atmospheric $CO_2$ (Schimel et al. 2001). However, the magnitude of the $CO_2$ response in woody plants can be constrained by the availability of soil nutrients, notably nitrogen (N) (McMurtrie and Commins 1996; Pan et al. 1998; Luo and Reynolds 1999; Zak et al. 2000a; Oren et al. 2001).

Rapid tree growth under elevated $CO_2$ increases plant demand for N (Norby et al. 1999). Increases in soil N availability via fertilization maintain high rates of tree growth under elevated $CO_2$ (Johnson et al. 1997; Prior et al. 1997; Murray et al. 2000; Zak et al. 2000a). It is un-

clear, however, if endogenous processes of N cycling will increase N availability when natural ecosystems are exposed to high $CO_2$ (reviewed in Zak et al. 2000b). If the N-uptake potential of vegetation under elevated $CO_2$ exceeds the rate of N replenishment to the available pool via mineralization (Rastetter et al. 1997) or exogenous inputs (Schlesinger 1997), vegetation may not acquire sufficient N to sustain the pulse of growth seen with initial exposure to elevated $CO_2$. In the absence of increases in N mineralization or exogenous N inputs, biogeochemical models predict little or no enhanced C storage in woody biomass as atmospheric $CO_2$ concentrations rise (McMurtrie and Commins 1996; Rastetter et al. 1997; Luo and Reynolds 1999).

In the absence of an increase in soil N availability, there are two mechanisms that could maintain rapid rates of plant growth under elevated $CO_2$: increases in nitrogen-use efficiency (NUE) and increases in the efficiency by which plants acquire available soil N. Increases in NUE imply greater C fixation per unit N acquired from soil (Birk and Vitousek 1986; Pastor and Bridgham 1999) and the maintenance of a C sink in woody biomass in N-limited ecosystems. NUE is rarely measured in elevated $CO_2$ studies. Following 2 years of $CO_2$ enrichment in a North Carolina pine forest, Finzi et al. (2001) found that NUE in aboveground litterfall increased ~5%, although this effect was not statistically significant.

According to mass balance, an increase in the efficiency with which soil N is acquired by plants should cause a shift in the distribution of N within existing soil pools or decreased losses of N from an ecosystem under elevated $CO_2$. A decline in the N content of microbial-biomass is a likely, initial response because of the rapid turnover time of microbes (Paul and Clark 1986). However, a relatively limited number of studies have shown variable responses of soil microbial biomass to elevated $CO_2$ (Allen et al. 2000; Zak et al. 2000b) and no consistent changes in the size of the N pool in microbial biomass (Diaz et al. 1993; Hungate et al. 1999; Allen et al. 2000; Zak et al. 2000c; Hu et al. 2001). Thus there is equivocal evidence for a shift in N distribution away from soil microbial biomass to support plant uptake under elevated $CO_2$. A decrease in soil N content associated with the process of net mineralization could occur under elevated $CO_2$, but this would be very difficult to measure accurately because net mineralization constitutes a very small flux out of this very large pool (Binkley and Hart 1989).

More efficient retention of N within ecosystems exposed to elevated $CO_2$ could maintain N capital and lead to long-term increases in N cycling between plants and soils. There are very few reports of nutrient losses below the rooting zone of plants growing under elevated $CO_2$. Hungate et al. (1999) and Johnson et al. (2001) found a small decrease in N losses below the rooting zone of a scrub oak forest in Florida exposed to elevated $CO_2$, implying greater retention of available soil N. Similarly, there are few observations reporting gaseous losses of N under elevated $CO_2$. Hungate et al. (1997) found that elevated $CO_2$ and nutrient fertilization decreased emissions of NO following the onset of the rainy season in California grasslands. In contrast, Ambus and Robertson (1999) found no significant difference in $N_2O$ fluxes in aspen stands exposed to elevated $CO_2$, and Smart et al. (1997) found higher gas fluxes of N in wheat systems exposed to elevated $CO_2$. Most studies do not place losses of N within the context of an overall ecosystem budget for N, making it difficult to interpret the importance of these losses as a mechanism maintaining or alleviating N limitation to net primary production (NPP) under elevated $CO_2$.

In this paper we present data on the pools and fluxes of dry matter and N for a young pine forest exposed to 4 years of elevated $CO_2$ using free-air $CO_2$ enrichment (FACE, Hendrey et al. 1999). With the dry matter and N budgets we address three questions: Does elevated $CO_2$ increase forest NPP and the demand for N by vegetation? Is demand for N met by greater uptake from soils, a shift in the distribution of N between plants, microbes, and soils, or increases in NUE under elevated $CO_2$? Will soil N availability constrain the NPP response of this forest as $CO_2$ fumigation continues?

## Materials and methods

### Site description

The FACE experiment in the Duke Forest (Orange County, N.C., 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 $l^{-1}$ above ambient (i.e., 565 µl $l^{-1}$). Three control plots are fumigated with ambient air only (365 µl $l^{-1}$). The experiment began 27 August 1996 and is continuous (24 h $day^{-1}$; 365 days $year^{-1}$). Additional details on FACE operation can be found in Hendrey et al. (1999).

The forest is derived from 3-year-old loblolly pine (*Pinus taeda*) seedlings that were planted in 1983 in a 2.4×2.4-m spacing. In 1996, the 13-year-old pine trees were approximately 14 m tall and accounted for 98% of the basal area of the stand. Since planting, a deciduous understory layer has recruited from nearby hardwood forests and stump sprouts. The most abundant understory tree species is sweet gum (*Liquidambar styraciflua*), with admixtures of red maple (*Acer rubrum*), red bud (*Cercis canadensis*), and dogwood (*Cornus florida*). The 32-ha site contains an elevation gradient of 15 m between the highest and lowest points, but topographic relief is less than 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 M $CaCl_2$ pH =5.75), and have well-developed soil horizons with mixed clay mineralogy. Additional site details can be found in Schlesinger and Lichter (2001) and Finzi et al. (2001).

### Plant biomass pools, increments and turnover

During road construction at the FACE site, a small number of the 13-year-old loblolly pine trees were removed and used to develop allometric regressions between stem diameter and wood, bark, and coarse root mass (Naidu et al. 1996). 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 1996. By using dendrometer bands to monitor di-

ameter growth for 40 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. Pool sizes, increments and turnover of woody biomass for the period 1997–1998 are taken from DeLucia et al. (1999). The 1999 and 2000 estimates are previously unpublished.

During the 2nd year of $CO_2$ fumigation, we noted that loblolly pine leaf litter production was greater than that predicted by the pre-treatment allometries (Naidu et al. 1996; Finzi et al. 2001). Therefore, loblolly pine and hardwood foliage pools and increments are based on the data collected from the leaf litter baskets (see below). 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 was not significantly different in any year of this study (Finzi et al. 2001 and unpublished data). Rather than multiplying litterfall mass by the ratio of green LMA and litter LMA, we used the simpler assumption that LMA was not different and that litterfall mass is the same as canopy mass. Similarly, there was no significant difference in the LMA of green leaves and leaf litter for the dominant hardwood species in this ecosystem (Finzi et al. 2001 and unpublished data). Thus we assumed that the mass of the deciduous leaves in the litter baskets was the same as that in the canopy.

Aboveground litterfall mass was collected from 5 June 1996 onward by placing 12 replicate 40×40 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 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 days, and weighed. The litter was sorted and subdivided into four categories: pine needles, deciduous leaves, reproductive structures, and bark + branch + "other." The "other" category consisted of small, difficult to identify fragments of aboveground litterfall and frass. The litterfall data for 1996 through 1998 are from Finzi et al. (2001); data for 1999 and 2000 are previously unpublished.

Characterizations of foliage production in loblolly pine are complicated by over-lapping generations of leaves, typically two. The longevity of loblolly pine foliage in the Piedmont of N.C. is 18 months (Finzi et al. 2001). A new cohort of leaves produced in 1 year does not abscise until the following year. Thus the mass 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 loblolly pine needles in the canopy in 1998 can be estimated from the sum of litterfall mass in 1998 and 1999. We therefore used litterfall mass data from 1996 through 2001 to calculate leaf biomass and increments for the period 1996–2000.

Fine root biomass for the period 1997–1999 was taken from Matamala and Schlesinger (2001) and Matamala (unpublished data); however, fine root increment and turnover data are only available for 1998. Fine root data are based on the sequential coring technique. The assumptions in estimating root production, increments and turnover using sequential coring are discussed in Matamala and Schlesinger (2001).

## Plant N analysis

Wood cores were extracted from a subset of ten canopy trees sampled in each plot in the autumn of 1997, 1999 and 2000. 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). We separated the 1996 and 1997 growth increments from the cores sampled in 1997 and analyzed them for N. Thus the 1997 core provided wood-N-concentration data for the 1996 and 1997 growing seasons. Similarly, the 1998 and 1999 growth increments were separated from the cores sampled in 1999 and analyzed for N separately. Thus the 1999 core provided data on the 1998 and 1999 wood N concentration. The cores taken in 2000 had only the 2000 growth increment separated and analyzed for N. No cores were extracted from sweet gum, the most abundant hardwood species in this forest. There is no difference in the concentration of N in the bolewood of sweet gum trees under ambient and elevated $CO_2$ at the sweet gum FACE experiment in Oak Ridge, Tennessee (R.J. Norby, personal communication). 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. Bark was assumed to have the same concentration as wood in loblolly pine. While bark N concentrations are generally higher than wood in loblolly pine (Naidu et al. 1996), the relatively small mass of bark on these trees ensures that the error associated with this assumption is relatively small.

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, Wis.). 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 are taken from Matamala and Schlesinger (2001).

## Soil pools and fluxes

Throughfall inputs of N were collected every 2 weeks from 1996 to 1998 and every 3 weeks in 1999 in 4-l bottles fit with 14.6-cm-diameter funnels, with 12 bottles per plot (Lichter et al. 2000). Concentrations of $NH_4^+$ in throughfall were measured on an autoanalyzer (TRAACS 800 Autoanalyzer, Bran + Leubbe, Buffalo Grove, Ill.) while concentrations of $NO_3^-$ were measured on an ion chromatograph (model 2010i, Dionex Corporation, Sunnyvale, Calif.). An in-depth discussion of the field and laboratory methodology is presented in Lichter et al. (2000). Throughfall data for 1998 are from Lichter et al. (2000); 1999 data are previously unpublished.

Soil-water $NH_4^+$ and $NO_3^-$ concentrations were measured at two sample locations per plot at each of four depths: the bottom of the O horizon and at 15, 70 and 200 cm. The O-horizon samples were collected gravimetrically. Soil water in the mineral soil horizons was collected under ~70 centibars of tension with Prenart lysimeters (Prenart Corporation, Copenhagen, Denmark) at 15- and 70-cm depth and Rhizon lysimeters (Rhizosphere Research Products, Wageningen, Netherlands) at 200-cm depth. Soil water was collected every 2 weeks from 1996 to 1998 and every 3 weeks in 1999. $NH_4^+$ and $NO_3^-$ concentrations were estimated by phenol-colorimetry and by ion chromatography, respectively, on the same analytical instruments as throughfall. We scaled lysimeter concentrations of inorganic N to g N $m^{-2}$ $year^{-1}$ according to Darcy's law, solving Richardson's equation in two dimensions (Clapp and Hornberger 1987; Katul et al. 1997).

Gaseous losses of $N_2O$ for 1998 and 1999 are taken from Phillips et al. (2001). In brief, nitrous oxide fluxes were measured, using the static-chamber method, bimonthly from January 1998 through December 1999. Four chambers were installed in each plot. Headspace gas was sampled with 5 or 10 ml SESI nylon syringes followed by analysis on a Shimadzu GC-14A $^{63}Ni$ electron gas capture gas chromatograph. Soil moisture and temperature were measured concurrently with each field measurement of $N_2O$.

Forest floor and mineral soil N content were measured in October 1999. Details of the field sampling and chemical analysis can be found in Schlesinger and Lichter (2001). 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 10×10 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 days and then ground to a fine powder for N analysis on an element analyzer. Stone mass was used to adjust final core mass and estimate soil bulk density.

Soil microbial-biomass N was measured in the forest floor and mineral soil. Microbial-biomass N was measured every year in April, June, August and October. Four replicate 10×10 cm forest

floor samples were collected in each plot. Similarly, 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. Within a plot, the replicate forest floor samples and the soil cores were then composited into two larger samples (a forest floor composite and a mineral soil composite). Two 20-g sub-samples were removed from each of the composite bags and hand picked to remove all fine roots. Each sub-sample was divided in half, placed into a 50-ml centrifuge tube and microbial biomass determined using the fumigation-extraction procedure (Brooks et al. 1985; Gallardo and Schlesinger 1991). The 1998 data on soil microbial biomass are from Allen et al. (2000). Microbial-biomass N in the forest floor was measured only during the 2000 calendar year and was assumed to be representative of pool sizes in 1999 (A.C. Finzi, unpublished data).

The rate of potential net N mineralization was measured four times per year – – in April, June, August, and October – – in each year of this study. Data from 1997 and 1998 are taken from Finzi et al. (2001). Data from 1999 and 2000 are from Finzi and Schlesinger (unpublished data). In brief, four replicate soil cores (4.78-cm diameter ×15-cm depth) were extracted from within the boundaries of each FACE plot. Each core was sieved through an 5-mm mesh opening to remove stones and course roots. Two replicate 20-g sub-samples 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 M KCl and the second bottle was incubated in the dark at 22°C for 28 days after which time it was extracted in 100 ml of 2 M KCl. The rate of potential net N mineralization was calculated as the difference in the accumulation of $NH_4^+$ and $NO_3^-$ in the incubated and initial sample.

Annual rates of net N mineralization were measured in the top 15 cm of mineral soil using the buried bag technique (Eno 1960). Soil cores were taken from 15 sampling locations on the outside perimeter of the plenum surrounding each of the six plots. Although these samples are technically outside the plots, their close proximity to the vertical $CO_2$-vent pipes and canopy trees within the plots (<2 m, Finzi personal measurement) ensures that they are representative of annual rates of mineralization within the plots. Estimates of N mineralization were made from 1 June 1997 through 31 May 1998. 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 sub-sample 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 1 month, after which they were removed and brought back to the laboratory 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 12 months.

### Calculations and statistical analysis

Dry matter and N budgets were calculated separately for each of the first 4 years under ambient and elevated $CO_2$. The budgets for dry matter and N in vegetation are divided into pools, increments and turnover. Pools (g $m^{-2}$) were calculated for the peak-growing season when both cohorts of loblolly pine needles were on the trees and the deciduous trees were foliated. Biomass increments (g $m^{-2}$ $year^{-1}$) were estimated from dimension analysis. Fine root increment was calculated from a regression of fine root biomass versus time determined by sequential coring over the period November 1997 – November 1998 (Matamala and Schlesinger 2000). Aboveground biomass turnover (g $m^{-2}$ $year^{-1}$) was calculated from the materials collected in litter baskets and throughfall. Fine root turnover was calculated as the sum of fine root mortality and decomposition for the period November 1997 – November 1998. Net primary production was calculated as the sum of biomass increments and turnover while annual N requirement was calculated as the sum of the N content in biomass increments and turnover (Schlesinger 1997). The quantity of N retranslocated prior to abscission was calculated as the difference between the content of N in pools and that in litter for loblolly pine needles and deciduous leaves. In this forest, there is no apparent N retranslocation from fine roots prior to senescence (Matamala and Schlesinger 2001). The annual uptake of N from soil was calculated as the difference between annual N requirement and N retranslocated prior to senescence.

NUE was calculated using two different methods. The first was based on the ratio of NPP and the annual N requirement. This calculation is qualitatively similar to that presented in Vitousek (1984) and Shaver and Melillo (1984). This definition of NUE measures the efficiency with which N allocation from storage and uptake from soils results in dry matter production and is analogous to the inverse of the weighted average tissue N concentration. The second method was based on the concepts presented in Pastor and Bridgham (1999), who defined nitrogen-response efficiency (NRE) as:

$$\mathrm{NRE} = \frac{\mathrm{NPP}}{N_{\mathrm{av}}} \quad (1)$$

where, $N_{av}$ is N availability from soils which we assumed to be the annual rate of net N mineralization (g $m^{-2}$ $year^{-1}$) measured in the soils collected just outside the FACE plots. Because the rate of potential net N mineralization (soils collected within each FACE plot, see above) was not significantly different between $CO_2$ treatments (Fig. 2), we assume that elevated $CO_2$ had no effect on the annual rate of net N mineralization. The annual rate of net N mineralization was measured during portions of the 1997 and 1998 growing seasons. We assumed that the average productivity response in 1997 and 1998 was the best estimate of the numerator in Eq. 1.

Initial measurements during 1996 demonstrated significant between-plot variation in most processes related to plant biomass and N pools and fluxes. This underlying between-ring variation often masked the effects of elevated $CO_2$ on biomass and N dynamics in response to $CO_2$ fumigation (e.g., Allen et al. 2000; Finzi et al. 2001; Schlesinger and Lichter 2001). We therefore used analysis-of-covariance (ANCOVA) and the 1996 pre-treatment data as the covariate in our tests for the effects of elevated $CO_2$ on variations in the components of the dry matter and N budgets (Underwood 1997; Finzi et al. 2001). We had pretreatment data for all biomass and N pools with the exception of fine roots and subcanopy hardwood biomass. In these cases we used one-way ANOVA with an $n$=6 (3 treatment and 3 control plots). There were no pretreatment data for the soil N pools and fluxes, so we used one-way ANOVA for tests of $CO_2$ effects. We averaged the rate of potential net N mineralization measured in April, June, August, and October to provide a single, integrated estimate of the rate of N mineralization under ambient and elevated $CO_2$ within a given year.

## Results

### Dry matter pools and fluxes

The biomass of loblolly pine needles was significantly greater under elevated $CO_2$ in all years (Table 1). The biomass of loblolly pine wood plus coarse roots increased throughout the 4 years of this study and was significantly higher under elevated $CO_2$ in years 3 and 4. The biomass of the remaining components – deciduous leaves, deciduous wood plus coarse roots, and fine roots – was not significantly different between ambient and elevated $CO_2$ in any year of this study. At the end of the 3rd growing season under elevated $CO_2$, the total biomass of vegetation in the plots under elevated $CO_2$ was

**Table 1** The pool sizes and fluxes (±1 SE) of dry matter from the 1st (1997) through the 4th (2000) year of forest growth under elevated $CO_2$. Superscript symbols to the right of the *%$CO_2$ Effect* indicates statistical significance at †=$P$<0.10; *=$P$<0.05, **=$P$<0.01, ***=$P$<0.001

| | 1997 | | | 1998 | | | 1999 | | | 2000 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | E | %$CO_2$ | A | E | %$CO_2$ | A | E | %$CO_2$ | A | E | %$CO_2$ |
| Compartment pools (g m$^{-2}$) | | | | | | | | | | | | |
| Pine foliage | 764 (112) | 825 (99) | 8** | 890 (123) | 1,043 (87) | 17** | 964 (124) | 1,106 (89) | 15* | 869 (102) | 1,006 (104) | 16* |
| Deciduous leaves | 108 (15) | 128 (47) | 18 | 117 (21) | 139 (48) | 19 | 117 (19) | 135 (47) | 15 | 96 (16) | 120 (44) | 25 |
| Pine wood + bark + coarse roots | 9,640 (1,360) | 9,526 (1,463) | –1 | 10,285 (1,398) | 10,334 (1,516) | 1† | 11,099 (1,416) | 11,357 (1,588) | 2* | 11,621 (1,417) | 12,094 (1,725) | 4* |
| Deciduous wood + bark + coarse roots | 727 (204) | 864 (336) | 19 | 818 (231) | 986 (384) | 21 (423) | 902 (255) | 1,086 (4232) | 20 | 1,001 (282) | 1,200 (468) | 20 |
| Fine roots | 222 (30) | 202 (25) | –9 | 241 (40) | 242 (19) | 0 | 243 (49) | 273 (49) | 12 | – | – | |
| Total biomass | 11,461 (1,244) | 11,545 (1,215) | 1† | 12,351 (1,256) | 12,744 (1,220) | 3† | 13,325 (1,248) | 13,956 (1,240) | 5* | 13,588 (1,255) | 14,419 (1,398) | 6† |
| Annual flux increments (g m$^{-2}$ year$^{-1}$) | | | | | | | | | | | | |
| Pine needle increment | 45 (9) | 126 (10) | 177* | 126 (11) | 218 (12) | 73* | 74 (6) | 62 (12) | –16 | –95 (23) | –100 (15) | –5 |
| Deciduous leaf increment | 15 (5) | 24 (13) | 63 | 8 (6) | 11 (2) | 33 | 0 (3) | –4 (3) | – | –21 (3) | –15 (5) | 31 |
| Pine wood + bark + coarse roots | 827 (71) | 1,024 (108) | 24* | 645 (56) | 809 (54) | 25* | 814 (18) | 1,023 (76) | 26** | 523 (2) | 736 (138) | 41† |
| Deciduous wood + bark + coarse roots | – | – | | 91 (27) | 122 (48) | 34 | 84 (24) | 100 (39) | 19 | 100 (28) | 114 (45) | 15 |
| Fine root increment | – | – | | 43 (7) | 80 (6) | 87* | – | – | – | | | |
| Total in increments | 887 (57) | 1,174 (92) | 32** | 941 (50) | 1,274 (52) | 35* | 996 (20) | 1,207 (32) | 21** | 506 (45) | 736 (123) | 45 |
| Turnover | | | | | | | | | | | | |
| Reproduction | 1 (1) | 4 (4) | 544 | 1 (1) | 5 (2) | 228 | 7 (5) | 9 (4) | 17 | 10 (2) | 26 (9) | 169 |
| Pine needle litterfall | 337 (57) | 350 (49) | 4* | 412 (55) | 490 (50) | 19** | 463 (68) | 568 (42) | 19* | 485 (57) | 552 (49) | 14*** |
| Deciduous leaf litterfall | 104 (16) | 120 (38) | 15 | 112 (22) | 131 (39) | 16 | 112 (19) | 126 (39) | 12 | 92 (16) | 113 (36) | 22 |
| Branch + bark + other litterfall | 79 (7) | 67 (15) | –15 | 80 (16) | 120 (19) | 50 | 143 (39) | 151 (19) | 6 | 144 (18) | 203 (57) | 41 |
| Fine root turnover | – | – | 37 (17) | 54 (21) | 47† | – | – | – | – | | | |
| Total turnover | 524 (49) | 541 (18) | 3† | 642 (43) | 800 (17) | 25** | 725 (81) | 854 (24) | 18* | 731 (59) | 894 (79) | 22* |
| Net primary production (g m$^{-2}$ year$^{-1}$) (increments + turnover) | 1,411 (94) | 1,715 (107) | 22** | 1,583 (61) | 2,074 (38) | 31** | 1,721 (70) | 2,061 (53) | 20** | 1,237 (32) | 1,630 (202) | 32† |

**Table 2** The average (± 1 S.E.) pool sizes and fluxes of N from the first (1997) through the fourth (2000) year of forest growth under elevated $CO_2$. See Table 1 for the description of symbols for statistical significance

| | 1997 | | | 1998 | | | 1999 | | | 2000 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | E | % $CO_2$ | A | E | % $CO_2$ | A | E | % $CO_2$ | A | E | % $CO_2$ |
| Compartment pools (g $m^{-2}$) | | | | | | | | | | | | |
| Pine foliage | 7.91 (1.03) | 8.21 (0.99) | 4 | 8.41 (1.56) | 9.42 (1.42) | 12* | 9.07 (1.52) | 9.88 (0.93) | 9* | 9.07 (1.36) | 10.10 (1.51) | 11 |
| Deciduous leaves | 1.63 (0.22) | 1.77 (0.62) | 9 | 2.00 (0.20) | 2.30 (0.73) | 15 | 1.97 (0.21) | 2.11 (0.64) | 8 | 1.72 (0.20) | 2.00 (0.69) | 16 |
| Pine wood + bark + coarse roots | 23.88 (3.44) | 23.34 (3.70) | –2 | 24.70 (3.52) | 24.29 (3.80) | –2 | 25.23 (3.53) | 24.93 (3.91) | –1 | 25.76 (3.51) | 25.69 (4.09) | –1 |
| Deciduous wood + bark + coarse roots | 1.45 (0.41) | 1.73 (0.67) | 19 | 1.64 (0.46) | 1.79 (0.77) | 21 | 1.80 (0.51) | 2.17 (0.85) | 20 | 2.00 (0.56) | 2.40 (0.94) | 20 |
| Fine roots | 1.76 (0.17) | 1.64 (0.14) | –7 | 1.91 (0.20) | 1.83 (0.06) | –4 | 1.82 (0.24) | 2.09 (0.28) | 15 | – | – | |
| Total in biomass | 36.63 (3.76) | 36.69 (3.41) | 1 | 38.65 (4.31) | 39.80 (3.83) | 3 | 39.90 (4.20) | 41.19 (3.33) | 3 | 38.55 (4.19) | 40.19 (4.23) | 4 |
| Annual flux increments (g $m^{-2}$ $year^{-1}$) | | | | | | | | | | | | |
| Pine needle | 1.01 (0.18) | 1.09 (0.83) | 8 | 0.50 (0.58) | 1.21 (0.73) | 142 | 0.66 (0.24) | 0.47 (0.52) | –30 | 0.00 (0.19) | 0.22 (0.63) | – |
| Deciduous leaf | – | – | | 0.37 (0.03) | 0.53 (0.11) | 42 | –0.04 (0.02) | –0.19 (0.11) | 375 | –0.25 (0.06) | –0.11 (0.10) | 56 |
| Pine wood + bark + coarse root | 0.97 (0.07) | 1.24 (0.20) | 28 | 0.82 (0.10) | 0.95 (0.10) | 16 | 0.54 (0.05) | 0.65 (0.12) | 21 | 0.53 (0.02) | 0.76 (0.19) | 44 |
| Fine root | – | – | 0.37 (0.07) | 0.67 (0.03) | 83* | – | – | – | – | | | |
| Total in increments | 1.97 (0.25) | 2.32 (0.63) | 18 | 2.29 (0.64) | 3.67 (0.81) | 60† | 1.38 (0.34) | 1.18 (0.41) | –14 | 0.48 (0.26) | 1.09 (0.80) | 127 |
| Turnover | | | | | | | | | | | | |
| Throughfall | – | – | 0.58 (0.01) | 0.51 (0.03) | –12 | 0.60 (0.06) | 0.51 (0.03) | –14 | – | – | | |
| Reproduction | 0.01 (0.01) | 0.02 (0.01) | 203 | 0.01 (0.01) | 0.05 (0.03) | 272 | 0.05 (0.03) | 0.08 (0.02) | 66 | 0.09 (0.02) | 0.23 (0.11) | 145 |
| Pine needle litterfall | 1.61 (0.33) | 1.72 (0.29) | 7* | 2.10 (0.39) | 2.42 (0.29) | 15** | 1.87 (0.42) | 2.20 (0.28) | 18* | 2.16 (0.35) | 2.38 (0.34) | 10* |
| Deciduous leaf litterfall | 1.02 (0.09) | 1.03 (0.21) | 2 | 0.88 (0.12) | 0.90 (0.19) | 2 | 0.90 (0.10) | 0.92 (0.20) | 1 | 0.93 (0.12) | 0.97 (0.21) | 4 |
| Branch + bark + other litterfall | 0.65 (0.07) | 0.53 (0.04) | –19 | 0.51 (0.08) | 0.64 (0.03) | 25† | 0.74 (0.14) | 0.80 (0.07) | 7 | 0.82 (0.11) | 1.03 (0.31) | 25 |
| Fine root turnover | – | – | 0.31 (0.14) | 0.44 (0.16) | 45 | – | – | – | – | | | |
| Total turnover | 3.29 (0.35) | 3.30 (0.12) | 1 | 3.81 (0.29) | 4.45 (0.27) | 17* | 3.56 (0.47) | 4.00 (0.10) | 12 | 4.00 (0.38) | 4.61 (0.57) | 16† |
| Annual requirement (g $m^{-2}$ $year^{-1}$)[a] | 5.26 (0.44) | 5.62 (0.52) | 7 | 6.10 (0.92) | 8.12 (0.93) | 33 | 4.94 (0.44) | 5.18 (0.33) | 5 | 4.48 (0.24) | 5.70 (1.37) | 27 |
| Retranslocation prior to abscission | 2.50 (0.09) | 2.70 (0.32) | 8 | 2.94 (0.37) | 3.17 (0.37) | 8 | 3.25 (0.27) | 3.44 (0.29) | 6 | 2.76 (0.24) | 3.30 (0.26) | 19 |
| Annual uptake from soil (g $m^{-2}$ $year^{-1}$)[b] | 2.76 (0.36) | 2.92 (0.30) | 6 | 3.16 (0.56) | 4.95 (0.92) | 57 | 1.69 (0.18) | 1.74 (0.23) | 3 | 1.72 (0.18) | 2.40 (1.37) | 40 |
| Nitrogen-use efficiency[c] | 268 (7) | 305 (46) | 14† | 259 (40) | 256 (24) | –1 | 348 (20) | 398 (39) | 14* | 276 (7) | 286 (36) | 4 |

[a] Defined as: Increments + turnover; excludes throughfall
[b] Defined as: Requirement–retranslocation
[c] Defined as: NPP/requirement

**Table 3** The average (1997–2000) responses of NPP and N-budget components to $CO_2$ treatment. All units are in g $m^{-2}$ $year^{-1}$ with the exception of nitrogen-use efficiency (*NUE*), which is dimensionless. Levels of statistical significance are as indicated in Table 1

| Component | A | E | %$CO_2$ |
|---|---|---|---|
| Dry matter production | | | |
| Total in increments | 820 (31) | 1,083 (66) | 32* |
| Total in turnover | 663 (55) | 765 (29) | 16** |
| NPP | 1,483 (60) | 1,848 (95) | 25** |
| N budget | | | |
| Total in increments | 1.50 (0.11) | 2.03 (0.15) | 36* |
| Total in turnover | 3.71 (0.36) | 4.05 (0.26) | 9* |
| Annual requirement | 5.22 (0.43) | 6.08 (0.36) | 16§ |
| Retranslocation | 2.87 (0.21) | 3.15 (0.25) | 10 |
| Uptake from soil | 2.33 (0.22) | 3.00 (0.54) | 28 |
| NUE | 290 (0.13) | 320 (10) | 10 |

§$P$=0.06

significantly higher than that under ambient $CO_2$. In other years, total biomass was higher in the plots under elevated $CO_2$, but only marginally statistically significant ($P$<0.10).

Biomass increments in loblolly pine wood plus coarse roots increased 24–41% under elevated $CO_2$ and were significantly higher in the first 3 years (Table 1). The increments in loblolly pine needle mass were significantly higher under elevated $CO_2$ in the 1st and 2nd years, but lower under elevated $CO_2$ in the 3rd and 4th years. In the 4th growing season, the increments to loblolly pine needle biomass under ambient and elevated $CO_2$ were negative resulting in a decrease in the biomass of foliage in the canopy relative to 1999 (Table 1). Neither the deciduous leaf nor the deciduous wood plus coarse root increments were significantly affected by growth under elevated $CO_2$. The increment in fine root biomass was significantly higher under elevated $CO_2$ in the 2nd growing season – the only year for which such data are available. The total mass of dry matter in increments was significantly higher under elevated $CO_2$ than ambient $CO_2$ in the first 3 years of $CO_2$ fumigation. Averaging across all 4 years, elevated $CO_2$ significantly increased the total dry matter in increments by 32% (Table 3).

Loblolly pine needle litterfall mass was significantly higher under elevated $CO_2$ in all years (Table 1), and fine root turnover was marginally higher under elevated $CO_2$ ($P$<0.10). The other components of turnover – reproductive structures, deciduous leaves and branches – were not significantly greater under elevated $CO_2$. However, the total return of dry matter to the soil surface was significantly higher under elevated $CO_2$ in years 2–4 and marginally higher ($P$<0.10) in the 1st year of this study. Averaging across all years, elevated $CO_2$ significantly increased the total turnover of organic matter by 16% (Table 3). NPP increased significantly under elevated $CO_2$ in years 1–3 and was marginally ($P$<0.10) higher in year 4. Averaging across years, elevated $CO_2$ increased NPP significantly by 25% (Table 3).

## N pools, fluxes and comparisons with dry matter

The quantity (g/$m^2$) of N in loblolly pine needles was significantly greater in the plots under elevated $CO_2$ in the 2nd and 3rd years but not in the 1st and 4th years of this study (Table 2). The quantity of N in deciduous leaves, deciduous wood plus coarse roots, and fine roots was not significantly different between ambient and elevated $CO_2$. The content of N in loblolly pine wood plus coarse root biomass was not significantly different between $CO_2$ treatments (Table 2). The total quantity of N in biomass under elevated $CO_2$ increased more rapidly than that under ambient $CO_2$ throughout the 4 years of this study, but in no year was this difference statistically significant.

Only the increment of N in fine root biomass was significantly higher under elevated $CO_2$ (Table 2). N increments in all other components increased under elevated $CO_2$ but the increases were not statistically significant. There was considerable inter-annual variation in the total quantity of N contained in the sum of all increments under elevated $CO_2$; this difference between control and experimental plots was much larger in years 2 and 4 (60% and 127% respectively), than in years 1 and 3 (18% and –14%, respectively) of this study. Only in year 2 was the sum of all N in increments marginally ($P$<0.10) higher under elevated $CO_2$. Averaging across years, however, elevated $CO_2$ significantly increased the quantity of N in all increments by 36% (Table 3).

The turnover of N in loblolly pine needle litterfall was significantly higher under elevated $CO_2$ in all years of this study (Table 2). Branch plus bark litterfall increased marginally ($P$<0.10) under elevated $CO_2$ in year 2 but was not significantly higher in other years. None of the remaining components of turnover category was significantly different between $CO_2$ treatments. There were no significant treatment differences in the quantity of N returned to the soil surface in throughfall precipitation (Table 2). The total quantity of N in the turnover category was significantly higher under elevated $CO_2$ in the 2nd year and marginally higher ($P$<0.10) in the 4th year of this study but not significantly different in the 1st and 3rd year (Table 2). Averaged across the 4 years, elevated $CO_2$ significantly increased the total quantity of N in turnover by 9% (Table 3).

Although not statistically significant, the annual requirement for N increased by 5–33% under elevated $CO_2$ (Table 2). There was considerable inter-annual variation in the stimulation of the N requirement under elevated $CO_2$; the stimulation was much larger in years 2 and 4 (33% and 27% respectively), than in years 1 and 3 (7% and 5%, respectively) of this study. Averaging across years, elevated $CO_2$ increased the annual requirement for N by 16% (P<0.06; Table 3).

The retranslocation of N prior to senescence was 6–19% higher under elevated $CO_2$, but this effect was not statistically significant in any year or when years were averaged together (Tables 2, 3). The annual uptake of N from soils was 3–57% higher, but not significantly

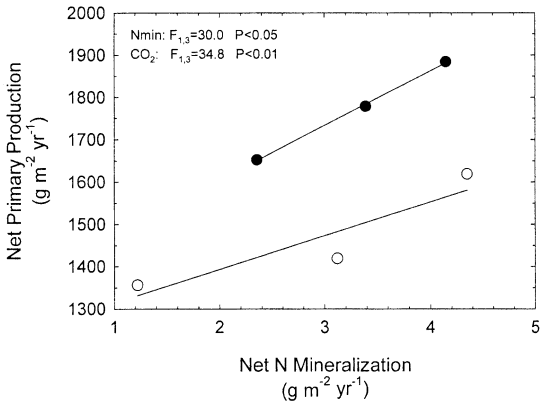


**Fig. 1** The average annual rate of net primary production (NPP) in 1997 and 1998 as a function of the annual rate of net N mineralization in 1998. The *open symbols* are the plots under ambient $CO_2$ and the *filled symbols* are elevated $CO_2$. The annual rate of net mineralization surrounding the ambient and elevated $CO_2$ plots is 2.85±0.89 and 3.18±0.60 g N $m^{-2}$ $year^{-1}$, respectively

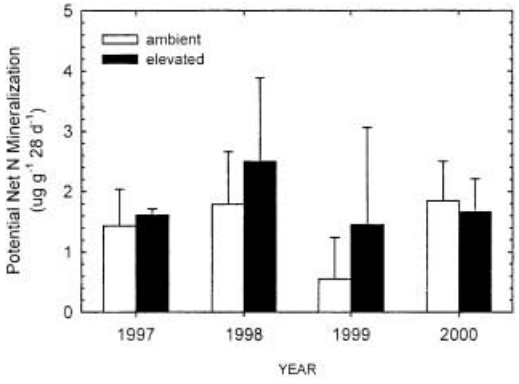


**Fig. 2.** The rate of potential net N mineralization (μg N $g^{-1}$ 28 $days^{-1}$) in the top 15 cm of mineral soil in each of the first 4 years under ambient and elevated $CO_2$. Each column is the within-growing-season average rate of potential net N mineralization for samples collected in April, June, August and October of each year. The 1997 and 1998 data are modified from Finzi et al. (2001). The 1999 and 2000 data are from Finzi and Schlesinger (unpublished data)

**Table 4** The mean (±1 SE) pool sizes and fluxes of N in soils in the 2nd (1998) and the 3rd year (1999) of $CO_2$ fumigation. See Table 1 for the description of symbols for statistical significance

| | 1998 | | | 1999 | | |
|---|---|---|---|---|---|---|
| | A | E | %$CO_2$ | A | E | % $CO_2$ |
| Pools (g $m^{-2}$) soils[a] | | | | | | |
| Forest floor | – | – | | 16.6 | 20.6 (0.8) | 24** |
| 0–15 cm mineral soil | – | – | | 105.8 (8.5) | 118.6 (13.6) | 12 |
| 15–30 cm mineral soil | – | – | | 40.8 (3.9) | 46.2 (1.7) | 13 |
| Microbial biomass forest floor[b,c] | – | – | | 0.45 (0.08) | 0.41 (0.08) | –9 |
| 0–15 cm mineral soil | 8.19 (1.14) | 8.05 (0.81) | –2 | 7.81 (0.56) | 8.56 (0.96) | 10 |
| Soil fluxes (g $m^{-2}$ $year^{-1}$) | | | | | | |
| $N_2O$[d] | 0.0070 (0.0006) | 0.0079 (0.0006) | 13 | 0.0055 (0.0010) | 0.0059 (0.0004) | 7 |
| Soil solution concentrations | | | | | | |
| O horizon | 0.2596 (0.0716) | 0.2759 (0.1085) | 6 | 0.3550 (0.0996) | 0.3562 (0.0364) | 1 |
| 15 cm | 0.0044 (0.0074) | 0.0052 (0.0056) | 18 | 0.0060 (0.0051) | 0.0067 (0.0019) | 12 |
| 70 cm | <0.001 | <0.001 | 0 | <0.001 | <0.001 | 0 |
| 200 cm | <0.001 | <0.001 | 0 | <0.001 | <0.001 | 0 |

[a] Data from Schlesinger and Lichter 2001 collected October 1999
[b] A.C. Finzi (unpublished data)
[c] The average of samples collected in June, August and October 2000
[d] Data from Phillips et al. 2001

so, under elevated $CO_2$ (Table 2). Averaged across all years, the uptake of N from soils was 28% higher under elevated $CO_2$ although this effect was not statistically significant.

NUE was significantly higher under elevated $CO_2$ in the third year of fumigation and marginally ($P$<0.10) higher in the 1st year of fumigation (Table 2). There was no significant difference in NUE in the 2nd or 4th year under elevated $CO_2$ or when averaged across the first 4 years (Table 3). NRE was significantly higher under elevated $CO_2$ (Fig. 1).

## Soil pools and fluxes

Forest-floor N mass increased significantly under elevated $CO_2$ (Table 4); however, the quantity of N in mineral soil to a depth of 30 cm was not significantly affected by elevated $CO_2$. Microbial-biomass N was not significantly different between ambient and elevated $CO_2$ in either the forest floor or the top 15-cm of mineral soil (Table 4). Soil $N_2O$ fluxes were not significantly different between treatment and control plots. Soil water concentrations of inorganic N were higher in the O horizon than in the

mineral soil, but the variation between treatment and control plots at any depth was not statistically significant (Table 4). There was no significant difference in the rate of potential net N mineralization between $CO_2$ treatments in any year of this study (Fig. 2).

## Discussion

A step-function increase in the concentration of atmospheric $CO_2$ significantly increased NPP during the first 4 years of this study (Table 1). Our multi-year measurements showed that different mechanisms operated to maintain high NPP in vegetation exposed to a step-function increase in atmospheric $CO_2$ (Table 2). In the 1st and 3rd growing seasons under elevated $CO_2$, significant increases in NUE under elevated $CO_2$ modulated the average annual requirement for N by vegetation; the average stimulation of NPP by elevated $CO_2$ in these years was 21% whereas the average annual stimulation of the N requirement was only 6%. However, in the 2nd and 4th growing seasons, increases in NPP increased the annual requirement for N by 27–33% (Table 2). Increases in the annual requirement for N under elevated $CO_2$ were met by 40–57% increases in N uptake from soils (Table 2). Retranslocation of nutrients prior to senescence played only a minor role in supplying the additional N required by trees growing under elevated $CO_2$ (Table 2). Although we failed to detect a statistically significant increase in the annual requirement for N and the uptake of N from soil on a year-by-year basis, we believe these processes are important to the long-term productivity of this forest under elevated $CO_2$.

NPP was highly correlated with both the $CO_2$ treatment and the annual rate of net N mineralization in each plot (Fig. 1). NRE (defined as NPP per unit of N mineralized from soil, Pastor and Bridgham 1999) was significantly higher under elevated $CO_2$ demonstrating that NPP in this ecosystem was initially limited by the availability of C from the atmosphere (Fig. 1). The strong positive correlation between NPP and the annual rate of net N mineralization also suggests that NPP is N limited. Experimental manipulations of soil N availability corroborate this conclusion. Oren et al. (2001) found a strong increase in forest production in this same forest with the application of N fertilizers. N fertilization increases the productivity of young loblolly pine stands throughout the piedmont region of the southeastern United States (Zhang and Allen 1996; Albaugh et al. 1998; Richter et al. 2000; Allen et al. 2002). Multiple resources often limit plant growth simultaneously (Bloom et al. 1985; Aerts and Chapin 2000), and co-limitation by C and N is common (cf. Curtis and Wang 1998). The correlation between NPP and N cycling and the increase in the requirement for N imply that soil N availability will affect the long-term rate of NPP in this ecosystem under elevated $CO_2$.

In this N-limited ecosystem, there are four mechanisms that could sustain a high rate of NPP under elevated $CO_2$: increases in NUE, increases in the rate of soil N cycling, redistributions of N among ecosystem pools, and decreased losses of N. NUE was significantly higher under elevated $CO_2$ in years 1 and 3 of this study (Table 2). Thus despite N limitation to NPP, there was enhanced C storage in woody biomass under elevated $CO_2$. An increase in NUE is a very important mechanism maintaining high NPP under elevated $CO_2$ in this ecosystem. The increase in NUE of woody plants growing under elevated $CO_2$ ranges from 9% to 21% (recalculated from Pregitzer et al. 1995; Johnson et al. 1997; Hattenschwiler and Korner 1998; Zak et al. 2000a); the 14% increase in NUE under elevated $CO_2$ in this ecosystem is similar in magnitude to those in the published literature. The inter-annual variation in NUE in this ecosystem was large (Table 2) but within the range of that reported for other southern United States pine forest ecosystems (Gholz et al. 1985; Birk and Vitousek 1986).

Increases in rates of soil N cycling could have a positive feedback on NPP if they increase plant N availability (Zak et al. 1993; Finzi et al. 2001; Fig. 1). There is little consensus on the magnitude and direction of the change in the rate of soil N cycling under elevated $CO_2$; there are reports of increases, decreases and no change in gross and net rates of N mineralization (Zak et al. 2000b and references therein). In this ecosystem, there are no detectable changes in the rate of potential net N mineralization in repeated measurements throughout the 1997 – 2000 growing season (Fig. 2). Net rates of N mineralization are assumed to indicate the supply of N to the plant-available pool. Therefore, we have little evidence to suggest that the rate of soil N supply to plants has increased during the first 4 years of $CO_2$ fumigation.

There is no evidence that the greater uptake of N by plants under elevated $CO_2$ shifted N away from other ecosystem pools. There was no decrease in the quantity of N in microbial biomass and an increase in the quantity of N in the forest floor and mineral soils under elevated $CO_2$ (Table 4). Our data support the hypothesis that the balance of competition for N between plants and microbes has not changed under elevated $CO_2$. In contrast, Hu et al. (2001) found that greater plant uptake of N was correlated with reduced soil microbial activity in California grasslands after 6 years of $CO_2$ fumigation. However, Hu et al. (2001) found no concomitant decrease in microbial biomass N content, and they suggested that the shift in microbial activity under elevated $CO_2$ could have been due to a change in the composition of the microbial community. Other studies have found increases, decreases or no change in microbial-biomass N content among diverse ecosystems exposed to elevated $CO_2$ (Diaz et al. 1993; Hungate et al. 1999; Allen et al. 2000; Zak et al. 2000c; Williams et al. 2001). Thus there is no consistent evidence that greater plant-N uptake under elevated $CO_2$ alters the balance of N between plants and microbes.

In this study, leaching losses of N were very small ($<0.001$ g $m^{-2}$ $year^{-1}$ at 70- and 200-cm depth, Table 4) and not significantly different between $CO_2$ treatments.

Differential retention of N from soil water does not explain greater plant uptake of N under elevated $CO_2$ (Table 3). Aggrading southern pine forests are very strong sinks for available N and negligible leaching losses of N have been reported in several studies (Johnson and Lindberg 1992; Richter and Markewitz 1995, 2001; Richter et al. 2000). Enhanced rates of plant growth under elevated $CO_2$ have been correlated with decreased leaching losses of N in other ecosystems. For example, Hungate et al. (1999) and Johnson et al. (2001) found reduced concentrations of $NO_3^-$ in resin lysimeters below the soil A horizon in a Florida scrub oak ecosystem exposed to 3 years of $CO_2$ enrichment. However, these results were not placed within the context of an ecosystem mass balance making it difficult to interpret the importance of this pathway as a mechanism alleviating N limitation to plant growth under elevated $CO_2$.

The annual loss of N as $N_2O$ was very small and not significantly different between $CO_2$ treatments (Table 4). Despite the small magnitude of this flux, Phillips et al. (2001) demonstrated significant seasonal variation in the evolution of $N_2O$ under elevated $CO_2$. Low soil temperature and high soil moisture content were correlated with significantly higher $N_2O$ fluxes under elevated $CO_2$ during the winter. Conversely, high soil temperature and low soil moisture were correlated with significantly lower soil $N_2O$ fluxes during the summer. Thus, growing season $N_2O$ losses were significantly lower under elevated $CO_2$ (Phillips et al. 2001). Lower $N_2O$ production during the growing season suggests that the greater plant demand for N under elevated $CO_2$ may control gaseous losses of N in this ecosystem.

## Conclusions

NPP in the Duke forest increased significantly in response to elevated $CO_2$ throughout the first 4 years of fumigation. High rates of gross primary production relative to respiration significantly increased net ecosystem production in this ecosystem in 1998 (Hamilton et al. 2002). Initially large increases in plant growth and NPP are common among ecosystems dominated by woody plant species following a step-function increase in atmospheric $CO_2$ (e.g., Johnson et al. 1997; Rey and Jarvis 1997; Tissue et al. 1997; Jach et al. 2000; Zak et al. 2000a). The duration and magnitude of the stimulation in plant growth and NPP under elevated $CO_2$ varies by plant species and ecosystem (Centritto et al. 1999; Idso 1999). Soil nutrient availability impacts plant responses to elevated $CO_2$ (Ceulmans and Mousseau 1994; Hattenschwiler and Korner 1997; Curtis and Wang 1998; Saxe et al. 1998), and soil resource availability can regulate long-term responses of terrestrial communities to high $CO_2$ (McMurtrie and Commins 1996; Rastetter et al. 1997; Kirschbaum et al. 1998; Pan et al. 1998; Luo and Reynolds 1999; Oren et al. 2001).

Inputs of N through atmospheric deposition (0.70 and 0.60 g $m^{-2}$ $year^{-1}$ in 1998 and 1999, respectively; D.D. Richter, unpublished data) are at least two orders of magnitudes larger than outputs at this site. The efficient retention of N within this ecosystem implies intense competition for N among plants, microbes and physical sinks in soils. There is no evidence that rates of net N mineralization have increased under elevated $CO_2$ (Fig. 2). Both N availability and C availability control NPP in the Duke Forest (Fig. 1). Ecosystem-simulation models show a down-regulation of NPP in response to a step-function increase in atmospheric $CO_2$ when plant uptake of N from the available pool exceeds the rate of replenishment via mineralization (McMurtrie and Commins 1996; Rastetter et al. 1997; Luo and Reynolds 1999). Similarly, Oren et al. (2001) found a steep decline in the stimulation of loblolly pine growth after 3 years of $CO_2$ fumigation in a prototype plot in the Duke forest. (The prototype plots is a single plot that was established prior to the fully replicated experiment to develop and test FACE technology.) In the absence of fertilization with N, loblolly pine growth under elevated $CO_2$ was not significantly different from that in a non-instrumented reference plot at the end of 4 years (Oren et al. 2001). We have no evidence suggesting that NPP is declining in response to >4-years of $CO_2$ fumigation in the fully replicated experiment. However, we have not measured any change in N-transformation processes that could increase plant N availability under elevated $CO_2$. If the annual requirement of N continues to be stimulated by elevated $CO_2$, we predict that the productivity response of this forest ecosystem will decline over time.

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