

Photosynthetic capacity of loblolly pine (*Pinus taeda* L.) trees during the first year of carbon dioxide enrichment in a forest ecosystem

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ABSTRACT

Our objective was to assess the photosynthetic responses of loblolly pine trees (*Pinus taeda* L.) during the first full growth season (1997) at the Brookhaven National Lab/Duke University Free Air CO₂ Enrichment (FACE) experiment. Gas exchange, fluorescence characteristics, and leaf biochemistry of ambient CO₂ (control) needles and ambient + 20 Pa CO₂ (elevated) needles were examined five times during the year. The enhancement of photosynthesis by elevated CO₂ in mature loblolly pine trees varied across the season and was influenced by abiotic and biotic factors. Photosynthetic enhancement by elevated CO₂ was strongly correlated with leaf temperature. The magnitude of photosynthetic enhancement was zero in March but was as great as 52% later in the season. In March, reduced sink demand and lower temperatures resulted in lower net photosynthesis, lower carboxylation rates and higher excess energy dissipation from the elevated CO₂ needles than from control needles. The greatest photosynthetic enhancement by CO₂ enrichment was observed in July during a period of high temperature and low precipitation, and in September during recovery from this period of low precipitation. In July, loblolly pine trees in the control rings exhibited lower net photosynthetic rates, lower maximum rates of photosynthesis at saturating CO₂ and light, lower values of carboxylation and electron transport rates (modelled from A–C_i curves), lower total Rubisco activity, and lower photochemical quenching of fluorescence in comparison to other measurement periods. During this period of low precipitation trees in the elevated CO₂ rings exhibited reduced net photosynthesis and photochemical quenching of fluorescence, but there was little effect on light- and CO₂-saturated rates of photosynthesis, modelled rates of carboxylation or electron transport, or Rubisco activity. These first-year data will be used to compare with similar measurements from subsequent years of the FACE experiment in order to determine whether photosynthetic acclimation to CO₂ occurs in these canopy loblolly pine trees growing in a forest ecosystem.

Key-words: chlorophyll fluorescence; elevated CO₂; forest ecosystem; loblolly pine; photosynthetic capacity; photosynthetic enhancement; temperature.

INTRODUCTION

Greenhouse and growth chamber studies have shown that elevated CO₂ increases photosynthesis and plant biomass compared with plants grown at lower CO₂ partial pressures (Eamus & Jarvis 1989; Poorter 1993; Ceulemans & Mousseau 1994; Gunderson & Wullschleger 1994; Curtis 1996). Most studies of trees, however, have been performed using seedlings or small saplings with non-limiting amounts of water and nutrients. While a few CO₂ experiments have been performed on mature trees, these studies either partially fumigated trees using branch bag techniques (Teskey 1995; Murthy, Zarnoch & Dougherty 1997) or were of short duration using a free-air CO₂ enrichment (FACE) prototype (Ellsworth *et al.* 1995). Many questions remain, however, whether the initial stimulation in photosynthesis and growth observed for seedlings and saplings with a doubling of CO₂ will be sustained for trees growing in a forest ecosystem under natural conditions.

Studies of leaf-level physiological responses to elevated CO₂ in crops, herbaceous and woody species have significantly increased our understanding of the processes regulating plant carbon balance (Bazzaz 1990). It is difficult to extrapolate these studies to predict the responses of mature trees and whole forest ecosystems. A number of recent studies have observed that leaf-level physiological responses of tree seedlings are different from the responses of mature trees of the same species (Grulke & Miller 1994; Hanson *et al.* 1994). In addition, seasonal variations in biotic and abiotic factors in a forest ecosystem may significantly affect the growth and photosynthetic responses to elevated CO₂ (Gunderson & Wullschleger 1994).

Temperature may be one of the most important abiotic factors affecting the photosynthetic response to elevated CO₂ (Long 1991). The relative degree of enhancement due to growth in elevated CO₂ has been shown to be positively correlated with needle temperature for loblolly pine (*Pinus taeda* L.) seedlings grown in open top chambers (Lewis, Tissue & Strain 1996). This strong correlation between temperature and CO₂ enhancement is due to the fact that the increase in specificity of Ribulose-1,5-bisphosphate

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carboxylase/oxygenase (Rubisco) for oxygen at higher temperatures decreases photosynthetic efficiency but is partially offset by high CO₂ partial pressures (Jordan & Ogren 1984). Determining if abiotic factors such as seasonal temperature fluctuations affect photosynthetic enhancement due to elevated CO₂ in large maturing trees has been greatly aided by the development of FACE technology.

A feature of many published reports is that the photosynthetic enhancement caused by exposure to elevated CO₂ abates with time. It is alternatively suggested that this is an artefact of the experimental conditions. A review of data from C3 crop species found that the initial enhancement of photosynthesis due to a doubling of CO₂ was 52%, but after long-term growth under elevated CO₂, photosynthetic enhancement declined to 29% (Cure & Acock 1986). Similarly, Gunderson & Wullschleger (1994) in an assessment of the effects of elevated CO₂ on photosynthesis of 39 tree species found that photosynthetic rates of plants grown in elevated CO₂ were enhanced by 44% on average but this stimulation was 21% lower than short-term measurements of the CO₂ response. One potential explanation for the decline of photosynthetic enhancement is the development of a source–sink imbalance within the plant. This occurs when the amount of carbon assimilation (the source) exceeds the capacity of the plant to store or use the carbon for growth or maintenance (metabolic sinks). Many studies have suggested that the acclimation of photosynthesis to long-term exposure of elevated CO₂ is linked to a lack of development of additional sinks (e.g. Thomas & Strain 1991). It has been postulated that feedback mechanisms may be triggered by starch and sugar accumulation with the end result of biochemical down-regulation of Rubisco activity (Stitt 1991) and some recent evidence supports this hypothesis (Sheen 1994; van Oosten & Besford 1995).

The objective of our study was to assess the photosynthetic responses of loblolly pine trees during the first full growth season at the Brookhaven National Lab/Duke University Free Air CO₂ Enrichment (FACE) experiment. This FACE experiment provides the opportunity to explore the responses of loblolly pine trees operating within the full suite of ecosystem processes. We hypothesized that CO₂ enrichment would stimulate photosynthesis but the degree to which CO₂ stimulated photosynthesis would vary seasonally due to abiotic factors such as seasonal fluctuations in air temperature. Thus, photosynthetic capacity of a single needle cohort was measured on five dates during the growing season, including air temperatures from 17.5 to 31.5 °C. Photosynthetic capacity was assessed by measuring net photosynthesis versus intercellular CO₂ partial pressure (A–C_i curves) and further examined by analysing these curves using a biochemical model that describes how photosynthesis is regulated by the amount, activity and kinetic properties of Rubisco and the rate of ribulose-1,5-bisphosphate regeneration (Harley & Sharkey 1991). In addition, pulse-modulated chlorophyll fluorescence quenching analysis was used to detect changes in energy dissipation and energy use by the chloroplasts.

Rubisco activity and activation state as well as leaf carbohydrate and nitrogen concentrations were also measured. This study of photosynthetic capacity of loblolly needles during the first year of the Brookhaven/Duke FACE experiment provides an important baseline of photosynthetic responses to which measurements from subsequent years of CO₂ exposure may be compared.

MATERIALS AND METHODS

Brookhaven/Duke free-air CO₂ enrichment (FACE) system

The Brookhaven/Duke FACE experiment in the Blackwood division of the Duke Forest (35°97' N 79°09' W) was established to examine the responses to elevated concentrations of atmospheric CO₂ in an intact forest ecosystem without disturbing the forest microclimate (Hendrey *et al.* 1998). The forest is dominated by loblolly pine (1733 stems ha⁻¹), with significant sweetgum (*Liquidambar styraciflua*, 620 stems ha⁻¹) and yellow poplar (*Liriodendron tulipifera*, 68 stems ha⁻¹) as secondary associates. Tree growth of loblolly pine in the plantation is remarkably uniform, with a median height of 13 m, a mean diameter of about 15 cm and a leaf area index of about 3.5 (Katul *et al.* 1997). The forest has not yet initiated self thinning. The clay-rich Alfisols are of the Enon series, a soil with low nitrogen and phosphorus availability that is typical of many upland areas in the southeast USA. This section of the Duke forest was farmed a century ago, and the current plantation was established in 1983 after a 45-year-old existing forest was clear cut in 1979.

The FACE system increases atmospheric CO₂ partial pressure in a 30-m-diameter experimental plots nested within this continuous pine forest. Each FACE ring consists of a large circular plenum that delivers air to an array of 32 vertical pipes. The pipes extend from the forest floor through the 13-m-tall forest canopy and contain adjustable ports at 50 cm intervals. These ports are tuned to control atmospheric CO₂ partial pressure through the entire forest volume. Fumigation with CO₂ in three experimental plots (ambient + 20.0 Pa or approximately 57 Pa CO₂ near the top of the central towers) began in August 1996. Three control plots receive the same volume of air to replicate any micrometeorological effects that the operation of the FACE facility has on the ecosystem. The corresponding ambient CO₂ partial pressure during the growing season was approximately 38 Pa. To control for topographic variation between plots (≈ 5 m) and potential gradients in site fertility, the three control and three elevated-CO₂ plots were arranged in a block design (three pairs). The FACE rings were operated continuously except when ambient temperature fell below 5 °C for more than 1 h.

The following measurements were made five times during the first year (1997) of the experiment: late winter (March), early spring (April), early summer (May), mid summer (July) and late summer (September). Monthly averages of maximum and minimum temperature and

monthly precipitation during the first full year of CO₂ treatment are shown in Fig. 1 (temperature data from NOAA weather station No. 1677, Carrboro, NC approximately 15 km south-west of the site). These measurement periods corresponded to periods of slow (March, September) and rapid (April, May and July) growth by the loblolly pine trees. A drought occurred during late July, August and early September due to a lack of precipitation (4.0 cm rain over the 58 day period). Drought-breaking rains occurred in mid-September (10.0 cm over the last 19 days of September). Measurements of soil moisture in the FACE rings began in June 1997 (Schäfer, unpublished results). During June, soil moisture averaged 23.3% (± 0.9) in the control rings and 26.2% (± 0.9) in the elevated CO₂ rings. In late July, August and early September, soil moisture averaged 16.0% (± 0.4) in the control rings and 16.4% (± 0.4) in the elevated CO₂ rings. During late September, soil moisture averaged 22.0% (± 0.8) in the control rings and 22.8% (± 0.9) in the elevated CO₂ rings.

Physiological parameters were measured on attached loblolly pine needles between 1000 and 1600 h on sunny days during periods of little or no cloud cover. Measurements were conducted using fully developed needles that were initiated during the later part of 1996 just before CO₂ fumigation in the FACE site began. Needles used for gas exchange and fluorescence analyses were chosen from sun-exposed branches of three trees per ring on trees located within 1–2 m of the central tower of each ring. Needles used for biochemical analyses were taken from sun branches of the same leaf age on trees adjacent to gas exchange trees in order to preserve the integrity of trees near the central tower.

Gas exchange analyses

Needle gas exchange was measured using an open-flow gas exchange system (LiCor 6400; LiCor Inc. Lincoln, NE, USA). Total needle surface area was calculated from a

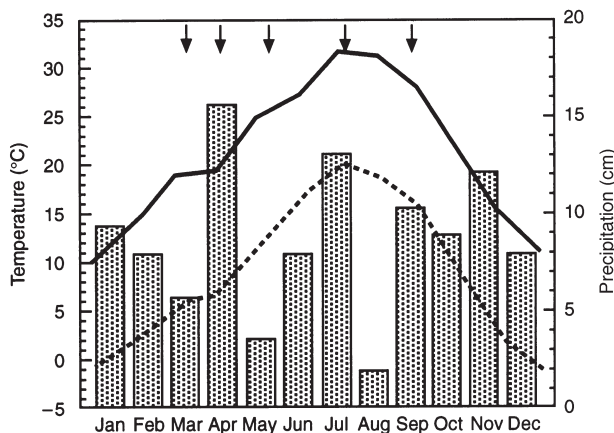


Figure 1. Maximum (solid line) and minimum (dashed line) temperature and precipitation (bars) during the first year of the FACE study. Arrows indicate time of measurement for assessing photosynthetic capacity in the ambient and elevated CO₂ rings.

regression of needle length to total needle surface area (Naidu, DeLucia & Thomas 1998) and was used as the basis for reporting gas exchange data. The relationship between net assimilation and the CO₂ partial pressure of the internal air space of needles ($A-C_i$ curve) was examined over a range of nine external CO₂ partial pressures (C_a) from approximately 5 to 175 Pa, including 38 Pa CO₂ and 57 Pa CO₂. Measurements were made with saturating light of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and at the ambient relative humidity. Cuvette temperatures were maintained at ambient air temperatures and averaged 17.5 °C in March, 20.5 °C in April, 28.0 °C in May, 31.3 °C in July and 27.0 °C in September. Gas exchange variables were calculated according to von Caemmerer & Farquhar (1981).

Net assimilation at the growth CO₂ partial pressures (A_{net}) was obtained from the $A-C_i$ curves where the measurement C_a equalled 38 Pa for the control needles and 57 Pa for the elevated CO₂ needles. Net assimilation data obtained at a C_i above 25 Pa was fitted to a second degree polynomial to estimate A_{max} , the net assimilation of the loblolly pine needles when given saturating light and CO₂. The maximum rate of carboxylation ($V_{c_{\text{max}}}$) was modelled from each $A-C_i$ curve by fitting data obtained at C_i below 25 Pa with a linear model and the maximum rate of electron transport (J_{max}) was modelled by linearization of the entire curve (Wullschlegel 1993; Long, personal communication).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a pulse modulated fluorometer (OS-500; OptiSciences Inc, Boston, MA) on the same day as the gas exchange measurements on a fascicle adjacent to the needles used for gas exchange. Quenching analysis was initiated by measuring the initial (F_o) and maximum (F_m) fluorescence prior to the start of a 5 min actinic light period. The actinic light intensity during quenching analysis was 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ red light (the maximum possible with the OptiSciences LED source). During exposure to actinic light, saturating flashes ($\approx 8000 \mu\text{mol m}^{-2} \text{s}^{-1}$) of 0.9 s duration, given at 20 s intervals, were used to cause transient reduction of QA, the electron acceptor of PSII, allowing measurement of the quenched state of F_m (F_m'). A weak 5 s far-red flash at the end of the measurement was used to determine the quenched state of F_o (F_o'). Photochemical and non-photochemical quenching parameters were calculated following the methods of van Kooten & Snel (1990).

Needle biochemistry

Needles for biochemical analyses were collected concurrent with gas exchange measurements. Needles for carbohydrate and N analyses were frozen with liquid N₂, stored on ice during transport to an oven, dried at 60 °C until achieving a constant mass and ground (Tecator Cyclotec 1093 sample mill; Tecator, Inc., Hoganas, Sweden).

Needle carbohydrate content was determined on a subsample (≈ 15 mg) using a spectrophotometric assay (Thomas & Griffin 1994). Carbohydrate analysis was performed on needles collected during April, May and September. Total carbon and total nitrogen were measured on a subsample (≈ 5 mg) following Dumas combustion with a Carlo Erba NA 1500 N,C,S elemental analyzer (Fisons Instruments, Valencia, CA, USA). Total C and N of loblolly pine needles was measured at all five sample periods of this study.

Needles collected for Rubisco activity analysis were ground within 3 min of collection in a grinding medium following the methods of Tissue, Thomas & Strain (1993). The resulting slurry was frozen in liquid N_2 and analysed at a later date. Initial Rubisco activity and total Rubisco activity (fully activated) was measured at 25 °C using the spectrophotometric method of Tissue *et al.* (1993) where the rate of oxidation of NADH was determined in a coupled assay. The activation state of Rubisco was calculated as the ratio of initial activity to total activity. Rubisco activity was assayed during all five sample periods of this study.

Statistical analysis

Needles from three trees from each ring were used for analyses of photosynthetic capacity. For each parameter measured, the FACE ring was treated as a replicate. All six rings (three control and three treatment) were used for measurements. A paired *t*-test was used at each measurement period to determine if the differences between paired ambient and control rings were significant at the $P < 0.05$ level. Because only one main effect was tested, no *post hoc* comparisons of means were performed.

RESULTS

Photosynthetic capacity

Net photosynthetic rates at growth CO_2 concentrations (A_{net}) of loblolly pine trees in the control and elevated CO_2 FACE rings are shown in Table 1. The enhancement ratio of photosynthesis due to elevated CO_2 (elevated/control, E/C) was calculated by dividing A_{net} of elevated CO_2 needles by A_{net} of control needles (Table 1). The smallest E/C was observed in March (0.98; no CO_2 stimulation) but E/C increased to 1.26 by late April. The maximum E/C was 1.52 and occurred in September. There was a strong positive correlation between E/C and leaf temperature during measurement ($r^2 = 0.67$, $P = 0.037$; Fig. 2).

Maximum rates of photosynthesis with saturating CO_2 and light (A_{max}) were significantly higher in needles from control loblolly pine trees in March and April when compared with needles from elevated CO_2 trees (Table 1). By the end of May and in July, A_{max} was higher in needles from trees in the elevated CO_2 rings than needles from trees in the control rings. There were no significant differences in A_{max} between loblolly pine trees from the control and treatment rings at the end of September.

Two potential biochemical limitations to photosynthesis, the maximum rate of carboxylation and the maximum rate of electron transport, were modelled from the $A-C_i$ curves (Fig. 3). Needles from trees in control rings had greater $V_{c,max}$ than needles in elevated CO_2 rings in March and April but there were no significant differences between treatments at the three measurement periods later in the season (Table 1). There were no significant differences in J_{max} between control and elevated CO_2 needles at any of the measurement periods (Table 1).

Table 1. Photosynthetic parameters derived from $A-C_i$ curves of needles from loblolly pine trees in control and elevated CO_2 rings. Elevated/Control represents the magnitude of enhancement of A_{net} by elevated CO_2 . Gas exchange was measured between 1000 and 1600 h on needles from an upper branch exposed to the sun. Measurements were performed ± 2 d of the date indicated in the table. Each value represents the mean of three FACE rings (± 1 SE). An asterisk indicates significant differences between control and elevated CO_2 rings determined by a paired *t*-test ($P < 0.05$)

Parameter		Date of measurement				
		March 15	April 15	May 30	July 30	September 30
Elevated/Control		0.98 \pm 0.09	1.26 \pm 0.06	1.28 \pm 0.07	1.45 \pm 0.05	1.52 \pm 0.07
A_{net} at growth CO_2 ($\mu mol m^{-2} s^{-1}$)	Control	6.47 \pm 0.55	6.01 \pm 0.32	6.40 \pm 0.22	5.24 \pm 0.26	5.87 \pm 0.62
	Elevated	6.38 \pm 0.44	7.58 \pm 0.14*	8.20 \pm 0.40*	7.58 \pm 0.28*	8.95 \pm 0.34*
Light and CO_2 saturated A_{max} ($\mu mol m^{-2} s^{-1}$)	Control	10.90 \pm 0.02	11.72 \pm 0.03	10.11 \pm 0.03	9.12 \pm 0.02	9.95 \pm 0.81
	Elevated	8.35 \pm 0.03*	9.87 \pm 0.03*	12.04 \pm 0.03*	11.28 \pm 0.05*	9.71 \pm 0.60
Maximum rate of carboxylation ($V_{c,max}$ $\mu mol m^{-2} s^{-1}$)	Control	34.07 \pm 1.40	35.27 \pm 1.00	36.01 \pm 0.43	30.91 \pm 2.33	39.25 \pm 0.34
	Elevated	27.53 \pm 1.90*	27.37 \pm 1.72*	29.71 \pm 1.81	31.36 \pm 2.71	33.08 \pm 2.97
Maximum rate of electron transport (J_{max} $\mu mol m^{-2} s^{-1}$)	Control	134.33 \pm 34.73	123.43 \pm 16.04	63.43 \pm 3.07	59.53 \pm 8.15	61.15 \pm 4.24
	Elevated	134.33 \pm 18.56	85.33 \pm 7.92	69.10 \pm 3.81	61.53 \pm 0.23	65.15 \pm 3.13

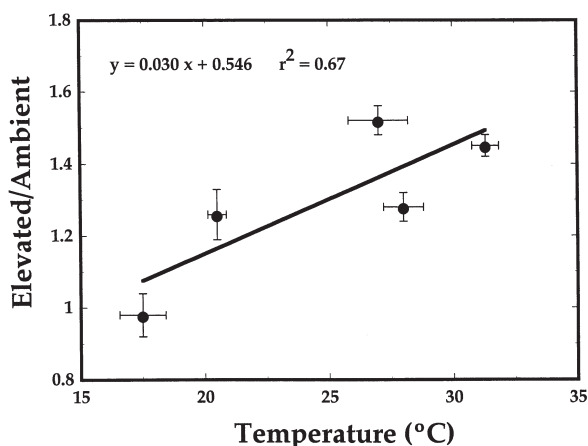


Figure 2. The relationship between the photosynthetic enhancement ratio (Elevated/Control) and leaf temperature measured during the first full growing season of the Brookhaven/Duke University FACE experiment. Each value represents the mean of three FACE rings (± 1 SE).

Quenching analysis

The photochemical efficiency of photosystem II, F_v/F_m , of loblolly pine needles was significantly greater in the control rings than in elevated CO_2 rings in March and April but in September, this trend was reversed (Table 2). There were no significant differences in F_v/F_m of needles from control rings and elevated CO_2 rings in May or July. Photochemical quenching (q_p), or light energy utilization through photochemistry, of loblolly pine needles showed no significant differences in q_p between treatments except in September when needles in elevated CO_2 were 11.6% lower than needles in control rings. Non-photochemical quenching of light energy (q_N), was significantly higher in elevated CO_2 needles than in control needles in March, April and September. There were no significant differences in q_N between treatments in May and July.

Rubisco activity

Spectrophotometric analysis of Rubisco activity from needles of loblolly pine generally agreed with the seasonal trends observed in maximum carboxylation rates modelled from $A-C_i$ curves (Table 3). In April and September, total Rubisco activity was significantly higher in control needles than that in elevated CO_2 needles but there were no significant differences between treatments in May and July. Rubisco activation state, ranging from 80 to 86%, varied little across the measurement periods and there were no significant differences between treatments in the activation state of Rubisco (Table 3).

Needle N concentration and non-structural carbohydrate content

Needle nitrogen concentration on a dry weight basis appears to be constant across the growing season and

there were no significant differences between control or elevated CO_2 needles with respect to N concentration or C/N ratio (Table 4).

Soluble sugar content of loblolly pine needles was not significantly different between treatments during all measurement periods (Table 4). Sugar content in all needles increased dramatically between late April and late May. As with soluble sugars, there were no significant differences in needle starch between control and elevated CO_2 rings (Table 4).

DISCUSSION

The rate at which CO_2 concentrations double in the atmosphere is strongly affected by the potential for accelerated CO_2 withdrawal from the atmosphere due to a stimulation of photosynthesis in terrestrial ecosystems (Vitousek 1991; Schimel 1995). During the first year of the Brookhaven/Duke FACE experiment, we found that canopy loblolly pine trees responded strongly to CO_2 enrichment. The magnitude of photosynthetic enhancement by elevated CO_2 was as high as 52%. The degree that elevated CO_2 stimulated photosynthesis, however, showed substantial seasonal variation that could be attributed primarily to the strong influence of the abiotic conditions occurring in the forest ecosystem.

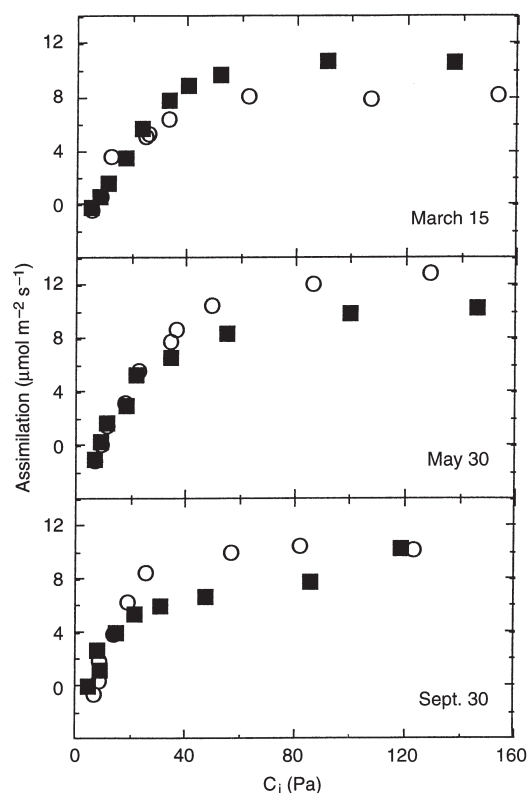


Figure 3. Representative $A-C_i$ curves of needles of loblolly pine trees from control rings (solid squares) and elevated CO_2 rings (open circles) measured in mid March (top) and late May (middle) and late September (bottom). $A-C_i$ response curves were also measured in April and July but are not shown.

Table 2. Chlorophyll fluorescence parameters of needles from loblolly pine trees in control and elevated CO₂ rings. Measurements were made with a pulse modulated fluorometer between 1000 and 1600 h on needles adjacent to gas exchange needles. Each value represents the mean of three FACE rings (± 1 SE). An asterisk indicates significant differences between control and elevated CO₂ rings determined by a paired *t*-test ($P < 0.05$)

Parameter		Date of measurement				
		March 15	April 15	May 30	July 30	September 30
F_v/F_m	Control	0.81 \pm 0.01	0.78 \pm 0.01	0.79 \pm 0.01	0.80 \pm 0.02	0.75 \pm 0.01
	Elevated	0.79 \pm 0.01*	0.74 \pm 0.01*	0.80 \pm 0.01	0.79 \pm 0.02	0.81 \pm 0.01*
Photochemical quenching	Control	0.93 \pm 0.01	0.93 \pm 0.01	0.94 \pm 0.01	0.81 \pm 0.02	0.95 \pm 0.02
	Elevated	0.96 \pm 0.02	0.94 \pm 0.01	1.00 \pm 0.02	0.86 \pm 0.03	0.84 \pm 0.01*
Non-photochemical quenching	Control	0.51 \pm 0.02	0.37 \pm 0.03	0.54 \pm 0.03	0.43 \pm 0.02	0.48 \pm 0.04
	Elevated	0.60 \pm 0.03*	0.49 \pm 0.03*	0.45 \pm 0.03	0.45 \pm 0.05	0.51 \pm 0.04*

A strong correlation was observed between leaf temperature and the enhancement ratio of photosynthesis by elevated CO₂ that explained 67% of the variation in magnitude of CO₂ stimulation. This result was probably due to the strong correlation between temperature and the specificity for oxygen by Rubisco (Jordan & Ogren 1984). The ratio of photorespiration to photosynthesis varies not only with the specificity of Rubisco for CO₂ and O₂, but also with partial pressures of both CO₂ and O₂ within the mesophyll air space (Woodrow & Berry 1988). Elevated CO₂, therefore, reduces the increase in photorespiration with higher temperatures. Based on the models of Farquhar, von Caemmerer & Berry (1980) and Farquhar & von Caemmerer (1982), Long (1991) provided a theoretical analysis of the correlation between the CO₂ enhancement of C3 photosynthesis and air temperature. Given a 20% reduction in $V_{c,max}$, a frequent effect of growth in elevated CO₂ (Sage, Sharkey & Seemann 1989), Long (1991) predicted a CO₂ enhancement ratio of 1.1 at a temperature of 17.5 °C (our March temperature) and an enhancement of 1.6 at 31.5 °C (our July temperature). These modelled estimates are close to our measured values in the field of 0.98 and 1.45 in March and July, respectively. The lower values of the CO₂ enhancement ratio that we observed are most likely related to the lower CO₂ treatment (57 Pa) in the FACE study compared with that used in Long's model (65 Pa).

Several studies have found a similar sensitivity to temperature by the photosynthetic responses of species to elevated CO₂ (Long 1991; Gunderson & Wullschleger 1994; Lewis *et al.* 1996; Idso, Kimball & Hendrix 1993). Lewis *et al.* (1996) expressed the magnitude of CO₂ stimulation of photosynthesis of loblolly pine saplings grown in open top chambers as a relative response based on the percentage increase of photosynthetic rates of pine saplings grown in ambient CO₂. When the data of Lewis *et al.* (1996) are converted to the enhancement ratio that we used, a comparable relationship to our own is found [$E/C = 0.038 \times \text{temperature} + 0.63$; see Fig. 2, Lewis *et al.* (1996)]. It is not clear if the relationship between temperature and CO₂ enhancement are different in these two studies of loblolly pine, but slight differences in the slopes may be related to the higher elevated CO₂ treatment used by Lewis *et al.* (1996). In addition, another difference between the two studies is the greater late season sink activity of the loblolly pine saplings in open top chambers with temperatures as high as 35 °C (Lewis, personal communication). The photosynthetic enhancement of loblolly pine saplings treated with elevated CO₂ for three years in open top chambers was as great as 110% during the summer but was less than 30% in the winter (Lewis *et al.* 1996). Our measurements with older canopy loblolly pine trees fumigated with elevated CO₂ for only 1 year indicated a smaller magnitude of enhancement by CO₂ enrichment.

Table 3. Biochemical measurements of Rubisco activity of needles from loblolly pine trees in control and elevated CO₂ rings. Needles were collected concurrent with gas exchange measurements between 1000 and 1600 h from an upper branch exposed to the sun. Needles were of a similar age as needles used in gas exchange measurements but were from a different tree. Each value represents the mean of three FACE rings (± 1 SE). An asterisk indicates significant differences between control and elevated CO₂ rings determined by a paired *t*-test ($P < 0.05$)

Parameter		Date of measurement			
		April 15	May 30	July 30	September 30
Total Rubisco activity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Ambient	17.96 \pm 3.80	22.16 \pm 4.85	14.03 \pm 1.53	17.35 \pm 0.92
	Elevated	10.15 \pm 0.98*	15.59 \pm 0.87	13.42 \pm 1.26	11.31 \pm 1.62*
Rubisco activation state (Initial/total \times 100)	Ambient	80.1 \pm 4.9	86.8 \pm 4.1	85.8 \pm 3.6	82.8 \pm 4.6
	Elevated	80.1 \pm 3.8	80.9 \pm 1.0	82.2 \pm 3.0	85.9 \pm 4.8

Parameter		Date of measurement		
		April 15	May 30	September 30
Nitrogen concentration (% of dry weight)	Control	1.30 ± 0.05	1.19 ± 0.05	1.15 ± 0.02
	Elevated	1.20 ± 0.03	1.10 ± 0.06	1.24 ± 0.06
Carbon/nitrogen	Control	38.73 ± 1.30	42.10 ± 1.88	43.78 ± 1.02
	Elevated	41.74 ± 0.97	45.70 ± 2.63	41.73 ± 1.73
Soluble sugar concentration (% of dry weight)	Control	3.94 ± 0.35	7.11 ± 0.30	5.56 ± 0.36
	Elevated	3.75 ± 0.35	7.62 ± 0.72	5.98 ± 0.39
Starch concentration (% of dry weight)	Control	7.81 ± 0.62	9.08 ± 1.65	6.74 ± 2.3
	Elevated	8.33 ± 1.03	7.78 ± 0.96	4.35 ± 0.28

Table 4. Needle chemistry from loblolly pine trees in control and elevated CO₂ rings. Needles were collected concurrent with gas exchange measurements between 1000 and 1600 h from an upper branch exposed to the sun. Needles were of a similar age as needles used in gas exchange measurements but were from a different tree. Each value represents the mean of three FACE rings (± 1 SE). An asterisk indicates significant differences between control and elevated CO₂ rings determined by a paired *t*-test (*P* < 0.05)

A second abiotic factor that may be involved in seasonal variation in photosynthetic enhancement by CO₂ enrichment is soil water availability. A drought occurred during the later part of the summer (late July, August, early September) due to low precipitation (4.0 cm over the 58 d period). Soil moisture during this time was approximately 16% in control and elevated CO₂ rings compared with approximately 25% soil moisture in the rings in June (Schäfer, unpublished results). The consequences of this drought on photosynthetic capacity can be seen in the measurements taken in July with a recovery period in September (Table 2). As a result of this drought, loblolly pine trees in the control rings exhibited lower net photosynthetic rates, lower maximum rates of photosynthesis at saturating CO₂ and light, lower modelled values of carboxylation and electron transport rates (from A–C_i curves), lower total Rubisco activity, and lower photochemical quenching of fluorescence than other measurement periods. While some symptoms of drought were found for trees in the elevated CO₂ rings, such as reduced net photosynthesis and photochemical quenching of fluorescence, there was little effect of drought on light- and CO₂-saturated rates of photosynthesis, modelled rates of carboxylation or electron transport, or Rubisco activity. In addition, in late September there was a greater recovery from drought in the trees in elevated CO₂ than in trees in the control rings. Thus, the enhancement ratio of photosynthesis by CO₂ enrichment during this drought and recovery period ranged from 45 to 52%. Several studies have shown greater photosynthetic enhancement by elevated CO₂ under drought conditions than with well-watered conditions (Morison 1993; Scarascia-Mugnozza *et al.* 1996).

A possible biotic influence on the enhancement ratio of photosynthesis by elevated CO₂ is plant growth rate. Responses to CO₂ enrichment are often affected by biochemical and physiological feedback mechanisms that regulate carbon assimilation to meet sink (growth) demand (Long & Drake 1992; Sage 1994). The relationship between photosynthetic capacity and sink demand in loblolly pine trees is complex because this tree species produces from one to seven distinct flushes of needle cohorts each growth season (Hellmers & Strain 1972). Growth of

the new cohorts depends on photosynthate from the preceding cohorts to synthesize the substances needed for growth (Chung & Barnes 1977). A compounding factor of sink demand in loblolly pine is that root growth is known to occur during two distinct periods, one in late spring–early summer and again in late summer–early autumn (Wahlenberg 1960). Sink demand and supply of loblolly pine trees therefore vary dramatically through the year. While it is difficult to assess in our study, the lack of photosynthetic enhancement by CO₂ enrichment in March, a measurement period before the expansion of the current year needles, may have been caused by either low sink demand or low temperatures or by a combination of these factors.

In a previous study where we experimentally manipulated source–sink balance of loblolly pine trees we found sharp increases in non-photochemical quenching in needles that had a strong sink limitation imposed on them (Myers, Thomas & DeLucia 1999). Non-photochemical quenching is a family of processes, dominated by the buildup of a transthylakoid pH gradient (ΔpH), that dissipate energy by means other than linear electron transport (Schreiber, Schliwa & Bilger 1986). Non-photochemical quenching provided a sensitive indicator of photosynthetic regulation in March before expansion of the current year needles, reinforcing the idea that early in the growing season, sink demand was limited and the requirement for excess energy dissipation greatly increased. In addition, in March and April, needles from the elevated CO₂ rings had lower F_v/F_m values than control needles. These data are in agreement with the hypothesis that elevated CO₂ can exacerbate photoinhibition of the photosynthetic apparatus, particularly during periods of low utilization of reducing equivalents (Scarascia-Mugnozza *et al.* 1996; Chaves & Pereira 1992). These results are also in agreement with Sharkey's (1990) interpretation that feedback regulation of photosynthesis requires increased amounts of q_N because photophosphorylation is limited by low phosphate levels in the stroma of chloroplasts.

The most often used technique for assessing sink limitations on photosynthesis is by measuring CO₂ response curves (A–C_i) (Farquhar & Sharkey 1982), and to further delimit biochemical restrictions on photosynthesis into

limitations imposed by carboxylation capacity and electron transport (von Caemmerer & Farquhar 1981; Sharkey 1985). Sink limitations on photosynthesis would be expected to induce reductions in $V_{c_{max}}$ and J_{max} indicative of feedback regulation of photosynthesis. In March, we found a strong CO₂-induced reduction in light- and CO₂-saturated photosynthesis as well as lower carboxylation rates. It is unlikely that this regulation of carboxylation rates was due to a greater portion of Rubisco in needles from the elevated CO₂ rings being maintained in an uncarbamyated state. Although activation state was not measured in March, this parameter was constant through all other measurement periods in both CO₂ treatments. Likewise, Lewis *et al.* (1996) found no effects of temperature and very little effect of elevated CO₂ on Rubisco activation state in loblolly pine saplings treated for 3 years with CO₂ enrichment. These data indicate that there was down-regulation of Rubisco activity during this period of low temperature and low growth in the canopy loblolly pine trees in the FACE experiment. During this measurement period, however, there were no effects of CO₂ on the modelled maximum electron transport rates. Since there were no reductions in both parameters these data contradict fluorescence data and suggest that the lack of photosynthetic enhancement by CO₂ enrichment in March was primarily an effect of temperature and secondarily a sink limitation due to slow growth by the loblolly pine trees.

In March, April and September we found either reduced $V_{c_{max}}$ or reduced total Rubisco activity. Otherwise we found no strong evidence for CO₂-induced down-regulation of Rubisco. Elevated CO₂ had no effect on maximum electron transport capacity and very little effect of elevated CO₂ on photochemical quenching from fluorescence measurements. In addition, we found no effects of CO₂ on needle nitrogen concentration, non-structural carbohydrate content (soluble sugar and starch) or C/N ratio. No accumulation of non-structural carbohydrate in response to CO₂ enrichment suggests that there were no sink limitations to limit photosynthesis at these measurements. The lack of an effect of elevated CO₂ on needle N concentration may be related to the fact that these needles were initiated just before CO₂ fumigation in the FACE rings started. Needle N concentrations across both treatments ranged from 1.1 to 1.3%, values that are less than optimal for loblolly pine (2–2.5% N; Fowells & Means 1990) and approaching the threshold at which N limits growth (< 1.1% N; Allen 1987). It remains to be seen if acclimation of photosynthetic capacity to growth at elevated CO₂ takes place over the time scale as more N in the forest ecosystem is sequestered in biomass.

The photosynthetic responses to elevated atmospheric CO₂ of canopy loblolly pine trees growing within the full suite of abiotic and biotic interactions of a forest ecosystem are complex. It is exceedingly hard to separate biotic and abiotic influences on photosynthesis in this system. This study clearly demonstrates that the photosynthetic enhancement of loblolly pine trees by CO₂ enrichment varies across the growth season. Air temperature is a primary influence on the photosynthetic responses of loblolly pine trees in the Brookhaven/Duke FACE experiment. Other abiotic factors,

such as soil water availability, and intrinsic biotic factors, such as needle flush development, may also influence the photosynthetic responses to elevated CO₂. During the first year of CO₂ fumigation in the FACE experiment, however, we believe that the effects of phenology on photosynthesis are secondary to abiotic influences. These first-year data will be used to compare to measurements from subsequent years of the FACE experiment in order to determine if photosynthetic acclimation to CO₂ occurs in these canopy loblolly pine trees in a forest ecosystem.

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