

Photosynthesis, light and nitrogen relationships in a young deciduous forest canopy under open-air CO₂ enrichment

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ABSTRACT

Leaf photosynthesis (P_s), nitrogen (N) and light environment were measured on *Populus tremuloides* trees in a developing canopy under free-air CO₂ enrichment in Wisconsin, USA. After 2 years of growth, the trees averaged 1.5 and 1.6 m tall under ambient and elevated CO₂, respectively, at the beginning of the study period in 1999. They grew to 2.6 and 2.9 m, respectively, by the end of the 1999 growing season. Daily integrated photon flux from cloud-free days (PPFD_{day,sat}) around the lowermost branches was 16.8 ± 0.8 and $8.7 \pm 0.2\%$ of values at the top for the ambient and elevated CO₂ canopies, respectively. Elevated CO₂ significantly decreased leaf N on a mass, but not on an area, basis. N per unit leaf area was related linearly to PPFD_{day,sat} throughout the canopies, and elevated CO₂ did not affect that relationship. Leaf P_s light-response curves responded differently to elevated CO₂, depending upon canopy position. Elevated CO₂ increased $P_{s,sat}$ only in the upper (unshaded) canopy, whereas characteristics that would favour photosynthesis in shade were unaffected by elevated CO₂. Consequently, estimated daily integrated P_s on cloud-free days ($P_{s,day,sat}$) was stimulated by elevated CO₂ only in the upper canopy. $P_{s,day,sat}$ of the lowermost branches was actually lower with elevated CO₂ because of the darker light environment. The lack of CO₂ stimulation at the mid- and lower canopy was probably related to significant down-regulation of photosynthetic capacity; there was no down-regulation of P_s in the upper canopy. The relationship between $P_{s,day,sat}$ and leaf N indicated that N was not optimally allocated within the canopy in a manner that would maximize whole-canopy P_s or photosynthetic N use efficiency. Elevated CO₂ had no effect on the optimization of canopy N allocation.

Key-words: A/C; analysis; global change; N allocation; Rubisco; trembling aspen.

INTRODUCTION

Determining the extent to which photosynthesis (P_s) of tree canopies may respond to atmospheric CO₂ enrichment is critical to predicting the role of temperate forests as sinks in the global carbon cycle. In recent years, we have learned

a great deal about photosynthetic responses to atmospheric CO₂ increase at the leaf level, but a better understanding of canopy-level responses is needed. Mechanistic models of forest carbon uptake are a reasonable opportunity to make canopy-level predictions of P_s because such models are based upon the processes underlying P_s at the leaf level, e.g. the interrelationships between P_s , leaf nitrogen (N) and light. Mechanistic predictions of canopy-level P_s generally include some means of scaling leaf P_s , N and light relationships because they occur at different canopy positions and these relationships may be modified by elevated CO₂ (Kull & Niinemets 1998; Norby *et al.* 1999; Constable & Friend 2000). Until recently, we have lacked the technology to evaluate P_s , N and light relationships as they occur in forest canopies under CO₂ enrichment. Without this ability, it is difficult to extrapolate leaf-level responses to whole-plant or canopy-level processes because canopy closure changes many aspects of tree growth and physiology such that only a small portion, if any, of the main canopy may respond as previous results with individual plants have indicated. While short-term experiments on isolated seedlings or small trees provide valuable information at the leaf level, they may lack the environmental and leaf variability associated with a closed canopy (Lee & Jarvis 1996). The paucity of data from intact, multi-species ecosystems is a serious shortcoming in modelling productivity and carbon sequestration of future forests and their role in the global carbon cycle (Norby *et al.* 1999).

The relationships between leaf P_s , N and daily integrated photosynthetic photon flux density (PPFD) have been characterized at leaf and canopy levels, but the subject continues to draw the interest of ecophysicologists. At the leaf level, decreasing PPFD may alter N partitioning within the photosynthetic apparatus to favour light-harvesting complexes, whereas N partitioning may shift to favour carboxylation and electron transport proteins under high irradiance (Evans 1989; Niinemets, Kull & Tenhunen 1998; Kull & Niinemets 1998; Kazda, Salzer & Reiter 2000). At the canopy level, higher concentrations of N are present in leaves exposed to greater daily PPFD (Niinemets, Kull & Koppel 1996; Niinemets *et al.* 1998; Kull & Niinemets 1998; Rosati *et al.* 1999; Gonzalez-Real & Baille 2000; Kazda *et al.* 2000). This vertical gradient of leaf N concentration helps to optimize N use in the canopy with respect to maximizing P_s (Field 1983; Sands 1995). The premise of this argument is that if shaded leaves in the canopy interior are

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light-and not N-limited, then decreasing their N content will not influence their daily C gain negatively. In theory, N could be reallocated to more sunlit canopy positions to the effect that all leaves within the canopy operate at the same N limitation with respect to daily P_s (Field 1983). Therefore, Field (1983) predicted that N would be optimally allocated within a canopy if $\delta P_{s_{\text{day}}}/\delta N$ were constant at all canopy microsites: in other words, if N were equally limiting of P_s at all canopy positions, regardless of light availability. This notion was mathematically supported by Sands (1995), and greatly simplifies the construction of mechanistic canopy P_s models (Kull & Niinemets 1998). However, the extent to which elevated CO_2 may modify the distribution of N within a forest canopy is not well understood.

Modification of canopy P_s and N may be further complicated by photosynthetic acclimation to elevated CO_2 (Constable & Friend 2000). Nearly all elevated CO_2 studies report some degree of photosynthetic acclimation as a change in photosynthetic biochemistry – primarily decreases in leaf N concentration and carboxylation capacity. (Curtis 1996). This ‘down-regulation’ can result from feedback inhibition caused by the restriction of internal carbohydrate transport from sources to sinks or nutrient limitations (Myers, Thomas & DeLucia 1999; Curtis *et al.* 2000; Liozon *et al.* 2000). Curtis & Wang (1998) asserted that no general conclusion can yet be drawn regarding photosynthetic acclimation by trees. They found that studies in which feedback inhibition was unlikely were split about evenly between those with down-regulation, no effect and up-regulation. This variation may reflect a mode of acclimation to elevated CO_2 that interacts strongly with other photosynthetic resource limitations, including light and N.

The purpose of this study was to examine how elevated atmospheric CO_2 affects the distribution and photosynthetic use of N within a developing tree canopy. We measured leaf P_s , N and PPFD characteristics within a *Populus tremuloides* canopy under free-air CO_2 enrichment (FACE) during the summer of 1999. We wanted to find out if the leaf photosynthesis–N relationship was altered by elevated CO_2 at different canopy positions, if those relationships would alter the ability of the canopy to optimize N distribution and whether leaf N partitioning plays a role in those canopy-level processes.

MATERIALS AND METHODS

Experimental design

This study was conducted at the free-air CO_2 enrichment (Aspen FACE) facility at Rhinelander, WI, USA (45.6°N, 89.5°W). The facility was constructed in 1997 and consisted of three replicate blocks of 12 30-m-diameter open-air plots fumigated with ambient air enriched by 200 p.p.m. CO_2 and/or O_3 and control plots exposed only to ambient air in a full factorial design. Only the CO_2 -enriched and control plots were utilized in the present study. Plots were spaced at least 100 m apart from their perimeters. A detailed description of the site and facilities was reported by Dickson *et al.*

(2000). Briefly, the fumigation system used a series of 32 vertical vent pipes forming a ring around the perimeter of each plot. A high-volume fan mixed ambient air with pure CO_2 gas and dispensed CO_2 -enriched air from the perimeter vent pipes on the upwind side of each ring at canopy height during daylight hours. Atmospheric CO_2 concentration and wind speed and direction were monitored continuously at plot centre and at canopy height (CO_2 monitored with a LI-6252 infrared gas analyser; Li-Cor, Lincoln, NE, USA). A microcomputer interfaced with these instruments and directed the opening and closing of valves on the vent pipes. Fumigation with CO_2 -enriched air was suspended at night for several reasons. Night-time subsidence reduced the effectiveness of the FACE delivery system, which depends upon air currents to disperse CO_2 across the study plots, and fumigation would greatly increase the risk of contamination for the control plots. Typically low night-time temperatures (5–20 °C, mean = 13 °C at night in August 1999), coupled with the small direct effects of elevated CO_2 on night-time respiration (Amthor 2000), suggest that the effects of night-time fumigation with CO_2 on tree or ecosystem C balance would be negligible. The control plots consisted of perimeter vent pipes and fans, but with no valve apparatus; every second perimeter pipe was vented continuously. Ambient CO_2 concentration was monitored with an infrared gas analyser (LI-6252, Li-Cor) at a single location outside the experimental plots at canopy height.

The plots were planted at 1 × 1 m spacing in 1997 with *P. tremuloides* Michx. clones in one half of each ring, a *P. tremuloides* clone (clone 216; Dickson *et al.* 2000) and *Betula papyrifera* seedlings in one quarter of each plot and *P. tremuloides* and *Acer saccharum* in the remaining quarter. The present study utilized only *P. tremuloides* clone 216, which was interplanted with *B. papyrifera*. Long-term fumigation with CO_2 enriched air during daylight hours began with bud burst in the spring of 1998. The soil was a mixed, frigid, coarse loamy alfic haplorthod that had been used for agricultural crops several years previously. Soil NO_3^- , NH_4^+ and total N prior to planting in 1997 were not significantly different between ambient and elevated CO_2 plots (Total N = 1.2 ± 0.2 and 1.4 ± 0.3 mg g⁻¹, respectively; details provided in Dickson *et al.* 2000). At the end of the 1999 growing season, total soil N did not differ between ambient and elevated CO_2 plots (1.4 ± 0.2 and 1.7 ± 0.4 mg g⁻¹ in ambient and elevated CO_2 plots, respectively; D.R. Zak, unpublished results).

The present study took place during the summer of 1999, which was the second full growing season of the fumigation treatments. The CO_2 -dispensing system operated during daylight hours from bud burst in early May through to leaf senescence in late September. The target CO_2 concentration of the elevated CO_2 plots was 560 $\mu\text{mol mol}^{-1}$. The season average CO_2 concentration at plot centres was 343 and 548 $\mu\text{mol mol}^{-1}$ for the control and elevated CO_2 plots, respectively. The 1 min integrated CO_2 concentration at the centre of the elevated CO_2 plots was within 10% of the target concentration 80% of the time, and within 20% of the target concentration 96% of the time.

Data collection

Canopy light and photosynthesis measurements were initiated in early July 1999. During any given week, measurements were concentrated on the ambient and elevated CO₂ plots in one of the three replicate blocks. Each measurement round consisted of measurements in all three blocks, so that each measurement round lasted for about 3 weeks, with brief interruptions because of the weather. We completed a total of three measurement rounds during the months of July, August and September. Any week-to-week variation during each measurement round was accounted for as the block effect in analysis of variance.

Upper canopy PPFD was monitored at a centralized weather station with a quantum sensor (model LI-190SA; Li-Cor) located 2 m above the ground and not shaded. Within-canopy PPFD was monitored in each plot with quantum sensors mounted on a vertical mast and positioned at mid- (120 cm above ground) and lower (40 cm above ground) canopy positions. The mast was located within 10 m of the plot centre, and was positioned in the centre of a 1 m square defined at the corners by the planting arrangement. Two of the four trees surrounding the mast served as the subjects for all physiology measurements. PPFD was logged (with a LI-1400 data logger; Li-Cor) as the 15 min mean of instantaneous measurements every 5 s from 0500 to 2100 h. The sensors were positioned permanently in each plot and only the data loggers were rotated among plots. All of the within-canopy quantum sensors were calibrated freshly by the manufacturer prior to the start of the experiment.

Photosynthesis light response (P_s /PPFD) curves were measured with a portable photosynthesis system (CIRAS-1; PP Systems, Hertfordshire, UK) on the leaf closest to each quantum sensor and on a leaf on the current terminal of each of the two study trees in each plot. The leaf cuvette was supplied with 360 $\mu\text{mol mol}^{-1}$ CO₂ in the ambient plots and 560 $\mu\text{mol mol}^{-1}$ CO₂ in the elevated CO₂ plots. Cuvette temperature was generally maintained within 2 °C of ambient by a Peltier block, but occasionally deviated from ambient by up to 4 °C. Photosynthetically active radiation was supplied by a bank of quartz-halogen lamps (PP Systems) attached to the leaf cuvette. The P_s /PPFD response was measured by decreasing PPFD from 1700 to 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in six increments. Following a 5 min equilibration period at each light intensity, three photosynthesis measurements were recorded in rapid succession. Each leaf was harvested and air-dried for total N concentration analysis using a combustion analyser (NA 1500; Carlo Erba, Milan, Italy). Additionally, a 2.2 cm² circular disk was extracted from each leaf and used for the determination of specific leaf area (SLA, m² g⁻¹) and a dry weight correction factor following oven-drying at 80 °C for 48 h.

Photosynthesis-leaf internal CO₂ response (P_s/C_i) curves were also measured twice in September with the CIRAS-1 system, on the leaf closest to each quantum sensor and on a leaf from the current terminal for each study tree. The cuvette light unit provided 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$

PPFD during these measurements. Cuvette CO₂ concentration was increased from 60 to 2000 $\mu\text{mol mol}^{-1}$ in six increments. Following a 5 min equilibration period, three photosynthesis measurements were recorded in rapid succession at each cuvette CO₂ concentration. Only six points were collected per curve to reduce the amount of time required for each. This facilitated the collection of data from each treatment ring within a 4 h time period around midday (approximately 1100–1400 h), but probably exacerbated experimental error in parameter estimates.

Leaves from these P_s/C_i measurements were then collected for the analysis of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) concentration. They were immediately frozen in liquid N and stored at -70 °C until analysis. Rubisco concentration was analysed using a Laemmli gel procedure (Brinegar & Peterson 1982). A 6 mg sample of leaf material was hand-ground in liquid N and sample buffer (0.6 mL) to extract protein. Extracted proteins were boiled for 5 min at 100 °C and centrifuged at 14 000 r.p.m. (15 000 g) for 5 min. Samples were separated with gels, which consisted of 12% separating gel and 4% stacking gel, by SDS-PAGE. The large subunit of Rubisco was identified based on pure spinach Rubisco standards and quantified using National Institutes of Health (NIH) image software (Scion Corporation, Frederick, MD, USA).

Data analysis

Photosynthesis-light response curves were analysed using SAS PROC NLIN (SAS Institute, Cary, NC, USA) to fit the rectangular hyperbolic model:

$$P_s = \frac{P_{s_{\text{sat}}} \times \text{PPFD}}{K + \text{PPFD}} - R_d, \quad (1)$$

where $P_{s_{\text{sat}}}$ is the light-saturated photosynthesis rate, K is the light saturation constant and R_d is the dark respiration rate (Givnish 1988). Light compensation point (LCP) and apparent quantum yield (ϕ) were estimated from the initial linear portion (PPFD < 300 $\mu\text{mol mol}^{-1}$) of each curve as the x -intercept and slope, respectively.

Logged PPFD values of each canopy level were factored in to the appropriate P_s /PPFD curves from Eqn 1 to estimate 15 min mean assimilation rates. Those 15 min means were integrated for an estimate of daily assimilation rates ($P_{s_{\text{day}}}$, 0500–2100 h) at each canopy position. Because measurements occurred at different plots on different days, and because PPFD_{day} can change from day to day, we conducted analyses on daily assimilation at each canopy position from all days, and also from light-saturating days only ($P_{s_{\text{day,sat}}}$) (as a standard reference). The light-saturating days were determined as follows: values of $P_{s_{\text{day}}}$ and PPFD_{day} from the upper canopy leaves of each plot during each measurement round were plotted to determine the days on which $P_{s_{\text{day}}}$ of the upper canopy were light-saturated ($P_{s_{\text{day,sat}}}$). Thus, values of $P_{s_{\text{day,sat}}}$ at each canopy position represent daily integrated photosynthesis on days for which the upper canopy leaves were light-saturated. Subsequent comparisons were then conducted on two sets of daily integrated values: the

mean daily P_s and PPFD from all measurements at each canopy position ($P_{s\text{day}}$ and PPFD_{day} , respectively, total of 46 d), and the mean daily P_s and PPFD from days on which the upper canopy leaves were light-saturated ($A_{\text{day,sat}}$ and $\text{PPFD}_{\text{day,sat}}$, respectively, total of 24 d).

The rates of photosynthesis rate-limiting reactions (maximum electron transport rate, J_{max} and maximum carboxylation rate, V_{cmax}) were derived from P_s/C_i curves using Photosyn Assistant software (Dundee Scientific, Dundee, UK).

Statistical analyses

The P_s/PPFD and P_s/C_i curves were compared between treatments and canopy positions for each measurement round according to Potvin, Lechowicz & Tardif (1990). All P_s/PPFD and P_s/C_i parameters, leaf N and Rubisco concentrations and daily integrated P_s and PPFD values were initially compared between measurement rounds and treatments within canopy positions using a repeated measures ANOVA model with date as a split-plot factor and CO_2 treatment as the main plot factor (Moser, Saxton & Pezeshki 1990). There were few statistical differences among measurement rounds and, where appropriate, subsequent analyses combined all sample dates using a split-plot, randomized block ANOVA with canopy position as the split-plot factor and CO_2 treatment as the main plot factor. Means were compared using Fisher's protected least significant difference (LSD) test at the 0.05 level of significance. All regression relationships were fitted using least-squares regression.

RESULTS

Light environment

Prior to bud burst in 1999, the canopies had mean heights of 156 ± 11 cm and 169 ± 20 cm in the ambient and elevated CO_2 plots, respectively. By the end of the 1999 growing season (October), their heights were 263 ± 19 cm and 293 ± 17 cm in the ambient and elevated CO_2 plots, respectively (detailed growth analysis was reported in Isebrands *et al.* 2001). At the time of leaf-out in May 1999, the tree crowns were not fully closed, but there was sufficient mutual shading to create a strong vertical light gradient. $\text{PPFD}_{\text{day,sat}}$ at the top of the canopy declined only slightly from July to September (Table 1). $\text{PPFD}_{\text{day,sat}}$ and PPFD_{day} both decreased significantly ($P < 0.05$) from upper to mid- and from mid- to lower canopy throughout the growing season. At mid-canopy, $\text{PPFD}_{\text{day,sat}}$ was similar under both CO_2 treatments in July, but was lower with elevated CO_2 thereafter. Both $\text{PPFD}_{\text{day,sat}}$ and PPFD_{day} at the lower canopy positions were significantly lower with elevated CO_2 than with ambient CO_2 during all measurement periods.

N and photosynthetic light response

Leaf N concentration on both leaf area and mass bases at all three canopy positions decreased significantly ($P < 0.05$) from the July to September sampling dates (Table 2). N concentrations decreased from upper to lower canopy across all dates, and were greater for leaves in ambient CO_2 than for those in elevated CO_2 at all three canopy positions

Table 1. Mean (\pm SE) daily integrated photosynthetic photon flux density (PPFD, $\text{mol m}^{-2} \text{d}^{-1}$) at the top of, and within, a *P. tremuloides* canopy under current ambient ($343 \mu\text{mol mol}^{-1}$) and elevated ($548 \mu\text{mol mol}^{-1}$) atmospheric $[\text{CO}_2]$ conditions. Data were from relatively cloud-free days on which upper-canopy leaves were photosynthetically light-saturated ($\text{PPFD}_{\text{day,sat}}$) and all days during the study period (PPFD_{day}). The upper canopy (156–293 cm above the ground) was unshaded for both CO_2 treatments; mid- and lower-canopy positions were fixed at 120 and 40 cm above the ground, respectively. $\text{PPFD}_{\text{day,sat}}$ means in the upper portion of the table represent 2–4 d within a 3 week timespan across three replicate plots ($n = 6$ –12). Means in the lower portion of the table are from the entire study period ($n = 18$ –36 for $\text{PPFD}_{\text{day,sat}}$ and $n = 63$ for PPFD_{day}). Means of a parameter within a row or column followed by the same letter were not statistically different ($P > 0.05$)

Canopy position	Ambient CO_2			Elevated CO_2		
	July	Aug.	Sep.	July	Aug.	Sep.
$\text{PPFD}_{\text{day,sat}}$						
Upper	42.1 ± 1.0^a	37.8 ± 0.9^a	38.3 ± 1.0^a	42.1 ± 1.0^a	37.8 ± 0.9^a	38.3 ± 1.0^a
Mid	16.1 ± 1.1^b	14.3 ± 0.6^b	14.8 ± 0.5^b	14.1 ± 1.4^b	11.0 ± 1.6^c	11.3 ± 1.8^c
Lower	6.4 ± 0.8^c	6.7 ± 0.2^c	6.7 ± 0.2^c	3.5 ± 0.4^d	3.4 ± 0.6^d	3.4 ± 0.7^d
Growing season means						
$\text{PPFD}_{\text{day,sat}}$		Ambient CO_2		Elevated CO_2		
Upper			39.4 ± 0.6^a			
Mid		15.1 ± 0.5^b		12.2 ± 0.9^c		
Lower		6.6 ± 0.3^c		3.4 ± 0.3^d		
PPFD_{day}						
Upper			31.4 ± 1.3^a			
Mid		12.2 ± 0.5^b		9.6 ± 0.6^c		
Lower		5.4 ± 0.3^c		3.8 ± 0.2^d		

Table 2. Mean (\pm SE) leaf N concentrations and specific leaf area (SLA) in a *P. tremuloides* canopy under open-air CO₂ enrichment during the 1999 growing season. Upper-canopy measurements were from unshaded leaves on the main terminals. Mid- and lower-canopy positions were fixed at 120 and 40 cm above the ground, respectively. Means of a parameter within a row or column followed by the same letter were not statistically different ($P > 0.05$)

Canopy position	Ambient CO ₂			Elevated CO ₂		
	July	Aug.	Sep.	July	Aug.	Sep.
<i>N</i> _{mass} (mg g ⁻¹)						
Upper	28.8 \pm 2.1 ^a	26.8 \pm 0.9 ^a	25.1 \pm 0.6 ^a	25.2 \pm 2.0 ^a	22.2 \pm 1.0 ^b	19.8 \pm 0.9 ^b
Mid	25.1 \pm 1.4 ^a	23.1 \pm 0.4 ^b	20.9 \pm 0.7 ^c	23.9 \pm 0.3 ^a	18.7 \pm 0.8 ^{bc}	17.1 \pm 0.4 ^c
Lower	22.6 \pm 0.2 ^{ab}	21.3 \pm 0.3 ^b	18.9 \pm 0.3 ^c	18.3 \pm 2.0 ^b	17.2 \pm 0.8 ^c	16.8 \pm 1.1 ^c
<i>N</i> _{area} (mg cm ⁻²)						
Upper	0.31 \pm 0.02 ^a	0.30 \pm 0.01 ^a	0.28 \pm 0.01 ^a	0.34 \pm 0.02 ^a	0.30 \pm 0.02 ^a	0.26 \pm 0.01 ^a
Mid	0.20 \pm 0.01 ^b	0.19 \pm 0.01 ^b	0.17 \pm 0.01 ^b	0.20 \pm 0.01 ^b	0.16 \pm 0.01 ^b	0.14 \pm 0.01 ^b
Lower	0.15 \pm 0.01 ^c	0.14 \pm 0.01 ^c	0.13 \pm 0.01 ^c	0.12 \pm 0.01 ^d	0.11 \pm 0.01 ^d	0.11 \pm 0.01 ^d
SLA (cm ² g ⁻¹)						
Upper			90 \pm 7 ^a			75 \pm 7 ^a
Mid			124 \pm 6 ^b			118 \pm 9 ^b
Lower			150 \pm 5 ^c			157 \pm 8 ^c

($P < 0.05$). Specific leaf area decreased ($P < 0.05$) from upper to lower crown leaves; there was no significant CO₂ effect on SLA. However, with few exceptions, CO₂ effects on mass-based leaf [N] were effectively eliminated by conversion to a leaf area basis. Leaf [N] was significantly correlated to PPFD_{day,sat} across all canopy positions and sampling dates, with no difference between CO₂ treatments (Fig. 1).

The only photosynthesis light-response parameter that

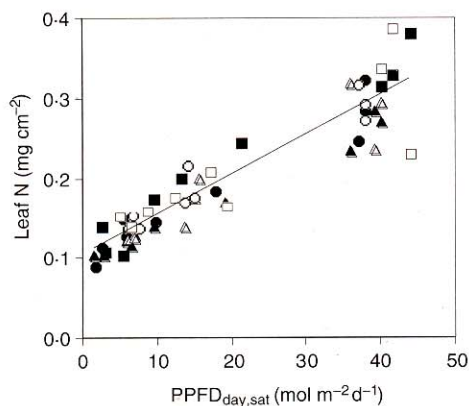


Figure 1. Leaf N concentration as a function of daily integrated photosynthetic photon flux density (PPFD) from relatively cloud-free days on which upper-canopy leaves were photosynthetically light-saturated (PPFD_{day,sat}). Measurements were from top, middle and bottom positions in *P. tremuloides* canopies under current ambient (343 μ mol mol⁻¹; open symbols) and elevated (548 μ mol mol⁻¹; closed symbols) atmospheric CO₂ concentrations ($n = 3$) at July (squares), August (circles) and September (triangles) sampling dates during the 1999 growing season. The least squares regression equation is $y = 0.50x + 10.27$ ($r^2 = 0.87$). Each data point represents a mean of two leaf N samples and PPFD from three to four treatment days during a 7–10 d sampling period from each treatment replicate.

changed significantly during the growing season was the light-saturated rate ($P_{s,sat}$) in elevated CO₂ (Fig. 2). In ambient CO₂, upper-canopy $P_{s,sat}$ decreased ($P < 0.05$) from 27.0 \pm 0.7 in July to 25.4 \pm 3.7 μ mol m⁻² s⁻¹ in September, and in elevated CO₂ it decreased ($P < 0.05$) from 36.5 \pm 1.0 in July to 28.2 \pm 1.0 μ mol m⁻² s⁻¹ in September. Similar $P_{s,sat}$ decreases ($P < 0.05$) occurred at the mid-canopy position in elevated CO₂ (from 30.7 \pm 1.5 in July to 21.0 \pm 0.3 μ mol m⁻² s⁻¹ in August). Other photosynthesis light-response parameters were statistically constant during the growing season and were subsequently combined across sampling dates (Table 3). Several photosynthesis light-response parameters, including $P_{s,sat}$, K , R_d and LCP all decreased from the upper to the lower canopy. The most consistent decrease in these parameters was from the upper to mid-canopy. Only $P_{s,sat}$ in elevated CO₂ decreased significantly from mid- to lower canopy. There were no changes in ϕ with canopy position. Elevated CO₂ affected $P_{s,sat}$ more than any other light-response parameter. $P_{s,sat}$ increased significantly with elevated compared with ambient CO₂ at the upper and mid-canopy positions, but not in the lower canopy. Also, elevated CO₂ significantly increased K in the upper canopy and ϕ at mid-canopy. $P_{s,sat}$ was related ($P < 0.05$) to leaf N concentration across all canopy positions and sampling dates (Fig. 3). The relationship between N_{area} and $P_{s,sat}$ was steeper ($P < 0.05$) under elevated than under ambient CO₂, and the linear regression fit was better in elevated ($r^2 = 0.77$) than in ambient CO₂ ($r^2 = 0.42$).

Daily assimilation rates and N/PPFD relationships

Daily integrated leaf assimilation rates at each canopy position were estimated using PPFD on cloud-free days ($P_{s,day,sat}$) (Table 4). $P_{s,day,sat}$ decreased from upper to lower canopy positions in both CO₂ treatments. Upper-canopy

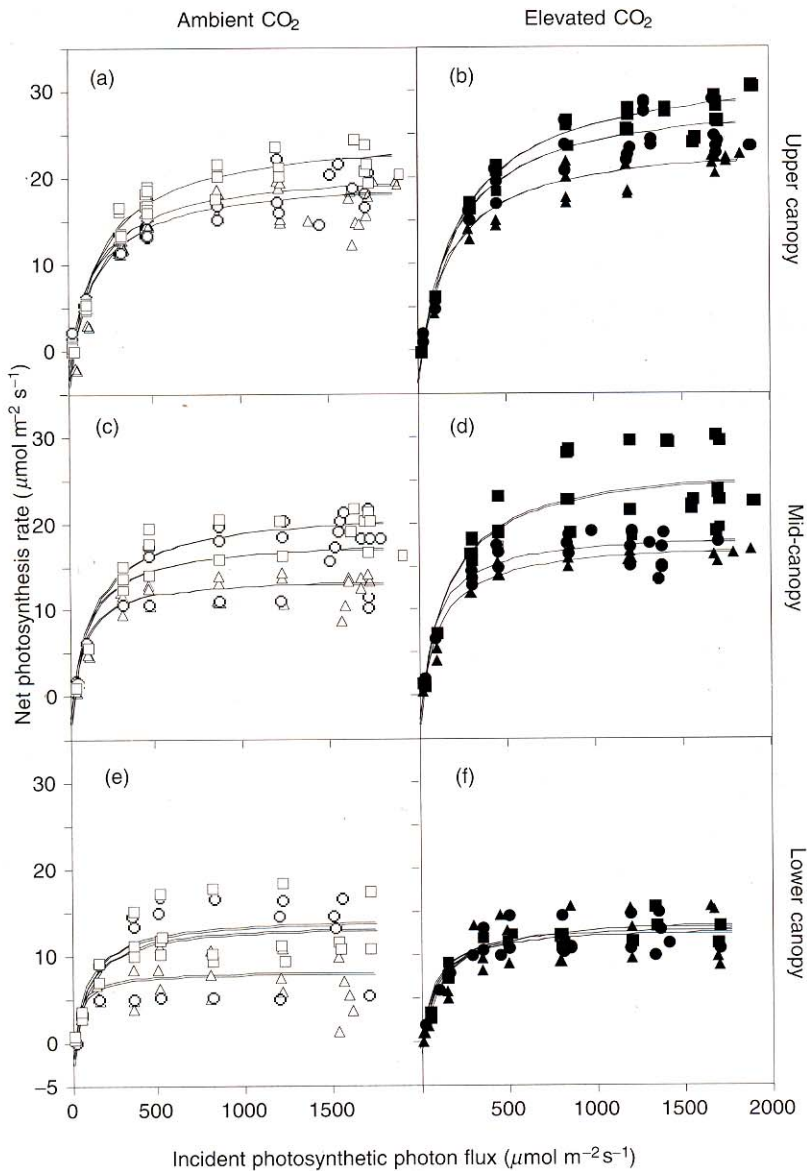


Figure 2. Photosynthesis light response curves from leaves in *P. tremuloides* canopies that developed under current ambient ($343 \mu\text{mol mol}^{-1}$; panels a, c and e) and elevated ($548 \mu\text{mol mol}^{-1}$; panels b, d & f) atmospheric CO_2 concentrations during July (squares), August (circles) and September (triangles) sampling dates in 1999. Measurements were recorded at upper (canopy top; panels a & b), middle (120 cm above ground; panels c & d) and lower (40 cm above ground; panels e & f) canopy positions, and growth CO_2 concentrations. Parameters are summarized in Table 3. Each curve was fitted to data from one fully expanded leaf from each of two trees.

foliage had higher ($P < 0.05$) $P_{s_{\text{day,sat}}}$ in elevated than in ambient CO_2 ; however, lower-canopy foliage had lower ($P < 0.05$) $P_{s_{\text{day,sat}}}$ in elevated than in ambient CO_2 . When viewed with respect to leaf light environment, the daily photosynthesis profiles differed significantly between ambient and elevated CO_2 (Fig. 4). Elevated CO_2 increased the linear parameter of the regression lines for both $P_{s_{\text{day}}}$ and $P_{s_{\text{day,sat}}}$, showing that $P_{s_{\text{day}}}$ and $P_{s_{\text{day,sat}}}$ increased with elevated CO_2 in high-light conditions, but not in the light conditions that occurred at the mid- and lower canopy.

Under both CO_2 regimes $P_{s_{\text{day,sat}}}$ was related to leaf N (Fig. 5a). The linear regression model was significant for both CO_2 treatments, and the slopes of the regression lines were different ($P < 0.05$) between CO_2 treatments. However, the residual plots clearly revealed systematic departures from linearity (Fig. 5b,c), indicating that the $P_{s_{\text{day,sat}}}/\text{N}$ relationship at different canopy positions was not truly linear. This conclusion was also supported by Fig. 5d, which

shows large variations in $P_{s_{\text{day,sat}}}/\text{N}$ at different canopy positions. The relationships in Fig. 4d differed significantly and $P_{s_{\text{day,sat}}}/\text{N}$ at the canopy top was significantly greater under elevated compared with ambient CO_2 . The regression lines in Fig. 5d crossed at $\text{PPFD}_{\text{day,sat}} = 2.8 \text{ mol m}^{-2} \text{ d}^{-1}$, which is less than the lowest $\text{PPFD}_{\text{day,sat}}$ measured at the very bottom of the canopies, indicating that leaf $P_{s_{\text{day,sat}}}/\text{N}$ was never lower under elevated CO_2 than under ambient CO_2 at a common light environment. Comparisons of the means at each canopy position indicated that $P_{s_{\text{day,sat}}}/\text{N}$ did not differ significantly between CO_2 treatments at the mid- and lower canopy positions.

Photosynthetic rate limitations and N partitioning

Photosynthesis–leaf internal CO_2 relationships (P_s/C_i curves) were measured twice during September. V_{cmax} and

Table 3. Mean (\pm SE) photosynthesis light response curve parameters and leaf N concentration in a *P. tremuloides* canopy under open-air CO₂ enrichment during the 1999 growing season. Photosynthesis parameters are light-saturated photosynthesis ($P_{s_{\text{sat}}}$), light saturation constant (K), light compensation point (LCP), apparent quantum yield (ϕ), and dark respiration rate (R_d). Upper-canopy measurements were from unshaded leaves on the main terminals. Mid- and lower-canopy positions were fixed at 120 and 40 cm above the ground, respectively. Means of a parameter within a row or column followed by the same letter were not statistically different ($P > 0.05$).

Canopy position	Ambient CO ₂	Elevated CO ₂
$P_{s_{\text{sat}}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
Upper	26.0 \pm 1.3 ^b	32.8 \pm 1.4 ^a
Mid	19.3 \pm 1.8 ^c	24.9 \pm 2.0 ^b
Lower	14.9 \pm 1.8 ^c	15.3 \pm 1.0 ^c
K ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
Upper	168.7 \pm 15.0 ^b	230.0 \pm 16.8 ^a
Mid	101.1 \pm 15.4 ^c	129.7 \pm 15.9 ^c
Lower	72.1 \pm 13.6 ^c	95.2 \pm 10.0 ^c
LCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
Upper	26.7 \pm 6.0 ^a	21.7 \pm 1.9 ^a
Mid	8.4 \pm 2.7 ^b	10.0 \pm 2.0 ^b
Lower	11.2 \pm 2.4 ^{bc}	5.1 \pm 1.3 ^c
ϕ (mmol mol ⁻¹)		
Upper	70 \pm 4 ^{ab}	72 \pm 3 ^a
Mid	63 \pm 3 ^b	75 \pm 4 ^a
Lower	65 \pm 5 ^{ab}	64 \pm 4 ^{ab}
R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
Upper	3.7 \pm 0.8 ^a	3.4 \pm 0.3 ^a
Mid	3.0 \pm 0.2 ^{ab}	3.0 \pm 0.3 ^b
Lower	2.3 \pm 0.5 ^{ab}	1.6 \pm 0.4 ^b

J_{max} decreased from the upper to the lower canopy in both CO₂ treatments (Table 5). V_{cmax} and J_{max} did not differ ($P > 0.05$) between CO₂ treatments, but the means were 21–28% lower under elevated CO₂ compared with ambient CO₂ at the mid- and lower canopy. The $J_{\text{max}}/V_{\text{cmax}}$ ratios decreased significantly from the upper to the lower canopy, but did not differ between CO₂ treatments.

Rubisco concentration, as determined by assay of the large subunit, decreased from upper to mid- and from mid- to lower canopy. It was also significantly lower under elevated than under ambient CO₂ in the upper, mid- and lower canopy by 29, 38 and 50%, respectively. The ratio of Rubisco to N decreased from the upper to the lower canopy, indicating that a smaller proportion of total leaf N was partitioned into Rubisco protein with increasing canopy depth. Similarly, Rubisco/N was lower under elevated compared with ambient CO₂ at all three canopy positions.

DISCUSSION

Photosynthesis of leaves within our *P. tremuloides* canopies increased under elevated CO₂, with the largest gains occur-

ring at the canopy top. Improvements in shade photosynthesis due to elevated CO₂ were insignificant, so that mid- and lower-canopy leaves did not exhibit an increase in daily photosynthetic C gain. Leaves at the bottom of the canopy had lower daily photosynthesis rates in elevated CO₂ because of decreased light availability. The range of leaf daily assimilation in elevated CO₂ was larger than in ambient CO₂ due to a higher maximum at the canopy top and a lower minimum at the canopy bottom. Therefore, the effect of elevated CO₂ was not simply a proportional stimulation of daily P_s at all canopy positions.

In our experiment, N per unit leaf area was related linearly to daily integrated PPFD, which reflected substantial inter-canopy gradients of leaf N concentration regardless of CO₂ treatment or time of year. Such gradients are typical of canopy environments and several authors have reported close coupling among $P_{s_{\text{sat}}}$, PPFD and leaf N (DeJong & Doyle 1985; Hirose & Werger 1987; Ellsworth & Reich 1993; Niinemets 1995; Hollinger 1996; Noormets *et al.* 1996; Niinemets *et al.* 1998; Kull & Niinemets 1998; Rosati *et al.* 1999; Schoettle & Smith 1999; Gonzalez-Real & Baille 2000; Kazda *et al.* 2000). Although relationships between $P_{s_{\text{sat}}}$, PPFD and N have been widely reported, the mechanisms controlling them are only partially understood (Kull & Niinemets 1998). It is clear that leaf N limits C fixation under saturating light because N limits the concentration of N-based photosynthetic proteins. However, co-ordination among daily assimilation, leaf N and PPFD in the canopy interior is much less clear. Does leaf N truly limit shade photosynthesis, or is N of shade leaves somehow regulated by environmental or physiological cues? Shade photosynthesis may be improved with increased N on a leaf-mass basis due to greater production of light-harvesting complexes [although those observations involved very shade

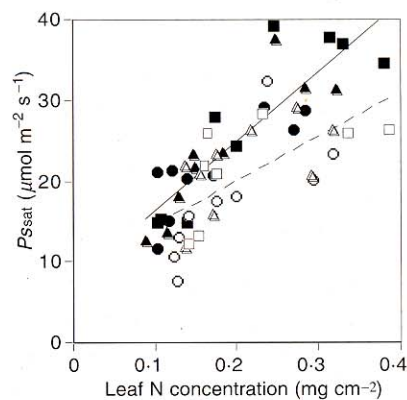


Figure 3. Light-saturated photosynthesis ($P_{s_{\text{sat}}}$) versus leaf N concentration from leaves in *P. tremuloides* canopies that developed under current ambient ($343 \mu\text{mol mol}^{-1}$; open symbols, dashed line) and elevated ($548 \mu\text{mol mol}^{-1}$; closed symbols, solid line) atmospheric CO₂. Measurements were taken under growth CO₂ during July (squares), August (circles) and September (triangles) sampling dates in 1999. Least squares regression lines are: ambient CO₂, $y = 54.5x + 9.2$, ($r^2 = 0.42$); elevated CO₂, $y = 86.5x + 7.7$, ($r^2 = 0.77$).

Table 4. Estimated mean (\pm SE) daily integrated net photosynthesis rates in a *P. tremuloides* canopy for days on which the main terminal leaves were light-saturated during the 1999 growing season ($P_{s_{day,sat}}$, $\text{mmol m}^{-2} \text{d}^{-1}$). The canopies developed and were measured under current ambient ($343 \mu\text{mol mol}^{-1}$) and elevated ($548 \mu\text{mol mol}^{-1}$) atmospheric CO_2 concentrations. Upper-canopy leaves were from the unshaded main terminal; mid- and lower-canopy leaves were at fixed positions 120 and 40 cm above ground, respectively. Means within a row or column followed by the same letter were not significantly different ($P > 0.05$)

Canopy position	Ambient CO_2			Elevated CO_2		
	July	Aug.	Sep.	July	Aug.	Sep.
Upper	857 \pm 22 ^a	713 \pm 30 ^{cd}	632 \pm 14 ^d	1044 \pm 17 ^b	890 \pm 24 ^{ab}	765 \pm 15 ^{ac}
Mid	503 \pm 29 ^c	387 \pm 24 ^c	343 \pm 12 ^{ef}	551 \pm 46 ^c	333 \pm 40 ^{ef}	297 \pm 37 ^f
Lower	261 \pm 16 ^d	192 \pm 24 ^b	180 \pm 11 ^{ab}	160 \pm 17 ^{ag}	141 \pm 17 ^g	102 \pm 24 ^e

tolerant species, *Acer saccharum* (Ellsworth & Reich 1993) and *Fagus sylvatica* (Niinemets 1995)]. We suggest that despite the clear linear relationship between leaf N and PPFD demonstrated here and elsewhere, the mechanisms that couple leaf daily P_s and N are not consistent at all canopy positions. Leaf N limits the maximum rate of photosynthesis at the canopy top, but not the non-light-saturated rates that occur most often. Pons & Bergkotte (1996) suggested that canopy N distribution is related to stomatal regulation of the transpiration stream that carries cytokinins into leaves and regulates protein synthesis. Whatever the mechanisms that couple canopy N distribution to PPFD are, they were unaffected by atmospheric CO_2 enrichment in our *P. tremuloides* canopy.

The gradient of leaf N concentration from upper- to lower-canopy foliage helps to optimize N use in the canopy with respect to maximizing P_s . In theory, the distribution of leaf N within a canopy is optimized with respect to P_s if $\delta P_{s_{day}}/\delta N$ is constant, representing a consistent photosynthetic N limitation at all canopy positions regardless of light environment (Field 1983; Sands 1995). If true, this is a valuable simplification for mechanistic modelling of canopy N and photosynthesis. In our study, we used this theory to test for optimization of canopy N allocation and to determine whether it was affected by elevated CO_2 . The linear regression of leaf N and $P_{s_{day,sat}}$ was significant under both CO_2 treatments, which would seem to satisfy the criterion for optimal allocation of N. However, closer examination of the model residuals, and of the $P_{s_{day,sat}}/N$ values themselves, indicated that the relationships departed from linearity and that daily photosynthetic N use efficiency was not constant across canopy positions. Similar results were reported by Hollinger (1996). Therefore, we conclude that leaf N in these *P. tremuloides* canopies was not distributed optimally, and that elevated CO_2 did not improve or diminish the optimization of canopy N allocation. Consequently, any change in canopy N use efficiency would be driven largely by the direct effects of elevated CO_2 on photosynthesis rates.

Photosynthetic light response curves at different canopy positions responded differently to elevated CO_2 . The largest photosynthetic stimulation occurred in $P_{s_{sat}}$ of the upper canopy leaves, while $P_{s_{sat}}$, LCP and ϕ of shade leaves were relatively unaffected (similar to other findings: Kubiske & Pregitzer 1996; Herrick & Thomas 1999, 2001; DeLucia &

Thomas 2000). Our results from a closed canopy are in clear contrast with other studies involving relatively open-grown *P. tremuloides* trees in open-topped chambers (Kubiske *et al.* 1997) or at our FACE facility prior to canopy closure

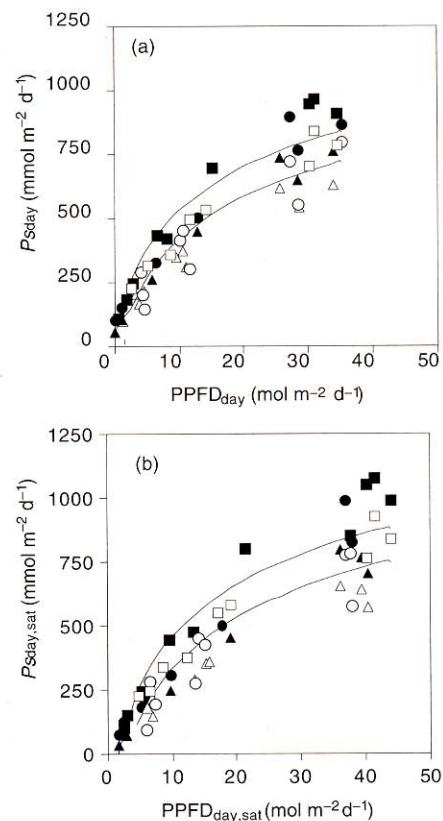


Figure 4. Daily integrated photosynthesis rates (P_s) versus daily integrated photosynthetic photon flux density (PPFD) of *P. tremuloides* leaves in young forest canopies that developed under current ambient ($343 \mu\text{mol mol}^{-1}$; open symbols) and elevated ($548 \mu\text{mol mol}^{-1}$; closed symbols) atmospheric CO_2 . (a) Data from all measurement days in the 1999 growing season ($P_{s_{day}}$ and PPFD_{day}). Least squares regression lines are: ambient CO_2 , $y = 63.6 + 27.4x - 0.3x^2$ ($r^2 = 0.88$); elevated CO_2 , $y = 2.9 + 43.1x - 0.6x^2$ ($r^2 = 0.95$). (b) Data from days on which upper-canopy leaves were light-saturated ($P_{s_{day,sat}}$ and $\text{PPFD}_{day,sat}$). Least squares regression lines are: ambient CO_2 , $y = 41.8 + 27.9x - 0.3x^2$ ($r^2 = 0.87$); elevated CO_2 , $y = 17.8 + 35.4x - 0.3x^2$ ($r^2 = 0.94$).

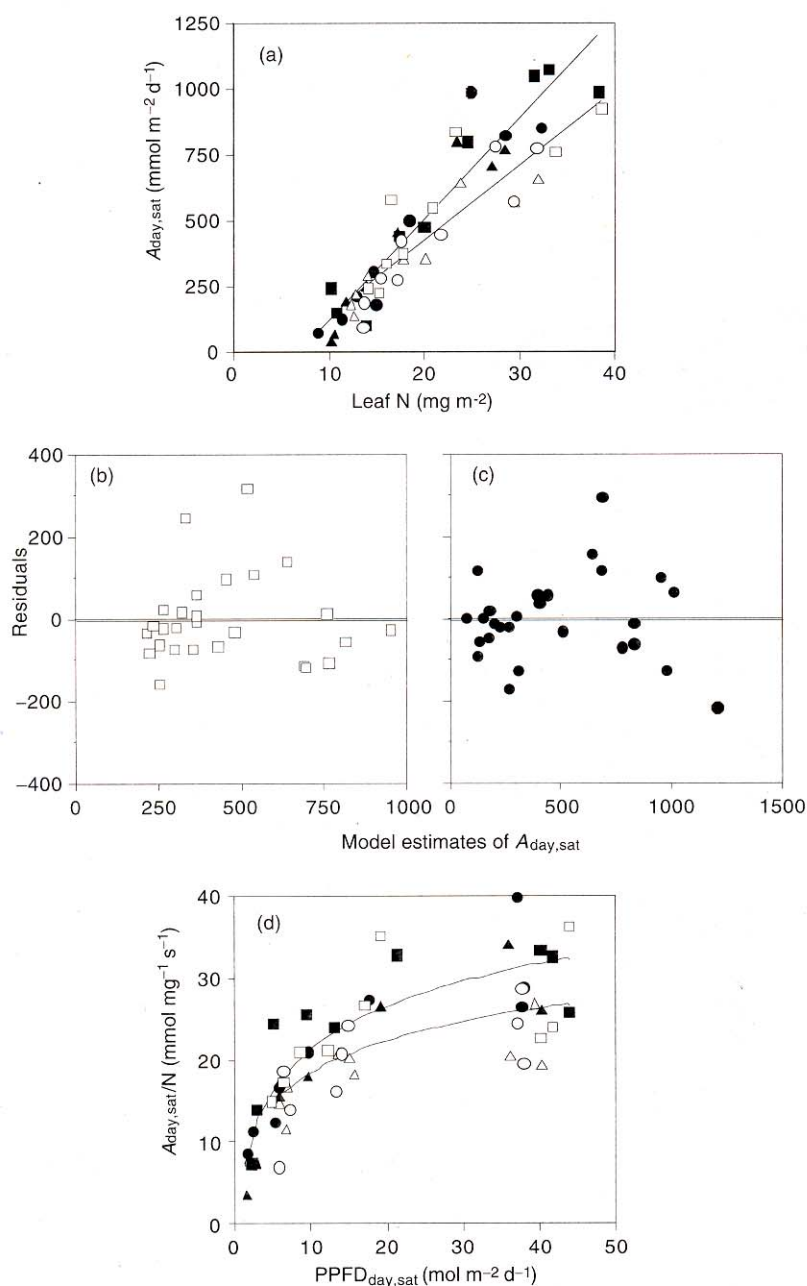


Figure 5. Leaf N, light and photosynthesis relationships from *P. tremuloides* canopies on relatively cloud-free days on which upper canopy leaves were photosynthetically light-saturated. The canopies developed under current ambient ($343 \mu\text{mol mol}^{-1}$; open symbols) and elevated ($548 \mu\text{mol mol}^{-1}$; closed symbols) atmospheric CO_2 ($n = 3$). Measurements were taken under growth CO_2 during July (squares), August (circles) and September (triangles) sampling dates in 1999. (a) Leaf N concentration versus daily integrated leaf photosynthesis ($P_{\text{day,sat}}$). Least squares regression lines: ambient CO_2 , $y = 2787x - 124$ ($r^2 = 0.79$); elevated CO_2 , $y = 3863x - 264$ ($r^2 = 0.91$). (b & c) Regression residuals (measured $A_{\text{day,sat}}$ - model predicted $A_{\text{day,sat}}$) from Fig. 4a. (d) Daily integrated photosynthetic photon flux density ($\text{PPFD}_{\text{day,sat}}$) versus daily integrated leaf photosynthetic N use efficiency ($P_{\text{day,sat}}/N$). Least squares regression lines: ambient CO_2 , $y = 13.2\log(x) + 5.2$ ($r^2 = 0.45$); elevated CO_2 , $y = 17.7\log(x) + 3.4$ ($r^2 = 0.83$).

in 1998 (Noormets *et al.* 2001; Sôber *et al.* 2001). In those studies, P_{sat} was stimulated by elevated CO_2 at all crown positions, and at all leaf ages. In open-topped chambers, the largest stimulation of assimilation occurred in the lower third of the crowns (Kubiske *et al.* 1997). These important differences illustrate that measurements from open-grown trees are not appropriate substitutes for the complexities of a closed-canopy environment.

In the present study, sun leaves had greater CO_2 stimulation of P_{sat} compared with shade leaves – probably because the depressed photosynthetic capacity that normally occurs in shade leaves muted the CO_2 response (Herick & Thomas 1999, 2001). Of the four forest understorey species, the magnitude of the P_{sat} response was related to the proportional size of the Rubisco pool (DeLucia & Tho-

mas 2000). P_{sat} should respond strongly to elevated CO_2 because the system is light-saturated and carboxylation should be stimulated by the increase in substrate availability. However, a reduction in the enzyme pool would counteract that stimulation. We found substantial CO_2 -related increases in P_{sat} at the upper canopy that quickly diminished with canopy depth, as would be expected given the large declines in photosynthetic capacity observed in the mid- and lower canopy. A similar response was reported for shade-intolerant *B. papyrifera*, in which sun plants had large increases in P_{sat} ; shade plants had no increases in P_{sat} (Kubiske & Pregitzer 1996). The rate of carboxylation in shade responds to elevated CO_2 in two ways. Firstly, C_3 carbon fixation by Rubisco is limited by the availability of the CO_2 substrate and by the competing effect of oxygenation

Table 5. Mean (\pm SE) photosynthetic leaf internal CO₂ response (P_s/C_i) parameters in *P. tremuloides* canopies in September 1999. The canopies developed under current ambient (343 $\mu\text{mol mol}^{-1}$) and elevated (548 $\mu\text{mol mol}^{-1}$) atmospheric CO₂ concentrations. Parameters are maximum rate of carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), leaf concentration of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the ratio of leaf Rubisco to N concentration (Rubisco/N). Upper-canopy measurements were from unshaded leaves on the main terminals. Mid- and lower-canopy positions were fixed at 120 and 40 cm above the ground, respectively. Means of a parameter within a row or column followed by the same letter were not statistically different ($P > 0.05$).

Canopy position	Ambient CO ₂	Elevated CO ₂
V_{cmax} ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)		
Upper	61.1 \pm 12.3 ^a	63.7 \pm 12.2 ^a
Mid	51.1 \pm 13.0 ^b	37.6 \pm 10.5 ^b
Lower	35.8 \pm 12.3 ^c	25.6 \pm 6.4 ^c
J_{max} ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)		
Upper	254.3 \pm 54.2 ^a	269.8 \pm 58.7 ^a
Mid	178.9 \pm 36.8 ^b	137.0 \pm 26.0 ^b
Lower	107.1 \pm 19.3 ^c	85.3 \pm 14.4 ^c
$J_{\text{max}}/V_{\text{cmax}}$		
Upper	4.6 \pm 0.7 ^a	4.4 \pm 0.5 ^{ab}
Mid	3.6 \pm 0.2 ^b	4.6 \pm 0.8 ^a
Lower	3.5 \pm 0.3 ^b	3.7 \pm 0.3 ^b
Rubisco (mg cm ⁻²)		
Upper	2.34 \pm 0.38 ^a	1.67 \pm 0.28 ^b
Mid	1.17 \pm 0.18 ^b	0.72 \pm 0.10 ^c
Lower	0.92 \pm 0.08 ^c	0.46 \pm 0.02 ^a
Rubisco/N (g g ⁻¹)		
Upper	10.5 \pm 0.9 ^a	7.2 \pm 1.2 ^b
Mid	6.7 \pm 0.6 ^b	4.9 \pm 0.5 ^a
Lower	7.2 \pm 0.6 ^c	4.1 \pm 0.2 ^a

of RuBP. Oxygenation of RuBP consumes a proportion of the RuBP pool and of absorbed light energy, and results in C expenditure in the photorespiration, or photosynthetic carbon oxidation (PCO), cycle. A shift in carboxylase : oxygenase activity due to CO₂ enrichment should increase net assimilation regardless of light, temperature or nutrient limitations (Drake *et al.* 1997). However, if the Rubisco pool is depressed, as often occurs in shade leaves (Kull & Niinemets 1998; Gonzalez-Real & Baille 2000; Liozon *et al.* 2000), CO₂ suppression of the PCO cycle will have a proportionally decreased effect (cf. Long 1991). In this way, a decrease in photosynthetic capacity can limit improvements in shade photosynthesis by elevated CO₂. Secondly, the direct response of Rubisco to an increase in CO₂ substrate is limited in shade because the energy limitation restricts the regeneration of RuBP. In our study, leaf Rubisco concentrations were depressed by the combined effects of shaded microsites in the canopy and by elevated CO₂. We believe that these factors combined to virtually eliminate any CO₂ stimulation of the photosynthetic light

response functions and our estimates of daily integrated leaf photosynthesis.

We found that photosynthetic down-regulation was more severe with decreasing canopy depth. Our findings are in contrast with those of others who found no interactive effects of crown position with photosynthetic down-regulation (Liozon *et al.* 2000; Herrick & Thomas 2001). Nonetheless, long-term loss of photosynthetic capacity has been reported for *P. tremuloides* (Curtis *et al.* 2000) and *P. trichocarpa* \times *P. nigra* (Ceulemans *et al.* 1997). In a previous experiment, *P. tremuloides* leaves only had significant decreases in both J_{max} and V_{cmax} at mid-crown positions where export to shoot and root extension would have been minimal (Kubiske *et al.* 1997). Carbohydrate sink restrictions because of plant phenology, nutrient limitations or physical space limitations may result in photosynthetic down-regulation (see reviews by Gunderson & Wullschlegel 1994; Curtis 1996; Drake *et al.* 1997; Saxe, Ellsworth & Heath 1998; Norby *et al.* 1999). The upper-canopy leaves clearly had the largest daily C budget and had no indication of down-regulation, but they were also in the closest proximity to rapidly growing sinks.

Some authors have compared the photosynthetic CO₂ acclimation response to the light acclimation response because both entail changes in the stoichiometric balance between photosynthetic rate-limiting reactions (Sage 1994; Kubiske & Pregitzer 1996; Kubiske *et al.* 1997; DeLucia & Thomas 2000). A number of leaf N use optimization models suggest that photosynthetic acclimation responses to shading and elevated CO₂ can help to optimize the distribution of nutrient resources (particularly N) between the rate-limiting reactions (Sage 1994; Woodrow 1994; Hikosaka & Terashima 1995; Medlyn 1996). For example, Woodrow (1994) calculated that as leaf internal CO₂ concentration increases from 350 to 700 p.p.m., the amount of carboxylating enzyme (Rubisco) needed to maintain constant P_s would decrease by 41%. Similarly, Medlyn (1996) estimated that $J_{\text{max}}/V_{\text{cmax}}$ should increase by an average of 38% under a doubling of atmospheric CO₂, depending upon N concentration. Similarities between the CO₂ and shade responses have led investigators to hypothesize that shading and elevated CO₂ should interact to drive a more complete acclimation response than either factor alone (Kubiske & Pregitzer 1996; DeLucia & Thomas 2000). In the present study, Rubisco concentration decreased from upper- to lower-canopy leaves by 61% under ambient CO₂ and by 72% under elevated CO₂. Looking at it another way, elevated CO₂ decreased the Rubisco concentration of upper-canopy leaves and lower-canopy leaves by 28 and 50%, respectively. The proportion of leaf N invested in Rubisco protein was significantly lower under elevated CO₂, particularly in the mid- and lower canopies. Elevated CO₂ clearly interacted with canopy shading to decrease photosynthetic capacity in this study, but we found no evidence for shifts in the $J_{\text{max}}/V_{\text{cmax}}$ ratio due to elevated CO₂.

We recognize the limitations in our methods of estimating daily leaf P_s . Variation in temperature and conductance within the canopies, within measurement days and between

measurement days probably combined to produce overestimates of $P_{s_{day}}$, particularly at the lower-canopy positions. However, because our $P_{s_{day,sat}}$ estimates were closely and predictably related to independent measurements of leaf N (as was $P_{s_{day}}$, not shown), we believe them to be reasonable. Moreover, Noormets *et al.* (2001) concluded that altered mesophyll processes were the primary mechanisms controlling photosynthetic responses to elevated CO_2 , while changes in stomatal conductance were secondary responses that maintained constant internal-to-external CO_2 ratios. The results of this study indicated several important points that should be considered in modelling tree canopy responses to elevated atmospheric CO_2 . Elevated CO_2 did not simply increase the daily assimilation of all canopy leaves by a constant proportion. Rather, the distribution of daily P_s throughout the canopy exhibited a larger maximum and a smaller minimum compared with ambient CO_2 . We found a significant acclimation of photosynthesis to elevated CO_2 that was modified by canopy depth. Leaves in the most shaded microsites had the largest decreases in photosynthetic capacity under elevated CO_2 . The effect of elevated CO_2 on the light response of photosynthesis was not constant throughout the canopy, but was driven by changes in photosynthetic capacity that interacted with the light environment. The stimulation of $P_{s_{sat}}$ by elevated CO_2 decreased progressively with canopy depth because of photosynthetic down-regulation of shade leaves. Changes in light-response parameters associated with shade photosynthesis were not sufficient to overcome the effects of shading so that the elevated CO_2 stimulation of daily leaf P_s decreased progressively with canopy depth. Leaf N within the canopies was not distributed optimally for photosynthesis. Although elevated CO_2 clearly stimulated daily photosynthetic N use efficiency at the canopy top, it did not improve the canopy N distribution. The relationship between leaf N and daily PPFD was not altered by elevated CO_2 . In these aspen canopies, the vertical patterns of photosynthetic characteristics, Rubisco concentration and leaf N have clearly changed with canopy development from 1998 (Noormets *et al.* 2001; Söber *et al.* 2001) to the present study. Further experiments are needed to determine if the patterns of light, N and photosynthesis interactions observed here during the initial stages of canopy closure will remain constant as canopy closure progresses at the Wisconsin aspen FACE facility.

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