

Canopy position affects photosynthetic adjustments to long-term elevated CO₂ concentration (FACE) in aging needles in a mature *Pinus taeda* forest

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Summary Few studies have examined the effects of elevated CO₂ concentration ([CO₂]) on the physiology of intact forest canopies, despite the need to understand how leaf-level responses can be aggregated to assess effects on whole-canopy functioning. We examined the long-term effects of elevated [CO₂] (ambient + 200 ppm CO₂) on two age classes of needles in the upper and lower canopy of *Pinus taeda* L. during the second through sixth year of exposure to elevated [CO₂] in free-air (free-air CO₂ enrichment (FACE)) in North Carolina, USA. Strong photosynthetic enhancement in response to elevated [CO₂] (e.g., +60% across age classes and canopy locations) was observed across the years. This stimulation was 33% greater for current-year needles than for 1-year-old needles in the fifth and sixth years of treatment. Although photosynthetic stimulation in response to elevated [CO₂] was maintained through the sixth year of exposure, we found evidence of concurrent down-regulation of Rubisco and electron transport capacity in the upper-canopy sunlit leaves. The lower canopy showed no evidence of down-regulation. The upper canopy down-regulated carboxylation capacity (V_{cmax}) and electron transport capacity (J_{max}) by about 17–20% in 1-year-old needles; however, this response was significant across sampling years only for J_{max} in 1-year-old needles ($P < 0.02$). A reduction in leaf photosynthetic capacity in aging conifer needles at the canopy top could have important consequences for canopy carbon balance and global carbon sinks because 1-year-old sunlit needles contribute a major proportion of the annual carbon balance of these conifers. Our finding of a significant interaction between canopy position and CO₂ treatment on the biochemical capacity for CO₂ assimilation suggests that it is important to take canopy position and needle aging into account because morphologically and physiologically distinct leaves could respond differently to elevated [CO₂].

Keywords: canopy CO₂ exchange, conifer forest, down-regulation, Forest Free-Air CO₂ Enrichment, leaf age, leaf carboxylation rate, leaf nitrogen, net photosynthesis.

Introduction

Plant canopies exhibit strong vertical patterns in photosynthesis, leaf nitrogen (N), leaf mass per area (LMA) and chlorophyll content that together ultimately regulate their ability to exchange CO₂ with the atmosphere (Baldocchi et al. 2002). Photosynthetic capacity varies greatly throughout the canopy, decreasing toward the bottom of the crown (Jurik 1986, Ceulemans and Mousseau 1994, Kellomäki and Wang 1997). Within-canopy variation in photosynthetic capacity is associated with decreases in photosynthetic photon flux (PPF) from the upper- to lower-canopy leaves (Ellsworth and Reich 1993, Kull and Niinemets 1998, Niinemets 1999). Compared with lower-canopy leaves, the higher leaf N, number of chloroplasts and amount of photosynthetic enzymes in the upper canopy confer high photosynthetic capacity for maximal use of high PPF at the canopy top (Ellsworth and Reich 1993, Griffin et al. 2000). Variation in leaf N has been correlated with variation in PPF throughout the tree crown, such that leaf N and photosynthetic capacity are both low in leaves grown at low PPF (Meir et al. 2002).

Because canopies mediate the magnitude of CO₂ uptake from the atmosphere, there is increasing interest in understanding the effects of rising atmospheric CO₂ concentration ([CO₂]) on forest canopy functioning (Saxe et al. 1998). Knowledge about the variations in photosynthetic capacity within tree canopies during the process of canopy closure and in response to changing environmental variables is important for accurate modeling of whole-canopy responses to elevated [CO₂], especially in the context of assessing the terrestrial CO₂ sinks that counteract fossil fuel C releases (Vitousek 1997). Nevertheless, few studies have exposed an intact forest tree canopy to elevated atmospheric [CO₂] for more than 2 years (Tissue et al. 2001, Marek et al. 2002). Even fewer studies have investigated the effects of long-term exposure to elevated [CO₂] on both sunlit and shaded portions of the canopy.

A major effect of elevated atmospheric [CO₂] on ecosystem C storage is mediated through adjustments in the physiological capacity for CO₂ assimilation (Sage 1994, Tissue et al. 1999, Evans and Poorter 2001). As trees are exposed to ele-

vated $[\text{CO}_2]$ for longer times, they may not sustain the initial stimulation of net CO_2 assimilation (Gunderson and Wullschleger 1994, Sage 1994, Curtis 1996, Drake et al. 1997). A reduction in photosynthetic capacity in response to long-term exposure to elevated $[\text{CO}_2]$, often termed down-regulation, has been observed in field-grown trees in several elevated $[\text{CO}_2]$ experiments (Tissue et al. 1999, Griffin et al. 2000, Jach and Ceulemans 2000, Rogers and Ellsworth 2002), although in most cases, a strong photosynthetic enhancement was observed. This reduction in photosynthetic capacity is frequently accompanied by a decrease in leaf N (Drake et al. 1997, Saxe et al. 1998, Stitt and Krapp 1999), or a decrease in the amount or activity of the carboxylating enzyme Rubisco (Rey and Jarvis 1998, Rogers and Ellsworth 2002) or both, because there is a close relationship between leaf carboxylation capacity and leaf N (Medlyn et al. 1999). The possible coordination of photosynthetic and leaf N responses to elevated $[\text{CO}_2]$ within canopies may be important for understanding CO_2 -induced changes in forest C cycle processes with limiting soil N supply (Oren et al. 2001).

It has been suggested that the decrease in leaf N is mediated by accumulated carbohydrates as a result of excess photosynthate production compared with growth demands (Tissue et al. 1999). However, down-regulation has also been related to an increased demand for N as a consequence of accelerated growth in elevated $[\text{CO}_2]$ (Tissue et al. 1993, Stitt and Krapp 1999). A reduction in photosynthetic capacity in elevated $[\text{CO}_2]$ can also be manifested as targeted decreases in Rubisco without changes in leaf N (Rogers and Ellsworth 2002). This protein-specific down-regulation suggests that N is reallocated within the leaf away from Rubisco toward other limiting components (Medlyn et al. 1999, Norby et al. 1999). This appears to be the case for *P. taeda*, a commercially important conifer species, where Rogers and Ellsworth (2002) found a 25% loss in Rubisco in 1-year-old needles but not in current-year needles. In general, the circumstances under which down-regulation occurs in canopies is still unclear. Because virtually all studies focus on upper-canopy leaves, it is also unclear whether the entire canopy responds similarly to elevated $[\text{CO}_2]$.

Given that down-regulation of photosynthesis in elevated $[\text{CO}_2]$ has been documented for pine species (Tissue et al. 1999, Rogers and Ellsworth 2002), we have extended these analyses across several years and to different canopy positions by studying the effects of elevated $[\text{CO}_2]$ on photosynthetic capacity of upper- and lower-crown foliage of *P. taeda*. The specific objectives of our study were to determine: (1) if there is evidence of down-regulation of photosynthesis in *P. taeda* in elevated $[\text{CO}_2]$; (2) if variation in down-regulation occurs among different crown positions in a temperate *P. taeda* canopy under Free-Air CO_2 Enrichment (FACE); and (3) whether the evidence of changes in biochemical parameters of photosynthesis in relation to leaf N are consistent with a nitrogen dilution-protein reduction hypothesis in elevated $[\text{CO}_2]$ or a targeted protein down-regulation hypothesis.

Materials and methods

FACE and site description

This study was conducted in the Blackwood Division of Duke Forest in Orange County, Chapel Hill in North Carolina (35°58' N, 79°05' W) at an elevation of 174 m. The climate in North Carolina is warm and humid with mean annual temperatures of 15.5 °C and mean annual precipitation of 1154 mm. The frost-free season lasts from about late March until mid-October. Measurements were made at different crown positions on 19-year-old (in 2002) *P. taeda* overstory trees. This forest type generally occurs on acidic, nutrient-poor soils in the region. The 32-ha site was planted (1733 trees ha⁻¹) in 1983 following clear-cutting and burning and has been described in detail by Ellsworth (1999).

Within the stand, three plots were fumigated with elevated $[\text{CO}_2]$ (ambient + 200 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) and three control plots were fumigated with ambient $[\text{CO}_2]$. Plots in both treatments were outfitted with blowers and vent pipes that were identically operated except that the air to the control plots was not injected with additional CO_2 . The FACE technique (Hendrey et al. 1999) releases jets of air enriched with CO_2 (elevated $[\text{CO}_2]$ treatment) according to wind speed and direction at a range of heights to maintain a uniform elevated $[\text{CO}_2]$ through the canopy. The trees in the treated plots had been exposed to elevated $[\text{CO}_2]$ for 24 h day⁻¹ since August 1996, except at times when the air temperature was below 5 °C for longer than an hour (Ellsworth 1999, Hendrey et al. 1999). Values of mean daily $[\text{CO}_2]$ of treatment plots are summarized in Table 1. The canopy was accessed for measurements to heights of 16 m from a central tower or by telescopic platform lifts (UL48, Upright, Charlotte, NC) within each plot.

Field gas exchange

We measured CO_2 and H_2O exchange with a portable infrared gas analyzer system (Model LI-6400, Li-Cor, Lincoln, NE). In each plot, two to three trees were measured during early- and mid-summer. Steady-state CO_2 response curve measurements at light-saturation were made in the upper and lower canopy of each tree. For the upper-canopy gas exchange measurements, we used foliage at ~90–95% of total tree height, and for the lower-canopy measurements, foliage at 65–70% of total tree height. Foliage in the upper canopy was completely sunlit, whereas foliage in the lower canopy was fully shaded. At both canopy levels, measurements were made on 1-year-old needles in May and June and on current-year needles in July through September. At least seven CO_2 concentrations were used for the CO_2 response curve measurements, including CO_2 concentrations around the CO_2 compensation point and ambient $[\text{CO}_2]$. Leaf temperatures were controlled in the cuvette to about 28 or 30 °C, depending on the month, with a mean vapor pressure of 1.7 kPa (range 0.7–2.5 kPa) and a saturating PPF of 1800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. At each $[\text{CO}_2]$, photosynthesis, stomatal conductance, leaf internal CO_2 concentration (C_i), leaf and air temperature and vapor pressure deficit were measured after conditions in the chamber stabilized (2–4 min).

Measurements from 1998, 1999, 2001 and 2002 comprised

Table 1. Description of climate parameters recorded at the Duke FACE site over the second through sixth years of exposure to elevated carbon dioxide concentration ([CO₂]) (1998–2002). Mean elevated [CO₂] data were from April 1 until September 30. Values for mean elevated [CO₂] are for $n = 3$ treatment plots only. The overall mean daytime ambient [CO₂] across these years was 374 $\mu\text{mol mol}^{-1}$. Data prior to 1998 are summarized in Ellsworth (1999).

Year	Mean annual temperature (°C)	Mean daily minimum January temperature (°C)	Mean daily maximum July temperature (°C)	Total annual precipitation (mm)	Precipitation during growing season (April–Sept) (mm)	Mean elevated [CO ₂] ($\mu\text{mol mol}^{-1}$)
1998	16.4	1.9 ± 1.1	34.5 ± 0.5	1294	427	574 ± 0.8
1999	15.8	1.3 ± 1.1	34.9 ± 0.9	1364	893	570 ± 1.1
2000	14.7	−1.3 ± 1.1	30.0 ± 0.6	1130	806	570 ± 0.7
2001	15.3	−1.1 ± 0.9	27.7 ± 0.5	884	485	571 ± 0.2
2002	16.0	0.8 ± 1.1	33.9 ± 0.6	1092	378	567 ± 0.5

the second through sixth year of CO₂ exposure for *P. taeda* at this site, with no data available in the fourth growing season. Results were analyzed in two data sets. One set included measurements from all 4 years and was analyzed to show how physiological functions changed over time in elevated [CO₂], extending earlier reports (Ellsworth 1999, Myers et al. 1999, Rogers and Ellsworth 2002). A second intensive data set, which included results for 2001 and 2002 only, gathered during the longest uninterrupted period of exposure to elevated [CO₂], was examined to assess long-term effects of elevated [CO₂]. The second data set was also used to test if the CO₂ response of photosynthetic parameters was similar between canopy positions. Both data sets comprised the highest observed maximum carboxylation capacity (V_{cmax}) values per plot, age class and canopy position ($n = 2$ or 3). It was necessary to use the highest observed V_{cmax} values for each age class and measurement year to minimize potentially confounding effects of drought-induced effects on photosynthetic capacity, given that a severe drought occurred in 2002 (see Table 1 and Results). This effectively eliminated measurements made when stomatal closure resulted in uncertainties in C_i calculations that caused errors in fitting photosynthetic CO₂ response curve data.

Needles were removed from the tree branch after each CO₂ response curve and cold-stored until placed in a drying oven. The diameter of the portion of each needle inside the leaf chamber was measured with digital calipers to calculate the area based on a geometric model (Niinemets et al. 2001). Needles were then dried at 70 °C. Homogenized subsamples of 6–8 mg of dried and ground needle tissue were analyzed for carbon and nitrogen concentration with an NA-1500 elemental analyzer (Carlo-Erba, Milan, Italy) after combustion. We present gas exchange parameters and nitrogen per area (N_{area}) on a needle surface area basis.

Parameter estimation and statistical analyses

Data from the photosynthesis–CO₂ response curve measurements were used to calculate the maximum in vivo carboxylation rate (V_{cmax}) and maximum electron transport rate for ribulose biphosphate regeneration (J_{max}) by fitting the equations of Farquhar et al. (1980) as described by Wullschlegler

(1993) and Ellsworth et al. (unpublished data), and using the temperature parameters in Bernacchi et al. (2001). As a result of the severe drought in 2002 (Table 1), data with minimum stomatal conductances (g_s) below 25 $\text{mmol m}^{-2} \text{s}^{-1}$ were omitted because of uncertainties in C_i calculations. Mean g_s in other years was about 35% higher than in 2002.

All statistical analyses were performed with SPSS v.11.5 (SPSS, Chicago, IL). The number of replicate rings per CO₂ treatment was three. Main effects and interactions were considered significant at $P < 0.10$ because of the low statistical power of the small number of replicates. A 3-way ANOVA with repeated measures including treatment, canopy position and age class as factors was done for both the 4-year data set for the years 1998 through 2002 and the 2-year data set with more intensive measurements in 2001 and 2002. In some cases, missing data necessitated the use of a reduced 3-way ANOVA model without canopy position interactions in the 4-year datasets, but not in the 2-year datasets. Where significant effects ($P < 0.10$) were detected in ANOVA for major photosynthetic parameters like net photosynthesis rate measured at light saturation and mean growth CO₂ concentration (A_{net}), maximal carboxylation rate (V_{cmax}), or J_{max} , within-year contrasts between CO₂ treatments were tested with a one-tailed Student's *t*-test.

We used regression analyses to examine relationships between A_{net} , V_{cmax} and J_{max} as a function of leaf N. All linear regressions were done on an area basis, to distinguish the effects of CO₂ treatments and needle age classes. Differences in slopes and intercepts between treatments were tested across age classes and for each age class separately.

Results

Gas exchange variables

Enhancement of light-saturated photosynthesis (A_{net}) by elevated [CO₂] was generally significant and maintained over the 4 years from the second to sixth year of CO₂ fumigation (Figures 1 and 2), with no data available for 2000. The mean stimulation in A_{net} over these years and across canopy positions was $60 \pm 11\%$ —an absolute enhancement of about 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figures 1 and 2). There was significant inter-annual variability

ity in photosynthesis, with A_{net} declining over the years, particularly in 1-year-old needles (Figure 1). Over the 4 years of measurements of the trees in FACE, there was a significant year effect for A_{net} ($P = 0.002$) and V_{cmax} ($P = 0.044$). There were generally no significant year interactions with treatment or canopy position for the variables examined ($P > 0.10$, data not shown), except for a significant year \times treatment interaction for A_{net} ($P = 0.014$). Severe drought may have contributed to this interaction because the 2002 growing season had the lowest total annual precipitation of the 5 years summarized in Table 1. In 2002, growing season rainfall was 40% lower than the 30-year mean of 622 mm (Table 1).

Over the 4 years of measurements, there were significant overall canopy position effects on the photosynthetic variables A_{net} ($P = 0.001$), V_{cmax} ($P = 0.002$) and J_{max} ($P = 0.017$), all based on the reduced 3-way repeated-measures ANOVA. Needle N_{area} declined by 35% from the upper to lower canopy ($P = 0.001$). Canopy position also had a large effect on shoot growth, with an upper-canopy shoot elongation of 52 cm in the third year of CO_2 exposure compared with a lower-canopy shoot growth of only 9 cm (D.S. Ellsworth, unpublished data).

We focused more detailed analysis, particularly for CO_2 treatment and interactive effects, on the 2001–2002 period because these years reflect the longest uninterrupted exposure to elevated $[\text{CO}_2]$ to date. There were large differences in photosynthetic parameters (e.g., A_{net} , V_{cmax} , J_{max} , N_{area}) between the upper and lower canopy (Figure 1, Table 2). The maximal carboxylation rate, was 25% lower in the lower canopy than in the upper canopy ($P = 0.002$). Similar reductions of 35 and 28% from upper to lower canopy were observed in N_{area} ($P < 0.001$) and LMA ($P < 0.001$) respectively, in a 3-way ANOVA. A significant enhancement effect of elevated $[\text{CO}_2]$ on A_{net} ($P = 0.001$) was found together with significant differences in A_{net} between the upper and lower canopy ($P = 0.003$). The magnitude of enhancement by elevated $[\text{CO}_2]$ varied from 12 to 51% in the upper canopy and from 35 to 120% in the lower canopy, although there was no interaction effect between CO_2 treatment and canopy position for A_{net} (Table 2). However, there was a significant interaction between CO_2 treatment and age class for A_{net} ($P = 0.021$). Enhancement of A_{net} by elevated $[\text{CO}_2]$ was about 30% less in 1-year-old needles than in current-year needles (Table 2).

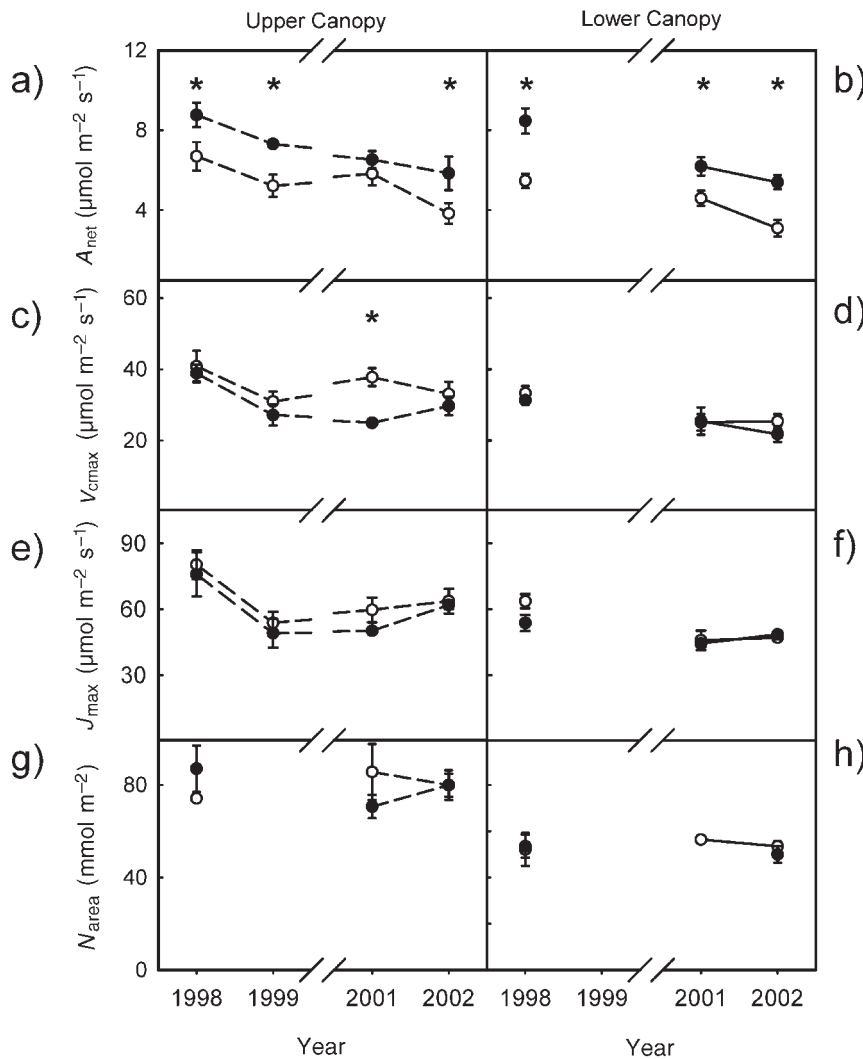


Figure 1. Interannual variability in the effects of elevated carbon dioxide concentration ($[\text{CO}_2]$) and canopy position on 1-year-old foliage of *Pinus taeda*. Parameters shown are: (a, b) net photosynthesis (A_{net}), (c, d) leaf maximum carboxylation capacity (V_{cmax}), (e, f) leaf maximum electron transport capacity (J_{max}), and (g, h) leaf nitrogen concentration per area (N_{area}) in the upper canopy (panels a, c, e) and the lower canopy (panels b, d, f). Within a given date and canopy position, an asterisk denotes significant ($P < 0.05$) treatment differences for each parameter. Symbols: \circ = ambient $[\text{CO}_2]$; and \bullet = elevated $[\text{CO}_2]$.

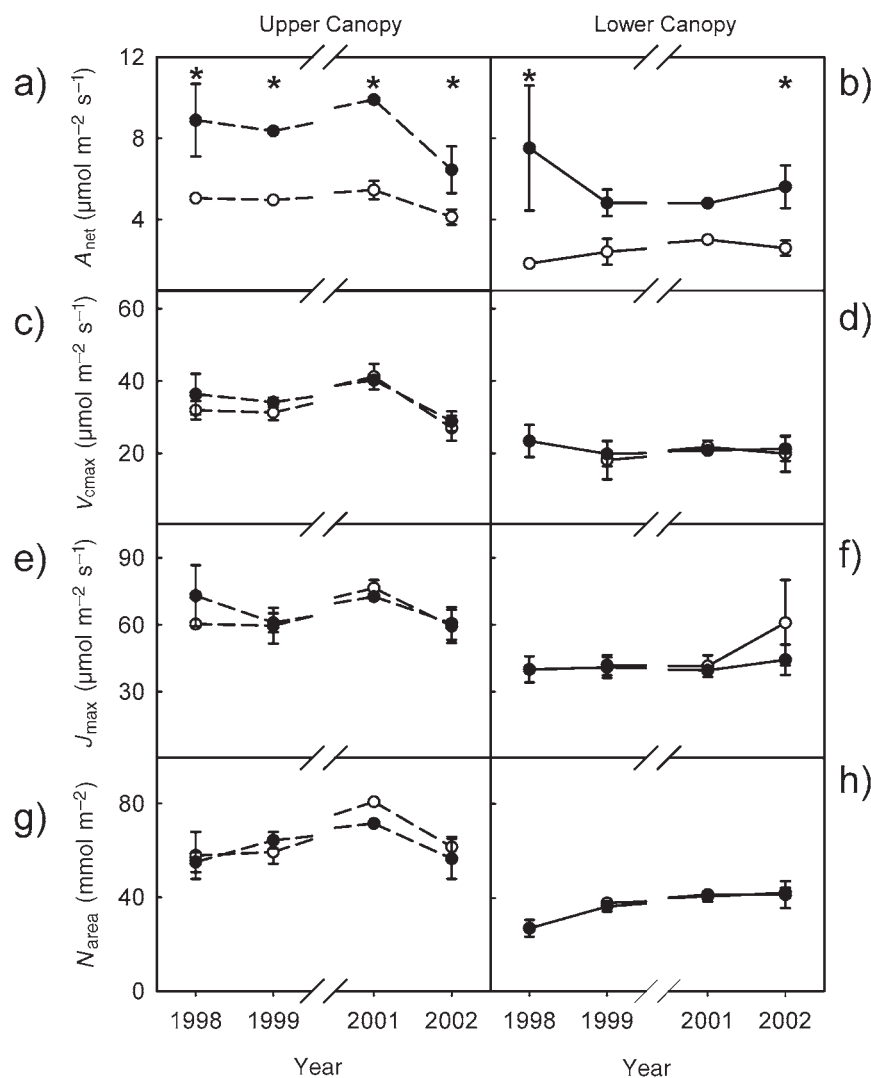


Figure 2. Interannual variability in the effects of elevated carbon dioxide concentration ([CO₂]) on gas exchange parameters and leaf nitrogen concentration per area (N_{area}) in current-year foliage of *Pinus taeda* by canopy position. Data are shown for the upper canopy (panels a, c, e) and the lower canopy (panels b, d, f) as in Figure 1. An asterisk denotes significant ($P < 0.05$) treatment differences for each parameter. Abbreviations: A_{net} = net photosynthesis; V_{cmax} = leaf maximum carboxylation capacity; and J_{max} = leaf maximum electron transport capacity. Symbols: \circ = ambient [CO₂]; and \bullet = elevated [CO₂].

In an attempt to explain the differences in photosynthetic enhancement with needle age and age \times canopy position, we analyzed the biochemical parameters underlying A_{net} (i.e., V_{cmax} and J_{max}). Although there was no significant effect of elevated [CO₂] across age classes ($P > 0.10$) for V_{cmax} , there was a significant reduction in J_{max} in elevated [CO₂] (11% reduction, $P = 0.019$). There were also marginally significant ($P = 0.10$) or significant ($P = 0.016$) 3-way interactions between CO₂ treatment \times canopy position \times needle age for A_{net} and J_{max} , respectively (Table 2). A possible component of this interaction may be related to the effects of elevated [CO₂] on the biochemical determinants of photosynthetic capacity. For V_{cmax} and J_{max} , there was a 17–20% reduction in 1-year-old needles in the upper canopy in elevated [CO₂] (Table 2). In a 2-way ANOVA with 1-year-old needles alone, there was a marginally significant CO₂ treatment \times canopy position interaction ($P = 0.06$), arising from the larger effect of elevated [CO₂] on V_{cmax} in the upper canopy (–20%, Figure 1) than in the lower canopy (–3%, Table 2). This effect was limited to 1-year-old needles and was not significant for current-year needles ($P > 0.10$, Ta-

ble 2), or for both age classes together (3-way ANOVA, $P > 0.10$, Table 2). There were similar findings for J_{max} as for V_{cmax} , although in this case, down-regulation of J_{max} was significant in current-year needles as well as in 1-year-old upper-canopy needles in elevated [CO₂] (CO₂ treatment effect in Table 2).

Across the years 2001 and 2002, the 3-way ANOVA indicated a significant age class effect ($P = 0.016$), with a 20% increase in N_{area} (Table 2) and a 28% increase in LMA (data not shown) in 1-year-old needles compared with current-year needles across treatments and canopy positions. In general, current-year needles showed no significant CO₂ treatment differences for the biochemical parameters V_{cmax} , J_{max} , N_{area} and LMA in the years 2001 and 2002 ($P > 0.10$ for all), though such differences were significant for A_{net} ($P = 0.006$).

Regression analysis of relationships as a function of nitrogen

Elevated [CO₂] altered some but not all photosynthetic–leaf N relationships. Because there was a strong age class effect on leaf N_{area} , with significantly more N_{area} in old needles (21% more N_{area} in 1-year-old needles than in current-year needles

Table 2. Analysis of variance (ANOVA) for photosynthetic parameters of *Pinus taeda* after 5 to 6 years of exposure to elevated carbon dioxide concentration ([CO₂]) (2001 and 2002 data combined). Data are means with standard error (SE), mean square (MS) error, and *F*-statistics from the CO₂ treatments at two canopy positions and for two age classes in a repeated-measures analysis. Effects by foliage age class are: canopy position effect, the proportional effect of canopy position as % increase from lower to upper canopy, and CO₂ treatment effect, the % change between ambient and elevated [CO₂] treatments for upper- and lower-canopy foliage, respectively. Positive effects are variables that increased from lower to upper canopy or ambient to elevated [CO₂]. Year was not significant ($P > 0.10$) for any parameter except A_{net} ($P = 0.002$). Abbreviations: V_{cmax} = maximum carboxylation rate of Rubisco ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); J_{max} = maximum electron transport ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); and N_{area} = amount of leaf nitrogen per area (mmol N m^{-2}). Symbols for statistical tests are: *** = $P < 0.005$; ** = $P < 0.025$; * = $P < 0.05$; + = $P < 0.10$; and ns = $P > 0.10$.

Parameter	Mean (\pm SE)	MS error	ANOVA effects (<i>F</i> -statistics)			% Canopy position effect						% CO ₂ treatment effect	
			CO ₂ treatment	Canopy position	Age class	CO ₂ × position	CO ₂ × age class	Age × position	CO ₂ × position × age	Current-year	1-year-old	Current-year upper, lower	1-year-old upper, lower
A_{net}	5.0 (\pm 0.3)	1.47	41.86 ***	14.89 ***	ns	ns	7.20 **	5.14 *	3.23 +	+44	+14	+63, +89	+32, +55
V_{cmax}	27.6 (\pm 1.1)	27.75	ns	18.00 ***	ns	ns	ns	ns	ns	+58	+21	-5, 0	-20, -3
J_{max}	55.3 (\pm 2.0)	40.98	7.79 **	31.33 ***	10.50 **	ns	ns	ns	8.30 **	+47	+29	-2, -16	-17, 0
N_{area}	61.2 (\pm 2.6)	1.43	ns	30.09 ***	8.81 **	ns	ns	ns	-	+61	+42	-5, -3	-20, 0

across treatments, $P = 0.016$, Table 2), we analyzed photosynthetic–leaf N relationships both across and within needle age classes (Table 3). The $A_{\text{net}}-N_{\text{area}}$ relationship did not show significant differences in slope between CO₂ treatments ($P > 0.10$) across age classes, but slopes were significantly different between CO₂ treatments in the current-year needle age class only. Current-year needles showed a steeper slope in the elevated [CO₂] treatment ($P = 0.025$) than in the ambient [CO₂] treatment (Table 3, Figure 3a), suggesting a higher nitrogen-use efficiency in elevated [CO₂]. In 1-year-old needles, there was no overall relationship between A_{net} and N_{area} nor was there a statistical relationship either in elevated [CO₂] or in ambient [CO₂] (Figure 3b).

Across age classes, there was a significant overall positive linear relationship between V_{cmax} and N_{area} ($P < 0.001$, Table 3 and Figure 4a). Additionally, although not statistically significant, there was a trend toward different slopes for the $V_{\text{cmax}}-N_{\text{area}}$ relationship between CO₂ treatments ($P = 0.114$ across age classes) (Table 3). The trend was indicated by a shallower slope and poorer fit across both age classes for the elevated [CO₂] treatment (slope = 0.189, $R^2 = 0.27$, $P = 0.03$) than for ambient [CO₂] (slope = 0.352, $R^2 = 0.63$, $P < 0.001$). In 1-year-old needles in particular, there was no significant relationship between V_{cmax} and N_{area} in needles in the elevated [CO₂] treatment ($P = 0.24$, Figure 4a), although the relationship was significant for all other age class × treatment combinations. For J_{max} , there was a significant overall relationship with N_{area} ($R^2 = 0.41$, $P < 0.001$, Figure 4b) but no differences in slopes between treatments or age classes (Table 3). As for V_{cmax} , there was no significant effect of elevated [CO₂] on the corresponding relationship between J_{max} and N_{area} either among or within age classes (Table 3, Figure 4b).

Discussion

Canopy light interception is maximized and photosynthesis is optimized by a pattern of vertical differentiation in leaf characteristics concurrent with the demand for resources (Kull and Kruijt 1999), resulting in higher metabolism in upper-canopy leaves than in lower-canopy leaves (Figure 1). A major issue is whether this pattern will be maintained in canopies subjected to long-term exposure to elevated [CO₂] or whether long-term exposure to elevated [CO₂] will alter canopy gradients in N and photosynthetic activity. Recent reviews and meta-analyses show that photosynthetic stimulation in elevated [CO₂] in sun leaves is maintained over the long-term, with stimulation ranging from 44 to 66% for a 50–100% increase in [CO₂] (Gunderson and Wullschlegler 1994, Saxe et al. 1998, Norby et al. 1999). We found that significant photosynthetic stimulation was maintained in the upper canopy as well as in the lower canopy in response to the elevated CO₂ treatment (mean A_{net} enhancement of 51–69% across years; Figures 1a, 1b, 2a and 2b). Canopy closure occurred around 1999–2000 (Ellsworth, unpublished data), which caused A_{net} ($P = 0.049$) and V_{cmax} ($P = 0.028$) to decrease in the lower canopy (Figures 1b and 2b), but similarly with respect to both CO₂ treatments. In the upper canopy, photosynthetic enhancement was maintained

Table 3. Summary of regression statistics of net photosynthesis (A_{net}), maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) across canopy positions as a function of leaf nitrogen concentration per area (N_{area}) in *Pinus taeda* after exposure to elevated carbon dioxide concentration for 5 to 6 years. Regression statistics are shown for both needle age classes combined, and for 1-year-old and current-year foliage age classes separately. The value of P_{slope} indicates the significance of differences in slope between the CO₂ treatments.

Relationship	Age class	Across treatments				P_{slope}
		Slope	Intercept	r^2	P -value	
$A_{\text{net}}-N_{\text{area}}$	Both ages	0.046	2.20	0.17	0.012	0.221
	Current-year	0.074	0.98	0.28	0.025	0.025
	1-year-old	0.029	3.15	0.08	0.237	0.630
$V_{\text{cmax}}-N_{\text{area}}$	Both ages	0.29	9.49	0.53	< 0.001	0.114
	Current-year	0.43	3.34	0.66	< 0.001	0.883
	1-year-old	0.22	13.2	0.40	0.003	0.153
$J_{\text{max}}-N_{\text{area}}$	Both ages	0.50	24.5	0.41	< 0.001	0.609
	Current-year	0.74	16.3	0.59	< 0.001	0.558
	1-year-old	0.49	21.5	0.47	0.001	0.167

for all years (Figure 1a), except in 2001, the year that significant down-regulation was observed ($P = 0.05$, Figure 1c). Our results show that the duration of photosynthetic enhancement

throughout the canopies of these field-grown *P. taeda* trees extends to 6 years of CO₂ enhancement, which at present is one third of the stand's total age.

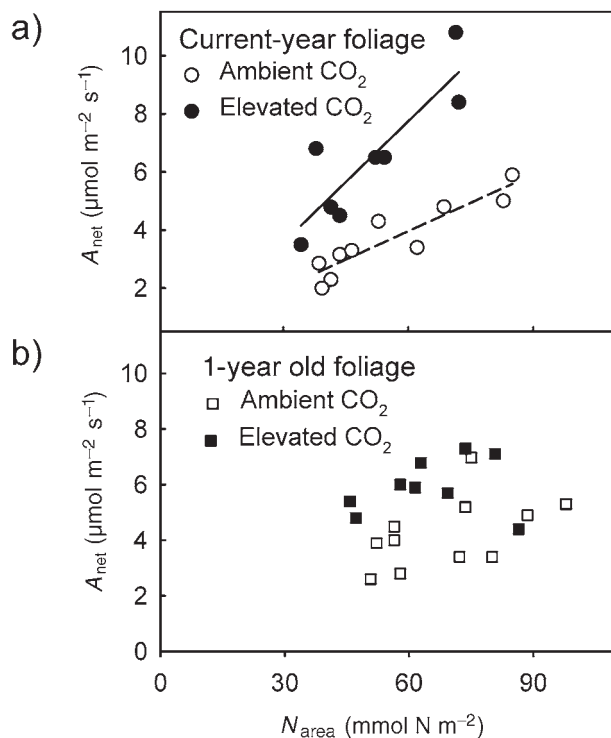


Figure 3. (a) Relationships between net photosynthesis (A_{net}) and leaf N concentration on an area basis (N_{area}) for current-year needles across canopy positions in ambient (○) and elevated carbon dioxide concentration ([CO₂]) (●). The dashed line denotes the ambient treatment ($R^2 = 0.84$, $P < 0.001$) and the solid line denotes the elevated [CO₂] treatment ($R^2 = 0.75$, $P < 0.001$). The regression equation for the ambient [CO₂] treatment is $A_{\text{net}} = 0.065N_{\text{area}} + 0.049$ and for the elevated treatment, $A_{\text{net}} = 0.139N_{\text{area}} - 0.613$. (b) There was no significant relationship between A_{net} and leaf N_{area} for 1-year-old needles across canopy positions. Data are shown for the ambient (□) and elevated [CO₂] treatments (■).

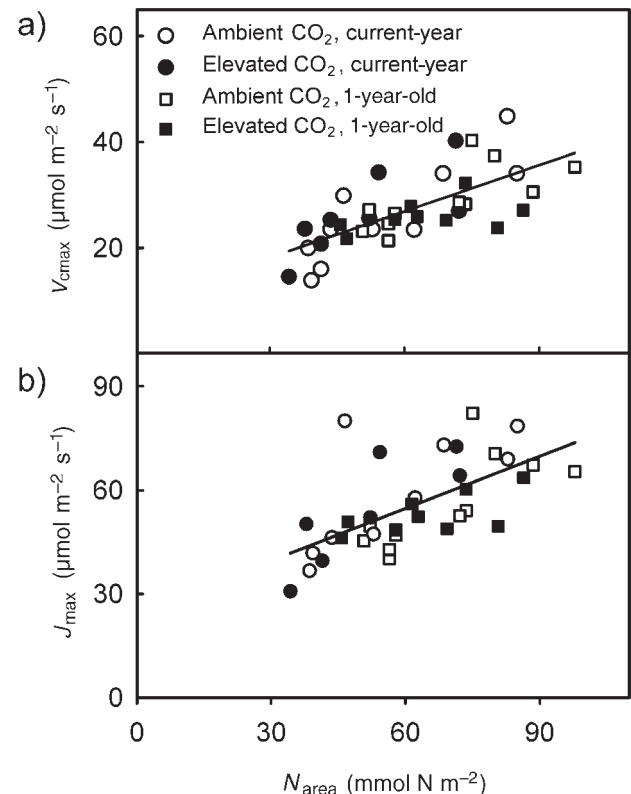


Figure 4. Regression analysis for (a) $V_{\text{cmax}}-N_{\text{area}}$ and (b) $J_{\text{max}}-N_{\text{area}}$ relationships, distinguishing between treatment and age class. Filled symbols (●, ■) represent the elevated [CO₂] treatment and open symbols (○, □) represent the ambient [CO₂] treatment. Squares denote 1-year-old foliage and circles denote current-year foliage. Regression statistics of data for foliage in both CO₂ treatments and foliage age classes (Table 3) are: $V_{\text{cmax}} = 0.29N_{\text{area}} + 9.5$ ($R^2 = 0.53$, $P < 0.001$). Regression statistics for the $J_{\text{max}}-N_{\text{area}}$ relationship in (b) are $J_{\text{max}} = 0.50N_{\text{area}} + 24.5$ ($R^2 = 0.41$, $P < 0.001$).

Many studies have shown that needle age in conifers influences the photosynthetic response to elevated $[\text{CO}_2]$ (Medlyn et al. 1999, Griffin et al. 2000). We found that the stimulation attributable to elevated $[\text{CO}_2]$ was maintained in both needle age classes, although the enhancement in 1-year-old foliage was 30% less than in current-year foliage (Figures 1 and 2). Although we cannot unequivocally infer that such effects are due to leaf aging in our study, because the measurements between different age classes were made at different times during the growing season, similar differences in photosynthetic enhancement were observed by Rogers and Ellsworth (2002) for current-year and 1-year-old needles measured concurrently. Differences in photosynthetic enhancement effects between needle age classes have been noted in several studies and attributed to differences in the biochemical efficiency of photosynthesis with needle aging (Tissue et al. 2001, Luomala et al. 2003).

The occurrence of photosynthetic down-regulation in response to elevated $[\text{CO}_2]$ is likely a major factor responsible for the CO_2 treatment \times needle age effects on A_{net} that we observed in the fifth and sixth years of exposure to elevated $[\text{CO}_2]$. At the same CO_2 supply rate to the sites of carboxylation inside leaves, photosynthetic down-regulation caused reductions in the stimulation of A_{net} by elevated $[\text{CO}_2]$. We found mixed evidence for photosynthetic down-regulation, with a significant overall CO_2 effect for J_{max} in the fifth and sixth years of exposure to elevated $[\text{CO}_2]$ ($P = 0.020$), and a marginally significant CO_2 treatment \times canopy position effect for V_{cmax} in 1-year-old needles ($P = 0.06$, Figure 1c). Down-regulation as a result of long-term exposure to elevated $[\text{CO}_2]$ has been documented for upper-canopy sun leaves, especially in conifers (Tissue et al. 1999, Griffin et al. 2000, Luomala et al. 2003). It is unclear whether such changes in photosynthetic proteins are truly long-term, and our data for the fifth and sixth seasons of CO_2 exposure are also equivocal in this respect (Figures 1 and 2).

We observed that current-year needles in both canopy positions showed large increases in A_{net} in response to elevated $[\text{CO}_2]$ with no evidence of photosynthetic down-regulation, either in V_{cmax} or N_{area} , which is consistent with other studies (Jach and Ceulemans 2000). Similar results have been found for upper-canopy needles of several other conifer species (Medlyn et al. 1999, Tissue et al. 1999, Griffin et al. 2000, Luomala et al. 2003). An often cited hypothesis is that down-regulation in aging conifer needles is a consequence of reduced local growth and utilization sinks for carbohydrates (Stitt and Krapp 1999, Tissue et al. 1999, Griffin et al. 2000, Jach and Ceulemans 2000). It is unclear if predictions from this hypothesis can be extended to the canopy because shoot growth in the lower canopy was 83% less than in the upper canopy of *P. taeda*, yet there was no evidence of down-regulation in the lower canopy associated with this reduced sink strength. Alternately, large nitrogen demands are apparent in the upper canopy of *P. taeda* relative to canopy biomass (Zhang and Allen 1996), and such demands may be accentuated in elevated $[\text{CO}_2]$ (Figure 1; also Finzi et al. 2002). Our evidence indicates preferential photosynthetic down-regula-

tion in the upper canopy rather than in the lower canopy (Figure 1), shown as $\approx 20\%$ reductions in V_{cmax} and J_{max} in the elevated $[\text{CO}_2]$ treatment compared with the ambient $[\text{CO}_2]$ treatment (Table 2).

Given the lack of a statistical CO_2 effect on N_{area} (Table 2), enhancement in A_{net} relative to leaf N in elevated $[\text{CO}_2]$ implies significant increases in nitrogen-use efficiency (Figure 2, Table 3) that may have important implications for the strength of coupling between carbon and nitrogen pools within plant canopies in future higher atmospheric CO_2 concentrations. Theory suggests that leaf aging may be closely tied to canopy N status (Field 1983). Because increased tree growth in elevated $[\text{CO}_2]$ can change plant demand for N as a result of increased photosynthetic nutrient-use efficiency (Norby et al. 1999), there has been speculation that the amount of stored N reserves influence the source-sink relationship for carbon (Luxmoore et al. 1995). If that is the case, the sink for N in new needles may prevent trees from sustaining initial photosynthetic stimulation in 1-year-old needles in elevated $[\text{CO}_2]$ because of reallocation demands for N, which are large in the upper canopy of *P. taeda* (Zhang and Allen 1996). Some experimental evidence suggests that large increases in the N pool available to conifer trees affects the magnitude of growth enhancement (Oren et al. 2001, Maier et al. 2002), although rigorous tests of physiological mechanisms for this response are lacking.

For *P. taeda*, possible age-related differences in the effect of elevated $[\text{CO}_2]$ on photosynthetic or carboxylation capacity were suggested by age differences in the slopes of the relationship between V_{cmax} and N_{area} . A weak trend toward a shallower slope of the $V_{\text{cmax}}-N_{\text{area}}$ relationship in elevated $[\text{CO}_2]$ (Table 3) suggests down-regulation of carboxylation capacity. Although we have no specific evidence that N was reallocated away from Rubisco in upper-canopy leaves in the elevated $[\text{CO}_2]$ treatment, given the high requirement for N in growing needles in the canopy during the summer (e.g., about 45% of the total N stock of 80–100 kg ha⁻¹ at this site; Finzi et al. 2002), we suggest that both internal repartitioning within this age class as well as reallocation to young needles occurred. However, this reallocation of N may not be specific only to Rubisco because no significant treatment differences were found in the $J_{\text{max}}:V_{\text{cmax}}$ ratio to indicate N reallocation within the photosynthetic apparatus. Overall, coordinated adjustments in V_{cmax} and J_{max} in elevated $[\text{CO}_2]$ appear to be common, even among coniferous species (Medlyn et al. 1999, Jach and Ceulemans 2000). Griffin et al. (2000) found photosynthetic down-regulation, indicated by a reduction in leaf N with significant decreases in V_{cmax} and J_{max} , in *Pinus radiata* D. Don grown in open-top chambers in elevated $[\text{CO}_2]$ for 4 years. Ultimately, our evidence argues against protein-specific down-regulation, contrary to Rogers and Ellsworth (2002). Given the mixed evidence of down-regulation in the *P. taeda* canopy in our study, we cannot conclusively resolve the nature of the CO_2 effects on biochemical parameters of photosynthesis in relation to leaf N.

This is one of the first canopy-level studies to demonstrate that the magnitude of the down-regulation response elicited by long-term elevated $[\text{CO}_2]$ is dependent on location within the

canopy (Table 2). Sun-exposed foliage in the upper canopy showed more down-regulation in response to elevated [CO₂] than shaded foliage in the lower canopy (Figures 1 and 2). In contrast, greater down-regulation was observed in the lower canopy than in the upper canopy of *Populus tremuloides* Michx. (Kubiske et al. 2002), although the difference between canopy positions became less apparent in the second year of the experiment in that study. We found significant reductions in J_{\max} and similar trends in V_{\max} after 5 to 6 years of growth in elevated [CO₂], indicating general biochemical adjustments to long-term exposure to elevated [CO₂] and different responses depending on canopy position (Figure 1, Table 2).

Taken together, this evidence supports previous work showing photosynthetic enhancement in response to elevated [CO₂], but also demonstrates down-regulation with needle aging in conifers through significant and consistent decreases in V_{\max} and J_{\max} (Figure 1, Table 2), although we have localized this phenomenon in the upper canopy only. Because the upper canopy is the most productive part of the crown on a leaf-area basis, our study suggests that the impact of needle aging in the upper canopy is associated with a demand for resources from photosynthetic components of old needles, resulting in a reduced photosynthetic capacity. Because down-regulation of photosynthetic capacity is associated with a change in the biochemical determinants of photosynthesis, analysis of photosynthetic-N relations could be a useful tool for modelers seeking to understand the effects of down-regulation on the magnitude of the global carbon sink for atmospheric CO₂. However, because we only examined two heights at endpoints in the canopy crown, caution must be taken when applying our findings to the whole canopy. Future studies should identify the specific differences in photosynthetic characteristics throughout the canopy in elevated CO₂ to determine the nature of the elevated CO₂ by canopy position effect.

In conclusion, substantial photosynthetic stimulation (e.g., +60% across age classes) was maintained through the sixth year of exposure to elevated [CO₂] in FACE. However, the increase was greater for current-year needles than for 1-year-old needles of pine. We obtained some evidence of moderate photosynthetic down-regulation, with a 20% decrease in V_{\max} and a 17% reduction in J_{\max} in 1-year-old needles across the fifth to sixth year of CO₂ exposure in FACE. This evidence is based both on analysis of variance of V_{\max} and J_{\max} as well as a trend toward changes in the V_{\max} -N relationship with leaf aging for *P. taeda* at a forest FACE site in North Carolina. Marginally significant interactive effects between CO₂ treatments and canopy vertical position were found for J_{\max} ($P = 0.02$), suggesting down-regulation in 1-year-old needles in the upper canopy, but not in any needle age class in the lower canopy. Our results are largely consistent with several previous studies in pines (Tissue et al. 2001, Rogers and Ellsworth 2002). Our evidence of down-regulation in 1-year-old needles in the upper canopy is not necessarily consistent with predictions based on the carbohydrate source-sink feedback hypothesis (Stitt 1991), suggesting that the mechanism underlying the effects of elevated [CO₂] on photosynthetic capacity in aging needles

in conifer species is not fully elucidated. Given that a major proportion of the annual carbon balance of *P. taeda* is contributed by 1-year-old needles (Ellsworth 2000) and the highest CO₂ assimilation rates occur at the top of the canopy, photosynthetic down-regulation could strongly affect the magnitude of net canopy CO₂ exchange in this species (Schäfer et al. 2003). The nature of age-related down-regulation in coniferous trees in elevated atmospheric [CO₂] has important implications for canopy carbon balance and the degree of coupling in canopy carbon-nitrogen relationships.

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