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Branch growth and gas exchange in 13-year-old loblolly pine (*Pinus taeda*) trees in response to elevated carbon dioxide concentration and fertilization

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Summary We used whole-tree, open-top chambers to expose 13-year-old loblolly pine (*Pinus taeda* L.) trees, growing in soil with high or low nutrient availability, to either ambient or elevated (ambient + 200 µmol mol^{-1}) carbon dioxide concentration ([CO_2]) for 28 months. Branch growth and morphology, foliar chemistry and gas exchange characteristics were measured periodically in the upper, middle and lower crown during the 2 years of exposure. Fertilization and elevated [CO_2] increased branch leaf area by 38 and 13%, respectively, and the combined effects were additive. Fertilization and elevated [CO_2] differentially altered needle lengths, number of fascicles and flush length such that flush density (leaf area/flush length) increased with improved nutrition but decreased in response to elevated [CO_2]. These results suggest that changes in nitrogen availability and atmospheric [CO_2] may alter canopy structure, resulting in greater foliage retention and deeper crowns in loblolly pine forests. Fertilization increased foliar nitrogen concentration (N_M), but had no consistent effect on foliar leaf mass (W_A) or light-saturated net photosynthesis (A_{sat}). However, the correlation between A_{sat} and leaf nitrogen per unit area ($N_A = W_A N_M$) ranged from strong to weak depending on the time of year, possibly reflecting seasonal shifts in the form and pools of leaf nitrogen. Elevated [CO_2] had no effect on W_A, N_M or N_A, but increased A_{sat} on average by 82%. Elevated [CO_2] also increased photosynthetic quantum efficiency and lowered the light compensation point, but had no effect on the photosynthetic response to intercellular [CO_2], hence there was no acclimation to elevated [CO_2]. Daily photosynthetic photon flux density at the upper, middle and lower canopy position was 60, 54 and 33%, respectively, of full sun incident to the top of the canopy. Despite the relatively high light penetration, W_A, N_A, A_{sat} and R_d decreased with crown depth. Although growth enhancement in response to elevated [CO_2] was dependent on fertilization, [CO_2] by fertilization interactions and treatment by canopy position interactions generally had little effect on the physiological parameters measured.

Keywords: foliar nitrogen concentration, leaf area, net photosynthesis, respiration.

Introduction

Loblolly pine (*Pinus taeda* L.) is the dominant species in forests in the southeastern United States where it grows on a wide variety of soil types. The species is widely used in plantation forestry because it is highly plastic with respect to resource availability such as soil nutrition. Hence, stand production can be manipulated by forest management activities that affect resources (e.g., bedding, weed control and fertilization) (Schultz 1997). For this reason, there is much interest in understanding and predicting loblolly pine stand responses to changes in environmental conditions caused by forest management activities and changing climate.

The effects of elevated [CO_2] on growth and physiology of loblolly pine seedlings and trees have been studied by means of a variety of experimental protocols including: controlled environmental chambers (Tschaplinski et al. 1993, Will and Teskey 1997), greenhouse chambers (Larigauderie et al. 1994, Groninger et al. 1995, 1996), outdoor open-top chambers (Tissue et al. 1996), branch chambers (Liu and Teskey 1995, Murthy et al. 1996) and by stand-level Free Air Carbon Enrichment (FACE) (Ellsworth et al. 1995, Ellsworth 1999). These studies have yielded three important results: (1) the photosynthetic response to elevated [CO_2] is large, especially in upper-canopy, well-illuminated foliage; (2) elevated [CO_2] has little influence on stomatal conductance; and (3) elevated [CO_2] increases growth of seedlings, saplings, branches, trees and stands, but the responses are small compared with the photosynthetic responses.

Growth rate generally increases dramatically in response to fertilization, especially in young loblolly pine stands. Increased productivity in response to fertilization is primarily a function of canopy structure. Empirical data show a strong linear relationship between aboveground productivity and leaf area index (Vose and Allen 1988, Albaugh et al. 1998, Samuelson et al. 2001). Fertilization may also increase productivity by increasing photosynthetic efficiency (Linder 1987). Fertilization usually results in significant increases in foliar nitrogen concentration; however, its impact on photosynthetic rates of loblolly pine trees ranges from negligible (Teskey et al. 1994*a*, Gough 2000, Samuelson et al. 2001, Will et al. 2001) to average increases of 30% when trees are provided with "optimum" nutrition (Murthy et al. 1996). Understanding the effects of nitrogen on photosynthetic capacity is important because the functional photosynthesis (or carboxylation efficiency)/nitrogen relationship (Evans 1989) is used for modeling photosynthesis at many scales (McMurtrie et al. 1992, Woodward et al. 1995, Aber et al. 1996, Medlyn 1996).

The combined effects of nutrient availability and [CO_2] on growth and physiology in mature loblolly pine trees are poorly understood. Potential increases in stand productivity in response to elevated [CO_2] may be constrained by nutrient limitations (Groninger et al. 1999, Oren et al. 2001). Seedling studies generally show that gas exchange (Tissue et al. 1993, Thomas et al. 1994) and growth (Griffin et al. 1995) responses to elevated [CO_2] are correlated with soil nitrogen supply. However, seedlings possess morphological, phenological and physiological characteristics that differ from those of mature trees (Cregg et al. 1989, Hanson et al. 1994, Johnson and Ball 1996), making the prediction of field responses in mature trees, on the basis of extrapolation of seedling data, problematic. In large trees, photosynthetic acclimation to long-term exposure to elevated [CO_2] appears to be minimal (Liu and Teskey 1995, Ellsworth et al. 1998, Myers et al. 1999). In a branch chamber study on large trees, Murthy et al. (1996) and Murthy and Dougherty (1997) found that the effects of fertilization and elevated [CO_2] on branch growth and photosynthesis were additive. However, the use of branch chambers is controversial because the treatment of individual branches does not allow for within-canopy or whole-tree feedbacks as a result of resource limitations caused by enhanced tree growth in response to elevated [CO_2] (Ceulemans and Mousseau 1994, Marek and Kalina 1996).

Whole-tree exposures are needed to determine field responses of mature trees to elevated [CO_2] and to test whether canopy feedbacks on growth and physiological processes are important. We used whole-tree open-top chambers to expose 13-year-old loblolly pine trees, growing in soil with high or low nutrient availability, to elevated [CO_2] to examine how [CO_2], foliar nutrition and crown position affect branch growth, phenology and physiology. We hypothesized that growth and physiological responses to elevated [CO_2] are greater at high nutrient availability than at low nutrient availability and that the responses vary with light availability within the crown. At the time of physiological measurements, all foliage had developed under elevated [CO_2] conditions.

Materials and methods

Site description

The study site is a loblolly pine plantation (USDA Forest Service, Southeast Tree Research and Education Site (SETRES); 34°48′ N, 79°12′ W) growing on an infertile sandy soil (Wakulla series, USDA Soil Classification Series). The climate is mild. Mean annual, summer and winter temperatures are 17, 26 and 9 °C, respectively. Mean annual rainfall is 121 cm, evenly distributed throughout the year. The stand consists of a mix of 10 "improved" (first generation seed orchard) North Carolina Piedmont loblolly pine families planted in 1985 at 2 × 3 m spacing. Stand density is 1161 trees per hectare.

In 1992, a 2 × 2 factorial study using a combination of fertilization (no addition and complete nutrition) and irrigation (no addition and well watered) was begun. Treatments were established on 0.25-ha plots replicated four times. To achieve "optimum" nutrition, fertilizer was applied annually to attain a foliar nitrogen (N) concentration ([N]) of 1.3% with other macro- and micronutrients in balance; control foliar [N] was about 0.9% (Murthy et al. 1996, Albaugh et al. 1998). In 1997, at the beginning of our study, fertilized trees had significantly greater heights, diameters and live crown lengths than unfertilized trees (Table 1). Fertilized trees had nearly double the foliage biomass and leaf area of unfertilized trees.

Two trees in the control (no fertilization or irrigation) and fertilized-only plots from three of the treatment blocks (12 trees total) were selected for the [CO_2] experiment. Whole-tree, open-top chambers, 12 to 14 m high and 3 m in diameter, were built to enclose the entire aboveground portion of the tree. One whole-tree chamber in each treatment plot received ambient air only, whereas the other chamber was supplemented with CO_2 to maintain chamber [CO_2] at about 200 μmol mol^{-1} above ambient. Air and added CO_2 were mixed within the chamber by two plenum fans. The [CO_2] treatments began in August 1996 and ended in February 1999.

Three south-facing branches in each chamber, representing the lower canopy (branch initiated in 1992), middle canopy (1994 branch) and upper canopy (1996 branch) positions, were selected for measurement. Carbon dioxide and water vapor at each canopy position were measured every 30 min with an automated sampling system and an infrared gas analyzer (LI-6262, Li-Cor, Lincoln, NE). Photosynthetic photon flux density (PPFD) and temperature were measured at each position every 6 s and averaged over each hour with a data logger (CR-7, Campbell Scientific, Logan, UT). Temperature was measured with a copper-constantan thermocouple and PPFD was measured with photodiodes (G1118, Hamamatsu, Bridgewater, NJ) calibrated with a quantum sensor (Li-Cor LI-190).

Branch phenology

All branches were assessed for growth characteristics during 1997 and 1998. Branch diameter, shoot extension, and fascicle number and length for each flush were measured monthly. Fascicle length was measured on a random subsample of three fascicles from the mid-section of each flush. At the end of the experiment, branches were harvested and separated into woody and foliage components, dried at 65 °C and weighed.

Leaf mass per unit area (W_A; g m^{-2}) was calculated as the ratio of needle dry mass to total surface area. The surface area

Table 1. Canopy characteristics of 13-year-old loblolly pine stands after 5–6 years of fertilization.[1] Abbrevations: LC = lower third of crown; MC = middle third of crown; UC = upper third of crown; LAI = leaf area index; numbers in parentheses denote percent of total foliage biomass in each crown level.

Treatment	Tree height (m)	Live crown length (m)	Mean branch diameter (cm)	Number of branches	Canopy density[2]	Foliage biomass ($kg\ ha^{-1}$) LC	MC	UC	Total	Peak LAI[3]
Unfertilized	7.0	5.1	1.68	47.5	0.08	1417 (41)	1410 (41)	615 (18)	3442	1.4
Fertilized	8.8	6.5	2.18	42.9	0.14	1974 (30)	3823 (58)	850 (13)	6447	2.9

[1] Data provided by Tim Albaugh, North Carolina State University.

[2] Canopy density = LAI/live crown length.

[3] Measured in August 1997.

was calculated on 10 needles per plot × [CO_2] × canopy position combination based on needle length, diameter and taper. Needles were then oven-dried at 65 °C and weighed.

Needle chemistry

Total carbon and nitrogen were measured monthly on three randomly selected fascicles (Carlo Erba NA 1500 N, C, S elemental analyzer, Fisions Instruments, Danvers, MA). Foliar nitrogen was expressed on a mass (N_M) and an area (N_A) basis, where $N_A = W_A N_M$. Needle carbohydrates were analyzed by an enzymatic assay modified for pine tissue (Schoeneberger et al. 1992).

Gas exchange

Light-saturated net photosynthesis (A_{sat}) and leaf conductance (g_l) were measured in first flush foliage in January, March, April, June, July and October 1998 in 1-year-old foliage (1997 cohort) and in July and October 1998 and January 1999 in current-year foliage (1998 cohort). Measurements were made with an open-flow gas exchange system (Li-Cor LI-6400).

Photosynthetic responses to PPFD (A/PPFD) and intercellular CO_2 concentration (A/C_i) were evaluated for foliage at each canopy position on detached needles. Needles were cut from branches and the cut ends placed in water immediately. Preliminary measurements showed that net photosynthesis and stomatal conductance were stable for up to 1 h after foliage detachment. All measurements were made between 0900 and 1200 h. Cuvette temperatures were maintained at the current ambient temperature and averaged 18.8 °C in January 1998, 24.9 °C in April, 26.4 °C in July, 26.8 °C in September and 21.6 °C in January 1999. Vapor pressure deficit (VPD) at the leaf ranged from 1.2 to 1.5 kPa. For A/PPFD curves, cuvette [CO_2] was fixed at the growth [CO_2] (370 or 570 µmol mol^{-1}) and PPFD was decreased from 2000 to 0 µmol $m^{-2}\ s^{-1}$ in seven steps (2000, 1600, 1000, 600, 300, 100 and 0). The response curves were fitted to a non-rectangular hyperbola (Hanson et al. 1987) by nonlinear least squares. The equation was:

$$P_n = A_{sat}(1-(1-R_d/A_{sat})^{((1-\mathrm{PPFD})/\mathrm{LCP})}), \qquad (1)$$

where A_{sat} is light-saturated net photosynthesis, R_d is dark respiration and LCP is the light compensation point. Apparent quantum yield (Q) was estimated from the first derivative of Equation 1:

$$Q = (A_{sat}/R_d)(1-\mathrm{LCP}/A_{sat})\ln(1-\mathrm{LCP}/A_{sat}). \qquad (2)$$

The photosynthetic response to internal [CO_2] was examined over a range of external [CO_2] concentrations (150, 200, 280, 370, 570, 800 and 1000 µmol mol^{-1}). All measurements were made at a PPFD of 1600 µmol $m^{-2}\ s^{-1}$ and cuvette temperature and VPD conditions were similar to those used for the A/PPFD curves. Gas exchange variables were calculated with the Farquhar biochemical model of photosynthesis (Farquhar and von Caemmerer 1982; David Ellsworth, University of Michigan, personal communication).

Foliage respiration was measured at each canopy position four times (May, July, October 1998 and January 1999) on fully expanded first-flush needles. In May and July, measurements were made on 1-year-old foliage and in July, October and January, measurements were made on current-year needles (1998). Measurements were made on detached needles at 20 °C beginning 2 to 3 h after sundown with a Li-Cor LI-6400 photosynthesis system equipped with a needle chamber.

Statistical analysis

All variables were subjected to analysis of variance in a split-split-plot design with fertilization treatment as the main-plot, [CO_2] treatment as the sub-plot and canopy position as the sub-sub-plot. Main or interactive effects of treatments and canopy position were considered significant at $\alpha = 0.05$. Tukey's studentized range test at $\alpha = 0.05$ was used to separate treatment means.

Results

Chamber environment

The effect of elevated [CO_2] on seasonal trends in mean daily chamber [CO_2] was similar at all canopy positions (Figure 1a). Mean chamber [CO_2] (± standard deviation) for each canopy position in the ambient and elevated treatments, respectively,

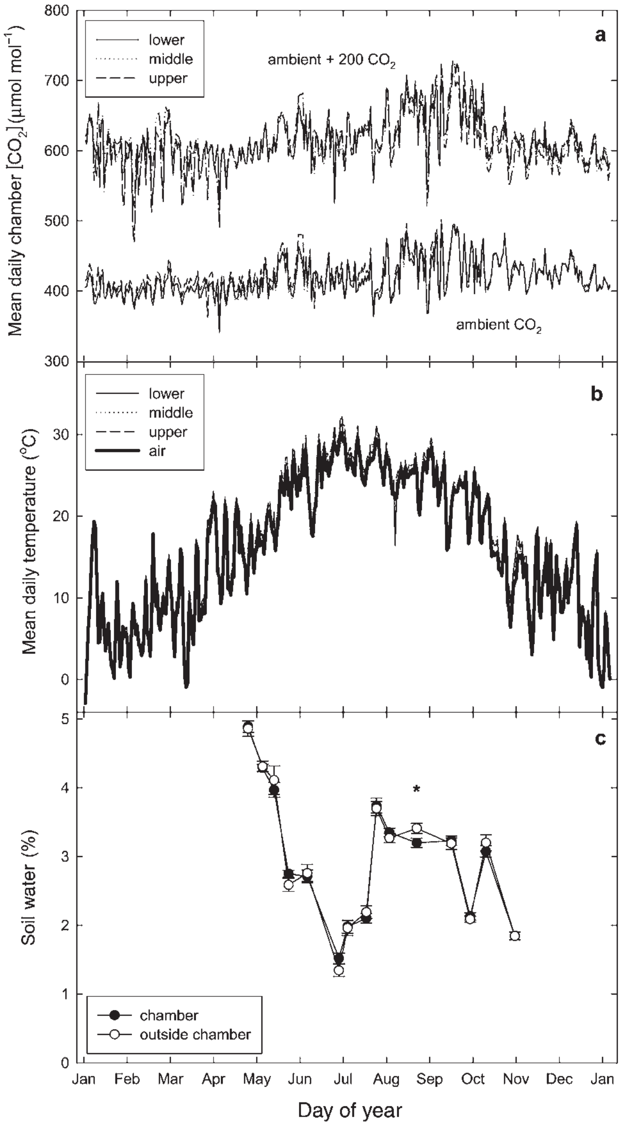

Figure 1. Seasonal course of mean daily chamber CO_2 concentration (a), air temperature (b) and per cent available soil water (c) (mean ± SE) in the upper 50-cm soil profile of field-grown 13-year-old loblolly pine trees enclosed in whole-tree, open-top chambers at the Southeast Tree Research and Education Site, Scotland County, North Carolina, USA.

Table 2. Mean daily photosynthetic photon flux density (mol m^{-2} day^{-1}) measured inside whole-tree, open-top chambers compared with above-canopy radiation in 13-year-old loblolly pine trees. Measurements are for January and July. Values are means ± 1 SE of $n = 16$ observations.

Position	January		July	
	Control	Fertilized	Control	Fertilized
Lower canopy	9.0 (2.1)	9.7 (2.3)	19.6 (1.0)	11.3 (4.1)
Middle canopy	14.8 (1.2)	13.4 (1.5)	30.3 (5.7)	24.7 (0.9)
Upper canopy	18.1 (1.4)	14.5 (1.9)	29.2 (6.8)	29.6 (4.9)
Above canopy	27.0		49.3	

was: lower 418 ± 26 and 619 ± 36, middle 416 ± 26 and 614 ± 41, upper 424 ± 29 and 606 ± 40. Environmental conditions were similar inside and outside the chambers. Mean daily air temperature was similar among canopy positions, and was within 2.5 °C of outside air temperature 99% of the time (Figure 1b). Mean hourly chamber air temperature was within 5 °C of outside air temperature 97% of the time. Soil water content inside the chambers was significantly lower than the plot average on only one sample date (Figure 1c). Mean daily PPFDs for January and July are given in Table 2. Daily PPFD at the upper, middle and lower canopy positions was 60, 54 and 33%, respectively, of full sun incident to the top of the canopy. The reduction in PPFD at each canopy level reflects the combined effects of the chamber wall and shading from overhead branches and surrounding trees.

Branch growth, phenology and structure

Fertilization increased branch diameter growth and number of flushes, whereas elevated [CO_2] had no effect on either variable (Table 3). Fertilization increased branch leaf area by 38% and elevated [CO_2] increased branch leaf area by 13%. The combined effects of fertilization and elevated [CO_2] were additive, increasing branch leaf area by 56% over that in unfertilized trees grown in ambient [CO_2]. Upper-canopy branches had significantly greater branch diameter growth, flush length, number of flushes, needle area and length and number of fascicles than lower-canopy branches. There were no significant treatment or treatment by canopy position interactions on branch diameter growth, number of flushes or leaf area. Because unfertilized, lower-canopy branches had only one flush, within-flush comparisons of growth attributes were limited to the first two flushes. Fertilization increased needle length in the first flush and fascicle number in the second flush, but had no effect on flush length (Table 3). In contrast, elevated [CO_2] increased flush length in both flushes and increased needle length and fascicle number, but only in the second flush. There was a significant fertilizer by canopy position interaction on needle length ($P = 0.013$), flush length ($P = 0.004$) and number of fascicles ($P = 0.015$) for the first flush. Flush density (needle area/shoot length) increased with fertilization and decreased with elevated [CO_2], but there were no canopy position or treatment interaction effects on flush density.

Neither fertilization nor [CO_2] treatment nor their interaction had a significant effect on leaf mass per unit area (W_A) (data not shown). However, W_A was significantly greater in upper-canopy foliage than in lower-canopy foliage (8 to 19% depending on time of year, Figure 2). Seasonal variations in W_A within a canopy layer were larger than vertical variations (15 to 25%) and W_A was always greater in 1-year-old foliage than in current-year foliage.

Foliar chemistry

Foliar N concentration on a dry mass basis (N_M) was 17 to 41% greater in fertilized trees than in unfertilized control trees (Figure 3) depending on time of year; however, these fertilizer ef-

Table 3. Mean shoot growth characteristics for loblolly pine branches in 1998. Measurements were made at three canopy positions on branches initiated in 1992 (lower), 1994 (middle) and 1996 (upper). Means within a treatment followed by a different letter are significantly different at α = 0.05. Values in columns for needle length, flush length and density refer to the first or second growth flush. Flush density was calculated as the ratio of flush leaf area and flush length.

Treatment/ canopy position	Diameter (mm)	Flush (no.)	Total leaf area (cm^{-2})	Needle length (cm)		Flush length (cm)		Fascicle no.		Flush density (cm^2 cm^{-1})	
				1	2	1	2	1	2	1	2
Unfertilized	3.0 a	1.7 a	1467.0 a	13.6 a	6.6 a	188.7 a	64.8 a	100.7 a	30.5 a	5.7 a	4.5 a
Fertilized	3.3 b	2.2 b	2031.5 b	15.6 b	8.8 a	200.2 a	55.4 a	110.1 a	40.2 b	7.3 b	9.3 b
Ambient [CO_2]	2.7 a	1.9 a	1640.5 a	15.2 a	6.6 a	169.8 a	41.9 a	99.6 a	25.6 a	7.2 a	7.6 a
Elevated [CO_2]	3.7 a	2.1 a	1858.0 b	14.0 a	8.8 b	219.1 b	78.3 b	111.1 a	45.1 b	5.8 b	6.5 a
Upper	6.2 a	2.4 a	2625.0 a	14.5 ab	12.1 a	279.9 a	94.3 a	157.7 a	58.3 a	6.4 a	7.5 a
Middle	3.2 b	2.1 a	1930.8 b	15.2 b	10.4 a	220.8 b	81.8 a	105.3 b	43.8 a	6.0 a	6.6 a
Lower	0.8 c	1.4 b	692.0 c	14.0 a	0.6 b	82.8 c	4.1 b	53.2 c	3.9 b	7.0 a	4.2 a

fects were not always significant at α = 0.05. Fertilizer effects on foliar N concentration on an area basis ($N_A = W_A N_M$) were similar to those on N_M because fertilization treatment had no effect on W_A. Elevated [CO_2] had no significant effect on N_M or N_A and there were no significant treatment interactions. Mean (± SE) foliar N_M was 9.56 ± 0.28, 9.66 ± 0.32, 12.04 ± 0.37 and 11.88 ± 0.34 mg g^{-1} for trees in the unfertilized + ambient [CO_2], unfertilized + elevated [CO_2], fertilized + ambient

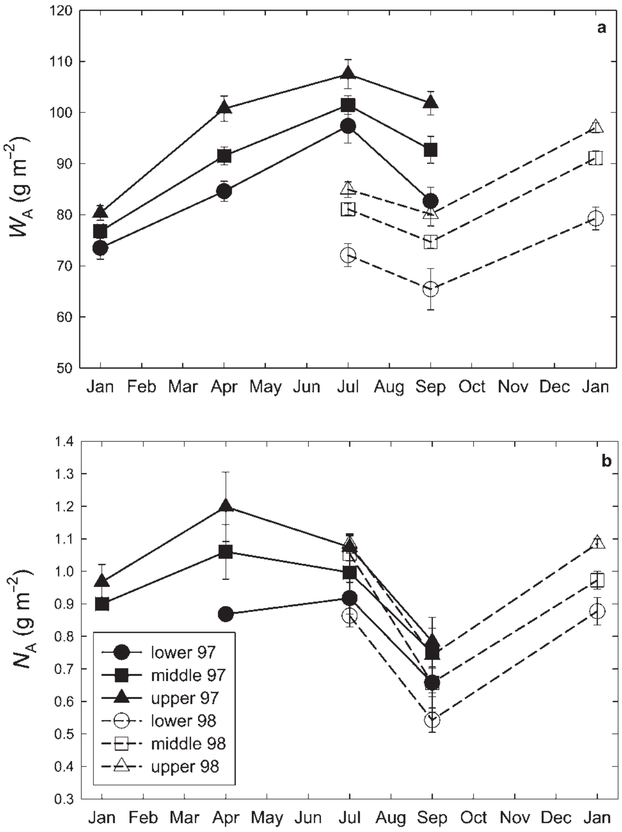

Figure 2. Seasonal changes in (a) leaf mass per unit area (W_A) and (b) foliar nitrogen concentration per unit area (N_A) for first flush 1-year-old (1997) and current-year (1998) foliage growing on branches initiated in 1992 (lower), 1994 (middle) and 1996 (upper) of 13-year-old loblolly pine trees. Current-year foliage (1998) was 95% fully expanded in July. Values are means ± 1 SE of n = 3 observations and represent the mean across fertilization and [CO_2] treatments.

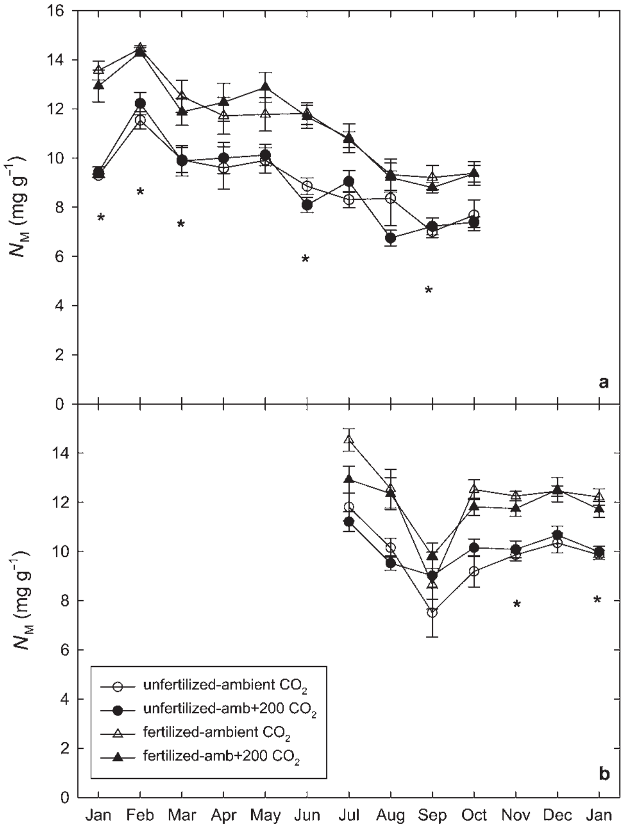

Figure 3. Seasonal trends in foliar nitrogen concentration (N_M) for first flush (a) 1-year-old (1997) and (b) current-year (1998) foliage of 13-year-old loblolly pine trees. Values are means ± 1 SE of n = 3 observations. An asterisk indicates a significant difference at α = 0.05 between the mean of the unfertilized and fertilized treatments.

[CO_2] and fertilized + elevated [CO_2] treatments, respectively. Canopy position had no effect on N_M and there were no treatment by canopy position interactions (data not shown). However, because of spatial differences in W_A, N_A varied throughout the crown and was significantly greater in upper-canopy branches than in lower-canopy branches (Figure 2b). Foliar N_A was also significantly higher in 1-year-old foliage than in current-year foliage.

Fertilization had little effect on foliar starch or soluble sugar concentration except in July and September, when it increased soluble sugars in 1-year-old foliage (Table 4). Elevated [CO_2] tended to increase starch and soluble sugar concentrations; however, these differences were significant only in September (both age classes) and for soluble sugars in July in current-year foliage. There was no significant fertilization by [CO_2] interaction for either variable except in July, when there was a slight interaction for soluble sugars ($P = 0.047$) in current-year needles. Starch and soluble sugars varied with crown position. Starch was significantly higher in the upper and middle crown than in the lower crown in July in current-year needles and in September in both age classes. Upper-canopy foliage always had significantly higher soluble sugar concentration than lower-canopy foliage. There were significant fertilization by position ($P = 0.037$), [CO_2] by position ($P = 0.046$) and fertilization by [CO_2] by position ($P = 0.020$) interactions in September for soluble sugars in current-year foliage.

Gas exchange

Fertilization did not significantly affect light-saturated net photosynthesis (A_{sat}), except in January in 1-year-old foliage (Table 5). Elevated [CO_2] significantly increased A_{sat} on all measurement dates except in 1-year-old foliage in September. Neither fertilization nor [CO_2] treatment had a significant effect on leaf conductance (g_l) or C_i/C_a (data not shown). Within a canopy position and fertilization treatment, the [CO_2] enhancement ratio, calculated by dividing A_{sat} of needles in the elevated [CO_2] treatment by A_{sat} of needles in the ambient [CO_2] treatment, varied between 1.2 and 2.5 with an overall mean of 1.82 ± 0.06 (Figure 4). There were no significant fertilizer, canopy position or season effects on the [CO_2] enhancement ratio. There was no significant fertilization by [CO_2] treatment interaction for any gas exchange variable, except for A_{sat} in 1-year-old foliage in June (Table 5, Figure 5a) and in current-year foliage in July and September (Table 5, Figures 5b and 5c)—at these times, elevated [CO_2] increased A_{sat} 93–127% in fertilized trees and 37–45% in unfertilized trees.

Upper-canopy branches always had significantly greater A_{sat} than lower-canopy branches (Figure 6a), with the largest difference occurring in April, coinciding with maximum A_{sat} and N_A. Leaf conductance was higher in the upper canopy than in the lower canopy; however, differences were smaller than for A_{sat} (Figure 6b). Canopy position generally did not affect C_i/C_a (Figure 6c). There was no significant treatment by canopy position interaction effect on A_{sat}, g_l or C_i/C_a at any time. Maximum A_{sat} and g_l occurred in April, although winter A_{sat} was 70% of the seasonal maximum. Seasonal variation in mean C_i/C_a ranged between 0.45 and 0.80 and averaged 0.65. In 1-year-old foliage, C_i/C_a was lowest in June and highest in July.

Table 4. Starch and soluble sugar concentrations of first flush 1-year-old (1997) and current-year (1998) foliage in 13-year-old loblolly pine trees. Measurements were made at three canopy positions on branches initiated in 1992 (lower), 1994 (middle) and 1996 (upper). Needles were collected between 0800 and 1200 h. Means within a treatment followed by a different letter are significantly different at $\alpha = 0.05$. Abbreviation: na = data not available.

Treatment/ canopy position	One-year-old foliage				Current-year foliage		
	January	April	July	September	July	September	January
Starch (mg g^{-1})							
Unfertilized	5.79 a	88.2 a	9.3 a	30.7 a	12.9 a	16.5 a	2.82 a
Fertilized	7.67 a	89.9 a	11.9 a	30.7 a	10.6 a	13.7 a	3.42 a
Ambient [CO_2]	5.78 a	86.6 a	5.96 a	18.1 a	7.0 a	8.2 a	2.34 a
Elevated [CO_2]	7.68 a	91.5 a	15.22 a	41.2 b	16.8 a	21.9 b	3.91 a
Upper	7.50 a	92.3 a	11.5 a	40.6 a	17.7 a	20.0 a	3.38 a
Middle	5.96 a	85.8 a	13.5 a	28.4 ab	10.0 ab	16.0 ab	3.18 a
Lower	na	na	6.8 a	23.0 b	7.9 b	9.1 b	2.81 a
Soluble sugar (mg g^{-1})							
Unfertilized	87.4 a	62.9 a	53.2 a	56.3 a	59.1 a	54.6 a	94.4 a
Fertilized	99.6 a	71.9 a	65.9 b	77.2 b	62.0 a	61.8 a	98.4 a
Ambient [CO_2]	89.8 a	66.8 a	52.3 a	55.4 a	54.1 a	51.6 a	96.1 a
Elevated [CO_2]	97.2 a	68.0 a	66.8 a	74.5 b	67.0 b	64.8 b	96.7 a
Upper	94.8 a	68.8 a	65.8 a	74.2 a	66.4 a	64.6 a	101.9 a
Middle	92.2 a	66.0 a	60.6 ab	61.2 b	60.6 b	59.3 a	97.2 ab
Lower	na	na	52.2 b	62.0 b	54.7 c	50.7 b	90.1 b

Table 5. Analysis of variance (*P*-values) for measurements of light-saturated net photosynthesis (A_{sat}) in 1-year-old (1997) and current-year (1998) foliage of 13-year-old loblolly pine trees. Abbreviations: F = fertilization treatment: unfertilized and fertilized; C = [CO_2] treatment: ambient [CO_2] and ambient + 200 µmol mol^{-1} CO_2; P = canopy position: lower, middle and upper.

Source		One-year-old foliage						Current-year foliage		
	df	Jan	Mar	Apr	Jun	Jul	Sep	Jul	Sep	Jan
F	1	0.015	0.449	0.999	0.734	0.121	0.564	0.182	0.203	0.496
C	1	0.009	0.031	0.001	0.001	0.038	0.057	0.001	0.001	0.032
F × C	1	0.870	0.642	0.796	0.018	0.969	0.298	0.047	0.023	0.926
P	2	0.011	0.013	< 0.001	0.003	0.018	0.045	0.002	0.184	0.029
F × P	2	0.656	0.679	0.282	0.433	0.951	0.767	0.908	0.437	0.688
C × P	2	0.687	0.848	0.474	0.366	0.934	0.405	0.441	0.398	0.443
F × C × P	2	0.102	0.761	0.390	0.830	0.378	0.827	0.876	0.486	0.819

Neither fertilizer nor elevated [CO_2] significantly affected foliage dark respiration (R_d) (Table 6). However, R_d of 1-year-old and current-year foliage was 19 and 27% higher, respectively, in the upper canopy than in the lower canopy.

Relationship between A_{sat} and N_A

Across growing seasons, A_{sat} was correlated with N_A in 1-year-old foliage (ambient [CO_2]: $r^2 = 0.45$, $P < 0.01$, $n = 22$; elevated [CO_2]: $r^2 = 0.41$, $P < 0.01$, $n = 22$), but not in current-year foliage (ambient: $P = 0.72$; elevated: $P = 0.52$). Within a growing season, the strength of the relationship between A_{sat} and N_A varied with time and treatment (Figure 7). For example, in 1-year-old needles grown in ambient [CO_2], A_{sat} was strongly correlated with N_A in January (Figure 7a), but not in July. In September, when current-year foliage growth had ceased, A_{sat} in 1-year-old foliage was again correlated with N_A. In foliage grown in elevated [CO_2], A_{sat} was significantly correlated with N_A only in January (Figure 7b). In current-year needles, A_{sat} was significantly related to N_A in July and Sep-

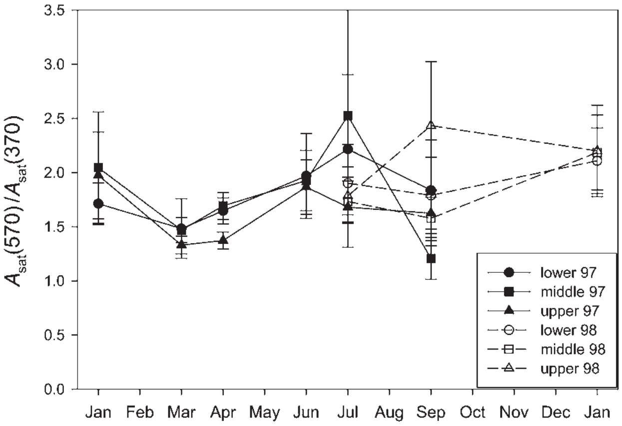

Figure 4. Seasonal changes in the [CO_2] enhancement ratio for 1-year-old (1997) and current-year (1998) foliage, by canopy position, of 13-year-old loblolly pine trees. The enhancement ratio is the ratio of light-saturated net photosynthesis (A_{sat}; µmol m^{-2} s^{-1}) of foliage grown and measured at 570 µmol mol^{-1} to that of foliage grown and measured at 370 µmol mol^{-1}. Values are means ± 1 SE of $n = 3$ observations and include both unfertilized and fertilized treatments.

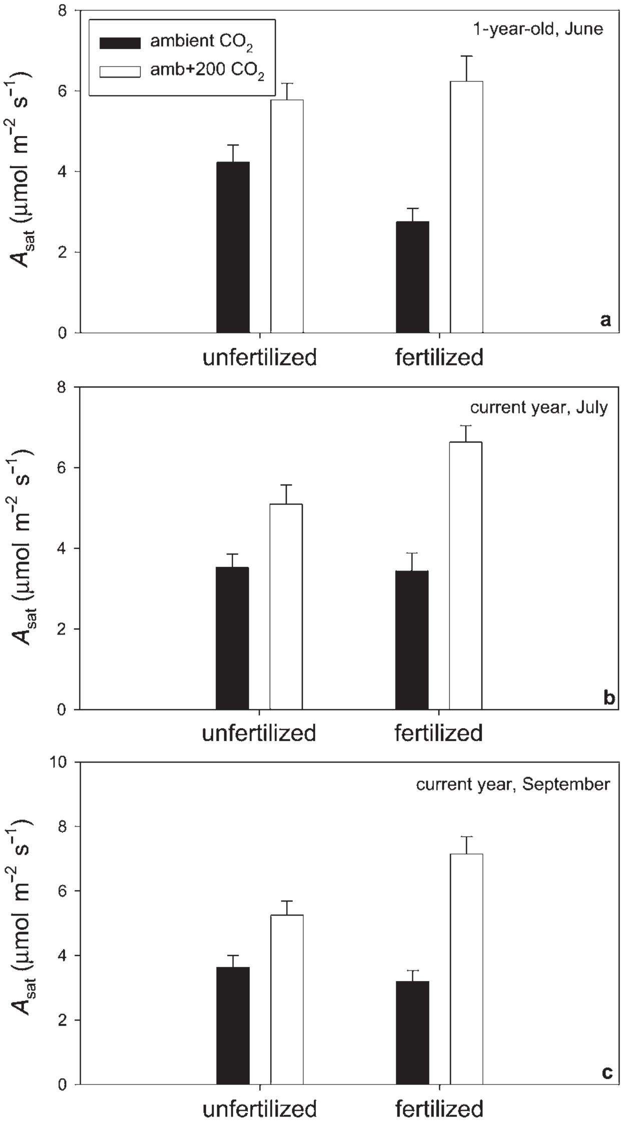

Figure 5. Light-saturated net photosynthesis (A_{sat}) as a function of treatment for 1-year-old foliage (1997) measured in June (a) and for current-year foliage (1998) measured in July (b) and September (c). Values are means ± 1 SE of $n = 3$ observations. Measurement conditions: PPFD =1600 µmol m^{-2} s^{-1}; chamber temperature = 26.5 °C; and vapor pressure deficit ≤ 1.5 kPa.

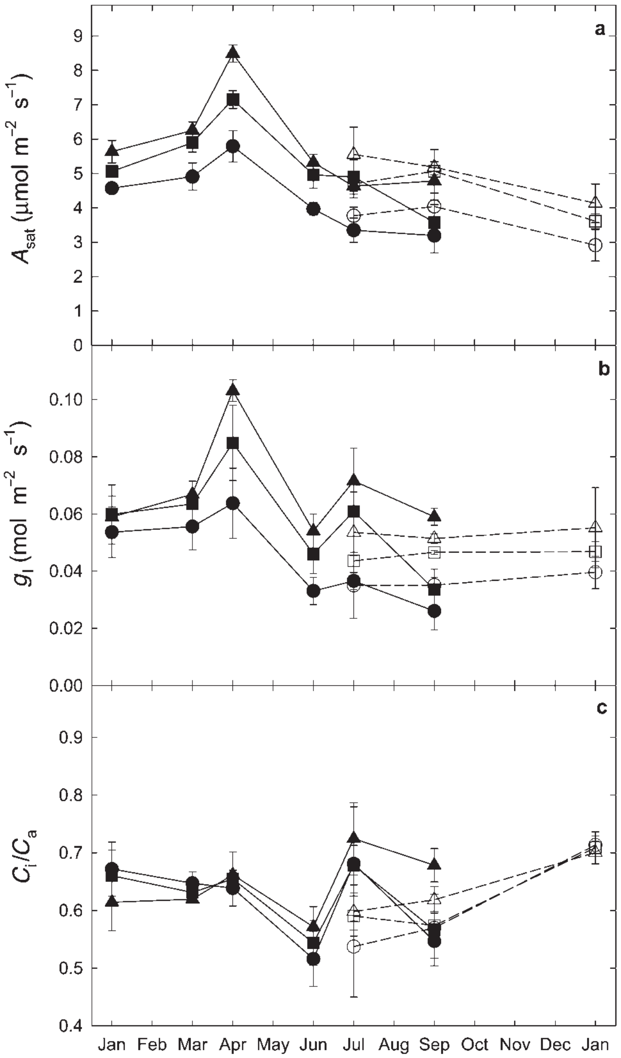

Figure 6. Seasonal changes in (a) light-saturated net photosynthesis (A_{sat}, measured at PPFD = 1600 μmol m^{-2} s^{-1}), (b) leaf conductance (g_l) and (c) the C_i/C_a ratio at A_{sat} for first flush 1-year-old (1997) and current-year (1998) foliage growing on branches initiated in 1992 (lower), 1994 (middle) and 1996 (upper). Values are means ± 1 SE of n = 3 observations and represent the mean across fertilization and [CO$_2$] treatments. Symbols: ● = lower, 1997; ■ = middle, 1997; ▲ = upper, 1997; ○ = lower, 1998; □ = middle, 1998; and △ = upper, 1998.

tember, but not in January, in foliage grown in ambient [CO$_2$] (Figure 7c), and in July and January, but not September, in foliage grown in elevated [CO$_2$] (Figure 7d). Within a growing season, A_{sat} of foliage grown in elevated [CO$_2$] was significantly higher for a given foliar N_A than that of foliage grown at ambient [CO$_2$].

Response curves

Equation 1 accounted for 80 to 95% of the variance in A/PPFD across all treatments. For this analysis, R_d was fixed at 0.35 μmol m^{-2} s^{-1}. Fertilizer generally increased modeled A_{sat}, but the increase was significant only in January for 1-year-old foliage and in October and January for current-year foliage (Table 7). During these periods, fertilization increased modeled A_{sat} by 14 to 23%. Fertilization generally had no effect on LCP or Q. Elevated [CO$_2$] almost always increased modeled A_{sat} and decreased LCP on three of the six sample dates, and increased Q on four of the six dates. Averaged across all dates, elevated [CO$_2$] increased modeled A_{sat} by 66 and 49%, decreased LCP by 16 and 29% and increased Q by 13 and 33% in 1-year-old and current-year foliage, respectively. There was no significant fertilization by [CO$_2$] treatment interaction for any A/PPFD variable. When both fertilization and elevated [CO$_2$] significantly increased modeled A_{sat} (1-year-old foliage in January, current-year foliage in September and January), the combined effect was additive, resulting in a doubling of modeled A_{sat} compared with unfertilized trees grown in ambient [CO$_2$]. The A/PPFD response varied within the crown. Upper- and middle-canopy foliage had significantly higher modeled A_{sat} than lower-canopy foliage. Canopy position generally had no effect on LCP or Q and there were no significant treatment by position interactions for any variable.

Table 6. Foliar dark respiration rates (R_d, μmol m^{-2} s^{-1}) for 1-year-old (1997) and current-year (1998) foliage of 13-year-old loblolly pine trees. Measurements were made at three canopy positions on branches initiated in 1992 (lower), 1994 (middle) and 1996 (upper). Within a treatment comparison, different letters following values indicate a significant difference at $\alpha = 0.05$. Analyses were performed on measurements made in April and July for 1-year-old foliage (1997) and July, October and January for current-year foliage (1998).

Treatment/ canopy position	One-year-old foliage	Current-year foliage
Unfertilized	0.32 a	0.33 a
Fertilized	0.36 a	0.35 a
Ambient [CO$_2$]	0.36 a	0.36 a
Elevated [CO$_2$]	0.32 a	0.33 a
Upper	0.38 a	0.38 a
Middle	0.33 b	0.34 b
Lower	0.32 b	0.30 c

The A/C_i curve analyses are summarized in Table 8. There were no significant fertilization or [CO$_2$] treatment effects on either V_{cmax} or J_{max}, except in 1-year-old foliage in January when both fertilizer and elevated [CO$_2$] treatments increased V_{cmax}. Because W_A did not vary with fertilization or [CO$_2$] treatment, V_{cmax} and J_{max} on a mass basis also did not change. Canopy position did not significantly affect either V_{cmax} or J_{max}, except in newly expanded current-year foliage in July when upper-canopy branches had significantly higher V_{cmax} and J_{max} than middle- and lower-canopy branches. Values of V_{cmax} ranged between 22.0 and 50.0 μmol m^{-2} s^{-1} and values of J_{max} ranged between 45.0 and 90.0 μmol m^{-2} s^{-1}. Both V_{cmax} and J_{max} were significantly higher in current-year foliage than in 1-year-old foliage in July ($P < 0.01$), but not in September ($P = 0.25$). There was no significant treatment or treatment by canopy position interaction effect on V_{cmax} or J_{max}.

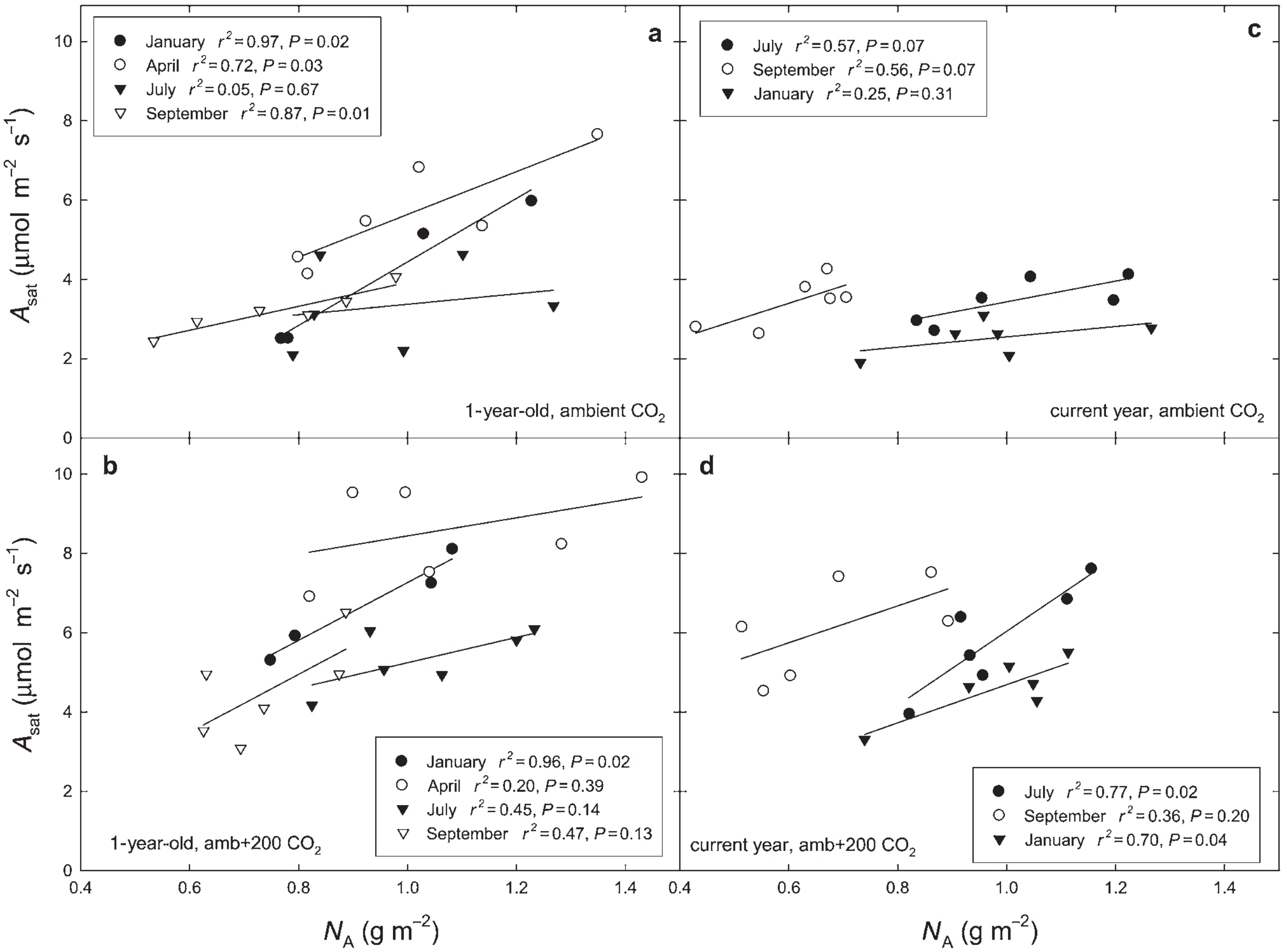

Figure 7. Relationship between light-saturated net photosynthesis (A_{sat}) and leaf nitrogen concentration (N_A) at different times of the year for first flush 1-year-old (1997) (a and b) and current-year (1998) (c and d) foliage in ambient [CO$_2$] (a and c) and elevated (ambient + 200 μmol mol^{-1}) [CO$_2$] (b and d). Data for each curve are pooled across unfertilized and fertilized treatments. Values are the means of three observations.

Discussion

We used whole-tree, open-top chambers to expose the aboveground portions of large loblolly pine trees to elevated [CO$_2$]. Compared with the branch chamber approach (Liu and Teskey 1995, Teskey 1995, Murthy et al. 1996), whole-tree open-top chambers allow exposure of the whole canopy to elevated [CO$_2$], thereby eliminating possible source–sink imbalances associated with exposing only a portion of the canopy to elevated [CO$_2$] (Groninger et al. 1999). Despite these advantages, open-top chambers may alter the canopy microenvironment with respect to temperature, humidity, light and wind. In our study, there was little difference in soil water availability between the inside and outside of the chamber. Outside and chamber air temperatures were also similar. We did not measure PPFD at similar canopy positions outside the chambers, so we do not know the absolute reduction in available light due to the chamber walls at these positions. However, W_A and the A/PPFD and A/C_i parameters for foliage at mid-canopy inside and outside of the chambers were similar, indicating that chamber foliage did not acclimate significantly to the chamber light conditions. Tree root systems extended well beyond the perimeter of the chambers and were not directly exposed to elevated [CO$_2$], thus we could not evaluate root responses (but see Saxe et al. 1998, Matamala and Schlesinger 2000, Andrews and Schlesinger 2001).

Changes in branch architecture (needle length, fascicle number and flush length) in trees grown in elevated [CO$_2$] were similar to those measured in branch chambers (Teskey 1995, Murthy and Dougherty 1997). Fertilization and elevated [CO$_2$] increased branch leaf area and the combined treatments were additive. Generally, the effect of elevated [CO$_2$] on flush length was comparable to or greater than the effect of fertilization, but fertilization and elevated [CO$_2$] differentially affected other branch characteristics. Foliage growth response to elevated [CO$_2$] was less than the response to nutrient amendments. Elevated [CO$_2$] increased needle number per branch but had either no effect or slightly decreased internode length. Foliage density, defined as leaf area per unit stem length, tended to increase in response to fertilization and decrease in response to elevated [CO$_2$].

Table 7. Photosynthetic parameters derived from A/PPFD curves (Equations 1 and 2). Measurements were made at three canopy positions on branches initiated in 1992 (lower), 1994 (middle) and 1996 (upper). Means within a treatment followed by a different letter are significantly different at $\alpha = 0.05$. See text for measurement conditions.

Treatment/canopy position	One-year-old foliage				Current-year foliage		
	January	April	July	September	July	September	January
Light saturated net photosynthesis (modeled A_{sat})							
Unfertilized	4.49 a	7.45 a	3.74 a	4.32 a	5.01 a	4.97 a	3.25 a
Fertilized	5.52 b	8.04 a	4.09 a	5.45 a	4.98 a	5.99 b	3.72 b
Ambient [CO_2]	3.84 a	6.36 a	2.72 a	3.51 a	3.74 a	3.96 a	2.74 a
Elevated [CO_2]	6.18 b	9.13 b	5.10 b	5.95 a	7.00 b	4.23 b	4.23 b
Upper	4.99 ab	8.58 a	4.75 a	5.80 a	5.50 a	6.45 a	3.45 ac
Middle	6.42 b	8.21 a	4.20 a	4.88 b	5.49 a	5.93 a	4.09 ab
Lower	4.62 a	6.44 b	2.78 b	3.53 c	3.99 b	4.07 b	2.91 c
Light compensation point (LCP)							
Unfertilized	14.9 a	20.0 a	35.9 a	32.4 a	24.1 a	31.2 a	35.3 a
Fertilized	16.9 a	20.5 a	47.6 a	36.9 a	21.8 a	29.9 a	36.1 a
Ambient [CO_2]	16.8 a	21.2 a	42.6 a	41.1 a	24.6 a	37.1 a	42.1 a
Elevated [CO_2]	15.1 b	19.3 a	40.8 a	28.4 a	21.2 a	24.0 b	29.4 b
Upper	16.5 a	19.6 a	62.7 a	29.9 a	24.1 a	26.9 a	36.7 a
Middle	16.0 a	20.7 a	23.8 b	28.8 a	21.9 a	28.0 a	31.0 a
Lower	15.4 a	20.5 a	38.7 ab	45.9 a	22.6 a	36.8 b	39.5 a
Apparent quantum yield (Q)							
Unfertilized	0.025 a	0.018 a	0.015 a	0.012 a	0.016 a	0.013 a	0.012 a
Fertilized	0.022 a	0.018 a	0.012 a	0.012 a	0.018 a	0.012 a	0.011 a
Ambient [CO_2]	0.022 a	0.017 a	0.012 a	0.010 a	0.015 a	0.011 a	0.010 a
Elevated [CO_2]	0.025 b	0.019 a	0.014 a	0.014 b	0.018 a	0.015 b	0.013 b
Upper	0.023 a	0.019 a	0.010 ab	0.012 a	0.016 a	0.014 a	0.011 a
Middle	0.023 a	0.017 a	0.017 a	0.013 a	0.018 a	0.014 a	0.012 a
Lower	0.025 a	0.018 a	0.013 b	0.011 a	0.017 a	0.011 b	0.011 a

Table 8. Photosynthetic parameters derived from A/C_i response curves using the Farquhar biochemical model (Farquhar and von Caemmerer 1982). Measurements were made at three canopy positions on branches initiated in 1992 (lower), 1994 (middle) and 1996 (upper). Means within a treatment followed by a different letter are significantly different at $\alpha = 0.05$. See text for measurement conditions.

Treatment/canopy position	One-year-old foliage				Current-year foliage		
	January	April	July	September	July	September	January
Maximum carboxylation rate (V_{cmax})							
Unfertilized	26.8 a	41.0 a	32.8 a	43.5 a	42.7 a	47.0 a	31.3 a
Fertilized	33.4 b	41.3 a	37.3 a	43.7 a	47.1 a	48.6 a	41.0 a
Ambient [CO_2]	27.1 a	41.0 a	33.8 a	42.4 a	46.4 a	44.9 a	37.4 a
Elevated [CO_2]	32.8 b	41.2 a	36.7 a	44.7 a	43.3 a	50.7 a	35.0 a
Upper	31.9 a	43.2 a	32.6 a	40.0 a	52.3 a	50.7 a	40.1 a
Middle	28.4 a	42.2 a	36.2 a	42.5 a	40.6 b	46.1 a	34.9 a
Lower	29.5 a	38.1 a	33.9 a	53.4 a	41.7 b	46.5 a	33.5 a
Maximum electron transport (J_{max})							
Unfertilized	46.5 a	62.8 a	51.4 a	70.5 a	65.6 a	78.5 a	57.2 a
Fertilized	53.5 a	63.4 a	59.1 a	72.2 a	73.5 a	81.0 a	72.0 a
Ambient [CO_2]	48.3 a	60.3 a	53.6 a	70.7 a	72.2 a	78.8 a	69.5 a
Elevated [CO_2]	51.6 a	65.8 a	57.5 a	71.8 a	66.9 a	80.6 a	59.7 a
Upper	52.7 a	67.2 a	50.3 a	69.2 a	83.2 a	86.8 a	70.4 a
Middle	49.4 a	65.5 a	53.6 a	70.1 a	63.1 b	75.5 a	63.6 a
Lower	47.7 a	61.7 a	53.1 a	85.2 a	62.3 b	76.9 a	59.8 a

Neither fertilization nor [CO_2] treatment altered W_A. Murthy and Dougherty (1997), who used branch chambers on trees in the same stand, measured a small but significant increase in W_A (~ 5%) in fertilized trees, but found that elevated [CO_2] had no effect. In other studies, elevated [CO_2] significantly increased W_A in loblolly pine seedlings (Tissue et al. 1993) and large trees (Ellsworth et al. 1998). Generally, elevated [CO_2] significantly reduces mass-based foliar nitrogen concentration (N_M) (Drake and Gonzalez-Meler 1996), which may have important consequences for long-term growth and carbon allocation (Curtis and Wang 1998). In our study, elevated [CO_2] had no effect on seasonal patterns of foliar N_M in either foliage age class. There was also no [CO_2]-induced dilution of foliar nitrogen on a mass (N_M) or area (N_A) basis, probably because roots extended well past the chamber edges and competed for nutrients with trees grown in ambient [CO_2] (Kurt Johnsen, unpublished data). Effects of elevated [CO_2] on foliar nitrogen in other studies vary. Tissue et al. (1993) reported 30% reductions in N_M in seedlings grown in elevated [CO_2] in open-top chambers. Teskey (1995), using branch chambers, reported no [CO_2]-induced dilution of foliar [N], whereas Murthy et al. (1996), in a similar experiment, found a 10% decrease in [N] in response to elevated [CO_2]. Ellsworth et al. (1998) also reported a 10% reduction in needle [N] in FACE-exposed loblolly trees.

In our study, fertilization caused a large increase in growth, but its effect on leaf physiology was less clear. Fertilization rarely had a significant effect on A_{sat}. However, the A/PPFD analysis indicated that modeled A_{sat} was higher in fertilized foliage than in unfertilized foliage. When data were pooled across the year, fertilization significantly increased modeled A_{sat} by 15 and 12% in 1-year-old and current-year foliage, respectively. Reports on the impact of fertilization on photosynthetic rates of loblolly pine are mixed. Murthy et al. (1996), who studied trees in the same stand as our study, measured a 20% increase in A_{sat} 2 years after the fertilization treatments began; however, in a follow-up study 4 years later, Gough (2000) found no effect of fertilization. Samuelson et al. (2001) and Will et al. (2001) also reported that fertilization had no effect on photosynthetic rates of loblolly pine despite relatively large differences in foliar [N]. However, in both of these studies, foliar [N] of unfertilized trees was ≥ 1.3%, which is similar to the foliar [N] of our fertilized trees. Fertilization-induced increases in net photosynthesis in loblolly pine trees will likely occur only on nutrient-poor soils where fertilization increases leaf nitrogen concentration 30% or more (Fife and Nambiar 1997, Samuelson et al. 2001), and may be ephemeral or seasonal in nature. The lack of a consistent fertilizer effect on A_{sat} may be associated with seasonal variation in the A_{sat}/N_A relationship. Variation in the slope of the A_{sat}/N_A relationship may reflect seasonal shifts in foliar N partitioning between photosynthetic proteins and forms that are more easily transported (e.g., amino acids) to growing sinks. For example, in July there was a weak or nonsignificant correlation between A_{sat} and N_A in 1-year-old foliage (97 cohort), whereas A_{sat} in current-year foliage (98 cohort) was significantly correlated with N_A. This difference would be consistent with developing foliage investing most assimilated nitrogen in chlorophyll and photosynthetic proteins. Changes in N nutrition may alter nitrogen partitioning among cellular components. Nitrogen in excess of that needed for photosynthetic biochemistry is likely stored as amino acids (Yoder et al. 1994). Keeping N in a mobile form would support subsequent flushes during the growing season, because loblolly pine is opportunistic and can have 2–4 flushes per year depending on soil resources and environmental conditions. Fertilization had no effect on foliage dark respiration rates (R_d), and R_d was not significantly correlated with N_A or N_M. We previously found that fertilization increases coarse root, branch and stem respiration (Maier et al. 1998, Maier 2001), but has no effect on fine root respiration (Maier 2000).

Effects of elevated [CO_2] on leaf physiological characteristics were consistent with other data for large loblolly pine trees after long-term exposure to elevated [CO_2] (Ellsworth et al. 1995, Liu and Teskey 1995, Teskey 1995, Murthy et al. 1996, Ellsworth et al. 1998, Ellsworth 1999, Myers et al. 1999). Elevated [CO_2] increased modeled A_{sat}, decreased LCP and increased quantum efficiency. Mean photosynthetic enhancement by [CO_2] enrichment was 82%, which is higher than the 50–60% enhancement measured in similar aged loblolly pine trees exposed by FACE to a similar elevated [CO_2] treatment (ambient + 200 μmol mol^{-1}) (Ellsworth 1999, Myers et al. 1999), but falls within the range reported by Liu and Teskey (1995) and Murthy et al. (1996). Theory and empirical evidence suggest that the stimulatory effects of elevated [CO_2] on net photosynthesis will increase with temperature (Drake and Gonzalez-Meler 1996). We detected no seasonal variation in the enhancement ratio, perhaps because of the broad photosynthetic temperature optimum for this species (Teskey et al. 1994*a*); however, photosynthetic enhancement by elevated [CO_2] was strongly temperature-dependent in loblolly pine saplings (Lewis et al. 1996) and large trees (Myers et al. 1999). Based on a series of studies, Groninger et al. (1999) concluded that loblolly pine exhibits minimal photosynthetic acclimation to elevated [CO_2]. Similarly, we found no significant downward acclimation to elevated [CO_2] in the capacity for [CO_2] carboxylation (V_{cmax}) or maximum electron transport (J_{max}) despite the large differences in soil nitrogen availability. The absence of an acclimation response may be partially explained by the lack of a [CO_2] effect on foliar [N] and the negligible effect on foliar nonstructural carbohydrates. Elevated [CO_2] had no effect on g_l (Liu and Teskey 1995, Murthy et al. 1996, Ellsworth 1999) or C_i/C_a. Mean C_i/C_a was 0.67, which is similar to the values reported by Liu and Teskey (1995) (0.58–0.65) and Ellsworth et al. (1995) (0.67). We observed some seasonal variation in C_i/C_a that was probably associated with a differential effect of increased soil water content on g_l and A_{sat} (Ellsworth 1999). We found no effect of long-term exposure to elevated [CO_2] on foliage dark respiration rates (R_d). These results are consistent with those reported for FACE-exposed loblolly pine (Hamilton et al. 2001).

Winter A_{sat} of loblolly pine was 70% of the seasonal maximum, which is similar to that reported for slash pine (*Pinus elliottii* Engelm.) (Teskey et al. 1994*b*), indicating that loblolly pine has the potential for substantial carbon gain during the non-growing season. Despite the relatively high penetration of light within the canopy (PPFD in the lower canopy was about 50% less than in the upper canopy), there were significant morphological and physiological differences attributed to canopy position. Leaf mass per unit area, N_A, A_{sat} and R_d decreased with crown depth. Mean W_A ranged between 65 and 108 g m^{-2}, which was relatively high compared with other studies (Tissue et al. 1997, Zhang et al. 1997, Ellsworth et al. 1998). Leaf mass per unit area is an important canopy trait that is often highly correlated with light availability (Niinemets et al. 1998) and is related to carbon and nitrogen allocation (Evans 1989, Reich and Walters 1994). Within the canopy, variation in A_{sat} was more likely a result of variation in W_A than of variation in leaf biochemistry because there was little difference in A/C_i within the canopy. Theoretically, carbon and nitrogen are allocated within the canopy to optimize light-use efficiency (Evans 1989, Jarvis 1993) and numerous studies have shown that, within the crown, W_A, nitrogen distribution and A_{sat} are strongly correlated with light availability (Hirose and Werger 1987, DeJong et al. 1989, Ellsworth and Reich 1993, Brooks et al. 1996, Hollinger 1996, Schoettle and Smith 1998, Bonds et al. 1999, Rosati et al. 1999). However, the relationship within coniferous canopies that maintain low LAI is less clear. In *Pinus pinaster* Ait. plantations, Porte and Loustau (1998) found a positive relationship between W_A, A_{sat} and light availability, but Warren and Adams (2001) found little or no relationship. Zhang et al. (1997) artificially induced changes in W_A in loblolly pine trees by differentially shading branches and found a strong correlation between A_{sat} and W_A.

Dark respiration (R_d) varied with canopy position. Because net photosynthesis and respiration are tightly coupled (Ceulemans and Saugier 1991), environmental changes that affect photosynthesis will likely cause a concomitant change in respiration such that a constant ratio of respiration and photosynthesis (R:P) is maintained (Amthor 1994). Zhang et al. (1997) found that R:P was similar for artificially shaded branches and unshaded controls. In our study, R:P was similar in upper- and middle-canopy foliage, but was higher in lower-canopy foliage.

Generally, [CO_2] by fertilization interaction effects on morphology and physiology were negligible. In the few cases where both fertilization and elevated [CO_2] had a significant effect (e.g., branch leaf area, modeled A_{sat}), the combined effect of fertilization and [CO_2] was usually additive. These results are consistent with previous studies showing that field-grown loblolly pine exhibits only minor photosynthetic acclimation after long-term growth in elevated [CO_2] (Saxe et al. 1998, Groninger et al. 1999). In our study and a related loblolly pine experiment using the same fertilization treatment under Free Air Carbon Enrichment (FACE), stem growth did not occur or was minor in unfertilized plots exposed to elevated [CO_2], but was substantial in fertilized plots exposed to elevated [CO_2] (Oren et al. 2001). Our data suggest that young- to mid-rotation loblolly pine stands exposed to elevated [CO_2] exhibit increased photosynthetic carbon gain; however, increases in total carbon gain do not translate into enhanced growth unless soil nutrition supply is adequate.

The extra carbon fixed in response to elevated [CO_2] must have been allocated to processes other than aboveground wood formation. Elevated [CO_2] generally increases secondary metabolites in needle extracts (Booker and Maier 2001). Although root growth did not appear to be enhanced by elevated [CO_2] in our trees (Kurt Johnsen, unpublished data), recent FACE studies indicate that elevated [CO_2] increases belowground carbon allocation by increasing fine root production and root turnover (Matamala and Schlesinger 2000), root and rhizosphere respiration (Andrews and Schlesinger 2001) and soil [CO_2] evolution (authors' unpublished data).

In conclusion, fertilization and elevated [CO_2] increased branch leaf area, and the combined effects were additive. However, fertilization and elevated [CO_2] differentially altered needle lengths, number of fascicles and flush length such that flush density increased with improved nutrition but decreased with exposure to elevated [CO_2]. We conclude that changes in nitrogen availability and atmospheric [CO_2] may alter canopy structure, resulting in greater foliage retention and deeper crowns in loblolly pine forests. This may be important because although loblolly pine generally has an open-crown structure and relatively low leaf area, the species exhibits significant variation in leaf morphological and physiological traits related to light flux density. These differences should be considered when modeling canopy assimilation. Elevated [CO_2] increased net photosynthesis and photosynthetic efficiency and lowered the light compensation point, whereas fertilization had little consistent effect on foliage gas exchange. We observed few significant interactions among fertilization, [CO_2] and canopy position on branch physiology, and there was no indication of long-term photosynthetic acclimation.

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