

Effects of CO₂ enrichment on the photosynthetic light response of sun and shade leaves of canopy sweetgum trees (*Liquidambar styraciflua*) in a forest ecosystem

JEFFREY D. HERRICK and RICHARD B. THOMAS

Department of Biology, West Virginia University, P.O. Box 6057, Brooks Hall, Morgantown, WV 26506, USA

Received October 28, 1998

Summary To investigate whether sun and shade leaves respond differently to CO₂ enrichment, we examined photosynthetic light response of sun and shade leaves in canopy sweetgum (*Liquidambar styraciflua* L.) trees growing at ambient and elevated (ambient + 200 $\mu\text{l l}^{-1}$) atmospheric CO₂ in the Brookhaven National Laboratory/Duke University Free Air CO₂ Enrichment (FACE) experiment. The sweetgum trees were naturally established in a 15-year-old forest dominated by loblolly pine (*Pinus taeda* L.). Measurements were made in early June and late August 1997 during the first full year of CO₂ fumigation in the Duke Forest FACE experiment. Sun leaves had a 68% greater leaf mass per unit area, 63% more leaf N per unit leaf area, 27% more chlorophyll per unit leaf area and 77% greater light-saturated photosynthetic rates than shade leaves. Elevated CO₂ strongly stimulated light-saturated photosynthesis of sun and shade leaves in June and August; however, the relative photosynthetic enhancement by elevated CO₂ for sun leaves was more than double the relative enhancement of shade leaves. Elevated CO₂ stimulated apparent quantum yield by 30%, but there was no interaction between CO₂ and leaf position. Daytime leaf-level carbon gain extrapolated from photosynthetic light response curves indicated that sun leaves were enhanced 98% by elevated CO₂, whereas shade leaves were enhanced 41%. Elevated CO₂ did not significantly affect leaf N per unit area in sun or shade leaves during either measurement period. Thus, the greater CO₂ enhancement of light-saturated photosynthesis in sun leaves than in shade leaves was probably a result of a greater amount of nitrogen per unit leaf area in sun leaves. A full understanding of the effects of increasing atmospheric CO₂ concentrations on forest ecosystems must take account of the complex nature of the light environment through the canopy and how light interacts with CO₂ to affect photosynthesis.

Keywords: elevated CO₂, FACE, Free Air Carbon Dioxide Enrichment, nitrogen, quantum yield, shade leaves, sun leaves.

Introduction

Numerous greenhouse, growth chamber and open-top chamber studies have shown that elevated atmospheric CO₂ enhances photosynthesis and plant biomass production

compared to that of plants grown at ambient CO₂ concentrations (Cure and Acock 1986, Eamus and Jarvis 1989, Poorter 1993, Ceulemans and Mousseau 1994, Gunderson and Wullschlegel 1994, Curtis 1996). Ceulemans and Mousseau (1994) reviewed many studies and found that a doubling of atmospheric CO₂ increased leaf-level photosynthesis by approximately 40% for conifers and 61% for deciduous trees. However, studies of the effects of elevated CO₂ on tree species are often conducted with seedlings or small saplings grown in controlled environments with non-limiting amounts of water and nutrients. As a consequence, many of these studies do not reflect the complex nature of trees growing in a forest ecosystem. If elevated CO₂ stimulates leaf area production and produces denser forest canopies, a greater percentage of foliage will be shaded and the role of the shaded branches will increase in importance with respect to whole-tree carbon balance. Thus, an understanding of the photosynthetic responses of sun and shade leaves growing in a natural canopy is critical to predictions of the effects of increasing atmospheric CO₂ on forest ecosystems.

Most studies on the effects of elevated CO₂ on plants have used relatively high irradiances, so that CO₂ supply and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity are the main limitations on C₃ photosynthesis (Bowes 1993). In a natural forest ecosystem, much of the photosynthesis in a canopy takes place in reduced light because of shading. Local light environments affect developmental, morphological and physiological attributes of individual leaves such as leaf thickness, nitrogen per unit area, chlorophyll per unit area, and net photosynthesis (Boardman 1977, Björkman 1981, McClendon and McMillen 1982, Hirose and Werger 1987, Ellsworth and Reich 1993). Pearcy and Björkman (1983) suggested that elevated CO₂ would improve carbon balance in light-limited as well as high-light environments through the CO₂ enhancement of quantum yield. The present CO₂:O₂ ratio of the air constrains photosynthesis by 30–40%, because of O₂ inhibition of carboxylation and associated photorespiration (Bowes 1993). As CO₂ concentrations increase, quantum yield is increased because the ratio of carboxylation to oxygenation by Rubisco increases and photorespiration decreases. Several studies have shown that CO₂ enrichment enhances photosyn-

thesis and growth under limiting irradiance conditions, and in some cases the relative enhancement was greater at low irradiances than at high irradiances (Sionit et al. 1982, Cure and Acock 1986, Allen 1990, Long and Drake 1991, Gifford 1992). Very few studies have examined the effect of CO₂ enrichment on photosynthesis in leaves shaded by a natural forest canopy (Osborne et al. 1997, Winter and Virgo 1998).

The Brookhaven National Laboratory/Duke University Free Air CO₂ Enrichment (FACE) experiment in North Carolina permits direct study of the effects of elevated CO₂ on large trees under natural field conditions. This experiment is being conducted in a piedmont forest dominated by loblolly pine trees (*Pinus taeda* L.) with sweetgum trees (*Liquidambar styraciflua* L.) as the dominant deciduous tree species. Sweetgum is an early successional tree species that commonly invades broomsedge (*Andropogon virginicus* L.) fields in the piedmont of North Carolina during secondary succession (Oosting 1942). Although an early successional tree species, sweetgum leaves have the ability to acclimate to shaded conditions (Zimmerman and Brown 1971, Harley et al. 1996). Sun leaves and shade leaves of sweetgum differ morphologically and physiologically with photosynthesis and stomatal conductance as much as three times greater in sun leaves than in shade leaves (Harley et al. 1996). In addition, elevated CO₂ has been shown to stimulate photosynthesis and biomass production of sweetgum seedlings under a variety of environmental conditions (Rogers 1983, Tolley and Strain 1984a, 1984b, 1985, Sionit et al. 1985, Groninger et al. 1995, 1996, Tschaplinski et al. 1995).

The objective of our study was to determine whether sun and shade leaves of canopy sweetgum trees in the Duke FACE experiment respond differentially to CO₂ enrichment. We tested the hypothesis that CO₂ enrichment will stimulate photosynthesis in sun and shade leaves in a manner that increases their daily carbon balance. We predicted that sun leaves would have the greatest absolute enhancement in response to CO₂ enrichment, because sun leaves have a greater photosynthetic capacity. Shade leaves, however, would have the greatest relative enhancement of net photosynthesis by elevated CO₂, because of very low net assimilation in the understory and a stimulation of quantum yield. Photosynthetic light responses and leaf characteristics were measured twice during the first full growing season of the Duke FACE experiment on sun and shade leaves of canopy sweetgum trees exposed to either elevated or ambient CO₂. To place the sun and shade leaf responses in the context of a natural light environment, a simulation of leaf-level daily carbon gain was constructed.

Materials and methods

Brookhaven National Laboratory/Duke University Free-Air CO₂ Enrichment (FACE) experiment

The Duke University FACE experiment in the Blackwood division of the Duke Forest (35°97' N, 79°09' W) was established to examine responses of an intact forest ecosystem to elevated concentrations of atmospheric CO₂. The forest is dominated by loblolly pine (1733 stems ha⁻¹), with significant

numbers of sweetgum (*Liquidambar styraciflua*, 620 stems ha⁻¹) and yellow poplar (*Liriodendron tulipifera* L., 68 stems ha⁻¹) as secondary associates. The loblolly pine trees in the plantation have exhibited remarkably uniform growth, with a median height of 14 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 Southeastern USA. This section of the Duke forest was farmed a century ago, and the current plantation was established in 1983 after the regenerating forest was clear-cut in 1979.

The FACE system increases atmospheric CO₂ concentrations in 30-m diameter experimental circular plots (or rings) nested within this continuous loblolly 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 14-m tall forest canopy and contain adjustable ports at 50-cm intervals. These ports are tuned to control atmospheric CO₂ concentration through the entire forest volume. Fumigation with CO₂ in three experimental rings (ambient + 200 ± 84 μl l⁻¹ or approximately 560 μl l⁻¹) began in August 1996. Three control rings (approximately 366 μl l⁻¹) receive the same volume of air to replicate any micrometeorological effects on the forest that occur during the operation of the FACE facility. To control for topographic variation (~5 m) and potential gradients in site fertility between rings, the three control and three elevated-CO₂ rings were arranged in a complete block design (three pairs).

Gas exchange and leaf chemistry

Measurements were made twice during the first growing season (1997) of the experiment. In June, mean maximum temperature was 27 °C and mean minimum temperature was 16 °C (Figure 1). Rainfall was 9.86 cm in June and soil water content averaged 23.3% across the three control rings and 26.2% across the experimental rings (K. Schäfer, Duke University, unpublished data). The second measurement period was in August, at which time mean maximum temperature was 31 °C and mean minimum temperature was 19 °C. In August, there was 3.26 cm of rainfall and soil water content averaged 15.5% across the three control rings and 16.1% across the experimental rings (Schäfer, unpublished data).

Two sweetgum trees approximately 7–11 m in height were selected in each FACE ring based on the proximity of trees to areas accessible from portable hydraulic lifts. All trees in this study had leaves exposed to full sunlight at the top of the canopy and deep shade at the bottom of the canopy.

Photosynthetic light response curves were measured at growth CO₂ concentrations on two sun and two shade leaves from each tree with an open flow infrared gas analyzer with an attached red LED light source (LI-6400, Li-Cor, Inc., Lincoln, NE). Measurements began with approximately 5 minutes of saturating light (1500 μmol m⁻² s⁻¹) followed by nine incremental reductions until the irradiance was 10 μmol m⁻² s⁻¹. Use of decreasing light rather than increasing light reduces the equilibrium time required for stomatal opening and photosyn-

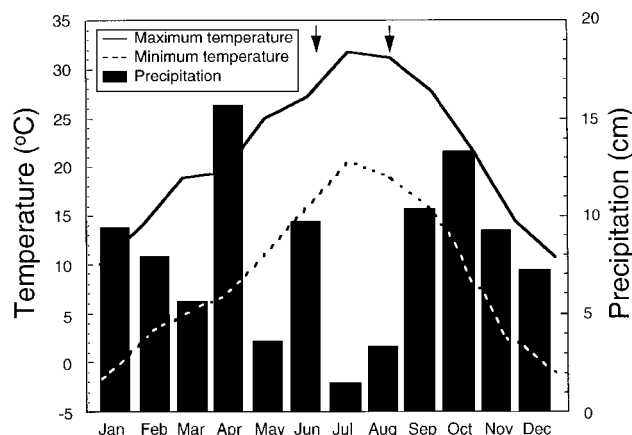


Figure 1. Mean monthly maximum and minimum temperatures and total monthly precipitation during the first full year (1997) of CO₂ treatment of the Duke Forest FACE experiment. Arrows indicate the two periods when sun and shade leaf photosynthetic parameters were measured.

thetic induction (Kubiske and Pregitzer 1996). Preliminary trials indicated that photosynthetic rates reached steady state within 2 minutes following each incremental decrease in light. Measurements were made on fully expanded leaves that were at least 2–3 weeks old. Leaf age was determined by a concurrent study of sweetgum leaf demography (Herrick, unpublished data). Gas exchange measurements were restricted to the hours between 1000 and 1500 h on sunny days to minimize diurnal effects on photosynthesis. Leaf temperatures averaged 28.12 ± 0.67 °C in June and 31.23 ± 0.58 °C in August. Within each measurement period, leaf temperatures were not significantly different between the CO₂ treatments or the leaf positions. Trees in one blocked pair of rings were measured each day so that slight differences in daily weather conditions could be included in the block effect in the analysis of variance. All ring pairs were measured within a one-week period during June and August.

Differences in light response curves due to CO₂ concentration and canopy position were examined by calculating and statistically comparing light-saturated photosynthesis (A_{sat}), dark respiration (R_d), light compensation point (Γ) (where $A = R_d$), and apparent quantum yield (ϕ). Apparent quantum yield (ϕ) and R_d were estimated from measured data. Values of ϕ were calculated as the slope of photosynthesis (A) versus incident irradiance between 10 and 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Light compensation points (Γ) were estimated by extrapolating between measured data.

The shape of the average light response curve in each CO₂ concentration and canopy position was modeled by fitting data to a non-rectangular hyperbola (Leverenz 1987, Leverenz 1995) by means of a nonlinear least squares curve-fitting program (JMP, SAS Institute, Inc., Cary, NC):

$$A + R_d = \left[I\phi + A_{\text{sat}} - \left[(I\phi + A_{\text{sat}})^2 - 4\phi A_{\text{sat}} C \right]^{0.5} \right] / 2C, \quad (1)$$

where A is the net photosynthetic rate, I is the photosynthetic photon flux density and C describes the convexity of the curve.

After gas exchange measurements were completed, each leaf was harvested and assayed for total nitrogen and chlorophyll on a weight and leaf area basis. Leaf tissue for nitrogen analysis was dried at 65 °C and assayed with a Carlo Erba CNS autoanalyzer (Fisons Instruments, Milan, Italy). Leaf tissue for chlorophyll analysis was immediately stored on ice and transported to the laboratory where chlorophyll was extracted with *N,N*-dimethyl formamide and analyzed spectrophotometrically according to the methods of Porra et al. (1989). Leaf mass per unit area (LMA, g m^{-2}) was calculated by measuring the dry mass of leaf disks of a known area.

Modeled diurnal net photosynthesis

A diurnal leaf-level simulation was performed in order to place the responses of sun and shade leaves to light and CO₂ enrichment in the context of daytime carbon gain (Kubiske and Pregitzer 1996). Twenty-four hour measurements of incident photosynthetic photon flux density (PPFD) were recorded for several days during June and July 1997 on one tree at three and eight meters above the ground. These positions correspond to the locations of shade leaves and sun leaves on the sweetgum tree. One-minute averages were taken with Li-Cor LI-190SA quantum sensors attached to a Li-Cor LI-1000 data logger. The light data were used to interpolate daytime carbon assimilation (A') from light response curves (as modeled by Equation 1) in each treatment for both measurement periods. These calculations rely only on the response of photosynthetic rate to light and ignore any possible changes in stomatal conductance to elevated CO₂, and photosynthetic induction response to sunflecks or diurnal effects. The relative CO₂ enhancement of sun and shade leaf carbon gain was compared.

Data analysis

Measurements made in June and August were analyzed separately. Two leaves were measured in June on each tree at both canopy positions. The June data were subjected to analysis of variance (ANOVA) with CO₂ treatment, leaf position and blocked ring pair as main effects and tree nested within the CO₂ treatment by ring pair interaction (JMP, SAS Institute, Inc., Cary, NC). Only one leaf was measured in August on each tree at both canopy positions. Sun and shade leaf measurements were averaged for each ring. Data from August were analyzed by an ANOVA model with CO₂ treatment, leaf position and blocked ring pair as main effects and CO₂ treatment by leaf position and CO₂ treatment by ring pair as interactions (JMP, SAS Institute). *Post hoc* comparison of parameter means was performed with the Tukey-Kramer HSD test (JMP, SAS Institute). Parameters were considered significantly different when $P < 0.05$.

Results

Photosynthetic light response

Photosynthetic light response curves are shown in Figure 2. Light-saturated net photosynthesis (A_{sat}) was greater in sun

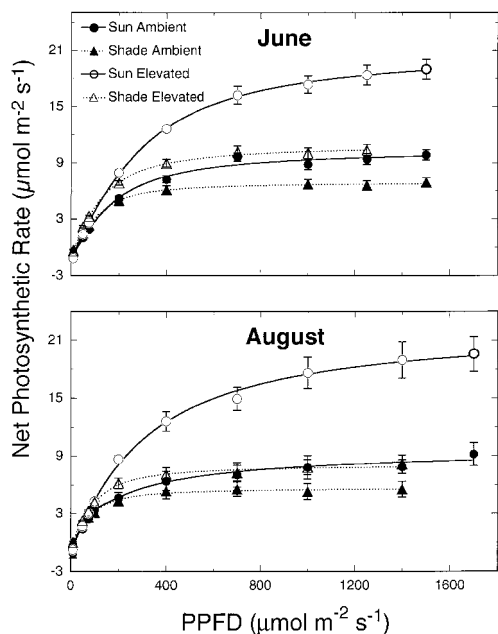


Figure 2. Net photosynthetic rate versus incident photosynthetic photon flux density (PPFD) of sun (circles) and shade (triangles) leaves of sweetgum trees growing at ambient CO_2 (closed symbols) and elevated CO_2 (open symbols). Measurements were made in June and August 1997. Error bars represent ± 1 SE ($n = 3$). Lines were predicted with Equation 1 and a nonlinear least squares curve fitting program (JMP, SAS Institute, Inc., Cary, NC).

leaves than in shade leaves during June ($P < 0.0001$) and August ($P < 0.001$) (Figure 3a). Elevated CO_2 resulted in higher rates of A_{sat} in June ($P < 0.001$) and August ($P < 0.001$) (Figure 3a). The enhancement of A_{sat} by CO_2 enrichment was significantly greater for sun leaves than for shade leaves ($\text{CO}_2 \times \text{leaf position}$, $P < 0.01$, Figure 3a). In June, elevated CO_2 enhanced A_{sat} by 92% in sun leaves and by 54% in shade leaves. During August, CO_2 enhancement of A_{sat} was 166% and 68% in sun and shade leaves, respectively. There were no blocked ring pair effects on any of the gas exchange parameters measured.

Apparent quantum yield, calculated from the initial slope of the light response curves, was slightly lower in shade leaves than in sun leaves during June ($P = 0.0569$). During August, sun leaves had a higher quantum yield than shade leaves ($P < 0.05$). Elevated CO_2 increased apparent quantum yield in sun leaves ($P < 0.01$) and shade leaves ($P < 0.01$) during both measurement periods, but there was no interaction between CO_2 and leaf position (Figure 3b). In June, quantum yield was enhanced by 29% in sun leaves and by 40% in shade leaves. In August, apparent quantum yield was enhanced by approximately 25% in both sun and shade leaves (Figure 3b).

Shade leaves had a lower light compensation point (Γ) and dark respiration rate (R_d) than sun leaves in June ($P < 0.01$) and August ($P < 0.01$) (Figures 3c and 3d). Elevated CO_2 had no significant effect on Γ or R_d , as estimated from the photosynthetic light response curves (Figures 3c and 3d). There were no interactions between CO_2 and leaf position for Γ or R_d .

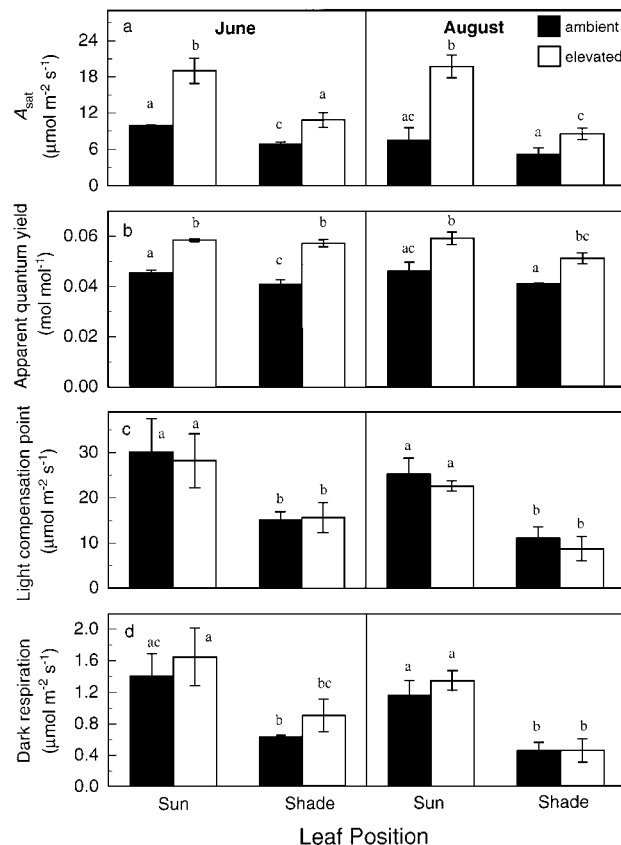


Figure 3. (a) Light-saturated photosynthesis (A_{max}), (b) apparent quantum yield, (c) light compensation point, and (d) dark respiration of sun and shade leaves in sweetgum trees grown at ambient CO_2 or elevated CO_2 . Parameters were calculated from light response curves measured in June and August 1997. Error bars represent ± 1 SE ($n = 3$). Within a measurement period, values that are designated by the same letter are not different at the 0.05 level of significance.

Leaf chemistry

Sun and shade leaves did not differ in N concentration on a dry mass basis (Table 1). However, sun leaves had greater N per unit area than shade leaves ($P < 0.01$), because leaf mass per unit area (LMA) was 68% greater in sun leaves than in shade leaves in June and August ($P < 0.01$). Leaf N per unit area was 67 and 61% greater in sun leaves than in shade leaves in June and August, respectively. Neither leaf N per unit mass nor leaf N per unit area was significantly affected by CO_2 enrichment, although there was evidence of a trend for CO_2 -induced reduction in N per unit mass in June ($P = 0.0762$) and August ($P = 0.0532$). There were no significant ring pair effects, or interactions between ring pair, CO_2 and leaf position, on leaf N per unit mass or per unit area. During June, there was a significant $\text{CO}_2 \times \text{leaf position}$ interaction on LMA because elevated CO_2 increased LMA in sun leaves, but not in shade leaves ($P < 0.0001$). However, in August, there were no effects of CO_2 and no interactions of CO_2 , leaf position or ring pair on LMA.

Shade leaves had more chlorophyll on a dry mass basis than sun leaves ($P < 0.05$; Table 1). In contrast, chlorophyll expressed on a leaf area basis was 35% greater in sun leaves than

Table 1. Biochemical and morphological parameters of sun and shade leaves from canopy *Liquidambar styraciflua* trees grown in ambient or elevated CO₂. Leaves were collected immediately after gas exchange measurements during the June and August sample periods. Each value is the mean of three rings (\pm SE) from both CO₂ treatments, ignoring block effects. Within a measurement period, values followed by the same letter are not different at the 0.05 level of significance.

		N/Leaf mass (mg g ⁻¹)	N/Leaf area (g m ⁻²)	Chl/Leaf mass (mg g ⁻¹)	Chl/Leaf area (g m ⁻²)	Leaf mass/Area (g m ⁻²)
<i>June 1997</i>						
Sun	Elevated	18.84 \pm 0.43 a	1.57 \pm 0.15 a	4.46 \pm 0.26 a	0.345 \pm 0.016 a	82.96 \pm 6.49 a
	Ambient	19.79 \pm 0.89 a	1.26 \pm 0.05 b	5.84 \pm 0.66 ab	0.352 \pm 0.034 a	64.23 \pm 4.76 b
Shade	Elevated	18.79 \pm 0.43 a	0.85 \pm 0.05 c	5.70 \pm 0.51 ab	0.260 \pm 0.016 b	45.44 \pm 2.31 c
	Ambient	19.94 \pm 0.57 a	0.84 \pm 0.03 c	6.16 \pm 0.61 b	0.256 \pm 0.014 b	42.19 \pm 2.79 c
<i>August 1997</i>						
Sun	Elevated	15.52 \pm 0.80 a	1.92 \pm 0.16 a	4.22 \pm 0.85 a	0.496 \pm 0.040 a	126.37 \pm 16.70 a
	Ambient	16.48 \pm 0.64 ab	1.61 \pm 0.13 ab	5.34 \pm 0.65 ab	0.479 \pm 0.041 a	96.23 \pm 6.73 ab
Shade	Elevated	15.75 \pm 0.16 ab	1.13 \pm 0.06 b	5.82 \pm 0.41 ab	0.399 \pm 0.034 a	71.32 \pm 3.70 b
	Ambient	17.84 \pm 1.44 b	1.07 \pm 0.05 c	7.10 \pm 0.61 b	0.430 \pm 0.019 a	61.42 \pm 3.58 b

shade leaves in June ($P < 0.05$) and, in August, sun leaves had 18% more chlorophyll per unit leaf area than shade leaves ($P < 0.05$, Table 1). Elevated CO₂ reduced leaf chlorophyll per unit dry mass in June ($P < 0.05$). In August, there was no significant effect of CO₂ on leaf chlorophyll per unit dry mass ($P = 0.0766$). Chlorophyll per unit leaf area was not affected by CO₂ enrichment. There were no significant effects of ring pair, or interactions between CO₂, leaf position, and ring pair, on leaf chlorophyll per unit dry mass or per unit leaf area.

Modeled diurnal net photosynthesis

The light environment of sun and shade leaves varied greatly on a daily basis (Figure 4). Photosynthetic photon flux density (PPFD), measured on June 29, 1997 at the top of a tree (approximately 8 m high), where sun leaves developed, and at the bottom of the same tree (approximately 3 m high), where shade leaves developed, is shown in Figure 4. These data were chosen because they represent a typical day during the mid-growing season with intermittent cloud cover. The irradiance data were used to estimate daytime photosynthetic rate (A') and provide a theoretical indication of the effects of elevated CO₂ on daily carbon gain of sun and shade leaves. Modeled results of leaf C gain for sun leaves and shade leaves for the June measurement period are shown in Figure 5. Daytime integrated net photosynthesis was strongly enhanced by elevated CO₂ in sun leaves ($P < 0.0001$), whereas there was only a small treatment effect in shade leaves (Figure 6). Sun leaves had a higher daytime integrated carbon gain than shade leaves ($P < 0.0001$). Elevated CO₂ stimulated daytime carbon uptake much more in sun leaves than in shade leaves (CO₂ \times leaf position, $P < 0.0001$). During June, CO₂ enrichment enhanced daytime carbon uptake of sun and shade leaves by 79 and 49%, respectively. The same irradiance data were also used to model daytime carbon gain for the sweetgum leaves measured in August. In August, elevated CO₂ enhanced daytime carbon gain by 120% in sun leaves ($P < 0.0001$) and 33% in shade leaves ($P < 0.0001$) (data not shown).

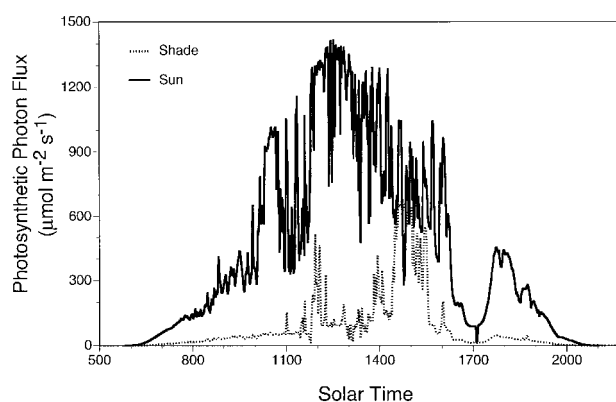


Figure 4. Representative light microenvironment on a sunny day (June 29, 1997) at the sun and shade canopy positions. The dark period at 1700 h was caused by cloud cover.

Discussion

Elevated CO₂ may stimulate leaf area production (Tolley and Strain 1984a, Sionit et al. 1985), producing denser forest canopies. As a result, a greater proportion of canopy foliage will be shaded and the role of shaded branches in whole-tree carbon balance will increase in importance. We hypothesized that elevated CO₂ would enhance photosynthetic rates of both sun and shade leaves of sweetgum trees growing in the canopy of the Duke Forest FACE experiment. During the first full year of fumigation with CO₂ at the FACE experiment, we found that light-saturated net photosynthetic rates (A_{sat}) of canopy sweetgum sun and shade leaves were strongly stimulated by CO₂ enrichment. We had predicted, however, that shade leaves would show a greater relative response to CO₂ enrichment than sun leaves because of the generally low photosynthetic rates of shade leaves and subsequent stimulation of quantum yield by high CO₂ concentrations. In contrast to our predictions, the relative photosynthetic enhancement by elevated CO₂ was greater in sun leaves than in shade leaves. The elevated CO₂-

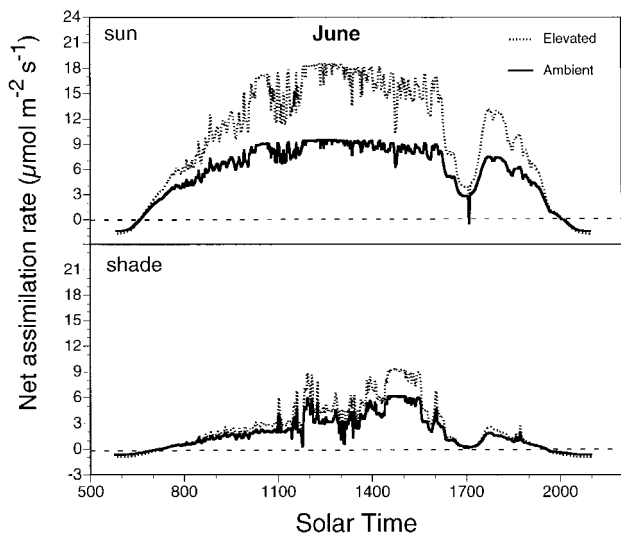


Figure 5. Daytime leaf photosynthetic rates (A') interpolated from June photosynthetic light response curves (shown in Figure 2) and the diurnal light environment (shown in Figure 4) of sun and shade leaves of sweetgum trees grown at elevated CO_2 (dashed lines) and ambient CO_2 (solid lines).

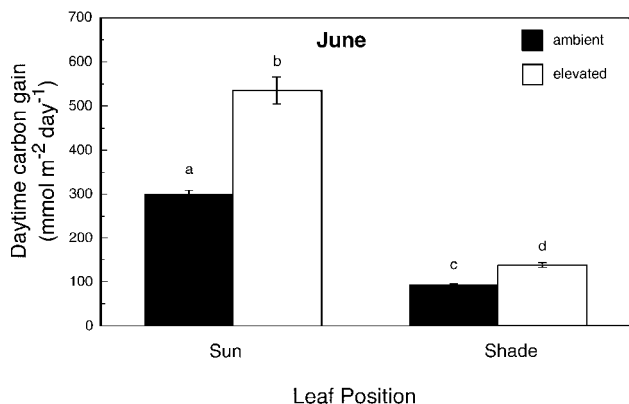


Figure 6. Daytime integrated leaf carbon gain of sun and shade leaves of large sweetgum trees grown at ambient CO_2 or elevated CO_2 . Values are means of three rings in each CO_2 treatment based on interpolated diurnal photosynthetic rates shown in Figure 5. Error bars represent ± 1 SE. Within a measurement period, values that are designated by the same letter are not different at the 0.05 level of significance.

induced stimulation of A_{sat} in sun leaves was 92% in June and 166% in August. In contrast, elevated CO_2 enhanced A_{sat} of shade leaves by 54% in June and by 68% in August (Figure 3a).

Many studies have suggested that shade leaves respond to atmospheric CO_2 enrichment to a greater extent than sun leaves as a result of increased quantum yields (Mortensen and Moe 1983, Lasko et al. 1984, Spalding and Portis 1985, Kirchbaum and Farquhar 1987, Idso et al. 1994, Kubiske and Pregitzer 1996). A small increase in quantum yield may increase daily carbon gain under low light conditions (Percy and Björkman 1983). In our study, elevated CO_2 increased apparent quantum yields of sun and shade leaves, but there was no difference in

the amount of CO_2 stimulation between the two leaf types (Figure 3b). In addition, in June, we found no significant differences between apparent quantum yield of sun and shade leaves, a result that is consistent with studies of shade-intolerant species (Kubiske and Pregitzer 1996), including sweetgum trees (Teskey and Shrestha 1985). During August, however, sun leaves had a greater apparent quantum yield than shade leaves.

Sun leaves of canopy sweetgum trees had a 68% greater leaf mass per unit area, 63% more N per unit leaf area and 27% more chlorophyll per unit leaf area than shade leaves (Table 1). Because the light-saturated portion of a light response curve (A_{sat}) is limited by Rubisco activity (Stitt 1991), we believe that the differential response of A_{sat} to CO_2 enrichment between the two leaf types was driven by greater amounts of N per unit leaf area in sun leaves than in shade leaves. A reduction in leaf N concentration has been observed in many CO_2 enrichment studies (Wong 1979, Norby et al. 1986, Williams et al. 1986). During the first year of the FACE experiment, however, we found no significant reductions in leaf nitrogen in response to CO_2 enrichment (Table 1). Several studies have reported leaf N concentrations of approximately 15.1 mg g^{-1} in forest-grown sweetgum trees (Blinn and Buckner 1989). We found leaf N concentrations that were greater than this value during both measurement periods suggesting that the sweetgum trees in the Duke Forest FACE experiment were not N limited (Table 1). However, these results are from the first year of a long-term study, and it may take longer for CO_2 -induced reductions in leaf N to become apparent in forest-grown trees. The FACE experiment is a perturbation of an intact forest system, which means that, as the elevated CO_2 treatment stimulates biomass production, nutrient availability may decrease causing a new equilibrium to become established.

Even if one documents a CO_2 -induced increase in A_{sat} and quantum yield, it is not clear how changes in these parameters will affect leaf carbon gain. We know that the light environment is highly variable above and below the canopy because of periodic cloud cover and shading through the canopy (Figure 4). Percy (1988) reported that 30 to 60% of the daily carbon gain in a forest understory may be contributed by sunflecks. To examine the effects of elevated CO_2 in a natural light regime, a simple daytime carbon uptake model was constructed. This model assumed that leaves respond immediately to changes in PPFD and photosynthetic induction and stomatal changes throughout the day were not taken into account. Results from this simple model indicated that enhancement of daytime C uptake by elevated CO_2 was greater for sun leaves than for shade leaves. In June, elevated CO_2 enhanced daytime carbon uptake of sun leaves by 79% and shade leaves by 49% (Figure 6). Between 1000 and 1500 h, PPFD averaged $130 \mu\text{mol m}^{-2} \text{ s}^{-1}$ below the canopy and $729 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at the top of the sweetgum canopy. The enhancement of modeled C uptake during this time period was 81% in sun leaves and 45% in shade leaves. Although stimulation of modeled C uptake by elevated CO_2 was lower than stimulation of A_{sat} , there was still a strong enhancement. Between 900 and 1000 h, PPFD in the shade averaged $39 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and modeled C uptake was enhanced 36% by elevated CO_2 . In contrast, between 1450 and

1550 h, PPFD in the shade averaged 536 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and modeled C uptake was enhanced 51%. These modeled data indicate the importance of considering patches of high irradiance when predicting the response of shade leaves to elevated CO₂. Kubiske and Pregitzer (1996) found similar results with red oak seedlings grown at elevated CO₂ in shaded open-top chambers.

Previous studies of sweetgum seedlings and saplings have reported that elevated CO₂ enhances photosynthesis by 0 to 71% (Rogers 1983, Tolley and Strain 1984a, 1984b, 1985, Sionit et al. 1985, Fetcher et al. 1988, Groninger et al. 1995, 1996, Tschaplinski et al. 1995). This range of response to CO₂, which is lower than that observed in our study, reflects variability in tree age, CO₂ treatments, duration of study, measurement techniques and experimental design. These factors make comparisons with our study difficult. We attribute the greater response to elevated CO₂ in sun leaves of canopy sweetgum trees of the FACE experiment to several of interactive factors including high leaf nitrogen and high air temperatures. A third factor that may have had a strong influence on the photosynthetic response to elevated CO₂ was the dry soil conditions resulting from low rainfall in August. Tolley and Strain (1985) found that elevated CO₂ significantly moderated the effects of water stress on sweetgum seedlings. Young seedlings in elevated CO₂ maintained high whole-plant assimilation rates during the soil drying cycle and, as a result, photosynthetic enhancement by CO₂ increased throughout the drying cycle until the last day of the cycle when there was no photosynthetic enhancement (Tolley and Strain 1985). In contrast, Tschaplinski et al. (1995) found that severe drought significantly reduced the enhancement of leaf photosynthetic rates by elevated CO₂. During August, at the FACE experiment, soil moisture was low and A_{sat} of sun leaves was enhanced by 166% by elevated CO₂. This high enhancement resulted from lower A_{sat} in August than in June in leaves grown in ambient CO₂ leaves, whereas A_{sat} of leaves grown in elevated CO₂ did not differ between the two sample periods.

In summary, elevated CO₂ significantly increased light-saturated photosynthetic rates, quantum yield and modeled daytime leaf carbon gain of sun and shade leaves of canopy sweetgum trees during the first full year of the Duke Forest FACE experiment. Elevated CO₂ enhanced A_{sat} and daytime carbon gain more in sun leaves than in shade leaves. Quantum yield was stimulated by CO₂ enrichment, but did not show a CO₂ \times leaf position interaction. Sun leaves had greater leaf mass per unit area, leaf N per unit leaf area, chlorophyll per unit leaf area and light-saturated photosynthetic rates than shade leaves. The greater CO₂ enhancement of light-saturated photosynthesis in sun leaves than in shade leaves was probably associated with a greater amount of leaf N per unit leaf area in sun leaves compared with shade leaves. Despite showing a smaller stimulation by elevated CO₂, shade leaves make up a large component of the sweetgum canopy and, as a result, are important to whole-canopy photosynthesis. If atmospheric CO₂ enrichment stimulates quantum yield, trees may be able to maintain greater leaf area in the shade, thus increasing LAI of the forest canopy to a new equilibrium. In order to understand the effects of increasing atmospheric CO₂ concentrations

on forest ecosystems, one must consider the complex nature of the light environment.

Acknowledgments

The authors thank Drs. David Myers and Evan DeLucia for guidance on the field and technical aspects of this project, Dr. James McGraw for statistical consultation and Jeff Pippen and Chris Nacci for assistance in data collection. We thank Christine Muth and Dr. David Myers for helpful comments on this manuscript. We thank Dr. Jerry Leverenz for assistance with curve fitting of the photosynthetic data. We acknowledge the Brookhaven/Duke Forest FACE site supported by the U.S. Department of Energy and the Duke University Phytotron staff. This research was supported through the NSF/DOE/NASA/USDA/EPA/NOAA Interagency Program on Terrestrial Ecology and Global Change (TECO) and by the U.S. Department of Energy Program for Ecosystem Studies Grant DE-FG02-95ER62124.

References

- Allen, L.H., Jr. 1990. Plant responses to rising carbon dioxide and potential interactions with air pollutants. *J. Environ. Qual.* 19:15–34.
- Björkman, O. 1981. Responses to different quantum flux densities. *In* Physiological Plant Ecology I. Responses to the Physical Environment. *Encycl. Plant Physiol. New Ser.*, Vol. 12A. Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler. Springer-Verlag, Heidelberg, pp 57–107.
- Blinn, C.R. and E.R. Buckner. 1989. Normal foliar nutrient levels in North American forest trees (Summary No. 590-1989). Minnesota Agricultural Experiment Station.
- Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Physiol.* 28:355–377.
- Bowes, G. 1993. Facing the inevitable: plants and increasing CO₂. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44:309–332.
- Ceulemans, R. and M. Mousseau. 1994. Effects of elevated atmospheric CO₂ on woody plants. *New Phytol.* 127:425–446.
- Cure, J.D. and B. Acock. 1986. Crop responses to carbon dioxide doubling: a literature survey. *Agric. For. Meteorol.* 38:127–145.
- Curtis, P.S. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell Environ.* 19:127–137.
- Eamus, D. and P.G. Jarvis. 1989. The direct effects of increase in the global atmospheric CO₂ concentration on natural and commercial temperate trees and forests. *Adv. Ecol. Res.* 19:1–55.
- Ellsworth, D.S. and P.B. Reich. 1993. Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* 96:169–178.
- Fetcher, N., C.H. Jaeger, B.R. Strain and N. Sionit. 1988. Long-term elevation of atmospheric CO₂ concentration and the carbon exchange rates of saplings of *Pinus taeda* L. and *Liquidambar styraciflua* L. *Tree Physiol.* 4:255–262.
- Gifford, R.M. 1992. Interaction of carbon dioxide with growth-limiting environmental factors in vegetation productivity: implications for the global carbon cycle. *In* Advances in Bioclimatology. Ed. G. Stanhill. Springer-Verlag, New York, pp 24–58.
- Groninger, J.W., J.R. Seiler, S.M. Zedaker and P.C. Berrang. 1995. Effects of elevated CO₂, water stress, and nitrogen level on competitive interactions of simulated loblolly pine and sweetgum stands. *Can. J. For. Res.* 25:1077–1083.
- Groninger, J.W., J.R. Seiler, S.M. Zedaker and P.C. Berrang. 1996. Photosynthetic response of loblolly pine and sweetgum seedling stands to elevated carbon dioxide, water stress, and nitrogen level. *Can. J. For. Res.* 26:95–102.

- Gunderson, C.A. and S.D. Wullschleger. 1994. Photosynthetic acclimation in trees to rising atmospheric CO₂: A broader perspective. *Photosyn. Res.* 39:369–388.
- Harley, P., A. Guenther and P. Zimmerman. 1996. Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum leaves. *Tree Physiol.* 16:25–32.
- Hirose, T. and M.J.A. Werger. 1987. Nitrogen use efficiency and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiol. Plant.* 70:215–222.
- Idso, S.B., B.A. Kimball and J. Nagy. 1994. Effects of free-air CO₂ enrichment on the light response curve of net photosynthesis in cotton leaves. *Agric. For. Meteorol.* 70:18–21.
- Katul, G.G., R. Oren, D. Ellsworth, C.I. Hsieh, N. Phillips, K. Lewin. 1997. A Lagrangian dispersion model for predicting CO₂ fluxes in a uniform pine (*Pinus taeda* L.) stand. *J. Geophys. Res.* 102:9309–9321.
- Kirchbaum, M.U.F. and G.D. Farquhar. 1987. Investigations of the CO₂ dependence of quantum yield and respiration in *Eucalyptus pauciflora*. *Plant Physiol.* 83:1032–1036.
- Kubiske, M.E. and K.S. Pregitzer. 1996. Effects of elevated CO₂ and light availability on the photosynthetic response of trees of contrasting shade tolerance. *Tree Physiol.* 16:351–358.
- Lasko, A.N., J.F. Bierhuizen and G.F.P. Martakis. 1984. Light response curves of photosynthesis and transpiration of two tomato cultivars under ambient and altered CO₂ and O₂. *Sci. Hort.* 23:119–128.
- Leverenz, J.W. 1987. Chlorophyll content and the light response curve of shade-adapted conifer needles. *Physiol. Plant.* 71:20–29.
- Leverenz, J.W. 1995. Shade shoot structure of conifers and the photosynthetic response to light at two CO₂ partial pressures. *Funct. Ecol.* 9:413–421.
- Long, S.P. and B.G. Drake. 1991. Effect of long-term elevation of CO₂ concentration in the field on the quantum yield of photosynthesis of the C₃ sedge, *Scirpus olneyi*. *Plant Physiol.* 96:221–226.
- McClendon, J.H. and G.G. McMillen. 1982. The control of leaf morphology and the tolerance of shade by woody plants. *Bot. Gaz.* 143:79–83.
- Mortensen, L.M. and R. Moe. 1983. Growth responses of some greenhouse plants to environment V. Effect of CO₂, O₂ and light on net photosynthetic rate of *Chrysanthemum morifolium* Ramat. *Sci. Hortic.* 19:133–140.
- Norby, R.J., E.G. O'Neill and R.G. Luxmoore. 1986. Effects of atmospheric CO₂ concentration on the growth and mineral nutrition of *Quercus alba* seedlings in a nutrient poor soil. *Plant Physiol.* 82:83–89.
- Oosting, H.J. 1942. An ecological analysis of the plant communities of Piedmont, North Carolina. *Am. Mid. Nat.* 28:1–126.
- Osborne, C.P., B.G. Drake, J. LaRoche and S.P. Long. 1997. Does long-term elevation of CO₂ concentration increase photosynthesis in forest floor vegetation? Indiana strawberry in a Maryland forest. *Plant Physiol.* 114:337–344.
- Pearcy, R.W. and O. Björkman. 1983. Physiological effects. In *CO₂ and Plants*. Ed. E.R. Lemon. Westview Press, Boulder, CO, pp 65–105.
- Pearcy, R.W. 1988. Photosynthetic utilization of lightflecks by understorey plants. *Aust. J. Plant Physiol.* 15:223–238.
- Poorter, H. 1993. Intraspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetatio* 104/105:77–97.
- Porra, R.J., W.A. Thompson and P.E. Kriedman. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* 975:384–394.
- Rogers, H.H., G.E. Bingham, J.D. Cure, J.M. Smith and K.A. Surano. 1983. Responses of selected plant species to elevated carbon dioxide in the field. *J. Environ. Qual.* 12:569–574.
- Sionit, N., H. Hellmers and B.R. Strain. 1982. Interaction of atmospheric CO₂ enrichment and irradiance on plant growth. *Agron. J.* 74:721–725.
- Sionit, N., B.R. Strain, H. Hellmers, G.H. Riechers and C.H. Jaeger. 1985. Long-term atmospheric CO₂ enrichment affects the growth and development of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can. J. For. Res.* 15:468–471.
- Spalding, M.H. and A.R. Portis. 1985. A model of carbon dioxide assimilation in *Chladomanas reinhardii*. *Planta* 164:308–320.
- Stitt, M. 1991. Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant Cell Environ.* 14:741–762.
- Teskey, R.O. and R.B. Shrestha. 1985. A relationship between carbon dioxide, photosynthetic efficiency and shade tolerance. *Physiol. Plant.* 63:126–132.
- Tolley, L.C. and B.R. Strain. 1984a. Effects of CO₂ enrichment on growth of *Liquidambar styraciflua* and *Pinus taeda* seedlings under different irradiance levels. *Can. J. For. Res.* 14:343–350.
- Tolley, L.C. and B.R. Strain. 1984b. Effects of CO₂ enrichment and water stress on growth of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can. J. Bot.* 62:2135–2139.
- Tolley, L.C., and B.R. Strain. 1985. Effects of CO₂ enrichment and water stress on gas exchange of *Liquidambar styraciflua* and *Pinus taeda* seedlings grown under different irradiance levels. 65:166–172.
- Tschaplinski, T.J., D.B. Stewart, P.J. Hanson and R.J. Norby. 1995. Interactions between drought and elevated CO₂ on growth and gas exchange of seedlings of three deciduous tree species. *New Phytol.* 129:63–71.
- Williams, W.E., K. Garbutt, F.A. Bazazz and P.M. Vitousek. 1986. The response of plants to elevated CO₂. IV. Two deciduous-forest tree communities. *Oecologia* 69:454–459.
- Winter, K. and A. Virgo. 1998. Elevated CO₂ enhances growth in the rain-forest understorey plant, *Piper codulatum*, at extremely low-light intensities. *Flora* 193:323–326.
- Wong, S.C. 1979. Elevated atmospheric partial pressure of CO₂ and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C₃ and C₄ plants. *Oecologia* 44:68–74.
- Zimmerman, M.H., C.L. Brown and M.T. Tyree. 1971. *Trees: structure and function*. Springer-Verlag, New York, 27 p.