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Photosynthetic responses to CO₂ enrichment of four hardwood species in a forest understory

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Abstract We compared the CO₂- and light-dependence of photosynthesis of four tree species (*Acer rubrum*, *Carya glabra*, *Cercis canadensis*, *Liquidambar styraciflua*) growing in the understory of a loblolly pine plantation under ambient or ambient plus 200 µl l⁻¹ CO₂. Naturally-established saplings were fumigated with a free-air CO₂ enrichment system. Light-saturated photosynthetic rates were 159–190% greater for *Ce. canadensis* saplings grown and measured under elevated CO₂. This species had the greatest CO₂ stimulation of photosynthesis. Photosynthetic rates were only 59% greater for *A. rubrum* saplings under CO₂ enrichment and *Ca. glabra* and *L. styraciflua* had intermediate responses. Elevated CO₂ stimulated light-saturated photosynthesis more than the apparent quantum yield. The maximum rate of carboxylation of ribulose-1,5-bisphosphate carboxylase, estimated from gas-exchange measurements, was not consistently affected by growth in elevated CO₂. However, the maximum electron transport rate estimated from gas-exchange measurements and from chlorophyll fluorescence, when averaged across species and dates, was approximately 10% higher for saplings in elevated CO₂. The proportionately greater stimulation of light-saturated photosynthesis than the apparent quantum yield and elevated rates of maximum electron transport suggests that saplings growing under elevated CO₂ make more efficient use of sunflecks. The stimulation of light-saturated photosynthesis by CO₂ did not appear to correlate with shade-tolerance ranking of the individual species. However, the species with the greatest enhancement of photosynthesis, *Ce. canadensis* and *L. styraciflua*, also invested the greatest proportion of soluble protein in Rubisco. Environmental and endogenous factors affecting N parti-

tioning may partially explain interspecific variation in the photosynthetic response to elevated CO₂.

Key words Acclimation · Atmospheric carbon dioxide · Climate change · Photosynthesis · Shade tolerance

Introduction

A synthesis of controlled-environment studies indicates that photosynthetic rates increase by approximately 54% when tree seedlings and saplings are grown under twice current levels of CO₂ (Curtis and Wang 1998). There is, however, considerable variation in this response, and the imposition of environmental heterogeneity common in native ecosystems can alter its magnitude (Bazzaz 1990). Low light limits photosynthesis in the forest understory and there is potentially a strong interaction between irradiance and the response of plants to CO₂ enrichment. For example, the average growth stimulation for woody plants to elevated CO₂ increases from 31% to 52% when plants were grown under limiting irradiance (Curtis and Wang 1998). For plants experiencing multiple environmental stresses under natural conditions, the magnitude of the photosynthetic response and the potential to acclimate to an increase in atmospheric CO₂ is uncertain.

Under steady-state conditions, C₃ photosynthesis is co-limited by the rate of carboxylation, the rate of ribulose bisphosphate (RuBP) regeneration, and the rate of triose phosphate utilization. This co-limitation represents an optimal partitioning of nitrogen between the component reactions of photosynthesis (Evans 1987, 1989). As human activities increase atmospheric CO₂ the capacity for carboxylation may exceed the rate of RuBP regeneration. Plants respond to this imbalance by decreasing the amount of ribulose-1,5-bisphosphate carboxylase (Rubisco), the primary carboxylating enzyme (Sage 1994). This is not, however, a universal phenomenon; changes in Rubisco activity are influenced by growth conditions, phenology, and nutrient status (Sharkey 1990; Gundersen and Wullschleger 1994; Thomas et al. 1994). The

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electron transport/carboxylation ratio provides one measure of acclimation, and a theoretical analysis by Medlyn (1996) indicated that this ratio should increase by 40% with a doubling of atmospheric CO₂. Medlyn (1996) found that empirical data for plants grown under high irradiance (Wullschlegler 1993) did not support this model.

Low irradiance also creates excess carboxylation capacity. Simultaneous exposure to elevated CO₂ and shade should therefore drive a more complete reinvestment of leaf nitrogen and acclimation than exposure to either variable independently. In shaded leaves of the woodland herb *Duchesnea indica*, Rubisco content decreased by 37% in plants grown under CO₂ enrichment (Osborne et al. 1997), and acclimation to elevated CO₂ decreased the ratio of Rubisco to light-harvesting complex in shaded wheat leaves (Osborne et al. 1998).

To determine if understory hardwoods acclimate to elevated CO₂, we examine the CO₂- and light-dependence of photosynthesis for four tree species in the southeastern United States growing in ambient and elevated atmospheric CO₂ concentration. Photosynthetic acclimation to CO₂ enrichment may vary among species with different life-history characteristics (Bazzaz and Miao 1993; Hättenschwiler and Körner 1996; Kubiske and Pregitzer 1996). We therefore selected species representing a range of shade tolerances and flushing strategies. *Acer rubrum* L. (intermediate to shade-tolerant) and *Carya glabra* (Mill.) Sweet (intermediate tolerance) are determinate, single-flushing species, presumably with limited ability to produce new sinks for carbohydrates late in the growing season; *Cercis canadensis* L. is shade-tolerant as a juvenile, and, under optimal conditions, continues stem elongation during the entire growing season; *Liquidambar styraciflua* L. (shade-intolerant) is an indeterminate, multiple-flushing species. Saplings established naturally in the understory of a loblolly pine plantation, where the CO₂ concentration in 30-m diameter plots was controlled at ~1.5×ambient levels with a free-air CO₂ enrichment (FACE) system. The FACE system controls [CO₂] in the entire volume of this 14-m-tall forest without altering other micrometeorological variables (Hendrey and Kimball 1994; Lewin et al. 1994).

Materials and methods

Experimental design and plant material

We selected naturally-established saplings growing in the understory of an unmanaged 15-year-old loblolly pine plantation (Duke Forest) in the Piedmont region of North Carolina (35°09'N, 79°09'W). In this plantation three 30-m-diameter plots are continuously fumigated with CO₂ to raise the atmospheric concentration to a target of 200 μl l⁻¹ above current ambient levels. Three additional fully instrumented plots that circulate air without added CO₂ serve as controls. Fumigation was initiated 10 months prior to our first measurements in June 1997, and all leaves developed under the CO₂ treatment. We restricted our measurements to saplings growing in the southern two control and two treatment plots.

The CO₂ concentration at 1 m height was sampled at 13 locations in each plot between May and August 1997. The spatially and temporally averaged daytime CO₂ concentration (0700–1900

hours EST) in the two “treatment” plots used in this study were 619 and 612 μl l⁻¹, with a coefficient of variation between sample locations within each plot of 4% (Hendrey et al. 1999). The average CO₂ concentration in the ambient plots over this interval was 366 μl l⁻¹.

Four individuals of each species between 0.5 and 2 m tall were selected in each of the ambient and elevated plots. Root sprouts and plants with damaged leaders were avoided. Because these saplings established naturally, it was not possible to balance the distribution of saplings among the different control and treatment plots. Additional individuals were chosen on subsequent sampling dates as saplings fell prey to herbivory.

Leaf area index (LAI) was measured above each sapling in June 1997 with a LI-2000 canopy analyzer (LiCor, Inc., Lincoln, Neb., USA). There was no systematic variation in LAI between species within each plot (data not shown) or between control and treatment plots (LAI control plots: 4.5±0.9 m² m⁻²; elevated plots: 4.7±0.9 m² m⁻², n=3). Using a Beer's law approximation of light attenuation through the forest canopy and assuming an extinction coefficient of 0.5, the average irradiance at sapling height was approximately 10% of the irradiance incident on the forest canopy.

Leaf gas exchange and fluorescence

The response of net photosynthesis to incident irradiance and to calculated intercellular CO₂ (c_i) at saturating irradiance was measured in situ on one healthy leaf per sapling in mid-June and mid-September 1997, and again in early June 1998. Measurements were made with an open gas-exchange system (LI 6400, LiCor, Inc.) between 1000 and 1600 hours (EST). For the light-response measurements the CO₂ concentration of air entering the cuvette was maintained at 350 or 550 μl l⁻¹ for saplings in the ambient and elevated CO₂ plots, respectively. We assumed that leaves were hypostomatous and used a boundary layer conductance (water vapor) of 1.42 mol m⁻² s⁻¹ to calculate stomatal conductance and intercellular CO₂ concentration. The water vapor content (RH) and cuvette temperature (°C) were uncontrolled but typically varied less than 8% and 3%, respectively, between samples.

For measuring the photosynthetic-light response, incident irradiance was provided by red light-emitting diodes (LEDs; λ_{max}=650 nm). Measurements were initiated after 1000 hours EST. By this time leaves had received several sun flecks and had become fully induced (E. Singaas, unpublished work). To examine a potential effect of the CO₂ treatment on the apparent quantum yield, photosynthesis was measured in June 1997 at incident levels of 10, 50, 75 and 125 μmol m⁻² s⁻¹ (photon flux density, PFD). However, the rate of photosynthesis increased non-linearly below 125 μmol m⁻² s⁻¹ PFD (data not shown). In September 1997 an additional measurement was made at an incident irradiance of 30 μmol m⁻² s⁻¹ and the apparent quantum efficiency was calculated as the slope of photosynthesis versus incident irradiance between 10 and 75 μmol m⁻² s⁻¹, PFD.

We measured the photosynthetic response to intercellular CO₂ concentration to assess the degree of acclimation to elevated CO₂. The photosynthesis versus CO₂ curves were analyzed with a mechanistic two-factor model derived by Farquhar et al. (1980) and modified by Harley et al. (1992). The light-saturated rate of carboxylation (V_{c,max}) and electron transport (J_{max}) were calculated using the kinetic assumptions in Wullschlegler (1993). The photosynthetic response to intercellular CO₂ was measured at a constant saturating irradiance of 1000 μmol m⁻² s⁻¹, PFD.

In September 1997, chlorophyll fluorescence from the adaxial leaf surface was measured with pulse-modulated fluorimeter (PAM-2000, Walz, Effeltrich, Germany). To perform a quenching analysis (Schreiber et al. 1986; Genty et al. 1989; Krause and Weis 1991), it was necessary to drive a transient reduction followed by oxidation of the primary electron acceptor of photosystem (PS) II. A 0.6-s exposure to an irradiance of ~14 mmol m⁻² s⁻¹ was used to reduce the plastoquinone (PQ) pool of PS II. Subsequently, the PQ pool was oxidized by stimulating PS I with a 0.6-s pulse of far red light. The leaf was darkened during exposure

to far-red light. Calculation of fluorescence variables follows van Kooten and Snel (1990). The quantum yield of PS II electron transport (Φ_{PSII}) was estimated as $(F_m - F_s)/F_m$, as in Genty et al. (1989), where F_m and F_s are the maximum and steady-state fluorescence yields, respectively. F_m was measured during a saturating pulse and F_o , the minimal fluorescence, was measured following exposure to far-red light.

To measure the light response of fluorescence variables, we used the red LED array in the fluorimeter to provide controlled irradiance levels at the leaf surface of 16–390 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The leaf was exposed to each actinic irradiance for 60 s prior to measurement of fluorescence. Preliminary experiments indicated that this duration was sufficient to achieve steady state at irradiances below 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Above 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, >90% of maximum levels was achieved within 60 s. Measurements were made on a single healthy leaf on each of the four saplings per species in the ambient and in the elevated CO_2 plots.

Leaf properties

Two disks (3.7 cm^2) per leaf were harvested and dried to calculate specific leaf area (SLA; leaf area/mass). Additional chopped leaf disks were extracted overnight with 10 ml N,N-dimethylformamide to determine chlorophyll contents as in Porra et al. (1989). Other samples were combusted in an elemental analyzer (NA1500, Carlo Erba, Milan, Italy) to determine leaf N content.

In June 1998, disks (~8 cm^2) were harvested with a freeze clamp (-196°C) from leaves used for gas exchange measurements. In some cases, depending on its size or condition, an adjacent leaf was selected. The disks were immediately stored in liquid N and then at -80°C. Soluble proteins were extracted as in Cheeseman et al. (1997). Following centrifugation, proteins were separated using 12.5% SDS-polyacrylamide gel electrophoresis and stained with Coomassie-blue. The Rubisco large subunit (LSU) band was identified by comparison to a 55-kDa molecular weight marker (Sigma Chemical Co.). The Coomassie-blue dye was quantified by removing and macerating the LSU band from the gel and eluting it in 25% pyridine (v/v with water) for 2 h (Fenner et al. 1975). Three known amounts of Rubisco were run on each gel as standards.

Statistical analyses

A power function, described in the legend of Fig. 1, was used to describe the responses of net photosynthesis to incident irradiance and calculated intercellular CO_2 concentration. The maximum apparent quantum yield was calculated with regression applied to the initial linear region of the photosynthetic-light responses (<100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, PFD). Within a species, the slopes of these regressions were compared between treatments with an analysis of covariance, with incident irradiance as the covariate (SYSTAT 7.0 for WINDOWS, SPSS Inc., Evanston, Ill., USA); a significant ($P < 0.05$) treatment \times PFD interaction indicated that the slopes were significantly different between the ambient and elevated CO_2 plots. The responses of fluorescence variables to incident irradiance were fit with second-order polynomials.

The chemical and structural attributes of leaves were compared with ANOVA (4 species \times 2 treatments \times 3 dates; June and September 1997, and June 1998). For statistical analyses we lumped saplings from the different control and treatment plots and we assumed that individual saplings within a plot were independent. This assumption was necessary because saplings of each species were unevenly distributed between plots. The spatial variation in CO_2 concentration within each plot was greater than the variation between plots, thus supporting our assumptions that saplings were independent. Where appropriate, data were log-transformed prior to analysis.

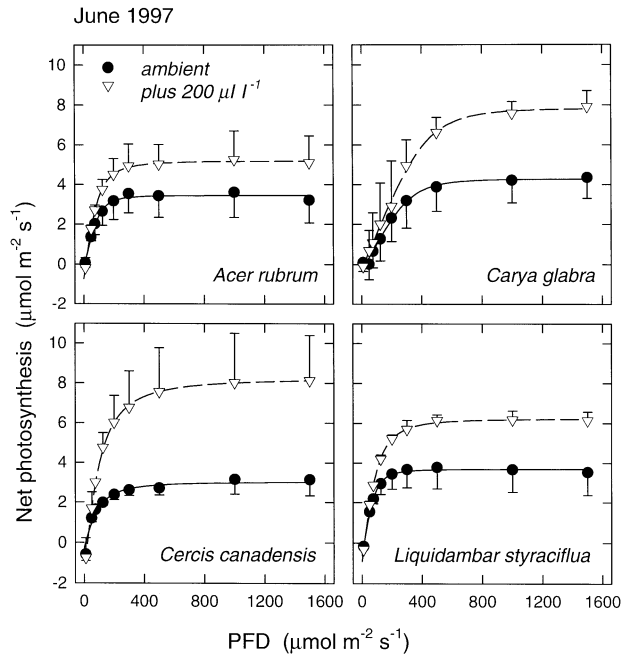


Fig. 1 The response of net photosynthesis to incident irradiance (photosynthetic photon flux density, PFD) measured in June 1997, for understory saplings growing under ambient (~350 $\mu\text{l l}^{-1}$; closed circles) and ambient plus 200 $\mu\text{l l}^{-1}$ atmospheric CO_2 (open triangles). Each point is an average (± 1 SD) of four independent measurements. The photosynthetic-light responses were fit with a power function in the form: $y = [a \times x / (1 + zc) / c] + d$; where $z = a \times x / b$, y is net photosynthesis, a is the initial linear slope, x is incident irradiance, c describes convexity, d is the y -intercept, and b is the asymptotic photosynthetic rate. The values for a , b , c , and d for each species and treatment are: *Acer rubrum* ambient: 0.03, 3.63, 2.61, -0.19; elevated: 0.05, 5.93, 2.05, -0.73; *Carya glabra* ambient: 0.01, 4.59, 3.50, -0.31; elevated: 0.02, 8.07, 3.62, -0.24; *Cercis canadensis* ambient: 0.03, 3.30, 1.60, -0.26; elevated: 0.07, 9.76, 1.58, -1.52; *Liquidambar styraciflua* ambient: 0.05, 4.31, 2.38, -0.61; elevated: 0.06, 7.14, 1.97, -0.90

Results

Light-saturated photosynthetic rates were 159–190% greater for *Ce. canadensis* saplings grown and measured under elevated than under ambient CO_2 (Figs. 1,2). This species had the greatest stimulation in response to the treatment. At the other extreme, the maximum photosynthetic rate under elevated CO_2 for *A. rubrum* saplings in June 1997 was only 59% greater than for saplings under ambient conditions and this stimulation was reduced by September. *Ca. glabra* maintained an 80–82% stimulation of light-saturated photosynthesis through the summer, and the 74% stimulation observed for *L. styraciflua* in June 1997 increased to a 126% enhancement by September.

There was no statistical effect of elevated CO_2 on the apparent quantum yield of photosynthesis for saplings of *Ce. canadensis* (the quantum yield for both ambient and elevated leaves was 0.038 ± 0.026 (SE) $\mu\text{mol CO}_2 \mu\text{mol}^{-1}$ PFD; September 1997). There was a trend for higher apparent quantum yield for *A. rubrum* grown in the elevat-

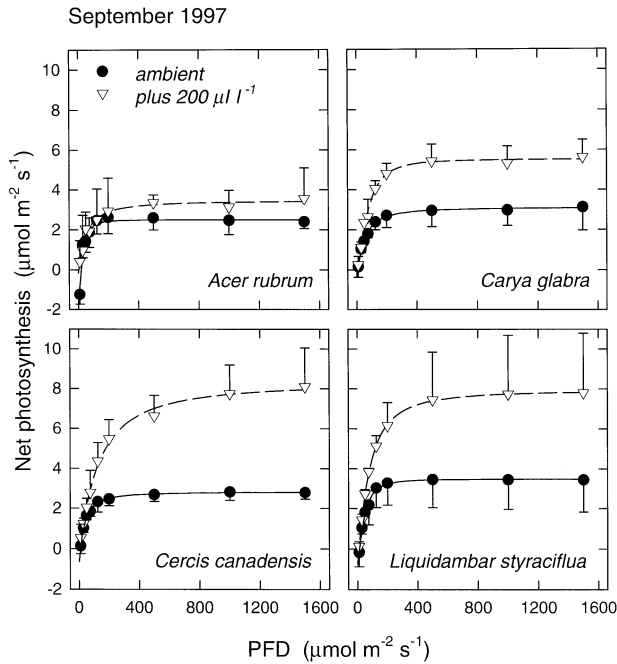


Fig. 2 The response of net photosynthesis to incident irradiance (PFD) measured in September 1997, for understory saplings growing under ambient ($\sim 350 \mu\text{l l}^{-1}$; closed circles) and ambient plus $200 \mu\text{l l}^{-1}$ atmospheric CO_2 (open triangles). The photosynthetic-light responses were fit with a power function as in Fig. 1, and the values for a , b , c , and d for each species and treatment are: *A. rubrum* ambient: 0.06, 3.63, 1.21, -0.17 , elevated: 0.10, 4.27, 2.06, -1.76 ; *Ca. glabra* ambient: 0.05, 3.43, 1.36, -0.33 , elevated: 0.05, 5.76, 2.03, -0.24 ; *Ce. canadensis* ambient: 0.09, 3.47, 1.23, -0.64 , elevated: 0.05, 8.46, 1.24, -0.11 ; *L. styraciflua*: ambient: 0.05, 5.76, 2.028, -0.24 , elevated: 0.08, 8.69, 1.45, -0.74

ed CO_2 plots (ambient: $0.047 \mu\text{mol } \mu\text{mol}^{-1}$; elevated: $0.067 \mu\text{mol } \mu\text{mol}^{-1}$) and this variable was significantly greater (33–66%) for the *Ca. glabra* (ambient: $0.032 \mu\text{mol } \mu\text{mol}^{-1}$; elevated: $0.053 \mu\text{mol } \mu\text{mol}^{-1}$) and *L. styraciflua* (ambient: $0.049 \mu\text{mol } \mu\text{mol}^{-1}$; elevated: $0.065 \mu\text{mol } \mu\text{mol}^{-1}$) under the CO_2 treatment. The average photosynthetic light-compensation point for all species and treatments was $11.5 \pm 6.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, PFD ($n=16$), and there was no consistent difference between control plants and those grown under elevated CO_2 .

Light-saturated Rubisco carboxylation ($V_{c_{\max}}$), measured in June and September 1997, varied by over two-fold across species, treatments, and dates. The highest $V_{c_{\max}}$, $30.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, was for *Ce. canadensis* in June 1997, and *Ca. glabra* measured at this same time had the lowest $V_{c_{\max}}$, $13.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 1). When examined across all species and dates, there was no effect of CO_2 enrichment on $V_{c_{\max}}$ (Table 2). Although not statistically significant, the difference in $V_{c_{\max}}$ for ambient and elevated plants varied seasonally. For example, $V_{c_{\max}}$ was substantially lower for *A. rubrum* in the elevated CO_2 plots in June 1997 but this trend reversed by September. Similarly, the strong reduction in $V_{c_{\max}}$ evident for *L. styraciflua* under CO_2 enrichment in June 1997 disappeared by September (Table 2). Across all dates and treatments *Ce. canadensis* (26.3) and *L. styraciflua* (26.5) had greater $V_{c_{\max}}$ than *A. rubrum* (23.0) and *Ca. glabra* (21.2).

$V_{c_{\max}}$ varied significantly between the three measurement dates. The highest rates were in late June 1997 and decreased by approximately 11% in September. The lowest values were measured in early June 1998 (Tables 1,2).

Table 1 Light-saturated Rubisco carboxylation ($V_{c_{\max}}$; $\mu\text{mol m}^{-2} \text{s}^{-1}$), electron transport (J_{\max} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), and the ratio of $V_{c_{\max}}/J_{\max}$ for *Acer rubrum* (ACRU), *Carya glabra* (CAGL), *Cercis canadensis* (CECA), and *Liquidambar styraciflua* (LIST) growing in ambient (Amb; $c. 360 \mu\text{l l}^{-1}$) or elevated CO_2 (Elev; ambient plus $200 \mu\text{l l}^{-1}$) plots. Measurements were made in June and September 1997 and in June 1998. Each value is the mean of 4 independent measurements (± 1 SD)

			$V_{c_{\max}}$	J_{\max}	$V_{c_{\max}}/J_{\max}$
ACRU	June 1997	Amb	29.8 (6.4)	91.8 (10.0)	0.328 (0.079)
		Elev	22.5 (3.0)	87.0 (9.9)	0.259 (0.017)
	Sept 1997	Amb	17.6 (5.0)	60.5 (9.8)	0.292 (0.099)
		Elev	24.5 (1.0)	78.2 (0.8)	0.313 (0.010)
	June 1998	Amb	23.7 (2.9)	67.6 (5.6)	0.349 (0.017)
		Elev	19.1 (2.7)	72.3 (7.9)	0.265 (0.029)
CAGL	June 1997	Amb	23.4 (5.7)	68.3 (15.5)	0.347 (0.077)
		Elev	27.7 (2.5)	102.3 (18.3)	0.276 (0.045)
	Sept 1997	Amb	13.8 (2.8)	55.1 (10.9)	0.251 (0.028)
		Elev	20.8 (1.0)	76.2 (20.2)	0.287 (0.069)
	June 1998	Amb	21.8 (5.2)	63.4 (13.7)	0.346 (0.063)
		Elev	19.6 (6.5)	64.9 (12.0)	0.303 (0.098)
CECA	June 1997	Amb	27.4 (5.7)	87.0 (18.0)	0.316 (0.024)
		Elev	30.8 (11.2)	87.8 (37.4)	0.358 (0.044)
	Sept 1997	Amb	21.8 (5.0)	57.2 (17.6)	0.395 (0.078)
		Elev	27.8 (3.1)	84.0 (26.6)	0.354 (0.110)
	June 1998	Amb	29.4 (3.0)	78.9 (12.1)	0.377 (0.052)
		Elev	20.5 (5.2)	76.5 (25.8)	0.307 (0.159)
LIST	June 1997	Amb	30.1 (3.8)	76.7 (13.9)	0.399 (0.064)
		Elev	19.1 (2.3)	63.9 (14.4)	0.316 (0.110)
	Sept 1997	Amb	28.0 (9.9)	75.3 (5.8)	0.366 (0.101)
		Elev	32.6 (8.6)	92.8 (23.6)	0.351 (0.022)
	June 1998	Amb	24.1 (5.1)	53.3 (6.3)	0.448 (0.051)
		Elev	25.0 (5.2)	64.1 (24.5)	0.414 (0.112)

The overall variance in J_{\max} was lower than for Vc_{\max} , and J_{\max} did not vary significantly between species. The maximum rate of electron transport was, however, significantly higher for saplings growing in elevated CO_2 (69.8 compared to 79.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$, for elevated and control plots, respectively). Seasonal changes in J_{\max} paralleled the changes in Vc_{\max} .

The ratio of Vc_{\max}/J_{\max} may provide evidence of acclimation of the component functions of photosynthesis (Medlyn 1996). When averaged across all dates, saplings in the elevated CO_2 plots had slightly lower Vc_{\max}/J_{\max} ratios than those in the ambient plots (0.317 compared with 0.352, $P<0.05$). This ratio also varied significantly between species. The species with the highest Vc_{\max} , *Ce. canadensis* and *L. styraciflua*, also had the highest Vc_{\max}/J_{\max} (Tables 1,2).

Table 2 Three-way ANOVA applied to the light-saturated Rubisco carboxylation (Vc_{\max}), the rate of electron transport (J_{\max}), and the ratio of Vc_{\max}/J_{\max} (Vc/J). The mean-square values are presented for the log-transformed Vc/J ratio and for untransformed values of the other variables

	df	Vc_{\max}	J_{\max}	Vc/J
Species (S)	3	160.04**	310.5	0.302**
Treatment (T)	1	0.065	2081.5**	0.289*
Date (D)	2	110.4*	1996.5**	0.041
S×T	3	29.8	237.2	0.002
S×D	6	74.5*	650.5	0.055
T×D	2	210.6**	674.2	0.102
S×T×D	6	61.1	352.2	0.053
Error	69	29.8	294.5	0.053

Factor was significant at * $P\leq 0.05$, ** $P\leq 0.01$

With the exception of *A. rubrum*, ΦPSII , which represents the efficiency of electron transport through PS II, consistently was greater for saplings in the elevated CO_2 plots (Fig. 3). The response of photochemical quenching (qP) to incident irradiance was identical to ΦPSII (data not shown), indicating that the higher ΦPSII under CO_2 enrichment could be attributed to a higher efficiency of electron transfer through the PS II reaction center rather than a higher proportion of functional reaction centers.

In addition to a significant CO_2 effect, specific leaf area (SLA) varied with the date of measurement and between species (Tables 3,4). The average ambient SLA across species and dates was 285 $\text{cm}^2 \text{g}^{-1}$ and this decreased to 257 $\text{cm}^2 \text{g}^{-1}$ for saplings in elevated CO_2 . Average SLA increased slightly from June (248 $\text{cm}^2 \text{g}^{-1}$) to September (257 $\text{cm}^2 \text{g}^{-1}$) 1997, and was highest in early June 1998 (320 $\text{cm}^2 \text{g}^{-1}$). Measurements were made about 10 days earlier in June 1998 than in June 1997 and the higher SLA in 1998 indicates that leaves had not yet fully developed. *Acer rubrum* and *Ca. glabra* had lower SLA (238 and 235 $\text{cm}^2 \text{g}^{-1}$) than *Ce. canadensis* and *L. styraciflua* (313 and 315 $\text{cm}^2 \text{g}^{-1}$). The CO_2 -induced decrease in SLA explained approximately 77% of the decrease in total chlorophyll expressed per unit leaf mass (6.7 to 5.8 mg g^{-1} , averaged across species and dates, ANOVA, $P<0.01$). There was no effect of the CO_2 treatment on total chlorophyll per unit area (Table 3). Chlorophyll per unit area varied across species; *A. rubrum* and *Ca. glabra* had the highest and lowest values, respectively, and values decreased consistently from June 1997 to September 1997 and June 1998.

The CO_2 treatment caused a small increase in the chlorophyll *a/b* ratio, which increased from an average

Table 3 Total chlorophyll (Chl_{area} , $\mu\text{g cm}^{-2}$; Chl_{mass} , mg g^{-1}), chlorophyll *a/b* ratio (Chl a/b), leaf nitrogen (N ; mg g^{-1}), and specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$) for *A. rubrum* (ACRU), *Ca. glabra* (CAGL), *Ce. canadensis* (CECA), and *L. styraciflua* (LIST) grown under ambient (Amb) or ambient plus 200 $\mu\text{l l}^{-1}$ (Elev) atmospheric CO_2 . Measurements were made in June and September 1997 and June 1998. Each value is the mean of 3–6 independent measurements (± 1 SD)

			Chl_{area}	Chl_{mass}	Chl a/b	N	SLA
ACRU	June 1997	Amb	36.5 (5.2)	8.5 (1.4)	1.9 (0.1)	18.4 (1.2)	231.6 (21.6)
		Elev	30.8 (3.4)	6.6 (1.7)	2.0 (0.1)	16.0 (2.7)	213.7 (45.2)
	Sept 1997	Amb	30.6 (5.6)	7.5 (1.8)	2.0 (0.1)	15.1 (2.3)	244.2 (22.5)
		Elev	27.5 (2.5)	5.7 (0.5)	2.1 (0.1)	12.7 (0.9)	208.0 (10.7)
	June 1998	Amb	28.5 (4.9)	4.0 (0.5)	2.3 (0.1)	19.1 (12.0)	295.9 (20.4)
		Elev	28.0 (3.1)	3.3 (0.7)	2.4 (0.1)	15.5 (8.0)	246.5 (27.8)
CAGL	June 1997	Amb	25.7 (7.6)	6.0 (2.2)	2.1 (0.1)	21.1 (0.9)	231.0 (41.4)
		Elev	30.4 (2.6)	6.0 (1.4)	2.2 (0.1)	19.1 (1.2)	196.1 (35.1)
	Sept 1997	Amb	17.7 (6.7)	4.3 (1.8)	2.2 (0.1)	16.2 (2.1)	240.2 (44.6)
		Elev	20.6 (6.1)	4.1 (1.3)	2.3 (0.2)	15.0 (1.5)	203.9 (32.8)
	June 1998	Amb	28.7 (7.1)	3.8 (0.5)	2.4 (0.3)	17.0 (10.6)	284.4 (37.3)
		Elev	28.9 (4.9)	3.8 (1.0)	2.4 (0.1)	17.1 (8.9)	277.5 (35.0)
CECA	June 1997	Amb	30.5 (7.0)	10.2 (0.6)	1.9 (0.1)	22.6 (3.1)	346.3 (86.4)
		Elev	34.3 (11.5)	9.7 (2.8)	2.0 (0.2)	23.6 (4.3)	285.4 (33.6)
	Sept 1997	Amb	29.7 (6.7)	8.5 (0.6)	2.0 (0.1)	19.4 (1.1)	298.3 (58.2)
		Elev	27.1 (8.6)	6.6 (2.7)	2.1 (0.2)	17.3 (2.9)	233.2 (45.4)
	June 1998	Amb	28.2 (5.6)	4.2 (0.3)	2.2 (0.1)	19.9 (9.1)	323.2 (65.1)
		Elev	28.6 (4.9)	5.6 (1.2)	2.2 (a)	16.3 (3.7)	416.7 (72.9)
LIST	June 1997	Amb	31.4 (1.6)	10.0 (1.4)	1.8 (0.1)	23.2 (3.3)	317.9 (44.9)
		Elev	27.6 (6.2)	6.9 (1.8)	1.9 (0.1)	18.3 (2.2)	242.9 (64.1)
	Sept 1997	Amb	29.3 (2.8)	9.4 (0.5)	1.9 (0.1)	17.9 (1.7)	322.0 (33.7)
		Elev	30.5 (3.5)	8.8 (2.9)	2.0 (a)	17.8 (3.3)	288.4 (91.7)
	June 1998	Amb	24.6 (1.2)	4.5 (0.3)	2.2 (a)	17.2 (8.8)	383.8 (33.1)
		Elev	21.9 (5.0)	3.6 (0.8)	2.3 (0.1)	8.7 (1.9)	351.1 (70.4)

^a Value < 0.05

Table 4 Three-way ANOVA applied to total chlorophyll per unit leaf mass (*Chl/mass*) and per unit leaf area (*Chl/area*), the chlorophyll *a/b* ratio (*Chl a/b*), specific leaf area (*SLA*), and leaf nitrogen content per unit mass (*N/mass*). *Chl a/b* was log transformed prior to the statistical analysis. Values represent the degrees of freedom (*df*) and the mean square

	<i>df</i>	<i>Chl/area</i>	<i>df</i>	<i>Chl/mass</i>	<i>df</i>	<i>Chl a/b</i>	<i>df</i>	<i>SLA</i>	<i>df</i>	<i>N/mass</i>
Species	3	140.7**	3	52.7**	3	0.063**	3	50969**	3	60.8
Treatment	1	4.8	1	17.6*	1	0.023**	1	22026**	1	156.6*
Date	2	179.5**	2	29.1**	2	0.191**	2	47251**	1	164.8**
S×T	3	42.7	3	7.4	3	0.001	3	1331	3	14.8
S×D	6	104.5**	6	9.0**	6	0.005	6	2762	3	33.5
T×D	2	0.3	2	3.9	2	0.002	2	5668	1	14.2
S×T×D	6	19.9	6	4.5	6	0.002	6	4417	3	12.7
Error	81	31.6	81	2.8	81	0.003	81	2237	59	4.9

Factors significant at * $P \leq 0.05$, ** $P \leq 0.01$

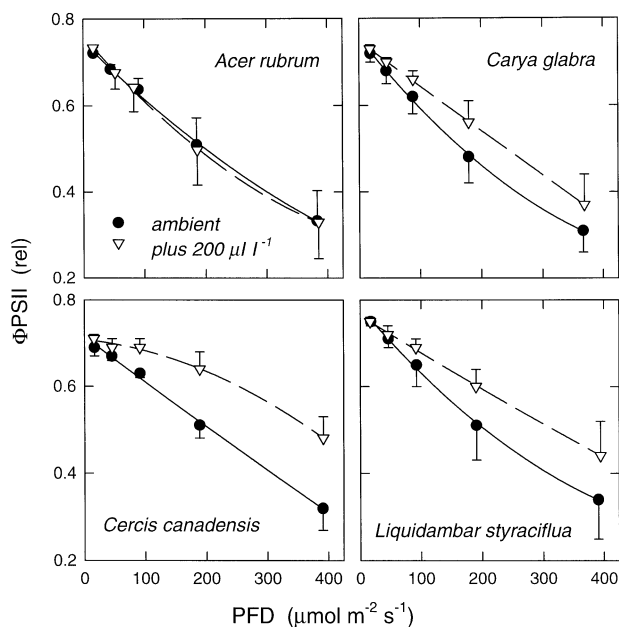


Fig. 3 Quantum yield of electron transport through photosystem II (Φ_{PSII}), calculated from chlorophyll fluorescence, as a function of incident irradiance for understory saplings of *A. rubrum*, *Ca. glabra*, *Ce. canadensis*, and *L. styraciflua* growing under ambient (~350 $\mu\text{l l}^{-1}$; closed circles and solid lines) and ambient plus 200 $\mu\text{l l}^{-1}$ atmospheric CO_2 (open triangles and dashed lines). Each point is an average (± 1 SD) of four independent measurements and the data were fit with a second-order polynomial

of 2.09 (ambient) to 2.16 (elevated) for saplings across species and dates. The chlorophyll *a/b* ratio was greatest for *Ce. canadensis* (2.27) and lowest for *L. styraciflua* (2.03), and varied across dates.

Leaf N expressed per leaf dry mass varied from 8.7 mg g^{-1} for *L. styraciflua* in June 1998 to >23 mg g^{-1} for *Ce. canadensis* in June 1997 (Table 3). Saplings growing in elevated CO_2 had approximately 12% lower leaf N_{mass} (16.3 compared to 18.5 mg g^{-1} , averaged across species and dates) than those growing in the control plots (Table 4). There was no difference between treatment and control saplings when N was expressed per unit leaf area (N_{area} : 66.8, elevated vs. 67.6 $\mu\text{g cm}^{-2}$, ambient, $P=0.93$). When averaged across dates and treatments, *Ce. canadensis* had the highest (76.7 $\mu\text{g cm}^{-2}$) and *L. styraciflua* had the lowest (N_{area} : 56.6 $\mu\text{g cm}^{-2}$). There was a marginal effect of species on N_{mass}

Table 5 Ribulose-1,5-bisphosphate carboxylase (Rubisco, $\mu\text{g cm}^{-2}$), total leaf protein (*Tot protein*, $\mu\text{g cm}^{-2}$), and the ratio of Rubisco/protein (*R/P*) for *A. rubrum* (*ACRU*), *Ca. glabra* (*CAGL*), *Ce. canadensis* (*CECA*) and *L. styraciflua* (*LIST*) growing under ambient (*Amb*) or elevated CO_2 (*Elev*; ambient plus 200 $\mu\text{l l}^{-1}$). Each value is the mean of three or four independent samples (± 1 SD). ANOVA is shown in Table 6

		Rubisco	Tot protein	R/P
ACRU	Amb	29.9 (26.5)	134.9 (56.5)	0.20 (0.09)
	Elev	23.6 (15.9)	165.1 (50.7)	0.18 (0.01)
CAGL	Amb	23.2 (17.9)	82.6 (25.4)	0.26 (0.11)
	Elev	20.9 (10.8)	89.4 (13.0)	0.23 (0.12)
CECA	Amb	42.8 (22.7)	187.6 (36.2)	0.48 (0.27)
	Elev	64.2 (34.8)	157.7 (33.7)	0.39 (0.15)
LIST	Amb	35.5 (11.0)	102.2 (3.5)	0.35 (0.10)
	Elev	54.7 (19.4)	134.0 (40.5)	0.40 (0.02)

Table 6 ANOVA applied to Rubisco, total protein, and the ratio of Rubisco to total protein shown in Table 5. Values are the degrees of freedom (*df*) and the mean square

	<i>df</i>	Rubisco	<i>df</i>	Tot Protein	<i>df</i>	R/P
Species	3	3914**	3	11425**	3	1.03**
Treatment	1	9	1	757	1	0.01
S×T	3	519	3	1657	3	0.05
Error	22	673	24	1336	22	0.14

Factor significant at * $P \leq 0.05$, ** $P \leq 0.01$

($P=0.067$) and a highly significant effect of species on N_{area} ($P < 0.01$). N_{area} decreased from June to September 1997, and was lowest in June 1998 (data not shown). The ratio of total chlorophyll/N was unaffected by growth in elevated CO_2 ($P=0.92$) but varied significantly with date ($P < 0.01$) and species ($P < 0.01$; data not shown). The chlorophyll/N ratio was highest in June 1998 (0.68) and lowest in June 1997 (0.39), and among the different species was lowest for *Ca. glabra* (0.37 compared to >0.48 for the other species).

Rubisco content per unit leaf area varied by more than two-fold among species (Table 5). *Ce. canadensis* and *Ca. glabra* had the highest and lowest content of Rubisco and total soluble protein, respectively. Those species with the highest absolute Rubisco contents also had the greatest proportion of total soluble protein invested in Rubisco. Because of the extremely high vari-

ance for estimates of Rubisco and total protein content, it was not possible to tell if there was a treatment effect on these variables (Table 6).

Discussion

Elevated atmospheric CO₂ caused a sustained increase in light-saturated photosynthesis for juvenile trees in the forest understory (Figs. 1,2). The treatment was ~1.5×ambient CO₂, yet the magnitude of the enhancements observed in this study, in most cases, exceeded the 44% (Gunderson and Wullschlegler 1994) to 54% (Curtis 1996) stimulation reported as means for woody plants grown at twice ambient CO₂. At their maximum, light-saturated photosynthetic rates for *C. canadensis* and *L. styraciflua* more than doubled under elevated CO₂. Environmental stresses can modulate the response to elevated CO₂. Nutrient limitations, for example, can reduce the CO₂-induced stimulation of photosynthesis, whereas low light may increase this response (Curtis 1996). Exposure to the modest increase in CO₂ in this study enhanced light-saturated photosynthetic rates, which may increase carbon acquisition in these saplings despite presumed resource limitations associated with competition in the forest understory.

The stimulation of photosynthesis appeared to be greatest at near-saturating irradiance. In September 1997, the increase in maximum photosynthetic rates consistently were greater than the enhancements in apparent quantum yield. *Ce. canadensis* and *L. styraciflua* were extreme examples; with our low sample size, we could not detect an effect of CO₂ enrichment on the apparent quantum yield of *Ce. canadensis*, but CO₂ enrichment caused a 2.9-fold increase in light-saturated photosynthesis. For *L. styraciflua*, the quantum yield increased by 1.3-fold compared to a 2.3-fold increase for maximum photosynthesis. Measurements in June 1999 indicated that the apparent quantum yield for *L. styraciflua* was 20% higher under elevated CO₂ (Singsaas, unpublished work), and, consistent with Osborne et al. (1997, 1998), this stimulation in quantum yield was caused solely by a reduction in photorespiration. There was no indication that the quantum yield acclimated to elevated CO₂. A strong effect of CO₂ on light-saturated photosynthesis in shade plants also was observed by Kubiske and Pregitzer (1996) and Lovelock et al. (1998). The greater stimulation of the maximum photosynthetic rate relative to the quantum yield may be related to the increasing importance of CO₂ as a limitation to photosynthesis at high irradiance. At low irradiance, net carbon uptake is limited by RuBP regeneration and the activation state of Rubisco, whereas the rate of CO₂ diffusion and Rubisco turnover become more limiting at high irradiance.

There was considerable variation in the magnitude of the photosynthetic response to elevated CO₂ and some portion of the variation may have been related to phenology. By September, deciduous species are approaching the end of their growing season in the Piedmont of North

Carolina, and maximum photosynthetic rates of *A. rubrum* saplings in ambient and elevated plots had declined (Fig. 2). The decrease in A_{\max} in control saplings indicates the initiation of Autumn senescence, which may have been accelerated in 1997 by a late-summer drought. For *A. rubrum*, the CO₂-induced stimulation of photosynthesis decreased from 59% in June to 48% in September. At the other extreme, the magnitude of the photosynthetic stimulation increased from 159% and 74% for *Ce. canadensis* and *L. styraciflua* in June to 190% and 126%, respectively, by September. Although measurements of photosynthesis at only these two dates is insufficient to draw firm conclusions, differences in leaf phenology may influence the photosynthetic response to elevated CO₂.

To maintain a balance between the major component reactions of photosynthesis (RuBP regeneration via electron transport versus the rate of carboxylation versus the rate of utilization of triose phosphates), a number of species “acclimate” to growth in elevated CO₂ by reducing the quantity and activity of Rubisco (Sage 1990; Gunderson and Wullschlegler 1994). In agronomic species, particularly those with high levels of cytoplasmic acid invertase activity, Rubisco content may decrease by >30% when grown at 1000 $\mu\text{l l}^{-1}$ CO₂ (Moore et al. 1997; B. Moore and J. Seeman, unpublished work). Theoretical analyses suggest that in shaded and high-CO₂ environments, “down-regulation” of Rubisco and increasing the rate of electron transport increases the capacity of the most limiting reaction under the new growth conditions (Sage et al. 1989; Sage 1990).

We expected that elevated CO₂, especially under low irradiance, would drive a substantial reinvestment of resources from carboxylation to electron transport. At least for $V_{c_{\max}}$ this was not the case. Across all species and dates there was no significant effect of CO₂ enrichment on $V_{c_{\max}}$ (Table 2). There were, however, substantial increases or decreases in this variable at specific times. For example, $V_{c_{\max}}$ was 31% lower for *Ce. canadensis* in June 1997 and 36% lower for *L. styraciflua* in June 1998 when grown in the elevated CO₂ than in ambient plots. At other times $V_{c_{\max}}$ increased by this amount (e.g., *Ca. glabra* in June 1997). This seasonal variation suggests that the response of $V_{c_{\max}}$ to CO₂ enrichment may vary with seasonal changes in sink strength or nutrient partitioning.

In contrast to $V_{c_{\max}}$, the average J_{\max} for all species and dates increased from 69.8 to 79.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for saplings grown at elevated CO₂ (Tables 1,2), causing a 10% increase in the ratio of $V_{c_{\max}}/J_{\max}$. Our analysis of the A/c_i curves did not incorporate estimates of the potential effect of triose phosphate feedback limitation on photosynthesis. Hence, we may have underestimated the effect of elevated CO₂ on J_{\max} .

The increase in J_{\max} suggests that shaded saplings acclimate to CO₂ enrichment by increasing the capacity to regenerate RuBP. In September 1997, the three species that maintained substantially higher light-saturated photosynthetic rates under elevated CO₂, *Ca. glabra*, *Ce.*

canadensis, and *L. styraciflua*, had higher levels of photochemical quenching (data not shown) and ΦPSII at high irradiances (Fig. 3). For these species, the greater efficiency of electron transport through PS II and possibly lower energy losses to antenna-based quenching mechanisms (e.g., xanthophyll intra-conversion), supported higher rates of electron transport at high irradiances. At sub-saturating irradiances representing the typical diffuse environment in the understory ($\sim 50 \mu\text{mol m}^{-2} \text{s}^{-1}$), photosynthesis is strongly limited by rate of RuBP regeneration, and there were no apparent differences in the energy partitioning or estimated rates of electron transport through photosystem II (Fig. 3). Higher J_{max} and instantaneous ΦPSII at high irradiance suggest that shaded saplings growing under elevated CO_2 may be more efficient at using sunflecks (Pearcy 1990) than those growing under current ambient conditions.

At least during their first full year of exposure, CO_2 enrichment increased the photosynthetic rate of saplings in the forest understory. Acclimation of Rubisco activity, measured as changes in $V_{\text{c,max}}$, was intermittent during the summer with no consistent response to elevated CO_2 . The disproportionate response of light-saturated photosynthesis and enhanced J_{max} , suggests that understory saplings may acclimate to CO_2 by increasing their capacity to regenerate RuBP, thus enhancing their capacity to utilize sunflecks. There was no clear correlation between the shade-tolerance ranking of these species and their photosynthetic response to CO_2 enrichment. *A. rubrum* and *Ce. canadensis*, both shade-tolerant species, had the smallest and largest response, respectively, of light-saturated net photosynthesis to CO_2 . It may be significant that the species showing the greatest stimulation of photosynthesis, *Ce. canadensis* and *L. styraciflua*, also had the highest SLA and greatest investment of leaf protein in Rubisco (Poorter et al. 1996; Poorter and Evans 1998). Environmental and endogenous factors affecting N partitioning (Farage et al. 1998) may partially explain interspecific variation in the photosynthetic response to elevated CO_2 .

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