

Persistent stimulation of photosynthesis by elevated CO₂ in a sweetgum (*Liquidambar styraciflua*) forest stand

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Summary

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- The photosynthetic response of trees to rising CO₂ concentrations ([CO₂]) can be affected by plant source–sink relations, in addition to seasonal changes in environmental conditions. Characterization of biochemical and morphological feedbacks is important for understanding ecosystem responses to elevated atmospheric [CO₂].
- The seasonal responses of leaf gas exchange and related biochemical parameters were measured during 3 yrs of exposure on established plantation sweetgum (*Liquidambar styraciflua*) trees at a Free-Air CO₂ Enrichment (FACE) facility in eastern Tennessee, USA.
- Net photosynthetic rates (A_{growth}) of upper-canopy leaves were 44% higher in trees grown in elevated [CO₂] compared with ambient [CO₂] over the 3-yr period. There were no significant CO₂ treatment effects on photosynthetic or biochemical capacity (i.e. no change in A_{max} , V_{cmax} or J_{max}) of *L. styraciflua* leaves, despite increased area-based leaf sugar (10%) and starch content (27%), and reduced mass-based leaf nitrogen concentration (N_M ; 10%).
- These results suggest that established *L. styraciflua* trees in closed-canopy forests might exhibit a long-term positive response to elevated [CO₂] without reductions in photosynthetic capacity.

Key words: biochemical capacity, deciduous trees, FACE, forest, *Liquidambar styraciflua*, nitrogen, photosynthetic adjustment, photosynthetic capacity.

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Introduction

Photosynthesis in trees is stimulated an average of 44–66% by growth in elevated CO₂ concentrations ([CO₂]) (see reviews: Gunderson & Wullschlegel, 1994; Curtis & Wang, 1998; Saxe *et al.*, 1998; Medlyn *et al.*, 1999; Norby *et al.*, 1999). The short-term response can be attributed largely to stimulation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation. Rubisco is CO₂ substrate-limited under current atmospheric conditions, but an increase in atmospheric [CO₂] stimulates carboxylation and suppresses oxygenation, thereby reducing photorespiration and increasing net photosynthesis (Sharkey, 1988; Long, 1991). Long-term photosynthetic responses to elevated [CO₂], however, may be affected by biochemical, morphological and physiological feedbacks that balance carbon assimilation with growth (i.e. sink) demand (Long &

Drake, 1991; Stitt, 1991; Sage, 1994; Griffin & Seemann, 1996; Peterson *et al.*, 1999; Rogers & Ellsworth, 2002; Körner, 2003). An understanding of these long-term, leaf-level responses may provide insight into ecosystem responses to elevated atmospheric [CO₂].

Trees exposed to CO₂ enrichment in field experiments generally show less evidence of the loss of photosynthetic stimulation after long-term exposure to elevated [CO₂], termed downregulation or acclimation, than had been suggested by earlier experiments conducted using seedlings grown in pots in growth chambers. Curtis & Wang (1998) found in a meta-analysis of 59 tree species that downregulation was minimal and inconsistent, with the greatest downregulation occurring in trees grown in small pots (–36%). However, a meta-analysis of 15 field-based elevated [CO₂] experiments on European forest tree species found a downregulation of

photosynthesis of 10–20% when measured at a common $[\text{CO}_2]$, in spite of a 51% enhancement of the light-saturated rate of photosynthesis when measured at growth $[\text{CO}_2]$ (Medlyn *et al.*, 1999). Although photosynthetic downregulation may reduce the positive response of photosynthesis to elevated $[\text{CO}_2]$, it rarely completely eliminates it. Therefore, continued photosynthetic enhancement might be expected in forest ecosystems exposed to long-term CO_2 enrichment, as is occurring in nature.

The lack of consistency with regard to the photosynthetic acclimation of trees can be partially explained by looking at plant-level source-sink relationships. The degree to which trees exhibit photosynthetic downregulation when grown in elevated $[\text{CO}_2]$ may be related, on a physiological level, to resource allocation within the plant, especially nitrogen, which often limits the productivity of forest ecosystems (Sage, 1994; Barnes *et al.*, 1998). Within plant photosynthetic tissue, resources are partitioned between light-harvesting and nonharvesting components. At a broader scale, resources must be allocated between those plant tissues that are net exporters of photosynthate (sources) and net importers (sinks), typically characterized as new roots, shoots and leaves or reproductive organs (Herold, 1980; Stitt, 1991). A reduction in photosynthetic capacity may occur in elevated $[\text{CO}_2]$ as biochemical adjustments cause reallocation of resources, particularly nitrogen, from nonlimiting processes to limiting processes (Sage, 1994; Sage & Reid, 1994).

Maintenance of active sinks is necessary for the effective stimulation of photosynthesis (Eamus & Jarvis, 1989; Farrar & Williams, 1991; Stitt, 1991). The increased production of carbohydrates induced by higher photosynthetic rates in elevated $[\text{CO}_2]$ can be sustained only with continued translocation of photosynthates to active sink material (Xu *et al.*, 1994; Wolfe *et al.*, 1998). An imbalance in the source-sink relationship can result in an accumulation of leaf carbohydrates, which may ultimately trigger a feedback mechanism that reduces photosynthetic capacity (Van Oosten & Besford, 1994; Webber *et al.*, 1994). Accumulation of large amounts of carbohydrates may lead to reductions in gene transcription and production of Rubisco protein, thereby reducing Calvin cycle activity and further sugar production (Sheen, 1990; Furbank & Taylor, 1995; Krapp & Stitt, 1995; Cheng *et al.*, 1998). Most commonly, both the maximum carboxylation rate of Rubisco (V_{cmax}) and maximum electron transport rate (J_{max}) are reduced in plants exhibiting photosynthetic downregulation (see reviews: Wullschleger, 1993; Gunderson & Wullschleger, 1994; Sage, 1994; Medlyn *et al.*, 1999; Norby *et al.*, 1999).

The regulation of photosynthetic rate and capacity, V_{cmax} and J_{max} , by source-sink relationships suggests that photosynthetic downregulation may be more prevalent in trees whose growth processes (i.e. sinks) are limited annually or seasonally by environmental factors. No single factor appears predominant in controlling photosynthetic downregulation (Gunderson &

Wullschleger, 1994; Medlyn *et al.*, 1999), but it is most common to observe photosynthetic downregulation at the end of the growing season, when sink strength is reduced and many environmental factors become more limiting. For example, in three deciduous trees, *Castanea sativa* (El Kohen & Mousseau, 1994), *Fagus sylvatica* (Epron *et al.*, 1996) and *Betula pendula* (Rey & Jarvis, 1998), the degree of photosynthetic downregulation in response to elevated $[\text{CO}_2]$ increased as the growing season progressed and sink strength declined. Improved soil nitrogen conditions through fertilization delayed photosynthetic downregulation in *Castanea sativa*, presumably due to increased growth and sink strength (El Kohen & Mousseau, 1994). In many pine species, downregulation is only observed in older needles, but not younger needles (Jach & Ceulemans, 2000; Tissue *et al.*, 2001; Rogers & Ellsworth, 2002), concurrent with a reduction in their sink strength. In a closed-canopy forest, leaf production is constrained, which may limit the continued development of carbon sinks in elevated $[\text{CO}_2]$.

The primary objective of this study was to characterize the effects of seasonal variation in plant source-sink relations on the photosynthetic capacity of upper-canopy leaves to respond to elevated $[\text{CO}_2]$ in a closed-canopy sweetgum forest. Sweetgum (*Liquidambar styraciflua*) is a canopy-dominant, deciduous tree with indeterminate growth (Bormann, 1953). Measurements of photosynthetic and biochemical responses of upper-canopy foliage were used to determine the degree of photosynthetic enhancement, and potential photosynthetic downregulation, to elevated $[\text{CO}_2]$ at different times during the growing season, which represent different levels of leaf and plant sink strength. We hypothesized that reductions in sink capacity would feed back on photosynthetic enzyme activity, thereby reducing the positive effects of elevated $[\text{CO}_2]$ on leaf carbon balance. A decline in the photosynthetic enhancement of elevated $[\text{CO}_2]$ would be associated with the following: (i) reduced A_{max} ; (ii) a decline in V_{cmax} and J_{max} ; (iii) increased leaf mass per unit leaf area (LMA); (iv) decreased mass-based leaf nitrogen concentration (N_M); and (v) an increase in leaf carbohydrate content, especially starch.

Measurements were conducted over three growing seasons at the Oak Ridge National Laboratory's (ORNL) Free-Air CO_2 Enrichment (FACE) site to determine whether photosynthetic responses to elevated $[\text{CO}_2]$ were persistent or transient. We hypothesized that canopy closure in this stand (i.e. leaf area index (LAI) not increasing) would constrain growth responses to elevated $[\text{CO}_2]$, in contrast to the responses of individual trees in an expanding system (Norby *et al.*, 1999). Net primary productivity in this stand has been about 21% higher in elevated CO_2 and there has been no sustained enhancement in aboveground growth (Norby *et al.*, 2002), in contrast to the much larger CO_2 responses sometimes reported for trees undergoing exponential growth (Norby *et al.*, 1999). Hence, sink limitation to photosynthetic responses might be more likely in this stand than has been reported in previous studies with CO_2 -enriched *L. styraciflua* (Herrick & Thomas, 2001).

Materials and Methods

Oak Ridge National Laboratory Free-Air CO₂ Enrichment (FACE) site

Research was conducted at the ORNL FACE facility in Roane County, Tennessee (35°54' N, 84°20' W). The FACE site was constructed within a sweetgum (*L. styraciflua*) plantation established in the autumn of 1988, at which time 1-yr-old trees were planted at a spacing of 2.3 m × 1.2 m. The FACE facility became operational in April 1998, at which time the live crown of the closed canopy extended from 8 to 9 m above the forest floor to a height of 13–14 m. Crown depth remained approximately the same each season, with 1 m of crown height at the top being added each year, but 1 m of crown height at the bottom being lost as lower branches died. Tree heights reached 14–15 m in spring 1999 and 15–16 m in spring 2000.

The ORNL FACE site consists of five circular plots (rings), each 25 m in diameter enclosing 80–90 trees inside a 2.5-m wide buffer zone. Two rings were treated with air enriched with CO₂ ('elevated CO₂'), while the remaining three rings ('ambient CO₂') served as controls. Two of the three control plots contain blower systems like those present in the elevated-CO₂ plots. Experimental treatment was applied 24 h d⁻¹ throughout the 1998–2000 growing seasons. The treatment set point for 1998 was a constant 565 μmol mol⁻¹ CO₂, which was approximately 200 μmol mol⁻¹ above the global average [CO₂]. The treatment protocol was adjusted in 1999 to incorporate the natural diurnal variation in [CO₂], with the set point for the night-time concentration increased to 645 μmol mol⁻¹. Actual mid-day [CO₂] in the elevated-CO₂ plots averaged 548, 556 and 555 μmol mol⁻¹ in 1998, 1999 and 2000, respectively, compared to mid-day concentrations in the ambient rings of 362, 365 and 366 μmol mol⁻¹ in 1998, 1999 and 2000, respectively (Gunderson *et al.*, 2002).

Gas-exchange measurements

The responses of net photosynthesis (*A*) to intercellular [CO₂] (*C_i*) (*A-C_i* curves) were measured during each field season, which for these *L. styraciflua* trees extended from leaf-out in mid April to leaf fall in late October (Norby *et al.*, 2003). All *A-C_i* curves (five to eight curves per ring per sampling period) were collected using fully expanded (mature), upper-canopy leaves in at least four trees, which were accessible from a stationary hydraulic lift located near the centre of each plot. A portable photosynthesis system (LI-6400, LiCor, Inc., Lincoln, NE, USA) was used to measure the rate of leaf assimilation at each of 11 external [CO₂] (*C_a*), ranging from 1500 to 0 μmol mol⁻¹ CO₂. An automated programme was utilized to log assimilation readings at each *C_a* set point when photosynthesis rates had equilibrated, which was typically 1–2 min after a stable *C_a* set point was reached. All gas-exchange measurements

were made at saturating light levels (1800 μmol m⁻² s⁻¹) provided by a blue-red light source mounted above the 6 cm² leaf cuvette. Chamber temperatures were set throughout the season to approximate the average mid-afternoon temperatures that occurred during the week that measurements were taken. Temperatures within the leaf cuvette typically ranged from 27 to 36°C. All gas-exchange measurements were taken between 10:00 and 16:00 h EST. *A-C_i* curves were collected in July and September 1998, monthly in May until September 1999, and in May, July and September 2000.

A-C_i curves were analysed using Photosyn Assistant (Dundee Scientific, Dundee, Scotland, UK). For each curve, this software uses equations that relate the effect of changing [CO₂] to photosynthetic rate. The equations estimate potential limitations to CO₂ assimilation due to the maximum Rubisco carboxylation rate (*V_{max}*), and the rate of ribulose biphosphate (RuBP) regeneration via electron transport (*J_{max}*). The program uses the Farquhar *et al.* (1980) model, as modified by von Caemmerer & Farquhar (1981), Sharkey (1985), Harley & Sharkey (1991), and Harley *et al.* (1992). Temperature-response functions were included in modelling these photosynthetic parameters to account for the wide range of mean leaf temperatures encountered throughout this study (Bernacchi *et al.*, 2001). The maximum CO₂- and light-saturated rate of photosynthesis (*A_{max}*) and net rate of photosynthesis at the respective growth [CO₂] (either *C_a* = 360 or 560 μmol m⁻² s⁻¹, *A_{growth}*) were also determined from *A-C_i* curves.

Leaf properties

All leaves used for *A-C_i* measurements were harvested for mensuration and biochemical analysis. The thickness of individual fresh leaves was measured in the field using digital calipers at the time of leaf collection beginning in July 1999. Leaves were then harvested and placed on ice until they could be returned to the laboratory for processing. Leaf area was determined for each individual leaf using a leaf-area meter (LI-3100). Following the measurement of individual leaf areas, all leaves were dried in a 70°C oven for at least 3 d, and then each leaf was weighed individually. These data were used to calculate leaf mass per unit area (*LMA*). Estimates of leaf volume and density were also calculated, because leaf function is affected by the interaction of leaf morphology (area and thickness) and composition (dry matter, liquid content, and fractional air space) (Niinemets, 1999; Roderick *et al.*, 1999a,b).

Leaf soluble sugar and starch contents were determined colorimetrically by a phenol-sulfuric acid technique (Tissue & Wright, 1995). Total nonstructural carbohydrate (TNC) was calculated as the sum of soluble sugar and starch. Total chlorophyll content was determined by immersing a 1.33 cm² leaf punch from each leaf in 10 ml of 95% ethanol for 2 d in a dark environment, followed by measurement of the absorbance of the extract at 665, 649 and 470 nm (Lichtenthaler & Wellburn, 1983). Total leaf nitrogen was measured in the

dried leaf samples using a CN Analyzer (NCS 2500, Carlo Erba Inc., Milan, Italy).

Statistical analyses

Treatment effects were calculated using the ring means of gas-exchange and leaf-property parameters for each sampling date. A two-way analysis of variance (ANOVA) was used to compare the effects of CO₂ treatment ($n = 3$ ambient [CO₂]; $n = 2$ elevated [CO₂]) and sampling date ($n = 10$) and the interaction of [CO₂] and sampling date on all measured and modelled parameters. Parameters were considered significantly different when $P < 0.05$. Least squares linear regression analysis was performed to analyse the relationship of V_{cmax} to J_{max} , leaf nitrogen per unit area (N_A) to the various photosynthetic parameters measured and modelled in this experiment, and total chlorophyll per unit area (Chl_A) to J_{max} . In those instances where statistically significant regression results were noted for both ambient- and elevated-CO₂ treatments, homogeneity of slopes and intercepts was tested using analysis of covariance (ANCOVA). All statistical analyses were performed using SYSTAT (Version 8.0, SPSS Inc. Chicago, IL, USA, 1998).

Results

Photosynthesis

The net photosynthetic rates (A_{growth}) of *L. styraciflua* in elevated [CO₂] were significantly higher than those of trees in ambient [CO₂] (Table 1, Fig. 1), with increases ranging from 33% to 93% (Fig. 2a). The average net photosynthetic rate was 44% higher with CO₂ enrichment (Fig. 1). Sample date also significantly affected the net rate of photosynthesis (Table 1).

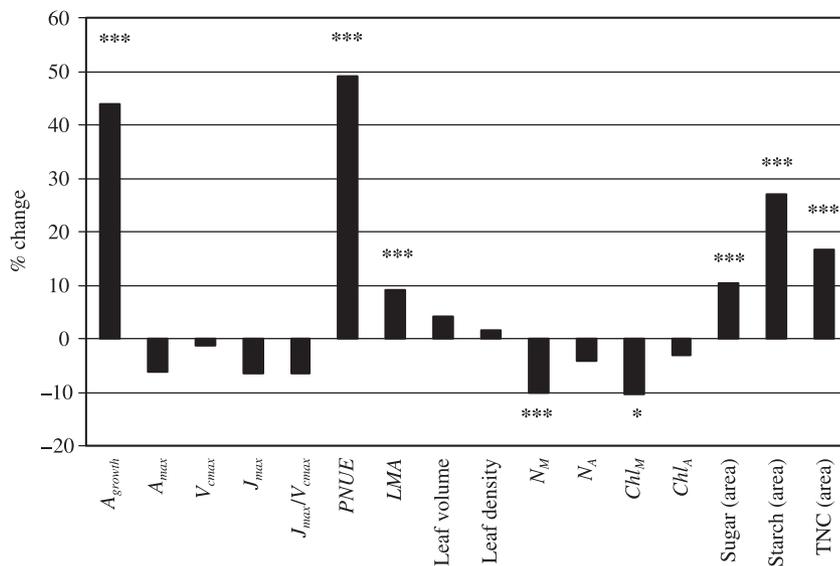


Fig. 1 Percent change of photosynthetic and leaf characteristic parameters measured in mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($\sim 364 \mu\text{mol mol}^{-1}$) or elevated ($\sim 553 \mu\text{mol mol}^{-1}$) CO₂. Values were calculated as $([\text{Elevated CO}_2 - \text{Ambient CO}_2]/\text{Ambient CO}_2 \times 100)$, with ring means serving as the experimental unit in each of 10 sampling periods ($n = 30$ ambient [CO₂]; $n = 20$ elevated [CO₂]) measured over the course of the project. The presence of asterisks denotes a significant CO₂ treatment effect (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) in the overall [CO₂] * Sampling Date ANOVA.

Table 1 ANOVA results for *Liquidambar styraciflua* leaf photosynthetic and biochemical parameters

Parameter	[CO ₂]	Sample date	[CO ₂] * Sample date
A_{growth} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	< 0.0001	< 0.0001	0.9397
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.2139	0.0001	0.7585
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.8580	0.0003	0.9603
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.2508	0.0004	0.9182
J_{max}/V_{cmax}	0.5324	0.0037	0.0921
PNUE ($\mu\text{mol g}^{-1} \text{N s}^{-1}$)	< 0.0001	< 0.0001	0.4657
LMA (g m^{-2})	0.0002	< 0.0001	0.9052
Leaf Density (g cm^{-3})	0.5449	< 0.0001	0.1443
N_M (mg g^{-1})	< 0.0001	< 0.0001	0.5545
N_A (g m^{-2})	0.2576	0.0084	0.7560
N_V (mg cm^{-3})	0.0463	< 0.0001	0.6167
Chl_M (mg g^{-1})	0.0291	0.0004	0.6827
Chl_A (g m^{-2})	0.4813	0.0675	0.2674
Chl_M (mg g^{-1})/ N_M (mg g^{-1})	0.8970	< 0.0001	0.7370
Sugar (g m^{-2})	0.0010	< 0.0001	0.1610
Starch (g m^{-2})	< 0.0001	< 0.0001	0.0006
TNC (g m^{-2})	< 0.0001	< 0.0001	0.0247

Mature upper-canopy leaves of *L. styraciflua* grown in ambient ($\sim 364 \mu\text{mol m}^{-2} \text{s}^{-1}$) and elevated ($\sim 553 \mu\text{mol m}^{-2} \text{s}^{-1}$) CO₂ were sampled in July and September 1998, monthly from May through September 1999, and in May, July and September 2000. Significance of treatment effect, as indicated by the P -value, is reported for CO₂ concentration, sample date, and the interaction between CO₂ treatment and date of sample collection ([CO₂] * Sample date).

There were seasonal and annual differences in A_{growth} response with the highest rates occurring in mid-season and lower rates in early and late-season (Fig. 2a). There was no significant CO₂ effect on A_{max} over the course of the experiment (Table 1, Fig. 2b). There was a significant effect of sample date on A_{max} (Table 1). There were seasonal and annual differences in A_{max}

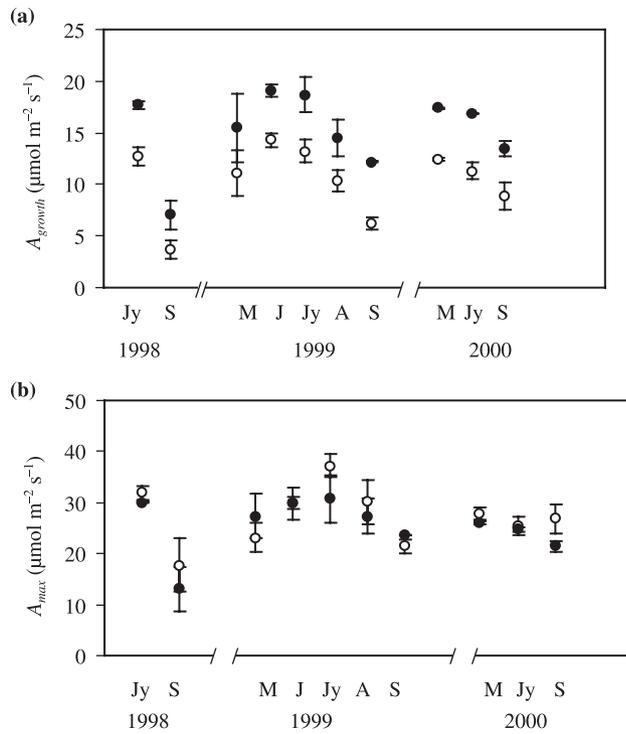


Fig. 2 Photosynthetic response of mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($\sim 364 \mu\text{mol mol}^{-1}$, $n = 3$, open circles) and elevated ($\sim 553 \mu\text{mol mol}^{-1}$, $n = 2$, closed circles) CO_2 . Photosynthesis at (a) growth [CO_2] (A_{growth}) and (b) CO_2 and light saturation (A_{max}) were determined using $A-C_i$ curves collected between May and September in 1998, 1999, and 2000 using a LiCor 6400 photosynthesis system. Values are presented as means \pm SEM.

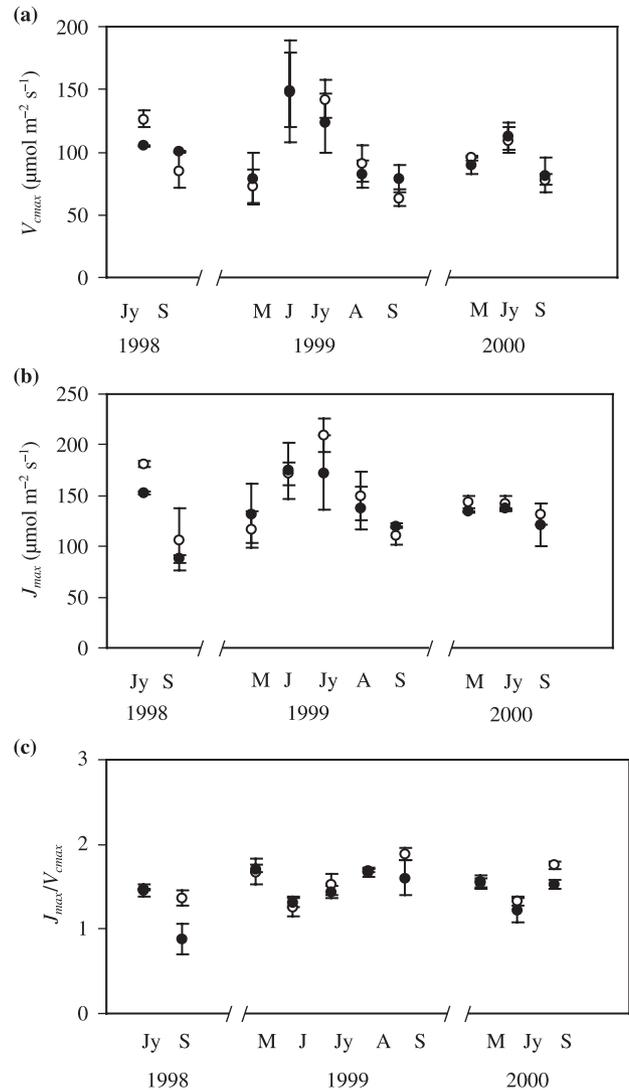


Fig. 3 Response of modelled photosynthetic parameters of mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($\sim 364 \mu\text{mol mol}^{-1}$, $n = 3$, open circles) or elevated ($\sim 553 \mu\text{mol mol}^{-1}$, $n = 2$, closed circles) CO_2 . $A-C_i$ curves were analysed using a C_3 model of photosynthetic biochemistry to determine: (a) V_{cmax} , the maximum carboxylation rate of Rubisco, and (b) J_{max} , the RuBP regeneration capacity mediated by electron transport rate. (c) The ratio of J_{max}/V_{cmax} was also calculated. Values are presented as means \pm SEM.

response with the highest rates occurring in mid-season and lower rates in early and late-season (Fig. 2b).

Growth in elevated $[\text{CO}_2]$ did not have a significant effect on V_{cmax} , J_{max} or the ratio of J_{max} to V_{cmax} (Table 1, Fig. 1). Sampling date had a significant effect on both V_{cmax} and J_{max} (Table 1), with rates generally higher in mid-season than early and late-season (Fig. 3a,b). Date of sampling also significantly affected the ratio of J_{max} to V_{cmax} (Table 1). This ratio was generally lower in mid-season when A_{growth} and A_{max} tended to exhibit their highest rates (Fig. 3c). In addition, there was a positive linear relationship between J_{max} and V_{cmax} for leaves grown in both ambient and elevated $[\text{CO}_2]$ ($R^2 = 0.87$ ambient; $R^2 = 0.78$ elevated) (Fig. 4). The regressions for both treatments were statistically significant ($P < 0.0001$ ambient, $P < 0.0001$ elevated), with similar slopes and intercepts.

The photosynthetic nitrogen-use efficiency ($PNUE$), defined as carbon assimilation per unit of leaf nitrogen (Lambers *et al.*, 1998), of *L. styraciflua* leaves was significantly greater in elevated $[\text{CO}_2]$ (Table 1, Fig. 1). Sample date was shown to significantly affect $PNUE$, with the greatest CO_2 effect occurring in mid-season (Table 1, Fig. 5).

Leaf properties

Leaf mass per unit leaf area (LMA) was consistently higher in leaves from trees grown in elevated $[\text{CO}_2]$ compared with ambient $[\text{CO}_2]$, with an average 9% increase over the 3-yr study period (Table 1, Fig. 1). The absolute increase in LMA due to elevated $[\text{CO}_2]$ ranged from 2.9 to 16.7 g m^{-2} , with the greatest differences occurring in mid-season, while the relative increase ranged from 3% to 19% (Fig. 6a). There were no differences in LMA on an annual basis. There was no CO_2

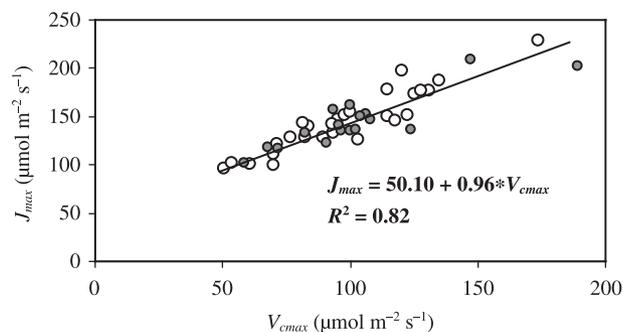


Fig. 4 Linear relationship between the maximum rate of electron transport (J_{max}) and the maximum rate of Rubisco carboxylation (V_{cmax}) of mature upper-canopy leaves of *Liquidambar styraciflua*. V_{cmax} and J_{max} were derived as best-fit estimates from individual A- C_i curves. A single regression line is presented because the regressions generated for each treatment were not statistically different. The regression for the combined data was statistically significant ($P < 0.0001$, $n = 45$). Open circles, ambient $[CO_2]$; closed circles, elevated $[CO_2]$.

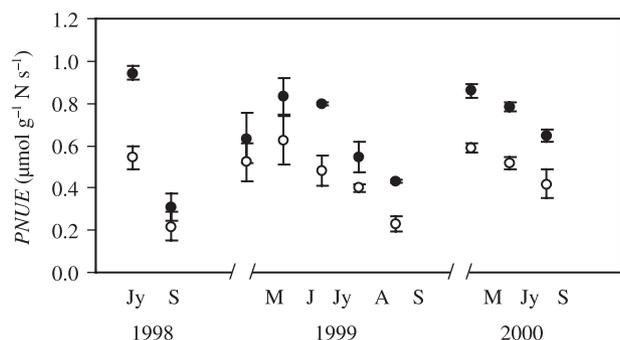


Fig. 5 Response of photosynthetic nitrogen-use efficiency ($PNUE$) of mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($\sim 364 \mu\text{mol mol}^{-1}$, $n = 3$, open circles) or elevated ($\sim 553 \mu\text{mol mol}^{-1}$, $n = 2$, closed circles) CO_2 . $PNUE$ was calculated as the rate of photosynthesis at growth CO_2 (A_{growth}) per unit leaf nitrogen concentration (N_A). Values are presented as means \pm SEM.

treatment effect on leaf density (g cm^{-3}), but date of sampling did have a significant effect (Table 1, Fig. 1) with greater mean leaf densities noted late in the growing season in 2000 (Fig. 6b).

Leaf biochemistry

There was no significant CO_2 treatment effect on nitrogen content when expressed on a leaf-area (N_A , g m^{-2}) basis (Table 1). Elevated $[CO_2]$ significantly decreased leaf nitrogen when expressed on a dry-mass (10%; N_M , mg g^{-1}) and leaf-volume (9%; N_V , mg cm^{-3}) basis (Table 1). The relative reduction in N_M due to elevated $[CO_2]$ ranged from 4% to 21%. Sampling date significantly affected N_A , N_M and N_V (Table 1). Foliar nitrogen was generally highest in 1999 and lowest in 2000 (Fig. 7).

Foliar total chlorophyll concentrations were significantly decreased by growth in enriched $[CO_2]$ when expressed on a

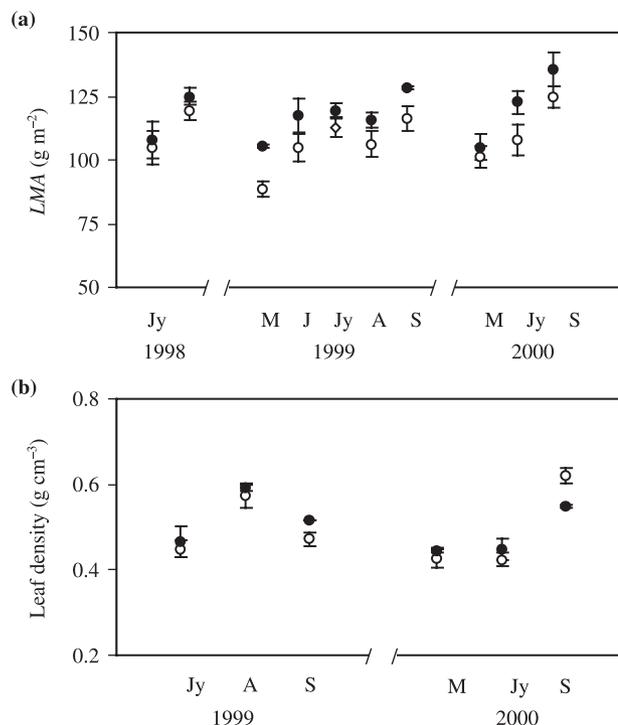


Fig. 6 Response of growth characteristics of mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($\sim 364 \mu\text{mol mol}^{-1}$, $n = 3$, open circles) or elevated ($\sim 553 \mu\text{mol mol}^{-1}$, $n = 2$, closed circles) CO_2 . Growth characteristics were determined for the leaves harvested following the collection of A- C_i curves. (a) Leaf mass per unit leaf area (LMA) measurements were collected in all 10 sample periods, while (b) leaf density measurements were limited to the latter half of the 1999 season and all of the 2000 season. Values are presented as means \pm SEM.

mass (Chl_M), but not area (Chl_A) basis (Table 1, Fig. 1). Chl_M was also significantly affected by sample date (Table 1). The relative decline in Chl_M due to elevated $[CO_2]$ ranged from 2% to 25%, with the greatest reduction occurring in mid-season (Fig. 8a). Chl_M did not vary annually. There was no significant effect of sample date on Chl_A (Table 1, Fig. 8b). The investment of plant nitrogen in chlorophyll was examined by calculating the ratio of Chl_M per unit N_M . Elevated $[CO_2]$ had no effect on this ratio, but there was a significant effect of sampling date (Table 1).

Area-based sugar, starch and TNC content of *L. styraciflua* leaves were significantly increased in elevated $[CO_2]$ (Table 1, Fig. 1). Sugar was increased an average of 10%, starch 27%, and TNC 17% over the 3-yr period of the study. The relative change in sugar ranged from -20% to 36% (Fig. 9a), while the relative change in starch ranged from -3% to 94% (Fig. 9b). Consistent seasonal CO_2 effects on sugar content were not as pronounced as those for starch, where the greatest CO_2 effects occurred in mid-season (Fig. 9). On a mass-basis, elevated CO_2 increased leaf starch (17%) and TNC (18%), but did not significantly affect leaf sugar concentration.

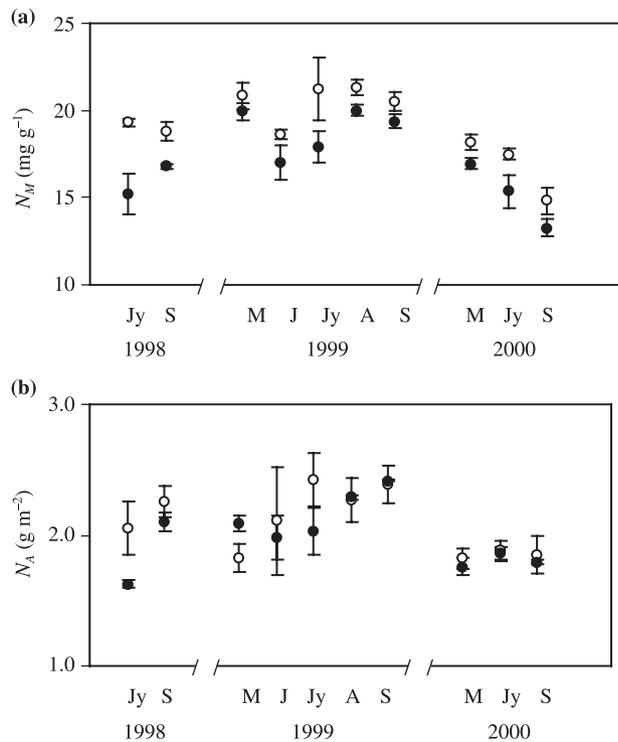


Fig. 7 Response of foliar nitrogen concentration of mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($\sim 364 \mu\text{mol mol}^{-1}$, $n = 3$, open circles) or elevated ($\sim 553 \mu\text{mol mol}^{-1}$, $n = 2$, closed circles) CO₂. Biochemical analyses were performed following the harvest of the mature upper-canopy leaves upon which photosynthesis measurements were taken. Leaf nitrogen results are reported (a) per unit leaf mass (N_M) and (b) per unit leaf area (N_A). Values are presented as means \pm SEM.

Discussion

Net photosynthetic rates (A_{growth}) of leaves in the upper canopy of the *L. styraciflua* forest stand varied seasonally, but they were consistently higher (44–54%) in elevated [CO₂] compared with ambient [CO₂] throughout the three seasons of this study. Similarly, Gunderson *et al.* (2002) reported a sustained 46% photosynthetic enhancement in mid- and upper-canopy foliage of *L. styraciflua* grown in this same stand. These values were similar to, or slightly lower than the levels of enhancement reported for other deciduous tree species exposed to a doubling of current atmospheric [CO₂] (Ceulemans & Mousseau, 1994; Saxe *et al.*, 1998; Norby *et al.*, 1999). Individual *L. styraciflua* trees that were expanding into the overstorey of a *Pinus taeda* stand in the Duke Forest FACE experiment were more responsive to CO₂ enrichment: Herrick & Thomas (2001) reported that sun leaves in elevated [CO₂] had net photosynthetic rates that were stimulated an average of 63% over the course of their 3-yr study. (Note that ‘sun leaves’ of the *L. styraciflua* trees in the mixed pine-sweetgum canopy of the Duke FACE experiment had morphological characteristics of the mid-canopy leaves in the ORNL FACE, and the upper-canopy leaves at the

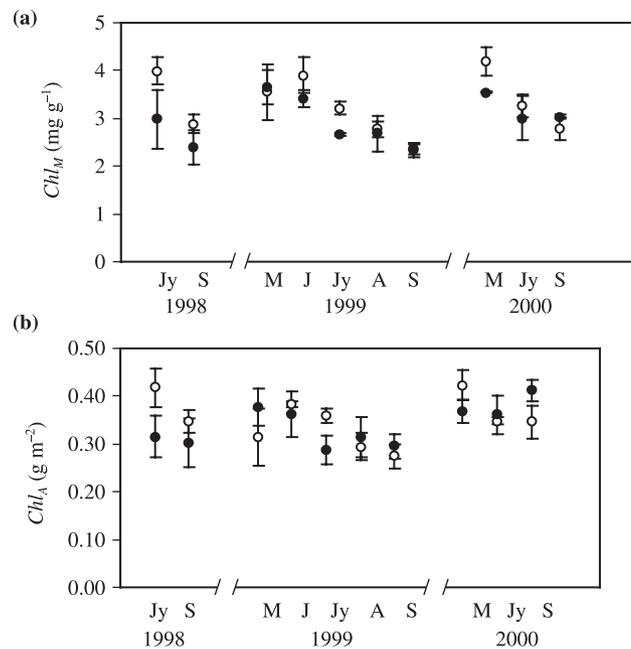


Fig. 8 Response of foliar chlorophyll concentration of mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($\sim 364 \mu\text{mol mol}^{-1}$, $n = 3$, open circles) or elevated ($\sim 553 \mu\text{mol mol}^{-1}$, $n = 2$, closed circles) CO₂. Biochemical analyses were performed following the harvest of the mature upper-canopy leaves upon which photosynthesis measurements were taken. Leaf chlorophyll results are reported (a) per unit leaf mass (Chl_M) and (b) per unit leaf area (Chl_A). Values are presented as means \pm SEM.

ORNL FACE, which were fully exposed to the sun, had higher LMA and N_A). Although the response to CO₂ enrichment is somewhat lower in the closed-canopy ORNL stand compared to that of individual emergent trees in the Duke FACE experiment, the photosynthetic response of *L. styraciflua* is strong and persistent, even in closed-canopy conditions.

Photosynthetic capacity (A_{max}) of *L. styraciflua* was not significantly affected by 3 yrs of exposure to elevated [CO₂]. During this period, net primary productivity was enhanced 21% by CO₂ enrichment (Norby *et al.*, 2002), indicating a sustained increase in carbon sinks in elevated [CO₂], even though growth responses were constrained by the nonexpanding canopy. Analyses of more than 350 $A-C_i$ curves collected in this experiment revealed no decrease in the rate of Rubisco carboxylation capacity (V_{cmax}) or RuBP regeneration mediated by electron transport (J_{max}), further indicating that photosynthetic acclimation to elevated [CO₂] did not occur during the experimental period in this forest stand. Similarly, Herrick & Thomas (2001) found that photosynthetic down-regulation did not occur for individual *L. styraciflua* trees after 3 yrs of [CO₂] enrichment at the Duke Forest FACE site. The ratio of J_{max} to V_{cmax} , which represents the balance between the rate of regeneration of RuBP by electron transport and the rate of Rubisco carboxylation, has been theorized to increase in plants grown in elevated [CO₂] as leaf N is reallocated to

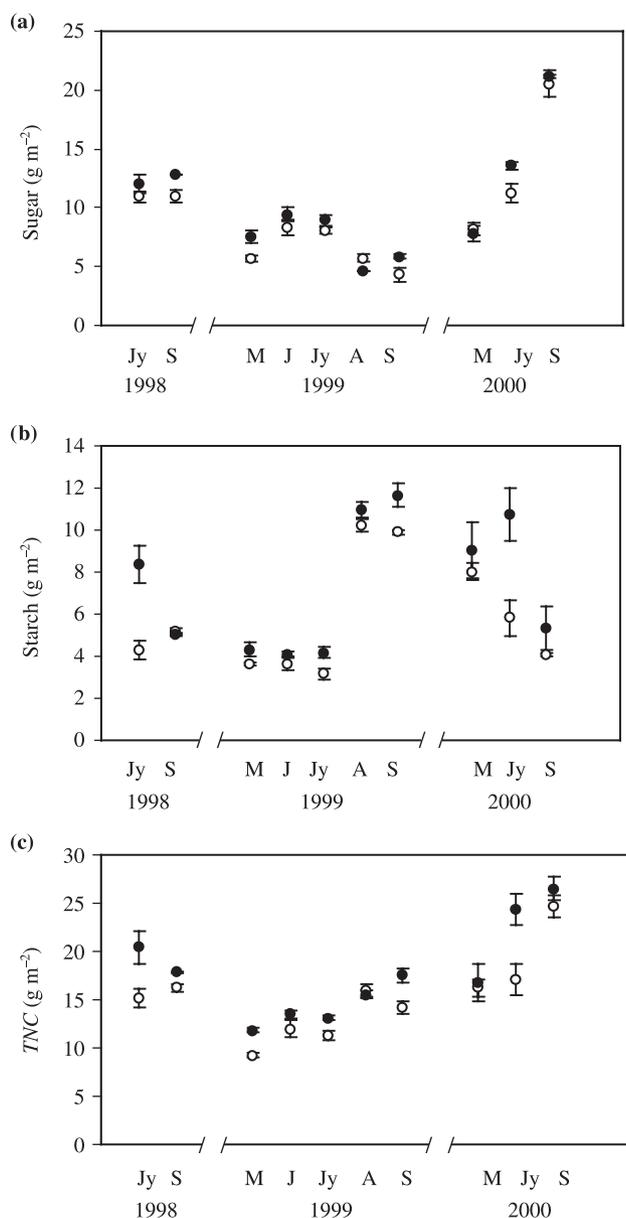


Fig. 9 Response of foliar carbohydrate content of mature upper-canopy leaves of *Liquidambar styraciflua* exposed to ambient ($-364 \mu\text{mol mol}^{-1}$, $n = 3$, open circles) or elevated ($-553 \mu\text{mol mol}^{-1}$, $n = 2$, closed circles) CO₂. Foliar (a) sugar (b) starch and (c) total nonstructural carbohydrate (TNC) content was determined for leaves harvested following the collection of A-C_i curves. Values are presented as means \pm SEM.

light-harvesting processes from carboxylation processes (Sage, 1994; Medlyn, 1996). However, this was not observed in *L. styraciflua* in our experiment, indicating that the relationship between carboxylation and light-harvesting processes, which varied seasonally, was not affected by CO₂ treatment. Similarly, no shift in the J_{max} to V_{cmax} ratio was observed in a meta-analysis of field-based experiments on European tree species

grown in elevated [CO₂] (Medlyn *et al.*, 1999), in *Populus tremuloides* and *Acer saccharum* (Kubiske *et al.*, 2002) or in *L. styraciflua* at the Duke FACE site (Herrick & Thomas, 2001).

Photosynthetic nitrogen-use efficiency (*PNUE*) was higher in *L. styraciflua* grown in elevated [CO₂]. Centritto & Jarvis (1999) noted a similar increase in *PNUE* in elevated-CO₂ plants, along with a reduction in V_{cmax} per unit leaf nitrogen. Significant positive correlations between V_{cmax} and J_{max} with leaf N_A have been shown for many plants (Evans, 1989; Sage *et al.*, 1990; McMurtrie *et al.*, 1992; Leuning *et al.*, 1995; Kellomäki & Wang, 1997), including *L. styraciflua* (Herrick & Thomas, 2001). Changes in these relationships would be suggestive of reallocation of nitrogen within the trees associated with improved nitrogen-use efficiency (Kellomäki & Wang, 1997). However, no such correlation between V_{cmax} and J_{max} with N_A was found for *L. styraciflua* in this study, which provides no further indication of N reallocation. Apparently, increased N demand in elevated [CO₂] was met by increased N uptake (Johnson *et al.*, 2003).

Although changes in leaf characteristics were observed in *L. styraciflua* grown in elevated [CO₂], this did not affect photosynthetic (A_{max}) and biochemical capacity (V_{cmax} and J_{max}). In many studies, a decline in N_M in elevated [CO₂] is associated with photosynthetic downregulation (Medlyn *et al.*, 1999; Peterson *et al.*, 1999). In *L. styraciflua*, N_M was generally lower in leaves grown in elevated [CO₂] in all sample periods. The average decrease in N_M over the 10 sample periods was 10%, compared to the 15% reduction reported by Medlyn *et al.* (1999) for 12 woody species exposed to elevated [CO₂] and 16% for many different species (Curtis & Wang, 1998). Despite the reduction due to growth in elevated [CO₂], N_M of leaves of *L. styraciflua* ranged from 15 to 20 mg g⁻¹, which is generally higher than the average N_M of forest-grown *L. styraciflua* (Blinn & Buckner, 1989). Therefore, the lack of photosynthetic downregulation in *L. styraciflua* may be partially explained by the lack of foliar N deficiency.

In *L. styraciflua*, there was no significant CO₂ effect on N_A . These results are in agreement with many studies that have shown significant reductions in N_M , but not N_A , because of reciprocal changes in N_M and LMA (Curtis, 1996; Curtis & Wang, 1998; Medlyn *et al.*, 1999; Norby *et al.*, 1999). Similarly, Chl_M , but not Chl_A , was significantly reduced in elevated [CO₂] in this study due to reciprocal changes in LMA . The reductions in N_M may be explained by the presence of increases in nonstructural, and potentially also structural, carbohydrates (i.e. increased LMA in elevated [CO₂]), which dilutes N_M ; Epron *et al.*, 1996). The enhanced LMA of *L. styraciflua* that was noted with growth in elevated [CO₂] could produce a secondary effect of increasing A_{growth} if it were partially due to the presence of additional photosynthetic tissue (Thomas & Harvey, 1983; Peterson *et al.*, 1999). However, linear regression analysis revealed that for *L. styraciflua* grown in elevated [CO₂] there was no significant relationship between A_{growth} and LMA (results not shown).

The photosynthetic response of *L. styraciflua* varied through the growing season, as has been observed in many other trees (Curtis *et al.*, 1995; Tissue *et al.*, 1997; Rey & Jarvis, 1998; Tissue *et al.*, 1999; Rogers & Ellsworth, 2002), due in part to varying environmental conditions. In this study, A_{growth} in both CO₂ treatments generally declined about 50% from a high in early season to a low at the end of our measurement period in September; however, the relative enhancement of A_{growth} due to elevated [CO₂] was maintained throughout this period. In a related study on *L. styraciflua* conducted during the same 3-yr period at the ORNL FACE site, light-saturated rates of photosynthesis and stomatal conductance were tightly correlated (Gunderson *et al.*, 2002). Reductions in photosynthesis and stomatal conductance observed during mid- and late-season (August to September) in 1998 and 1999 were primarily associated with drought conditions (Gunderson *et al.*, 2002). During these drought conditions, the most significant variable reducing photosynthesis was increased vapour pressure deficit (VPD), followed by reduced soil water potential (Gunderson *et al.*, 2002). Under extreme conditions of dry soil and high VPD, as experienced in late 1998, stomatal conductance and photosynthesis were reduced sharply in both ambient- and elevated-CO₂ trees, and differences between the CO₂ treatments were small. At other times, CO₂ stimulation of photosynthesis was sustained despite late-season reductions in gas exchange. Likewise, in the Duke Forest, *L. styraciflua* exhibited a pattern of seasonal decline in A_{growth} , which was attributed to a reduction in stomatal conductance; similarly, a significant elevated-CO₂ effect on photosynthesis was maintained until leaf senescence in early November (Herrick & Thomas, 2003).

In some deciduous tree species, this seasonal variation in photosynthetic response may also be linked to seasonal variations in source activity and sink capacity. For example, Epron *et al.* (1996) noted that tree species, such as *Fagus sylvatica*, that continue to produce new sinks beyond the first flush of leaves in spring in response to increased carbohydrate availability, do not show a decrease in early season A_{max} , in contrast to the decline in A_{max} observed in species with determinate growth, such as *Quercus petraea* (Epron *et al.*, 1994). However, when aboveground growth slows dramatically, thus limiting sink capacity, trees show a late-season decline in photosynthesis (Epron *et al.*, 1996). In the herbaceous species, *Lolium perenne*, growing in the Swiss FACE site, the ratio of carbon sink activity to source activity was greatly increased by removing 89% of the canopy using a defoliant (Rogers *et al.*, 1998). Photosynthetic acclimation in *L. perenne* was not observed until much of the canopy had regrown and the ratio of sink to source activity had greatly declined (Rogers *et al.*, 1998).

In *L. styraciflua*, which exhibits indeterminate growth (Kuers & Steinbeck, 1998), general bud set marks a major decline in leaf production and branch extension. Bud set was noted in this experiment (over three growing seasons) to occur

around 20 July, which is also the time of peak LAI (Norby *et al.*, 2003). Thus, the marked decline in aboveground sink capacity of these trees was accompanied by a decrease in A_{max} in the second half of the growing season, which was more pronounced in leaves grown in elevated [CO₂] as compared to those grown in ambient [CO₂]. The monthly data collected in 1999 for A_{max} , V_{cmax} and J_{max} illustrate this decline in photosynthetic capacity in elevated [CO₂] at the time of bud set, and hence decreased sink capacity, in July. These data suggest that even transient reductions in sink capacity may lead to reduced photosynthetic capacity, thereby reducing, but not eliminating, the positive effects of elevated [CO₂] on leaf photosynthesis.

As in many previous studies, growth of *L. styraciflua* in elevated [CO₂] resulted in enhanced leaf TNC concentration (Xu *et al.*, 1994; Van Oosten & Besford, 1996; Saxe *et al.*, 1998; Stitt & Krapp, 1999; Curtis *et al.*, 2000). Starch accumulation in leaves grown in elevated [CO₂] was especially pronounced, with an average enhancement of 27% ± 10. There were no consistent seasonal trends as starch was highest in mid-season in 1998, late-season in 1999 and early- to mid-season in 2000. However, in all three growing seasons the greatest effect of elevated [CO₂] on starch accumulation occurred in July, during the period of bud set (i.e. reduced sink strength), suggesting a negative relationship between sink strength and carbohydrate accumulation. Nonetheless, starch accumulation in elevated [CO₂] did not affect photosynthetic capacity or biochemical capacity.

The lack of photosynthetic acclimation in canopy-dominant *L. styraciflua* in our experiment in the ORNL FACE site over a 3-yr period is consistent with observations for *L. styraciflua* during the first 3 yrs of exposure to elevated [CO₂] at the Duke Forest FACE site (Herrick & Thomas, 2001). However, these results were not consistent across all species sampled at the Duke FACE site. For example, photosynthetic acclimation (i.e. decrease in Rubisco activity) was observed in 1-yr-old, but not current-year needles of canopy-dominant *Pinus taeda* (loblolly pine) trees following 2.5 yrs of exposure to elevated [CO₂] (Rogers & Ellsworth, 2002). It had been previously shown that *P. taeda* growing in an adjacent FACE ring in Duke Forest displayed both photosynthetic enhancement (32%) and downregulation (15–20%) following 3 yrs of exposure to elevated [CO₂] (Ellsworth *et al.*, 1998). Photosynthetic downregulation has also been observed in open-top chambers after multiple-year exposure to elevated [CO₂] in *P. taeda* (Lewis *et al.*, 1996; Tissue *et al.*, 1996, 1997), *P. ponderosa* (Tissue *et al.*, 1999) and *P. radiata* (Turnbull *et al.*, 1998; Griffin *et al.*, 2000).

A possible explanation for the differing responses of *L. styraciflua* and the various pine species to long-term exposure to elevated [CO₂] involves differences in the phenology of deciduous versus evergreen trees. Evergreen gymnosperms are typically characterized by their maintenance of photosynthesizing green leaves (i.e. needles) throughout the entire year.

Evergreen leaves generally have lower rates of light-saturated photosynthesis and experience seasonal changes in photosynthetic capacity more gradually than do leaves of deciduous species. In addition, evergreen species have the capacity to maintain needles for more than one growing season, which results in trees having different-aged needles with different physiological characteristics (e.g. source-sink relations). As a result, differences have been noted in CO₂ effects on current-year and 1-yr-old needles in evergreen species. Photosynthetic acclimation has been shown to be limited to 1-yr-old needles in *P. taeda* (Rogers & Ellsworth, 2002) and *P. radiata* (Turnbull *et al.*, 1998; Griffin *et al.*, 2000). Current-year needles of *P. radiata* grown in elevated [CO₂] have exhibited greater increases in net photosynthesis than 1-yr-old needles, and had enhanced photosynthetic capacity, while 1-yr-old needles showed no CO₂ effect (Tissue *et al.*, 2001). It should be noted, however, that deciduous trees exposed to long-term elevated [CO₂] may also exhibit photosynthetic downregulation, including *Betula pendula* (Rey & Jarvis, 1998; Juurola, 2003), *Populus tremuloides* and *Acer saccharum* (Kubiske *et al.*, 2002).

In rapidly growing deciduous angiosperms, such as *L. styraciflua*, the rate of photosynthesis typically accelerates rapidly in the spring as trees re-leaf, remains high during the summer, and declines rapidly in late summer as leaves senesce before abscising (Kozlowski & Pallardy, 1997). As a consequence, deciduous trees, which have only current-year leaves, accomplish all of their growth before the dormant season, whereas evergreens are capable of continuing to accumulate dry matter during the dormant period. These differences in phenology result in significant differences in the overall source-sink relations of trees, which reduce the likelihood of downregulation of fast-growing deciduous species such as *L. styraciflua*.

In conclusion, photosynthetic enhancement (44–54%) of *L. styraciflua* was maintained for 3 yrs at the ORNL FACE site. There were no significant CO₂ treatment effects on photosynthetic (A_{max}) or biochemical (V_{cmax} and J_{max}) capacity, despite increased sugar and starch accumulation and reduced N_M in elevated [CO₂], indicating that photosynthetic downregulation did not occur. Elevated [CO₂] also increased PNUE in leaves that are not apparently limited by N in this forest. These results suggest that the enhancement of photosynthesis in *L. styraciflua* in elevated [CO₂] is likely to continue for many years, thereby providing more carbon for growth in this closed-canopy deciduous forest.

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