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Canopy N and P dynamics of a southeastern US pine forest under elevated CO_2

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Abstract. Forest production is strongly nutrient limited throughout the southeastern US. If nutrient limitations constrain plant acquisition of essential resources under elevated CO_2, reductions in the mass or nutrient content of forest canopies could constrain C assimilation from the atmosphere. We tested this idea by quantifying canopy biomass, foliar concentrations of N and P, and the total quantity of N and P in a loblolly pine (*Pinus taeda*) canopy subject to 4 years of free-air CO_2 enrichment. We also used N:P ratios to detect N versus P limitation to primary production under elevated CO_2. Canopy biomass was significantly higher under elevated CO_2 during the first 4 years of this experiment. Elevated CO_2 significantly reduced the concentration of N in loblolly pine foliage (5% relative to ambient CO_2) but not P. Despite the slight reduction foliage N concentrations, there were significant increases in canopy N and P contents under elevated CO_2. Foliar N:P ratios were not altered by elevated CO_2 and were within a range suggesting forest production is N limited not P limited. Despite the clear limitation of NPP by N under ambient and elevated CO_2 at this site, there is no evidence that the mass of N or P in the canopy is declining through the first 4 years of CO_2 fumigation. As a consequence, whole-canopy C assimilation is strongly stimulated by elevated CO_2 making this forest a larger net C sink under elevated CO_2 than under ambient CO_2. We discuss the potential for future decreases in canopy nutrient content as a result of limited changes in the size of the plant-available pools of N under elevated CO_2.

Introduction

The relationship between soil nutrient availability, foliage biomass production and canopy nutrient content is a key determinant of C storage in terrestrial ecosystems exposed to elevated CO_2. Photosynthesis is strongly dependent upon the concentration of nitrogen (N) and phosphorus (P) in leaves (Pearcy et al. 1987; Peterson et al. 1999) and gross primary production depends on the photosynthesis – nutrient relationship throughout the plant canopy. If elevated CO_2 or limiting quantities of soil nutrients alter the production of leaves or the distribution of N and P within plant canopies, the magnitude of the C influx via photosynthesis will change (Luo and Reynolds 1999).

Foliage biomass and net primary production (NPP) are highly correlated across a broad range of forest types (Gholz 1982; Webb et al. 1983; Vose and Allen 1988; Piatek and Allen 2000). In young forest ecosystems, foliage biomass increases rapidly during stand development (Marks and Bormann 1972). With their high

nutrient contents, leaves are an important sink for available nutrients in plants. For example, the production of new foliage in young loblolly pine forests (15–25 years old) sequesters between 30 and 70% of the total annual nutrient uptake (Switzer and Nelson 1972), ranging between 50–83 kg N ha^{-1} $year^{-1}$ and 5–11 kg P ha^{-1} $year^{-1}$ (Piatek and Allen 2000 and references therein). This large annual requirement for N and P accounts for widespread nutrient limitation to NPP throughout southeastern US forests (Richter and Markewitz 2001).

The available data suggest a wide range of canopy responses to elevated CO_2. Most short-term experiments (<4 years) on tree seedlings and saplings demonstrate increases in foliage biomass production and canopy N content under elevated CO_2 (e.g., Murray et al. 1996; Rey and Jarvis 1997; Norby et al. 1999; Jach et al. 2000). Notable exceptions report that foliage production and canopy-N content do not respond to elevated CO_2 in the absence of increases in soil nutrient availability (Hättenschwiler and Körner 1998; Zak et al. 2000; Oren et al. 2001). These may represent extreme cases of nutrient limitation where nutrient supply prohibits even a transient response to elevated CO_2. Very little data are available on plant P pools under elevated CO_2 (Johnson et al. 1997; Walker et al. 2000).

Primary production in southeastern US forests is commonly nutrient limited (e.g., Piatek and Allen 2000; Richter et al. 2000). While much of the focus on nutrient limitation in the temperate zone focuses of N (c.f. Vitousek and Howarth 1991), the soils of the piedmont region of the southeastern US are highly weathered with the potential for P limitations due to the depletion of P-containing primary minerals and the occlusion of P into unavailable forms for plant growth (Walker and Syers 1976; Crews et al. 1995; Cross and Schlesinger 1995; Vitousek and Farrington 1997). Foliar nutrient status is commonly used to detect the nature and extent of soil nutrient limitations to NPP (e.g., Valentine and Allen 1990). Foliar N:P ratios have been used to assess N versus. P limitation to primary production in grassland and heathland ecosystems (Verhoeven et al. 1996) and N saturation in ecosystems of the western United States (Fenn et al. 1996).

Biogeochemical models predict that the magnitude and direction of the CO_2 stimulation of NPP is controlled by the rate of nutrient mineralization from soil organic matter or weathering from source pools (McMurtrie and Comins 1996; Rastetter et al. 1997; Luo and Reynolds 1999). These models suggest that if soil nutrients limit NPP under elevated CO_2, then the initial increase in canopy biomass and nutrient content under elevated CO_2 should attenuate through time, and eventually no longer be significantly different from that under ambient CO_2. We have studied the productivity response of a rapidly aggrading pine forest grown at elevated CO_2 in the Duke Forest, NC (DeLucia et al. 1999; Hamilton et al. 2002). Both atmospheric CO_2 concentrations and soil N availability limit NPP in this ecosystem (Finzi et al. 2002). The potential for P limitation to NPP has not been explored in this ecosystem. Thus, the objectives of this study were to (1) assess the effect of elevated CO_2 on the concentration of N and P throughout the loblolly pine canopy, (2) use N:P ratios to assess the degree of N versus P limitation to NPP, and (3) determine the temporal pattern in canopy biomass production and nutrient content under ambient and elevated CO_2.

Methods and materials

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 $\mu l\, l^{-1}$ above ambient. Three control plots are fumigated with ambient air only. The experiment began 27 August 1996 and is continuous (24 h day^{-1}; 365 days $year^{-1}$). 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 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 $\leq 1^{\circ}$ throughout. Soils are classified in 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. These soils are very deep (>15 m) but organic C, N and P are concentrated in the upper 15 cm of the soil profile (Schlesinger and Lichter 2001). Additional site details can be found in Schlesinger and Lichter (2001) and Finzi et al. (2001).

Canopy sampling and chemistry

The longevity of loblolly pine foliage in the Piedmont of NC is 19 months (Zhang and Allen 1996) so that at any time there are needles of two different ages on a single branch (e.g., 2 cohorts). We collected foliage samples from both needle cohorts–those produced in the current year and those produced in the previous year–at three heights within the canopy; the bottom 25%, the middle 50%, and the top 25% of the crown. The canopy divisions were based on the large variation in the specific-leaf area (SLA) of projected foliage with crown depth (DeLucia et al. 2002). SLA was 33.5 $cm^2\, g^{-1}$ at the top of the canopy, 38.2 $cm^2\, g^{-1}$ in the middle of the canopy and 45.6 $cm^2\, g^{-1}$ at the bottom (DeLucia et al. 2002).

We collected canopy samples 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 5–8 fascicles of current and year-old foliage along a primary branch. Foliage samples were collected in June and September of each year from 1997 through 2000. The June sample represents the initial concentration of N and P in the first fully expanded flush of new needles, and the

September sample represents the peak canopy biomass N and P pools (Zhang and Allen 1996).

We compared the biomass of foliage predicted from pretreatment allometric relationships with the biomass predicted from the leaves collected in litter baskets (*see below*, Finzi et al. 2002). Foliage biomass predicted allometrically was consistently smaller than that predicted from the litter baskets. We cannot harvest trees from the replicated experiment at this time, leading to uncertainties in the allometric relationship between tree diameter and foliage biomass under elevated CO_2. Thus, we opted to calculate foliage biomass from collections of litterfall (cf. Finzi et al. 2002).

Aboveground litterfall mass was collected from 5 June 1996 onward by placing 12 replicate 40 cm × 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 into seven categories, including pine needles. Given the longevity of loblolly pine foliage, a new cohort of leaves produced in 1 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 loblolly pine needles in the canopy in 1998 can be 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 2001 to calculate leaf biomass for the period 1997–2000. Repeated measures ANOVA 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 during the first 4 years of this experiment (Finzi et al. 2002). 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 the mass of leaves for a given year in the canopy is the same as that collected as litterfall.

We measured the N and P concentration of green leaves in a sulfuric–salicylic acid Kjeldahl digestion (Lowther 1980) followed by colorimetric analysis on an automated ion analyzer (Lachat QuickChem FIA+ 8000 Series, Zellweger Analytics, Milwaukee, WI). We calculated the quantity of N and P throughout the entire pine canopy by multiplying the concentration of N or P at a given canopy position (bottom, middle, or top) by the foliage biomass in that canopy position. We assumed that 25% of the foliage biomass was in the lower canopy, 50% was in the mid-canopy and 25% was in the upper canopy.

Statistical analysis

We used repeated-measures ANOVA to determine the influence of atmospheric CO_2 (2 levels: ambient, elevated), canopy position (3 levels: bottom, middle, top)

and needle age class (2 levels: new, old) on N and P concentrations and their ratio. There was large pretreatment variation in the mass and the quantity of N and P in the loblolly pine canopy across the six plots that comprise this experiment (Finzi et al. 2001). Thus we used analysis of covariance to determine the influence of atmospheric CO_2 on the mass of foliage and the content of N and P in the loblolly pine canopy on a year-by-year basis. All data were assessed for normality and homogeneity of variance following ANOVA or ANCOVA. We used Tukey's test to compare means among the different levels of the fixed effects.

Results

Pine needle N and P concentrations and ratios

Elevated CO_2 slightly but significantly decreased the concentration of N in loblolly pine needles (Table 1). Concentrations of N were $10.6 \pm 0.1\ mg\ g^{-1}$ under ambient CO_2 and $10.1 \pm 0.1\ mg\ g^{-1}$ under elevated CO_2. Concentrations of N in loblolly pine foliage under elevated CO_2 were consistently lower across all canopy positions (Figure 1). There were large differences in N concentrations in the different age classes, and there was a significant age class *x* position-within-the-canopy interaction (Table 1). Concentrations of N were lower in 1-year-old needles than in needles produced within the current year. Concentrations of N were lowest in the bottom of the canopy and highest at the top of the canopy in the needles produced within the current year (Figure 2). Conversely, concentrations of N were highest in the bottom of the canopy and lowest in the top of the canopy in the 1-year-old needles. Concentrations of N in foliage were significantly higher early in the growing season ($11.1 \pm 0.1\ mg\ g^{-1}$ in June) than in the late growing season (September, $9.6 \pm 0.1\ mg\ g^{-1}$), when canopy N reaches its peak (Table 1). The concentration of N in foliage was significantly higher in 1997 and 2000 than in 1998 and 1999 (Table 1).

Elevated CO_2 had no effect on the concentration of P in pine needles (Table 1, Figure 1). The concentration of P in foliage was significantly higher at the top of the canopy ($1.15 \pm 0.04\ mg\ g^{-1}$) than the middle of the canopy ($1.05 \pm 0.03\ mg\ g^{-1}$) but not significantly different than in the bottom of the canopy ($1.06 \pm 0.03\ mg\ g^{-1}$, Table 1). Concentrations of P in loblolly pine foliage were significantly lower in the 1-year-old needles ($0.89 \pm 0.01\ mg\ g^{-1}$) than in the current year needles ($1.29 \pm 0.03\ mg\ g^{-1}$, Table 1). Concentrations of P were significantly higher early in the growing season ($1.26 \pm 0.03\ mg\ g^{-1}$ in June) than late in the growth season ($0.92 \pm 0.01\ mg\ g^{-1}$ in September, Table 1). Although P concentrations varied from year-to-year and between years and needle age classes, seasons and canopy positions, there were no significant year *x* CO_2 or higher order interactions (Table 1).

Pine needle N:P ratios were generally lower under elevated CO_2 (Table 2), but the effect of elevated CO_2 was not statistically significant (Table 1). N:P ratios were significantly higher in 1-year-old foliage (11.0 ± 0.2) than in current year foliage (9.2 ± 0.2, Table 1), and significantly lower in June (9.5 ± 0.2) than September

Table 1. The results of repeated measures ANOVA for N and P concentrations and the N:P ratio of loblolly pine foliage under ambient and elevated CO_2 ('CO_2'), at three heights within the canopy ('position'), across needle age classes ('age class'), in June and September ('season') during the first 4 years of this experiment ('year'). *F* statistics with superscript symbols indicate statistical significance at * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$.

Source of variation	df	N concentration		P concentration		N:P ratio	
		MS	*F*	MS	*F*	MS	*F*
Between subjects							
CO_2	1	25.49	11.83**	0.007	0.09	38.37	2.26
Position	2	10.78	5.00**	0.258	3.27*	15.29	0.90
CO_2 × position	2	0.49	0.33	0.004	0.06	0.23	0.01
Age class	1	265.69	123.33****	11.749	148.74****	241.26	14.21***
CO_2 × age class	1	0.10	0.05	0.027	0.34	11.83	0.70
Age class × position	2	10.21	4.74*	0.004	0.05	9.40	0.55
CO_2 × age class × position	2	0.15	0.07	0.018	0.22	0.36	0.02
Season	1	163.31	75.81****	7.977	100.99****	116.97	6.89*
CO_2 × season	1	3.00	1.39	0.014	0.18	0.23	0.01
Season × position	2	3.97	1.84	0.042	0.53	0.21	0.01
CO_2 × season × position	2	1.36	0.63	0.001	0.01	0.88	0.05
Age class × season	1	0.11	0.05	3.597	45.54****	157.24	9.26**
CO_2 × age class × season	1	1.04	0.49	0.002	0.03	2.98	0.18
Age class × season × position	2	1.02	0.47	0.003	0.03	0.62	0.04
CO_2 × age class × season × position	2	0.05	0.02	0.005	0.06	0.04	0.01
Error	48	2.16		0.079		16.97	
Within subjects							
Year	3	26.41	76.27****	0.394	67.86****	31.30	85.79****
Year × CO_2	3	0.64	1.85	0.001	0.11	0.56	1.53
Year × position	6	0.15	0.42	0.035	6.03****	1.18	3.24**
Year × CO_2 × position	6	0.06	0.16	0.001	0.24	0.17	0.48

year × age class	3	5.11	14.76****	0.158	27.27****	2.37	6.48**
Year × CO_2 × age class	3	0.08	0.22	0.001	0.23	0.47	1.29
Year × age class × position	6	0.40	1.15	0.011	1.86	0.13	0.35
Year × CO_2 × age class × position	6	0.13	0.37	0.006	0.95	0.46	1.27
Year × season	3	0.31	0.91	0.059	10.16****	2.41	6.61***
Year × CO_2 × season	3	0.32	0.93	0.002	0.34	0.50	1.37
Year × season × position	6	0.24	0.68	0.019	3.22**	0.49	1.33
Year × CO_2 × season × position	6	0.25	0.71	0.001	0.13	0.28	0.76
Year × age class × season	3	1.74	5.01	0.083	14.26****	2.35	6.44***
Year × CO_2 × age class × season	3	0.08	0.23	0.001	0.14	0.18	0.50
Year × age class × season × position	6	0.16	0.45	0.009	1.61	0.35	0.95
Year × CO_2 × age class × season × position	6	0.15	0.42	0.002	0.32	0.23	0.64
Error	144	0.35		0.006		0.36	

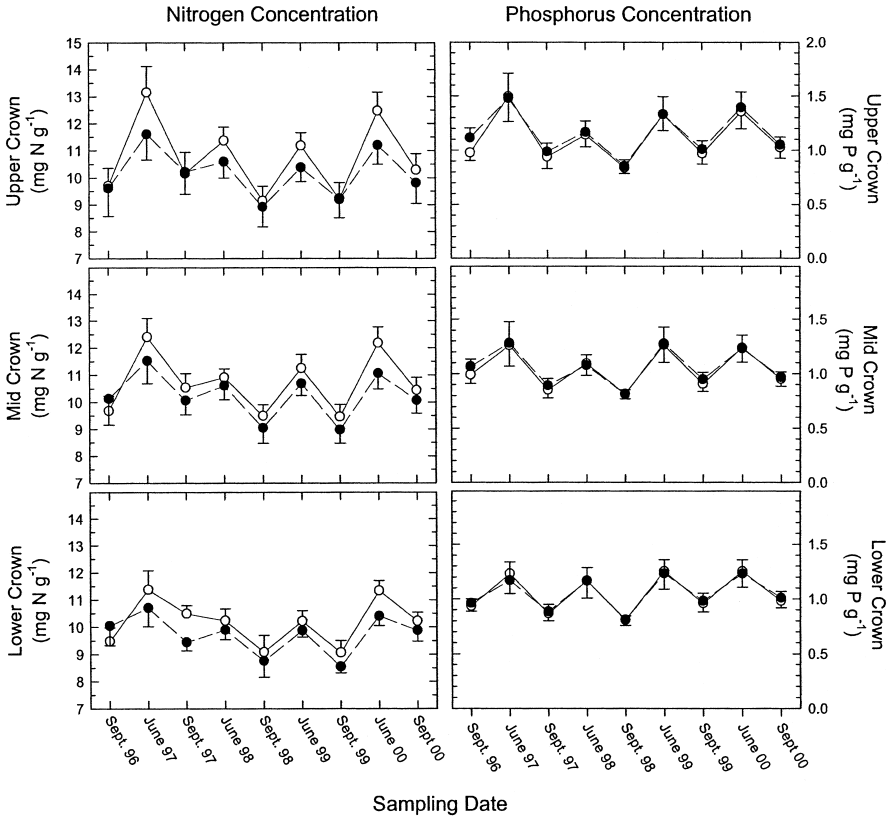

Figure 1. The average (±1 S.E.) concentration of N and P at three different heights in the canopy under ambient (open symbols) and elevated CO_2 (filled symbols). The September 1996 data represent pre-treatment differences in the N and P concentration of loblolly pine needles.

(10.7 ± 0.2, Table 1). There was a significant age class *x* season interaction (Table 1). Foliar N:P ratios were highest in 1-year-old needles in June (11.1 ± 0.3) and lowest in current year foliage in June (7.8 ± 0.1). There was significant inter-annual variation in foliar N:P ratios and significant variations between years and canopy position and age class (Table 1). However, there were no significant year *x* CO_2 or higher order interactions (Table 1).

Canopy biomass and N and P content

The mass of foliage in the loblolly pine canopy was significantly higher under elevated CO_2 throughout the first 4 years of fumigation (Figure 3). The mass of N in the loblolly pine canopy was significantly higher under elevated CO_2 in the second and third years of fumigation but not during the first and fourth years of fumigation.

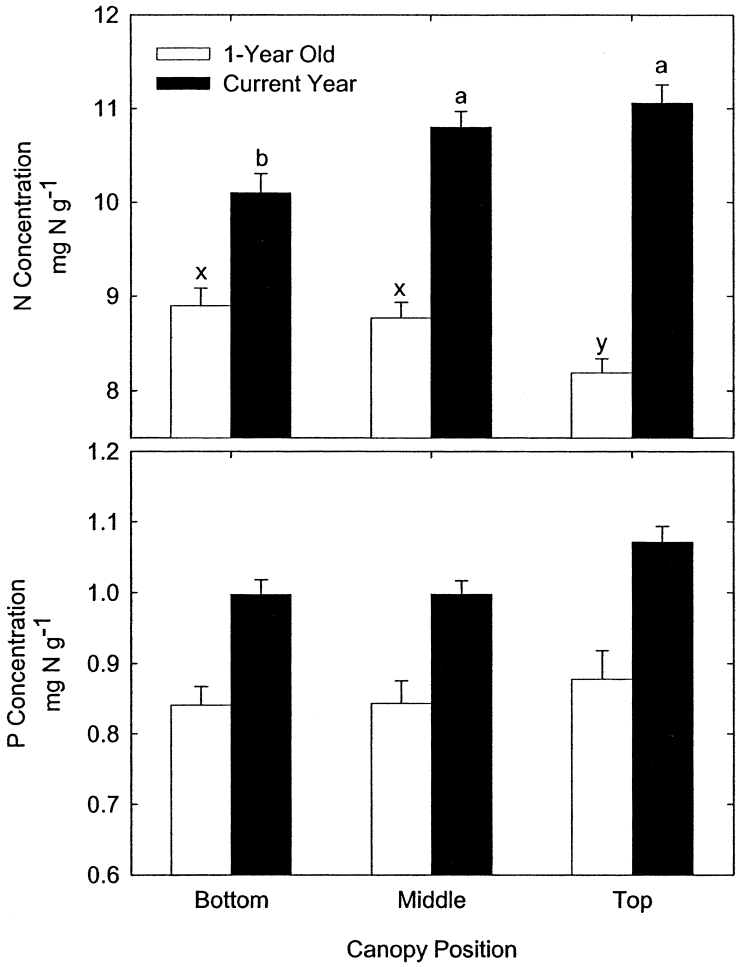

Figure 2. The concentration of N and P in current year and 1-year old needles at three different heights within the canopy. Bars of the same color with different superscript letters are significantly different from one another at $P < 0.01$.

Table 2. Foliar N:P ratios (weight:weight) by needle age class under ambient and elevated CO_2 during the first 4 years of CO_2 fumigation.

Year	N:P ratio			
	1-Year-old needles		Current-year needles	
	Ambient	Elevated	Ambient	Elevated
1997	12.6 (0.6)	11.1 (0.7)	9.7 (0.5)	9.3 (0.5)
1998	11.8 (0.6)	10.7 (0.6)	9.8 (0.4)	9.8 (0.6)
1999	10.6 (0.6)	9.8 (0.5)	8.6 (0.4)	8.1 (0.4)
2000	11.3 (0.6)	10.0 (0.6)	9.3 (0.3)	8.9 (0.4)

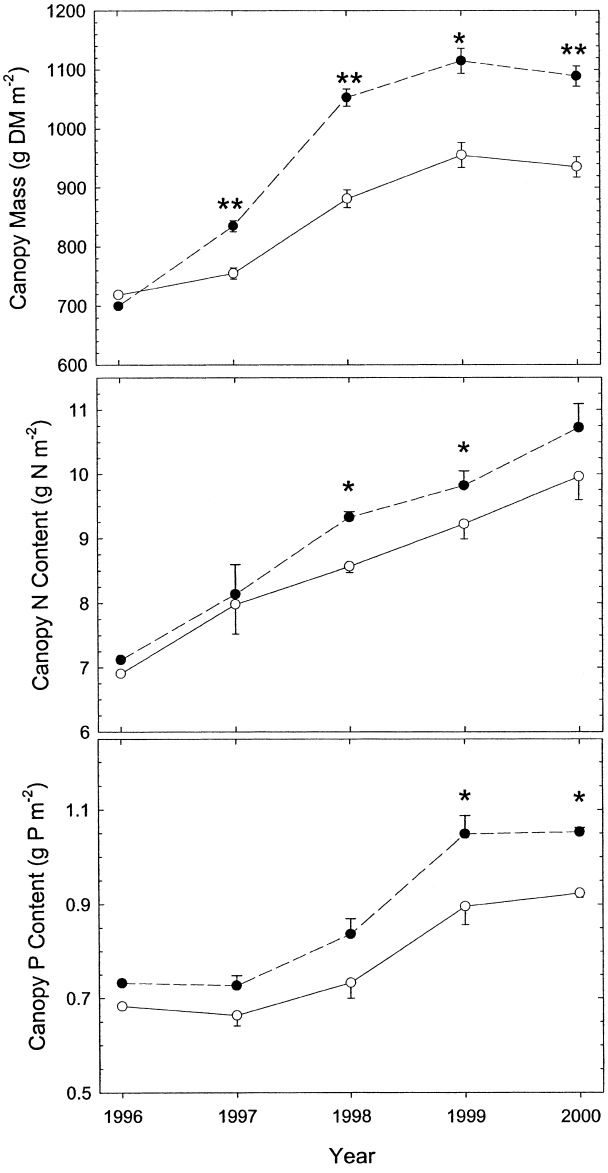

Figure 3. The mass, N content and P content of the loblolly pine canopy under ambient (open symbols) and elevated CO_2 (filled symbols). 1996 was the pre-treatment year. Significant differences between CO_2 treatments within a given year are denoted by: $* = P < 0.05$, $** = P < 0.01$.

The mass of P in the loblolly pine canopy was higher under elevated CO_2 throughout the experiment, but only significantly higher following the fourth year of CO_2 fumigation (Figure 3).

Discussion

The mass and nutrient content of forest canopies is highly correlated with NPP (Gholz 1982; Vose and Allen 1988; Piatek and Allen 2000). If nutrient limitations constrain plant acquisition of essential resources under elevated CO_2, reductions in the mass or nutrient content of forest canopies may constrain C assimilation from the atmosphere. As a result, the mass and the nutrient content of forest canopies are critical parameters for the forecasting of the productivity response of forests to rising concentrations of atmospheric CO_2.

Elevated concentrations of atmospheric CO_2 reduced needle N concentrations but had no effect on the concentration of P during the first 4 years of this experiment (Figure 1). At the same time, however, there was a significant increase in the production of foliar biomass (Figure 3). This resulted in a significant increase in the quantity of N and P in the loblolly pine canopy under elevated CO_2 (Figure 3). The production of loblolly pine foliage in this ecosystem sequesters $\sim$53% of the total annual nutrient requirement (Finzi et al. 2002). During the first 4 years of this study, the quantity of N in the loblolly pine canopy increased by 3.7 g m^{-2} under elevated CO_2 whereas it increased by 2.9 g m^{-2} under ambient CO_2. Similarly, the quantity of P in the loblolly pine canopy increased by 0.34 g m^{-2} under elevated CO_2 but only 0.22 g m^{-2} under ambient CO_2. This represents a 26 and 50% increase in the quantity of N and P, respectively, in the loblolly pine canopy under elevated CO_2. A recently completed N budget for this ecosystems shows that increases in N-use efficiency (NUE) and increases in N uptake from soils maintain the productivity response of this ecosystem under elevated CO_2 (Finzi et al. 2002). We do not have a complete budget for P in this ecosystem, however the same basic mechanisms are likely to be in operation for P.

Four lines of evidence suggest that primary production is more strongly N limited than P limited in this ecosystem. First, foliar N:P ratios suggest N limitation. Foliar N:P ratios have been successfully used to demonstrate N versus. P limitation of temperate grassland and heathland productivity (Verhoeven et al. 1996). Similarly, Valentine and Allen (1990) summarized diameter growth and foliar nutrient responses to factorial additions of N and P fertilizers for loblolly pine forests throughout the southeastern US. Based on (i) the initial concentration of N and P in current year needles in the top third of the loblolly pine canopy, and (ii) the increase in tree diameter growth following N and P fertilization, their results suggest that N:P ratios $<$10.5 indicate N limitation, N:P ratios between 10.5 and 12.5 indicate joint limitation by N and P, and N:P ratios $>$12.5 indicate P limitation. N:P ratios for current year needles averaged across the canopy during the first 4 years of this study were $\leq$9.8 suggesting that N limits forest NPP (Table 2). A similar pattern was observed for the current year needles in the top third of the canopy in this study (data not shown). Second, Finzi et al. (2002) reported a highly significant positive relationship between NPP and the annual rate of net N mineralization at this site under ambient and elevated CO_2 in 1998, the second year of CO_2 fumigation. There was no correlation between NPP and bicarbonate-extractable P pools in mineral soils at this site for the same time period (A. Finzi and

A. Gallardo, unpublished data), although base extractions of acid soils can under recover plant-available pools of P. Third, foliar N concentrations were significantly lower under elevated CO_2 (Table 1) and they showed a strong early-growing season decline in the top third of the canopy whereas no such patterns were observed for P (Figure 1). The reduction and seasonal lag in foliar N concentrations suggests that demand for N early in the growing season exceeded the rate of N uptake from soils and/or reallocation from storage pools to the canopy. These patterns do not appear to be due to a dilution of N by carbohydrate buildup in leaves; if it were, the dilution effect would have also manifested itself in foliar P concentrations but no such effect was observed (Figure 1). Fourth, although N:P ratios were not statistically significantly lower under elevated CO_2, they were consistently lower than those under ambient CO_2 (Table 2). This reduction in N:P ratio suggests that rapid forest growth under elevated CO_2 drives the systems towards greater N limitation than P limitation.

One major assumption in scaling nutrient concentrations from individual leaves at selected heights in a forest canopy to an estimate of the nutrient content of the entire canopy is the distribution of foliage biomass throughout the forest canopy. In our initial analysis we assumed that 25% of the foliar biomass was in the lower third of the canopy, 50% of the foliar biomass was in the middle third of the canopy and the remaining 25% of the foliar biomass was in the top third of the canopy. We then calculated the mass of N and P in the forest canopy by multiplying the N and P concentration of needles at each height in the canopy by the mass of foliage at these heights. We performed a sensitivity analysis of this assumption by evenly redistributing foliar biomass across the canopy heights (i.e., 33, 34, and 33% of the foliar biomass in the bottom, middle and top of the canopy, respectively). There was no evidence that an even distribution of foliar biomass altered our estimate of the quantity of N (Figure 4(A)) or P (data not shown) in the plant canopy. The insensitivity of total N and P content in the canopy reflects the inverse pattern in the N concentration of different age-class needles throughout the forest canopy and the very subtle variation in P concentrations (Figure 2).

A second major assumption in our scaling is that the morphology of needles (i.e., LMA) is not significantly different between abscised needles and green needles. Theoretically the LMA of green needles should be greater than that of abscised needles because the retranslocation of carbohydrates and nutrients from senescing tissues decreases leaf mass (assuming no shrinkage of needles area). Interestingly, our data show that the LMA of abscised needles was slightly higher than that of green needles in 1997 (+5%), 1999 (+6%) and 2000 (+7%) whereas it was lower in 1998 (−5%). We propagated the difference between green needle LMA and abscised needle LMA through our calculation of the content of canopy N (Figure 4(B)). This propagation shows that the absolute quantity of N estimated for the canopy is highly sensitive to our assumption of no change in LMA. However, repeated measures ANOVA shows that the difference in LMA between green and abscised needles is not statistically significant under ambient or elevated CO_2 (analysis not shown). This ANOVA suggests that there is little reason to use the difference in LMA between green and abscised needles when calculating canopy

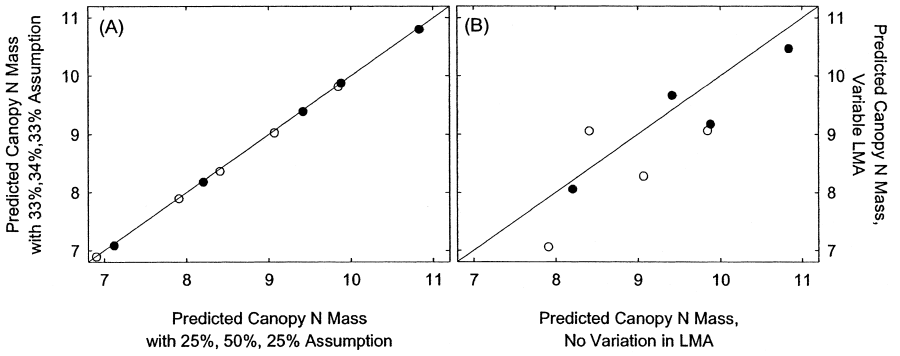

Figure 4. A sensitivity analysis of (A) the distribution of foliage biomass throughout the canopy, and (B) the effect of leaf-mass to area ratios for the content of N in the canopy. In (A) the *X*-axis is the predicted canopy N content given our original assumption that 25, 50, and 25% of the foliage biomass is distributed in the bottom, middle and top of the canopy, respectively. The *Y*-axis is the predicted canopy N content assuming that foliage biomass is evenly distributed across the plant canopy. There are 10 points total reflecting the predicted canopy biomass from 1996 through 2000 in the ambient (open symbols) and elevated CO_2 (filled symbols) plots. In (B) the *X*-axis is the predicted canopy N content assuming that there is no difference in the LMA of green needles and abscised needles. The *Y*-axis is the predicted canopy N content assuming that the mass of foliage per unit area differs between green and abscised needles (see text for details). There are 8 points total reflecting the predicted canopy biomass from 1997 through 2000 in the ambient (open symbols) and elevated CO_2 (filled symbols) plots. In both plots the solid line is the 1:1 line.

nutrient content. Moreover, the relative difference in LMA between green and abscised leaves is smaller than the relative difference in foliar biomass production under ambient and elevated CO_2. Therefore, while the absolute content of the N in the canopy would change with a different assumption for LMA, this difference would not fundamentally alter our conclusion regarding the effect of elevated CO_2 on canopy nutrient content.

Biogeochemical models predict a progressive decline in forest production under elevated CO_2 in nutrient-limited ecosystems (Rastetter et al. 1997; Luo and Reynolds 1999). This occurs because rapid plant growth under elevated CO_2 immobilizes nutrients in long-lived plant tissues and soils more rapidly than their mineralization from soil pools or weathering from soil minerals. Chronosequence studies show that aggrading forests actively assimilate N from mineral soil horizons and that this N is stored in woody biomass and O horizon pools (Gholz et al. 1985; Magill et al. 2000; Richter et al. 2000; Hooker and Compton 2003). If the majority of the N is stored in woody biomass, then the reduction in the size of labile N pools in mineral soils could result in a rapid attainment of severe N limitation under elevated CO_2 (cf. Richter et al. 2000). Conversely, if the majority of the N is returned to the O horizon of soils in litterfall, then the rate of N release from the decomposition of the O horizon relative the rate at which labile N is removed from mineral soil will dictate the severity of nutrient limitation under elevated CO_2. In particular, if the O horizon decomposes rapidly, then the reduction in N uptake as a

result of the mining of labile soil N from mineral soil horizons by plants could be offset by the uptake of N from the O horizon.

Despite the clear limitation of NPP by N at this site (Finzi et al. 2002; Oren et al. 2001), this limitation has not precluded a response to elevated CO_2 during the first 4 years of this experiment (Finzi et al. 2002). Similarly, there is no evidence that the mass of N in the canopy is declining through the first 4 years of CO_2 fumigation (Figure 2). As a consequence, whole-canopy C assimilation is strongly stimulated by elevated CO_2 making this forest a larger net C sink under elevated CO_2 than under ambient CO_2 (DeLucia et al. 2002; Hamilton et al. 2002). However, there is little evidence that the rate of N supply for plant growth has increased under elevated CO_2 and N is being immobilized in woody biomass and the O horizon more rapidly under elevated than ambient CO_2 (Finzi and Schlesinger 2003). Given that NPP in this ecosystem is N limited (Finzi et al. 2002) and that foliar N:P ratios are consistently lower under elevated CO_2 and declining in a direction suggesting greater N limitation (Table 2), we hypothesize that limited soil N availability will eventually curtail the initial increase in productivity under elevated CO_2 (see Oren et al. 2001). The time scale for such a down-regulation, however, is not known. One of the first indications of such a response, however, should be a convergence in the pool of N in the loblolly pine canopy under ambient and elevated CO_2.

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