

Short-term effects of fertilization on photosynthesis and leaf morphology of field-grown loblolly pine following long-term exposure to elevated CO₂ concentration

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Summary We examined effects of a first nitrogen (N) fertilizer application on upper-canopy needle morphology and gas exchange in ~20-m-tall loblolly pine (*Pinus taeda* L.) exposed to elevated carbon dioxide concentration ([CO₂]) for 9 years. Duke Forest free-air CO₂ enrichment (FACE) plots were split and half of each ring fertilized with 112 kg ha⁻¹ elemental N applied in two applications in March and April 2005. Measurements of needle length (*L*), mass per unit area (LMA), N concentration (*N*_i) on a mass and an area basis, light-saturated net photosynthesis per unit leaf area (*A*_a) and per unit mass (*A*_m), and leaf conductance (*g*_L) began after the second fertilizer application in existing 1-year-old foliage (*F*₀) and later in developing current-year first-flush (*F*_{C1}) and current-year second-flush (*F*_{C2}) foliage. Elevated [CO₂] increased *A*_a by 43 and 52% in *F*₀ and *F*_{C1} foliage, respectively, but generally had no significant effect on any other parameter. Fertilization had little or no significant effect on *L*, LMA, *A* or *g*_L in *F*₀ foliage; although *N*_i was significantly higher in fertilized trees by mid-summer. In contrast, fertilization resulted in large increases in *L*, *N*_i, and *A* in *F*_{C1} and *F*_{C2} foliage, increasing *A*_a by about 20%. These results suggest that, although both needle age classes accumulate N following fertilization, they use it differently—current-year foliage incorporates N into photosynthetic machinery, whereas 1-year-old foliage serves as an N store. There were no significant interaction effects of elevated [CO₂] and fertilization on *A*. Elevated [CO₂] increased the intercept of the *A*:*N*_i relationship but did not significantly affect the slope of the relationship in either foliage age class.

Keywords: FACE, global change, *J*_{max}, nitrogen availability, photosynthetic capacity, *Pinus taeda*, stomatal conductance, *V*_{cmax}.

Introduction

Temperate forests ecosystems are predicted to increase net primary productivity (NPP) in response to increased atmospheric carbon dioxide concentration ([CO₂]), but it is uncertain whether increased NPP will play a significant role in mitigat-

ing increases in atmospheric [CO₂] (IPCC 2007). Across a broad productivity range, exposure to elevated [CO₂], in the range expected for the mid 21st century, increased NPP by about 24% in young fast-growing temperate zone forests (Norby et al. 2005). However, increased forest growth in elevated [CO₂] is highly variable (Oren et al. 2001, Finzi et al. 2002) and recent data and theory suggest that sustained increased productivity over the long term may be constrained by nutrient limitations (Luo et al. 2004, Reich et al. 2006).

Results from the prototype ring of the Duke Forest free-air CO₂ experiment (FACE) (Oren et al. 2001) showed that large initial increases in stem growth (~40%) in loblolly pine (*Pinus taeda* L.) plantations following a step-increase in atmospheric [CO₂] disappeared after 3 years. Stimulation of stem growth by elevated [CO₂] recovered after fertilization (Oren et al. 2001). The fully replicated Duke FACE experiment showed that the magnitude of the growth stimulation to elevated [CO₂] was a function of soil nitrogen (N) availability (Finzi et al. 2002, 2006, McCarthy et al. 2006). Moreover, the rate of N accumulation in biomass, litter and soil organic matter in response to elevated [CO₂] exceeded atmospheric deposition, indicating increased N uptake from mineral soil (Finzi et al. 2002, 2007). These results suggest that soil fertility restrains the potential of forests to sequester carbon as atmospheric [CO₂] increases. Current models frequently fail to simulate the effect of elevated [CO₂] on ecosystem carbon fluxes, partly because the interactive effects of [CO₂] and leaf nitrogen (*N*_i) on leaf and whole-plant physiology are inadequately represented (Albani et al. 2006). There is thus an urgent need to provide parameter estimates for diagnostic and prognostic modeling of carbon sequestration.

Canopy carbon and N cycles are strongly coupled, driven primarily by the relationship between light-saturated photosynthetic capacity (*A*) and *N*_i (Evans 1989, Kull and Kruijt 1999). Based on their mechanistic turnover model, Kull and Kruijt (1999) argued that the strong relationship between *A* and *N*_i within a canopy is not because most *N*_i is in the photosynthetic apparatus but because a certain amount of energy (carbon) from photosynthesis is necessary to retain N in

the leaf. Accordingly, this model predicts proportionality between the leaf carbohydrate pool and N_l . Thus, the amount of photosynthetic machinery (quantified as N in the photosynthetic machinery) should increase with increasing carbohydrate availability under elevated $[CO_2]$ conditions, unless the common N pool in the plant is diluted because N uptake does not keep up with the intake of carbon. The relationship between A and N_l is often used as an indicator of $[CO_2]$ effects on canopy carbon and N interactions (Peterson et al. 1999). Many studies have shown that elevated $[CO_2]$ increases A in field-grown loblolly pine; for example, a 50% increase in $[CO_2]$ over ambient $[CO_2]$ increased A by 40 to 60% (Ellsworth et al. 1995, Myers et al. 1999, Maier et al. 2002). Although the enhancement appears to be sustained over the long term, some down-regulation ($< 15\%$) occurs (Crous and Ellsworth 2004) that may be related to N limitations (Rogers and Ellsworth 2002). However, only a few studies have examined how changes in soil N availability of these forests interact with elevated $[CO_2]$ to affect canopy physiology.

The interactive effects of elevated $[CO_2]$ and fertilization on A in loblolly pine trees are poorly understood. Murthy et al. (1996) found that elevated $[CO_2]$ and fertilization increase A by 60 and 40%, respectively, and that the combined effects were additive. However, Maier et al. (2002), working in the same stand, later found a similar $[CO_2]$ response, but despite large differences in N_l , no differences were observed in A . Fertilization generally increases N_l in field-grown loblolly pine (Allen 1987); however, the effects of increased N_l on A are less clear (Teskey et al. 1994). Variations are attributed to differences in native soil N supply, foliar nutrient imbalances and stage of stand development, i.e., growing tissue has a higher priority for N than photosynthetic processes. The majority of studies showed no effect of fertilization on A (Teskey et al. 1994, Samuelson et al. 2001, Will et al. 2001, Gough et al. 2004a), although there are exceptions (Murthy et al. 1996). In addition, loblolly pine growth appears to be N -limited when N_l is below 1.2% (Allen 1987). Fertilizing stands to bring N_l into the optimal range (exceeding 1.2%) may increase N_l but not the investment of additional N_l into photosynthetic proteins, and thus will have no effect on photosynthetic rates (Fife and Nambiar 1997, Samuelson et al. 2001). The response to N fertilization may also be transient (Fife and Nambiar 1997), suggesting phenological and ontogenetic regulation of N_l partitioning among photosynthetic and non-photosynthetic pools (Maier et al. 2002). A study on growth and physiological events following fertilization in loblolly pine seedlings showed early increases in N_l , leading to dramatic increases in A , followed by increased leaf area and growth (Gough et al. 2004b). The enhancement in A was ephemeral and disappeared as N_l decreased, presumably because of retranslocation to support new growth. These studies indicate that the photosynthetic response to elevated $[CO_2]$ and N supply can be highly dynamic. A better understanding of the mechanisms underlying short- and long-term physiological adjustments are necessary to adequately model canopy CO_2 assimilation.

We examine the effects of a first N fertilizer application on the photosynthetic capacity and leaf morphology of mid-rotation loblolly pine trees at the Duke FACE facility. The Duke FACE rings were split, and half of each ring was N fertilized. At the time of fertilization, trees were 21 years-old and had been exposed to elevated $[CO_2]$ for 9 years. Elevated $[CO_2]$ had increased canopy leaf area by 13% (McCarthy et al. 2006), increased NPP by 20–30% (Finzi et al. 2002, Hamilton et al. 2002, McCarthy et al. 2006) and had minimal effect on soil organic matter (Lichter et al. 2005). We predicted the following: (1) N from fertilization would rapidly accumulate in existing and developing foliage, and because of higher availability of carbohydrates (Kull and Kruijt 1999), foliage in elevated $[CO_2]$ treatments would accumulate proportionately more N than foliage in ambient $[CO_2]$; (2) A would increase concurrently with N_l concentration, and based on observed divergence in $A:N_l$ between ambient and elevated $[CO_2]$ (Crous and Ellsworth 2004), the increase in A from increased N_l would be greater in trees exposed to elevated $[CO_2]$; and (3) the relative increases in N_l and A in new foliage would be transitory, declining over the growing season as N is distributed to new growth.

Materials and methods

Site description

The Duke FACE facility is located in a loblolly pine plantation in Orange County, NC (35°58' N, 79°08' W). The climate is warm and humid in summer and moderate in winter with a mean annual temperature of 15.5 °C. Annual precipitation is 1145 mm distributed evenly throughout the year. The stand is a mid-rotation loblolly pine plantation established in 1983 after clear-cut harvesting. Seedlings from a North Carolina Piedmont provenance were hand planted at 2 × 2.4 m spacing. Total stand density in 2003 was 3700 trees ha⁻¹ with the dominant pines contributing about 1320 trees ha⁻¹. Soils are Enon series characterized as a moderately low fertility acidic clay-loam. Soil pH is around 6.0, and pine foliage N (~1.1%) and phosphorus (~0.3%) tend to be at the middle range for mid-rotation loblolly pine in the region. The site index is between 20 and 21 m at age 25.

The FACE system consists of eight 30-m-diameter plots. Four of the plots are fumigated with CO_2 to maintain atmospheric $[CO_2]$ at ambient + 200 $\mu l l^{-1}$, and the other four serve as controls receiving ambient air only. The elevated $[CO_2]$ treatment began in August 1996 (1994 in a prototype plot) and is continuous except when wind speed exceeds 5 m s⁻¹ or when air temperature is below 6 °C. See Hendrey et al. (1999) for details on FACE operation and protocols for quality control and assurance.

Following a successful mid-course adjustment tested since 1998 in the prototype plot and its control, in late 2004, after 9 years of exposure to elevated $[CO_2]$ exposure, each of the other six FACE plots was divided in half and one half fertilized in a split-plot design. Partitioning was done on a N–S or E–W axis such that each half had similar annual pine biomass incre-

ment and litter production rates. The halves were physically separated by trenching to a depth of 70 cm, which is greater than twice the depth of the fine root layer (Oren et al. 1998). An impermeable plastic sheet was inserted into the trench to prevent lateral root growth and nutrient movement between treatment halves. Fertilization with 112 kg ha⁻¹ elemental N (NH₄NO₃) occurred in two 56 kg ha⁻¹ applications in March and April 2005. These rates are typical of silviculture management practiced by the forest industry in the region (Allen 1987) and an order of magnitude greater than local atmospheric deposition (~7 kg ha⁻¹; Finzi et al. 2002).

Measurements

A central tower in each FACE plot allowed access to the upper third of the canopy of one to five trees in each [CO₂] by fertilization treatment. Accessibility from the central tower determined the number of trees sampled per FACE ring. Light-saturated photosynthetic rates and leaf conductance (g_L) were measured on detached foliage (two fascicles) with an LI-6400 (LiCor). Gas exchange measurements were completed within 30 min of fascicle removal. Tests showed that A of detached foliage remained stable within this time period with only small changes in g_L or internal leaf [CO₂] (C_i) (Figure 1). We measured A at both 370 and 570 $\mu\text{l l}^{-1}$ CO₂. Chamber conditions were maintained at 20 °C (spring) and 25 °C (summer and fall), 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR), and a vapor pressure deficit less than 1 kPa. Measurements were made on existing 1-year-old foliage (F_O) which expanded during the 2004 growing season once before the fertilization treatment and on 5 days through early July. Developing current-year (2005) first-flush (F_{C1}) foliage was measured on 4 days from late June to mid October. Current-year second-flush (F_{C2}) foliage was measured once in October. Each FACE ring was sampled once per day over a 5-day period beginning at about 0900 h. To reduce bias associated with diurnal variation in A or g_L , the order of measurement of the rings and trees within the ring was random.

After gas exchange measurements, needle length (L , mm) and diameter (mm) were measured and dry mass determined. Fascicles were oven-dried to constant mass at 65 °C, weighed and ground in a Wiley mill. Leaf mass per unit area (LMA, g m⁻²) was calculated as the ratio of needle dry mass to total surface area, where surface area is based on needle length, diameter and taper (Maier et al. 2002). We expressed A on a total area (A_a ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and mass (A_m ; $\text{nmol g}^{-1} \text{s}^{-1}$) basis. Fascicle N concentration was determined with a Carlo-Erba analyzer (Model NA 1500, Fison Instruments, Danvers, MA). Nitrogen concentration was expressed on a leaf area ($N_{l,a}$; g m⁻²) and a leaf mass ($N_{l,m}$; mg g⁻¹) basis.

The biochemical parameters of photosynthesis were calculated from A - C_i curves. Curves were developed on detached shoots (Maier et al. 2002) of F_{C1} foliage in July on one tree per [CO₂] by fertilization treatment. Measurements were completed by early afternoon to avoid stomatal closure. Two days were required to measure all treatment combinations. Measurements of A and C_i were made over eight external CO₂ con-

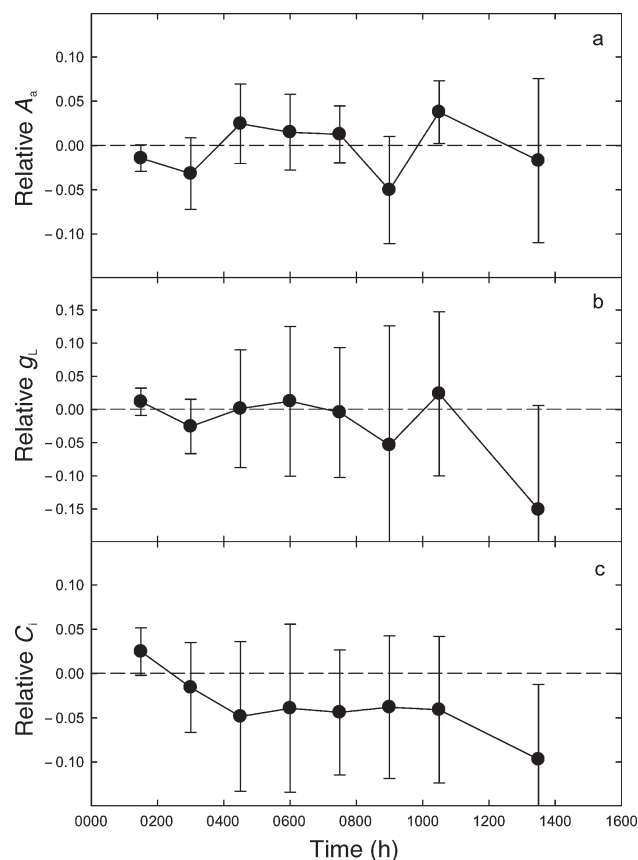


Figure 1. Relative changes in (a) area-based light-saturated net photosynthetic rate (A_a), (b) leaf conductance (g_L) and (c) internal CO₂ concentration (C_i) in detached loblolly pine (*Pinus taeda*) foliage. Values are normalized to the initial measurement after reaching stable A_a and indicate the relative changes in gas exchange parameters over time. Data are means (\pm standard error) averaged over 150-s intervals of 3–5 samples, except for the last time period, which is the average over a 500-s interval. Chamber measurement conditions: CO₂ concentration = 380 $\mu\text{l l}^{-1}$; photosynthetically active radiation = 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$; temperature = 20 °C; and vapor pressure deficit < 1.0 kPa.

centrations (100, 180, 280, 370, 570, 800, 1500 and 2000 $\mu\text{l l}^{-1}$) and required about 30 min to complete. Leaf cuvette conditions were maintained near 25 °C, 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and vapor pressure deficit less than 1 kPa. Leaf g_L changed less than 20% during measurements. Maximum rates of carboxylation (V_{cmax}) and electron transport (J_{max}) were determined for each shoot with the Farquhar biochemical model of photosynthesis (Farquhar et al. 1980) as described by Ellsworth et al. (2004).

Statistical analysis

All variables (L , LMA, foliar $N_{l,a}$, $N_{l,m}$, A , g_L , V_{cmax} and J_{max}) were subjected to analysis of variance (ANOVA) in a split-plot design where [CO₂] and fertilization were the main and split-plot effects, respectively. Individual plots were used as replicates. Data were examined graphically to confirm normality of distribution and homogeneity of variance. Main and interactive effects were considered significant at $\alpha = 0.05$. If treat-

ment effects or interactions were significant, multiple comparisons of adjusted means were performed with Tukey's test. Measurements made on multiple dates were analyzed by repeated measures ANOVA where ring (treatment) was considered a random variable. Tests were done on each age class separately based on the combined data over all dates.

Specific response functions of A to N_i were tested by analysis of covariance (ANCOVA) with A_m or A_a as the dependent variable, $[CO_2]$ as the treatment variable and foliar $N_{l,m}$ or $N_{l,a}$ as the quantitative linear covariate. Full and reduced models were developed to test for significant effects ($\alpha = 0.05$) of treatment, covariate and treatment \times covariate interaction. Regression estimates were generated after selection of the appropriate model.

Results

Foliar characteristics

Elevated $[CO_2]$ had no significant effect on L or LMA in F_{C1} or F_{C2} foliage (Table 1). Fertilization increased L by 17% in both foliage cohorts and marginally increased LMA in F_{C1} foliage (Tables 1 and 2). Fertilization had no significant effect on fascicle diameter (F_{C1} : $P = 0.38$; F_{C2} : $P = 0.98$), so increased L translated into a similar increase in total fascicle area. There were no $[CO_2] \times$ fertilization interaction effects on any parameter in F_{C1} or F_{C2} foliage.

In F_O foliage that had developed before fertilization, there

was a marginal $[CO_2] \times$ fertilization interaction ($P = 0.06$) and date of measurement (DOY) effect ($P = 0.07$) on L , and a significant $[CO_2] \times$ fertilization interaction on LMA (Table 1). Mean L was shorter and LMA lower in fertilized trees in ambient $[CO_2]$ than in fertilized trees in elevated $[CO_2]$ (Table 2). These interactions are difficult to interpret. However, it is unlikely that fertilization affected L , because the F_O foliage cohort had completely expanded the previous year before fertilization began. Treatment interactions on L likely result from large variation in this trait within the upper canopy (mean plot CV = 14). No treatment or treatment \times time interactions were observed in L in F_{C1} or F_{C2} foliage, probably because of the strong growth response to fertilization (17%).

Growth $[CO_2]$ had no significant effect on $N_{l,a}$ or $N_{l,m}$ in any foliage cohort (Table 1). Fertilization increased $N_{l,a}$ by 10, 19 and 17% and $N_{l,m}$ by 13, 13 and 14% in F_O , F_{C1} and F_{C2} foliage, respectively. There were no $[CO_2] \times$ fertilization interactions on N_i . However, there was a significant fertilization \times DOY interaction in F_O foliage caused by diverging patterns of $N_{l,a}$, where in late spring, $N_{l,a}$ declined in unfertilized trees and increased in fertilized trees resulting in a 25–28% difference in $N_{l,a}$ of F_O by mid summer (Figure 2). In F_{C1} foliage, the relative difference in $N_{l,a}$ between unfertilized and fertilized trees was constant across the season, although the absolute concentration declined slightly in the fall (Figure 2).

Gas exchange

Net photosynthesis varied among needle age classes and treat-

Table 1. Analysis of variance (ANOVA) probability values for carbon dioxide concentration ($[CO_2]$, C), fertilization treatment (F) and date of fertilizer application (D) effects in 1-year-old (F_O), current-year first-flush (F_{C1}) and current-year second-flush (F_{C2}) foliage (n = number of measurement days) on: needle length (L); leaf mass per unit area (LMA); leaf nitrogen per unit leaf area ($N_{l,a}$) and per unit mass ($N_{l,m}$); light-saturated net photosynthesis per unit leaf area (A_a) and per unit mass (A_m); leaf conductance (g_L); and the ratio of internal leaf $[CO_2]$ to ambient $[CO_2]$ (C_i/C_a).

Effect	df	L	LMA	$N_{l,a}$	$N_{l,m}$	A_a	A_m	g_L	C_i/C_a
<i>F_O (n = 6)</i>									
C	1	0.983	0.068	0.897	0.284	< 0.001	0.006	0.686	0.072
F	1	0.837	0.068	0.011	0.005	0.741	0.567	0.265	0.135
C \times F	1	0.062	0.038	0.851	0.111	0.182	0.068	0.552	0.327
D	5	0.073	0.038	0.054	0.221	0.145	0.644	< 0.001	0.187
C \times D	5	0.578	0.023	0.073	0.091	0.156	0.457	0.832	0.035
F \times D	5	0.810	0.507	0.020	0.006	0.545	0.567	0.136	0.896
C \times F \times D	5	0.670	0.896	0.136	0.061	0.932	0.828	0.263	0.772
<i>F_{C1} (n = 4)</i>									
C	1	0.747	0.338	0.608	0.417	0.013	0.017	0.085	0.865
F	1	0.016	0.072	0.009	0.017	< 0.001	0.088	0.223	0.417
C \times F	1	0.462	0.260	0.945	0.332	0.420	0.759	0.283	0.507
D	3	< 0.001	< 0.001	0.010	< 0.001	0.030	0.002	0.003	0.006
C \times D	3	0.847	0.607	0.089	0.063	0.060	0.914	0.066	0.325
F \times D	3	0.100	0.304	0.186	0.198	0.872	0.699	0.937	0.784
C \times F \times D	3	0.817	0.767	0.734	0.865	0.759	0.763	0.334	0.536
<i>F_{C2} (n = 1)</i>									
C	1	0.427	0.870	0.718	0.640	0.034	0.020	0.261	0.840
F	1	0.062	0.336	0.021	0.008	0.030	0.029	0.020	0.752
C \times F	1	0.694	0.841	0.365	0.263	0.963	0.694	0.558	0.295

Table 2. Least square means for needle length (L , mm), leaf mass per unit area (LMA, g m^{-2}), leaf nitrogen per unit leaf area ($N_{l,a}$, g m^{-2}) and per unit mass ($N_{l,m}$, mg g^{-1}), light-saturated net photosynthesis per unit leaf area (A_a , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and per unit mass (A_m , $\text{nmol g}^{-1} \text{s}^{-1}$), leaf conductance (g_L , $\text{mmol m}^{-2} \text{s}^{-1}$) and the ratio of internal leaf CO_2 concentration ($[\text{CO}_2]$) to ambient $[\text{CO}_2]$ (C_i/C_a) for fertilized (F) and non-fertilized (NF) 1-year-old (F_0), current-year first-flush (F_{C1}) and current-year second-flush (F_{C2}) foliage of loblolly pine (*Pinus taeda*) for all treatments (n = number of measurement days). Measurement conditions for A: photosynthetically active radiation = $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$; temperature = 25°C ; and vapor pressure deficit < 1.2 kPa.

Treatment	L	LMA	$N_{l,a}$	$N_{l,m}$	A_a	A_m	g_L	C_i/C_a
<i>F₀</i> ($n = 6$)								
Ambient $[\text{CO}_2]$, NF	164.2	109.7	1.32	12.0	5.03	46.1	105.7	0.63
Ambient $[\text{CO}_2]$, F	154.6	100.6	1.44	14.3	5.25	52.5	81.8	0.56
Elevated $[\text{CO}_2]$, NF	163.6	114.2	1.45	12.7	7.20	63.9	111.4	0.67
Elevated $[\text{CO}_2]$, F	155.7	113.3	1.34	11.9	7.55	67.5	113.1	0.68
SE	8.2	2.7	0.09	0.6	0.20	2.8	10.2	0.03
<i>F_{C1}</i> ($n = 4$)								
Ambient $[\text{CO}_2]$, NF	172.6	83.2	1.02	13.2	5.15	66.8	113.1	0.66
Ambient $[\text{CO}_2]$, F	201.8	83.5	1.21	15.3	6.11	77.8	140.3	0.57
Elevated $[\text{CO}_2]$, NF	208.2	94.4	1.18	14.0	9.24	111.2	124.7	0.62
Elevated $[\text{CO}_2]$, F	178.7	83.4	0.99	12.8	7.88	103.0	116.2	0.63
SE	7.6	3.6	0.05	0.7	0.51	7.0	15.3	0.06
<i>F_{C2}</i> ($n = 1$)								
Ambient $[\text{CO}_2]$, NF	135.8	87.3	1.02	11.6	4.8	54.6	48.1	0.52
Ambient $[\text{CO}_2]$, F	165.0	90.7	1.25	13.7	6.0	66.9	67.8	0.49
Elevated $[\text{CO}_2]$, NF	173.8	89.7	1.22	13.7	10.4	116.6	97.8	0.56
Elevated $[\text{CO}_2]$, F	152.9	87.4	1.10	12.5	9.3	107.1	70.0	0.50
SE	12.5	2.8	0.06	0.7	1.0	9.9	14.6	0.07

ments, but there were no significant $[\text{CO}_2] \times$ fertilization interactions on A . Averaged across all days, elevated $[\text{CO}_2]$ increased A_a by 43 and 52% in F_0 and F_{C1} foliage, respectively (Tables 1 and 2); on any particular day the enhancement

ranged from 33 to 88% (data not shown) and was significant for all days. Fertilization had no significant effect on A_a in F_0 foliage even when there were large differences in N_l in late spring (Figure 2). In contrast, fertilization increased mean A_a

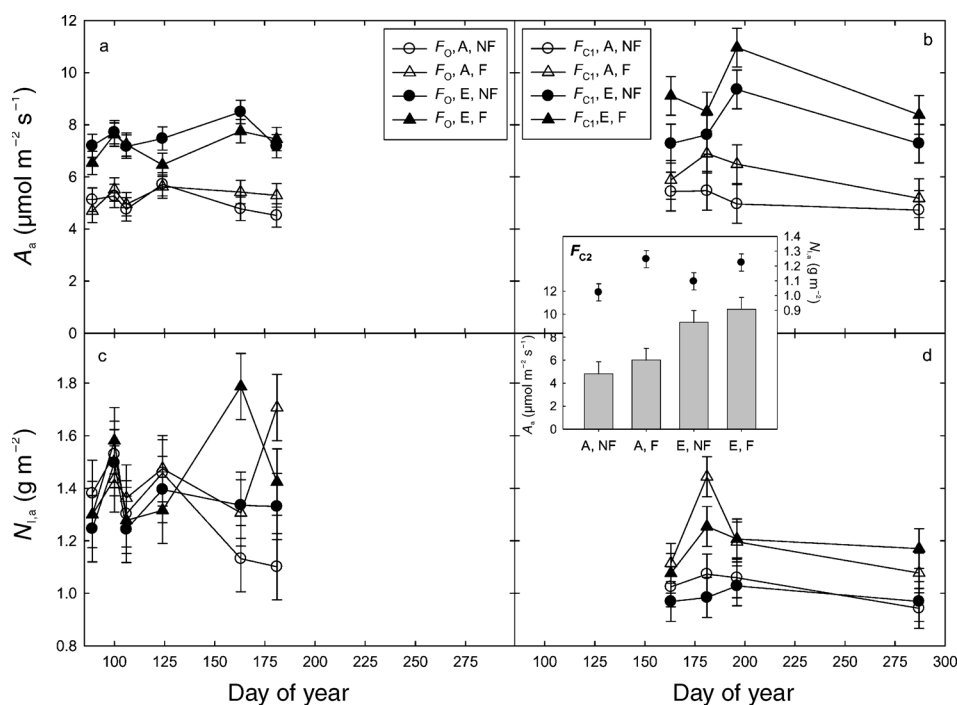


Figure 2. Seasonal trends in area-based light-saturated net photosynthetic rate (A_a) and area-based leaf nitrogen concentration ($N_{l,a}$) for 1-year-old (F_0) (a and c) and current-year first-flush (F_{C1}) (b and d) and current-year second-flush (F_{C2}) (inset) foliage of loblolly pine (*Pinus taeda*) for all treatments: A and E are ambient and elevated CO_2 concentrations, respectively; NF = non-fertilized; and F = fertilized. Values are least square means ($n = 3$ rings) with standard error.

and A_m in F_{C1} foliage by 18 and 11%, respectively. The relative difference in A_a between fertilization treatments was significant for all measurement days, even in the fall after the second flush (F_{C2}) had completely expanded. In the fall, differences in A in F_{C2} foliage were similar to F_{C1} foliage during the summer (Figure 2, inset). There was a marginal $[CO_2] \times DOY$ interaction on A and g_L in F_{C1} caused by an increase in A in response to elevated $[CO_2]$ in late July (Figure 2), which may indicate a differential treatment response to dry conditions. There were no significant treatment effects on g_L , except in F_{C2} foliage in the fall when elevated $[CO_2]$ significantly increased g_L . There were no treatment or treatment interaction effects on C_i/C_a for any foliage cohort.

Analysis of $A-C_i$ curves on F_{C1} foliage in mid July (DOY 201) indicated partial acclimation of net photosynthesis under elevated $[CO_2]$ conditions. Values of V_{cmax} and J_{max} decreased by 19% ($P = 0.0574$) and 13% ($P = 0.0955$), respectively, in the elevated $[CO_2]$ treatments (Table 3). Fertilization had no significant effect on V_{cmax} ($P = 0.9907$), but increased J_{max} ($P = 0.0021$) by 33%. The ratio $J_{max}:V_{cmax}$ increased from 1.15 in unfertilized trees to 1.57 in fertilized trees ($P = 0.004$). There were no significant $[CO_2] \times$ fertilization interactions on either parameter (V_{cmax} : $P = 0.628$; J_{max} : $P = 0.878$). Acclimation to elevated $[CO_2]$ was evident in the measurements of A made on DOY 201. For example, at a common measurement $[CO_2]$ of $570 \mu l l^{-1}$, mean A_a was 6.42 and $5.38 \mu mol m^{-2} s^{-1}$ ($P = 0.0765$) for foliage grown in ambient and elevated $[CO_2]$, respectively, a 17% reduction. However, these results contrast with similar measurements made on F_{C1} foliage on other days. Across all dates, there was no difference in mean A_a measured at a $[CO_2]$ of $570 \mu l l^{-1}$ (ambient $[CO_2]$: $8.28 \mu mol m^{-2} s^{-1}$, elevated $[CO_2]$: $8.56 \mu mol m^{-2} s^{-1}$, $P = 0.47$).

Assimilation rate–leaf nitrogen relationships

Analysis of covariance indicated that, within an age class, there was no significant $[CO_2]$ treatment \times foliar N interaction ($P > 0.10$) on $A_m:N_{1,m}$ or $A_a:N_{1,a}$. Removal of the interaction term in the reduced model indicated a significant linear relationship between $A_m:N_{1,m}$ and $A_a:N_{1,a}$ ($P < 0.001$) and significant $[CO_2]$ treatment effects on A_m and A_a ($P < 0.01$). Generally, A increased linearly with N_i with similar slopes for each $[CO_2]$ treatment, but significantly different intercepts (Fig-

ure 3). The slope of $A:N_i$ was greater for current-year foliage than for older foliage, although these differences were not statistically tested. The correlation for $A_m:N_{1,m}$ was generally greater than for $A_a:N_{1,a}$.

Discussion

Foliar characteristics

Canopy leaf area and leaf area duration will largely determine forest NPP under predicted elevated atmospheric $[CO_2]$ conditions (McCarthy et al. 2006). In this study, 9 years of exposure to elevated $[CO_2]$ had little effect on needle length or total fascicle area. Thus, the ~13% increase in canopy leaf area in the elevated $[CO_2]$ rings (McCarthy et al. 2006) was not associated with larger individual fascicles. Elevated $[CO_2]$ generally has only small effects on needle morphology in field-grown loblolly pine trees. Instead, elevated $[CO_2]$ primarily increases leaf area through increased shoot extension and fascicle number (Teskey 1995, Murthy and Dougherty 1997, Maier et al. 2002). In contrast, fertilization increased needle length and total fascicle area by 17% in F_{C1} and F_{C2} foliage. This increase is less than that reported for other loblolly pine stands (Murthy and Dougherty 1997), probably because the advanced stage of stand development at the Duke FACE site limited its response to fertilization (Jokela et al. 2004). Nevertheless, increased fascicle growth will likely have a significant effect on canopy leaf area and NPP in subsequent years (McCarthy et al. 2006).

Generally, long-term exposure to elevated $[CO_2]$ decreases $N_{1,m}$ (Stitt and Krapp 1999), which may have important consequences for growth and carbon allocation and sequestration in forests (Curtis et al. 2000). However, several studies of field-grown loblolly pine indicate that long-term exposure to elevated $[CO_2]$ induces only small reductions (generally $< 10\%$) in $N_{1,m}$ (Liu and Teskey 1995, Murthy et al. 1996, Ellsworth et al. 1998, Oren et al. 2001, Finzi et al. 2002, Maier et al. 2002, Springer et al. 2005). In our study, although the initial significant increase in N_i ($N_{1,m}$ or $N_{1,a}$) in all foliage age classes following fertilization matched Prediction 1, in contrast to expectations based on the results of Kull and Kruijt (1999), the higher availability of carbohydrates under elevated $[CO_2]$ conditions did not increase N_i in upper-canopy foliage of fertilized trees, and did not result in lower N_i in unfertilized trees (elevated $[CO_2]$ reduced $N_{1,a}$ and $N_{1,m}$ by 5–9%, but the effect was not significant). Such a response suggests that the relationship between the size of the photosynthetic machinery and that of the carbon pool saturates, as proposed by Kull and Kruijt (1999).

Treatment differences in N_i in F_O foliage in late spring were the result of both an accumulation of N_i in fertilized trees and a reduction of N_i in unfertilized trees. The diverging patterns indicate that, in non-fertilized trees, older foliage is an important source of N for the development of current-year foliage, and that N supplied from fertilization exceeded that required for F_{C1} growth and as expansion of F_{C1} foliage neared completion, excess N was stored in older foliage. This is similar to patterns observed with other mobile nutrients (Oren et al. 1988). Fur-

Table 3. Least square means and standard errors (SE) ($n = 3$ rings) for photosynthetic parameters derived from $A-C_i$ response curves. Measurements were made in current-year first-flush foliage (F_{C1}) in July. Abbreviations: V_{cmax} = maximum carboxylation rate ($\mu mol m^{-2} s^{-1}$); J_{max} = maximum electron transport ($\mu mol m^{-2} s^{-1}$); NF = non-fertilized; F = fertilized.

Parameter	Treatment (carbon dioxide/fertilization)				SE
	Ambient NF	Ambient F	Elevated NF	Elevated F	
V_{cmax}	33.43	34.90	28.44	26.89	3.02
J_{max}	38.01	49.93	32.74	43.94	2.40

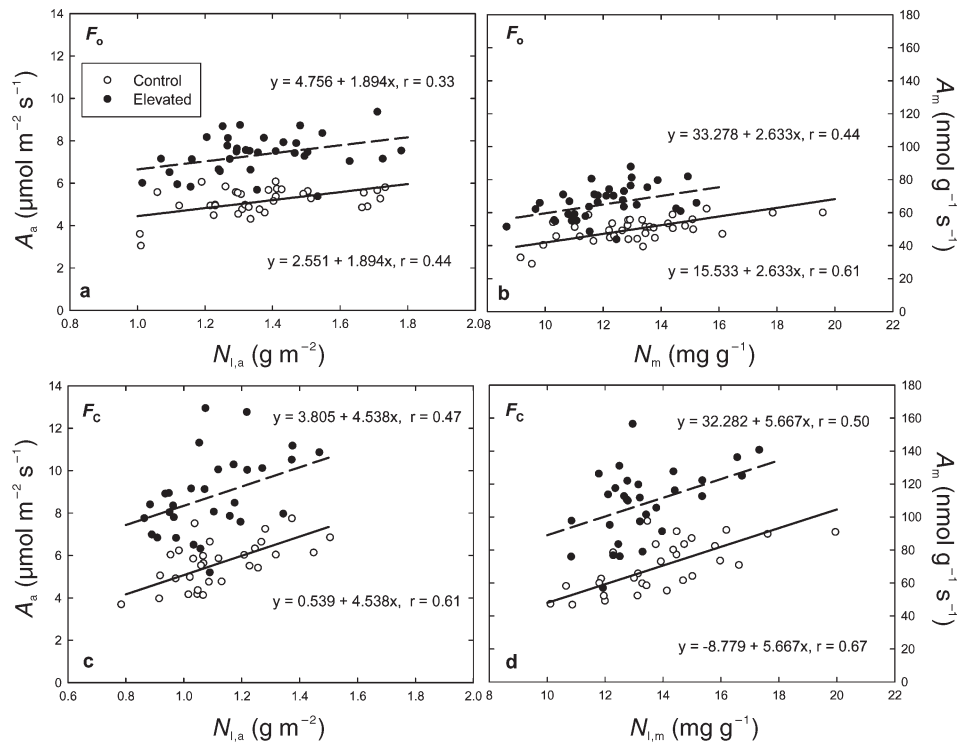


Figure 3. Relationships between needle area-based light-saturated net photosynthetic rate (A_a) at the growth $[\text{CO}_2]$ and foliar nitrogen expressed on a unit leaf area ($N_{l,a}$) or mass basis ($N_{l,m}$) for 1-year-old foliage (F_0) (a and b) and current-year first-flush (F_{C1}) and current-year second-flush (F_{C2}) foliage (c and d). Data are combined across the growing season, and each value represents an individual measurement.

thermore, the lack of a response of A to increased N in F_0 foliage indicates that excess N is not allocated to photosynthetic enzymes. We saw no large reductions in N_l over the growing season in F_{C1} foliage as predicted (Prediction 3), likely because of efficient retranslocation of N from senescing F_0 foliage to developing foliage (Zhang and Allen 1996) in the fall.

Gas exchange

We predicted (Prediction 2) that fertilization would increase A , and that the response to N fertilization would be greater in the elevated $[\text{CO}_2]$ treatments. During the 10th year of exposure to elevated $[\text{CO}_2]$, there was still a strong enhancement ($> 50\%$) of A across age classes, and the stimulation was 28% greater for current-year foliage than for F_0 foliage. These results are generally similar to those reported in earlier Duke FACE studies (Ellsworth et al. 1995, Myers et al. 1999, Crous and Ellsworth 2004) and other studies in field-grown loblolly pine trees (Murthy et al. 1996, Maier et al. 2002). Fertilization increased A in developing F_{C1} and F_{C2} foliage by about 20%, but had no significant effect on F_0 foliage.

The $A-C_i$ curves indicated that fertilization increased J_{max} , but had no influence V_{cmax} . This result contrasts with theory (Evans 1989) and previous results with loblolly pine seedlings (Tissue et al. 1993, Gough et al. 2004a), indicating that improved nutrition increases photosynthetic capacity because of preferential allocation of N_l to Rubisco (reflected in V_{cmax}) rather than thylakoid proteins associated with light harvesting and electron transport (reflected in J_{max}). There were marginal fertilization effects on LMA in F_{C1} foliage ($P=0.07$) in the ele-

vated $[\text{CO}_2]$ plus fertilized treatment, so it is unclear whether enhanced photosynthetic capacity was a result of improved photochemistry or changes in leaf mass (Peterson et al. 1999). There were no $[\text{CO}_2] \times$ fertilization interaction effects on A . Thus, the effects of elevated $[\text{CO}_2]$ and fertilization on A are additive, as found by Murthy et al. (1996). The stimulatory effect of fertilization on A persisted in F_{C1} foliage over the growing season, because there was only a small reduction in N_l .

There was some evidence of acclimation of A to $[\text{CO}_2]$ in current-year foliage. Analysis of $A-C_i$ curves indicated that V_{cmax} and J_{max} were reduced by 13–19% and A_a by 17%. These results are similar to those for current-year needles in other field-grown pines (Tissue et al. 1999, Luomala et al. 2003). Crous and Ellsworth (2004) reported a 17–20% down-regulation in V_{cmax} and J_{max} for the Duke FACE trees, but only in 1-year-old foliage. Crous et al. (2008) also found that fertilization in this stand resulted in foliage grown in elevated $[\text{CO}_2]$ having similar or higher net photosynthetic rates the following year. It is unlikely that N_l mediated photosynthetic acclimation in current-year needles in our study, because there were only small ($< 10\%$) nonsignificant reductions in N_l ($N_{l,m}$ or $N_{l,a}$) in response to elevated $[\text{CO}_2]$. Furthermore, whereas increased N_l in fertilized trees increased J_{max} , it did not prevent reductions in J_{max} and V_{cmax} in elevated $[\text{CO}_2]$; however, these results are based on only a single measurement campaign (mid July). When averaged across all measurements, there were no differences in A , C_i/C_a or g_L between foliage grown at elevated or ambient $[\text{CO}_2]$ when measured at a common $[\text{CO}_2]$. This inconsistency may reflect subtle short-term variation in stomatal limitations to A or transient changes in gas exchange that are difficult to control.

Assimilation rate–leaf nitrogen

We found that A was linearly correlated with $N_{1,a}$ and $N_{1,m}$ in upper-crown sunlit foliage. Elevated $[\text{CO}_2]$ increased the intercept, but not the slope, of the $A:N_1$ relationship, indicating that increased photosynthetic N-use efficiency under elevated $[\text{CO}_2]$ conditions is consistent across N_1 . This suggests that the partitioning of N_1 into photosynthetic and non-photosynthetic constituents was similar between fertilization treatments (Reich et al. 1995). In contrast to our findings, other studies at Duke FACE have shown that elevated $[\text{CO}_2]$ increases the slope of the $A_a:N_{1,a}$ relationship (Crous and Ellsworth 2004, Springer et al. 2005), although Crous and Ellsworth (2004) observed this increase in current-year needles only. Cao et al. (2007) found that elevated $[\text{CO}_2]$ increased A in white birch seedlings, but had no effect on the slope of the $A:N_1$ relationship, either on a mass or area basis. The contrast between our parallel slopes and the diverging slopes observed by others may be caused by the inclusion in these studies of lower-canopy foliage that developed in lower light regimes. Growth irradiance affects the energy available for turning over the photosynthetic machinery (Kull and Kruijt 1999) and alters the partitioning of N_1 among photosynthetic components (Evans 1989). Therefore, the increased slope under elevated $[\text{CO}_2]$ conditions may reflect a differential $[\text{CO}_2]$ effect on N partitioning among photosynthetic constituents. However, LMA also differs between sun and shade foliage. Peterson et al. (1999) demonstrated that when LMA is controlled, elevated $[\text{CO}_2]$ generally increases the intercept but has no significant effect on the slope of the $A:N_1$ relationship.

Photosynthetic capacity of loblolly pine foliage is affected by both age and position within the canopy. Photosynthetic capacity decreases as foliage ages (Maier et al. 2002, Crous and Ellsworth 2004), which is likely related to N_1 dynamics (Lavigne et al. 2001). Age-related differences in N-use efficiency, i.e., the intercept and slope of $A:N_1$, may reflect changes in the partitioning of N_1 where developing foliage allocates more N to photosynthetic constituents, whereas fully expanded foliage stores N in non-photosynthetic compounds. The partitioning of N into mobile forms (i.e., amino acids) as foliage ages facilitates reallocation to new sinks. Furthermore, there are strong vertical gradients in A , N_1 , and LMA (Maier et al. 2002, Springer et al. 2005) and potential crown position by $[\text{CO}_2]$ interactions on the biochemical capacity (Crous and Ellsworth 2004). Elevated $[\text{CO}_2]$ and soil N availability may also differentially affect branch flush density and alter crown structure (Maier et al. 2002). Thus, although our data suggest that including age-related changes in photosynthetic capacity and LMA is required in modeling whole-canopy net photosynthesis, the results must be combined with data throughout the canopy to determine how $[\text{CO}_2]$ and fertilization interact to control the vertical distribution of N and carbon uptake, and thereby whole-canopy carbon gain.

It is unclear whether the observed increases in A will persist over time even with repeated fertilization. Large increases in canopy leaf and woody growth of loblolly pine plantations generally occur in the year(s) following N fertilization

(Albaugh et al. 1998, Jokela et al. 2004). Initial increases in A may disappear in subsequent years as nutrients are preferentially allocated to sustain increased canopy leaf area and wood biomass production rather than photosynthetic capacity. For example, studies in a young loblolly pine plantation have shown that increased photosynthetic capacity and N availability following fertilization lead to large increases in canopy leaf area and above- and belowground wood. Following the establishment of increased canopy leaf area in fertilized stands, photosynthetic capacity returned to pre-fertilization rates even though N_1 remained elevated (Gough et al. 2004a).

We found few fertilization \times $[\text{CO}_2]$ interaction effects on leaf physiology and morphology. In older foliage, fertilization increased N_1 but had no significant effect on gas exchange. In contrast, in developing foliage, fertilization resulted in large increases in leaf area, N_1 and A , and the increases in N_1 and A were sustained over the growing season. These results suggest that, although both needle age classes accumulate N following fertilization, the N is used differently in the two cohorts. Current-year foliage incorporates N into photosynthetic components increasing photosynthetic capacity, whereas older pre-fertilized foliage serves as a N store. The effects of $[\text{CO}_2]$ and fertilization on A were additive, thus, the synergistic $[\text{CO}_2] \times N_1$ effect on stem biomass accumulation observed by Oren et al. (2001) in the Duke FACE prototype study was likely because of large increases in canopy leaf area (Albaugh et al. 1998, McCarthy et al. 2006) or fertilization-induced shifts in above- and belowground carbon allocation (Palmroth et al. 2006).

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