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## ***Populus tremuloides* photosynthesis and crown architecture in response to elevated CO<sub>2</sub> and soil N availability**

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**Abstract** We tested the hypothesis that elevated CO<sub>2</sub> would stimulate proportionally higher photosynthesis in the lower crown of *Populus* trees due to less N retranslocation, compared to tree crowns in ambient CO<sub>2</sub>. Such a response could increase belowground C allocation, particularly in trees with an indeterminate growth pattern such as *Populus tremuloides*. Rooted cuttings of *P. tremuloides* were grown in ambient and twice ambient (elevated) CO<sub>2</sub> and in low and high soil N availability (89 ± 7 and 333 ± 16 ng N g<sup>-1</sup> day<sup>-1</sup> net mineralization, respectively) for 95 days using open-top chambers and open-bottom root boxes. Elevated CO<sub>2</sub> resulted in significantly higher maximum leaf photosynthesis ( $A_{\max}$ ) at both soil N levels.  $A_{\max}$  was higher at high N than at low N soil in elevated, but not ambient CO<sub>2</sub>. Photosynthetic N use efficiency was higher at elevated than ambient CO<sub>2</sub> in both soil types. Elevated CO<sub>2</sub> resulted in proportionally higher whole leaf  $A$  in the lower three-quarters to one-half of the crown for both soil types. At elevated CO<sub>2</sub> and high N availability, lower crown leaves had significantly lower ratios of carboxylation capacity to electron transport capacity ( $V_{c_{\max}}/J_{\max}$ ) than at ambient CO<sub>2</sub> and/or low N availability. From the top to the bottom of the tree crowns,  $V_{c_{\max}}/J_{\max}$  increased in ambient CO<sub>2</sub>, but it decreased in elevated CO<sub>2</sub> indicating a greater relative investment of N into light har-

vesting for the lower crown. Only the mid-crown leaves at both N levels exhibited photosynthetic down regulation to elevated CO<sub>2</sub>. Stem biomass segments (consisting of three nodes and internodes) were compared to the total  $A_{\text{leaf}}$  for each segment. This analysis indicated that increased  $A_{\text{leaf}}$  at elevated CO<sub>2</sub> did not result in a proportional increase in local stem segment mass, suggesting that C allocation to sinks other than the local stem segment increased disproportionately. Since C allocated to roots in young *Populus* trees is primarily assimilated by leaves in the lower crown, the results of this study suggest a mechanism by which C allocation to roots in young trees may increase in elevated CO<sub>2</sub>.

**Key words** Carbon allocation · Elevated CO<sub>2</sub> · Nitrogen · Photosynthesis · *Populus tremuloides*

### **Introduction**

Photosynthetic carbon assimilation ( $A$ ) is the primary transducer for energy flow from the abiotic to the biotic environment. A fundamental change in the efficient assimilation of C by plants would likely affect a number of plant, community, and ecosystem processes (Bazzaz 1990; Field et al. 1992). In addition to being limited by soil N availability, C3 photosynthesis is presently limited by atmospheric CO<sub>2</sub> largely because O<sub>2</sub> and CO<sub>2</sub> compete for the primary photosynthetic enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco; Tolbert and Zelitch 1983). An increase in the ratio of atmospheric CO<sub>2</sub>:O<sub>2</sub> favors carboxylation by rubisco, enabling a greater amount of N invested in photosynthetic enzymes to be utilized in C fixation (Tolbert and Zelitch 1983; Sage 1994).

Ample evidence exists for increased resource-use efficiency and photosynthetic carbon assimilation by leaves and increased productivity in trees with an increase in atmospheric CO<sub>2</sub> concentration (Poorter 1993; Ceulemans and Mousseau 1994; Gunderson and Wullschlegel 1994). In addition, studies indicate a change in C alloca-

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tion patterns within individual trees in response to elevated CO<sub>2</sub> that is highly dependent on soil N availability. Such a change in allocation could significantly affect community and ecosystem processes, including species composition (Williams et al. 1986), belowground C storage (Dixon et al. 1994), and ecosystem C and N cycling (Zak et al. 1993; Pregitzer et al. 1995).

At current CO<sub>2</sub> concentrations, trees often exhibit a continual reallocation of N from lower, shaded leaves to upper, sunlit leaves as the stem grows that is related to light availability and photosynthetic capacity (Field 1983; Field and Mooney 1983; Sands 1995; Noormets et al. 1996). In trees with an indeterminate growth pattern, there is a mechanistic link between the location of assimilating leaves and the direction of C export from those leaves (Dickson 1986, 1989). Most studies of tree response to elevated CO<sub>2</sub> focus closely on factors affecting growth (C sinks) or on photosynthetic characteristics of individual leaves (C sources), while we understand little about how the activity of C sources may vary throughout the plant canopy in CO<sub>2</sub> enrichment. This is particularly important because lower crown leaves are the sources for C allocation to belowground growth (Dickson 1986, 1989). Because elevated CO<sub>2</sub> often results in higher photosynthetic N use efficiency, we hypothesized that (1) lower crown leaves would retain a greater proportion of their original N, (2) there would be greater leaf mass in the lower crown due to longer leaf retention, and (3) the distribution of whole-crown *A* would shift downward on the crown.

To test these hypotheses, we measured leaf photosynthesis, N, and mass throughout the crowns of *Populus tremuloides* trees grown at ambient and elevated CO<sub>2</sub> and in low and high soil N availability. To examine the effects of a redistribution of *A* on C allocation, we measured the architecture of the main stem and related those measurements to the distribution of *A* through the crown.

## Methods

### Experimental design

Rooted cuttings of wild clones of *Populus tremuloides* Michx. were planted in June 1994 in either low-N or high-N soil ( $89 \pm 7$  and  $333 \pm 16$  ng N g<sup>-1</sup> day<sup>-1</sup> net mineralization, respectively) using buried, open-bottom root boxes (0.5 m<sup>2</sup> × 1.3 m deep) at the University of Michigan Biological Station in northern lower Michigan, United States. While four clones were planted into each root box, a single clone was selected from each box for this study. The high-N soil was the A-horizon of Kalkaska Sand (Typic Haplorthod), and the low-N soil was a mixture of Kalkaska A-horizon and C-horizon of Rubicon Sand (Entic Haplorthod; 1:4 by volume). Soil N availability was representative of the range of sites on which *P. tremuloides* typically occurs in Lake States forests. Transparent, open-top chambers were placed over the trees (16 total chambers, 1 tree per chamber) and half of the chambers received supplemental CO<sub>2</sub> through a blower system to elevate the atmospheric CO<sub>2</sub> concentration to approximately twice that of the ambient air ( $373 \pm 1$  ml l<sup>-1</sup> and  $713 \pm 3$  ml l<sup>-1</sup> season means for ambient and elevated chambers, respectively). Blower volume was sufficient to supply two exchanges of air per minute in the chambers (Curtis and Teeri 1992).

### Photosynthesis and crown architecture measurements

Beginning in early September 1994, after 95 days of growth in the treatments, two ADC LCA-2 portable photosynthesis systems were used to measure instantaneous light-saturated photosynthesis per unit leaf area (*A*<sub>area</sub>) on every leaf attached to the main stem ( $n = 4$  per CO<sub>2</sub>/fertility treatment) at 7- to 10-day intervals. There was not a substantial amount of self shading of the lower crown leaves in any treatment. On the same days that *A* was measured, a 1.7 cm<sup>2</sup> disk was punched from every third leaf on the main stem of the tree for determination of N concentration (with a Carlo Erba NA1500 series II elemental analyzer), and specific leaf area (cm<sup>2</sup> g<sup>-1</sup>). Leaf area was determined non-destructively by applying leaf length and width measurements to a predetermined regression equation:

$$\text{area} = 3.38 - 0.20(\text{length}) + 0.18(\text{width}) + 0.01(\text{length})(\text{width}) \quad (r^2 = 0.99) \quad (1)$$

A CIRAS-I portable photosynthesis system (PP Systems, Hitchin, UK) was used to construct *A* versus internal CO<sub>2</sub> (*C*<sub>i</sub>) relationships for leaves in the upper (first fully expanded leaf), mid, and lower portions of the crown. The CO<sub>2</sub> concentration in air supplied to the leaf cuvette was increased from 100 to 1700 μmol mol<sup>-1</sup> in seven increments under saturating irradiance (2250 μmol m<sup>-2</sup> s<sup>-1</sup>).

### Photosynthesis analysis

Photosynthesis per unit leaf area (*A*<sub>area</sub>) was calculated according to von Caemmerer and Farquhar (1981). The maximum *A* measured for each tree on each sample date was determined for comparison with leaf N concentration. Whole leaf photosynthesis (*A*<sub>leaf</sub>) for every leaf was calculated from predicted leaf area and measured *A*<sub>area</sub>. We focused our analysis on *A*<sub>leaf</sub> because it was a better indication of total assimilation than *A*<sub>area</sub>. Whole leaf *A*, in turn, would be affected by leaf N content and leaf mass. Leaf mass of every third leaf was calculated from predicted leaf area divided by specific leaf area. Whole leaf N content was calculated from N concentration and estimated leaf mass for every third leaf. Instantaneous N-use efficiency (NUE) was calculated as μmol CO<sub>2</sub> mol<sup>-1</sup> N s<sup>-1</sup> for leaves in the upper, mid and lower portions of the crown. *A/C*<sub>i</sub> relationships were analyzed by fitting the biochemical models of Farquhar and von Caemmerer (1982) and solving for maximum carboxylation capacity (*V*<sub>c,max</sub>) and maximum rate of electron transport (*J*<sub>max</sub>). Values of *V*<sub>c,max</sub>, *J*<sub>max</sub> and NUE were analyzed as a split-plot ANOVA with crown position treated as a sub-unit in the split-plot design. Means were compared with Fisher's protected LSD procedure. Significance for all statistical analyses was accepted at  $P \leq 0.05$ .

### Crown photosynthesis analyses

Previous research has demonstrated that leaf initiation and development in young *Populus* trees occurs in proportion to the total number of leaves on the stem (Dickson and Shive 1982). Due to large treatment differences in stem height and leaf numbers in this study, leaves and stem segments were compared between treatments by relativizing the vertical position on the stem, with 100 representing the apex. The relative position of each leaf was rounded to the nearest 5% for *A*<sub>leaf</sub> (since *A* was measured on every leaf), and to the nearest 10% for leaf N, leaf mass, and stem section parameters (since these measurements were conducted on every third leaf). Where more than one leaf or stem segment fell into a 5% or 10% section, the mean value was used. Thus, we reconstructed the distribution of *A*<sub>leaf</sub>, leaf N and leaf mass into theoretical trees of the same size with the same number of leaves. Analysis of variance with the relativized crown position as a co-variate was used to test for CO<sub>2</sub> and N effects on *A*<sub>leaf</sub>, leaf N and leaf mass. A *t*-test was used to compare *A*<sub>leaf</sub>, leaf N and leaf mass among treatments for each 25% of the crown.

## Architecture and allocation analyses

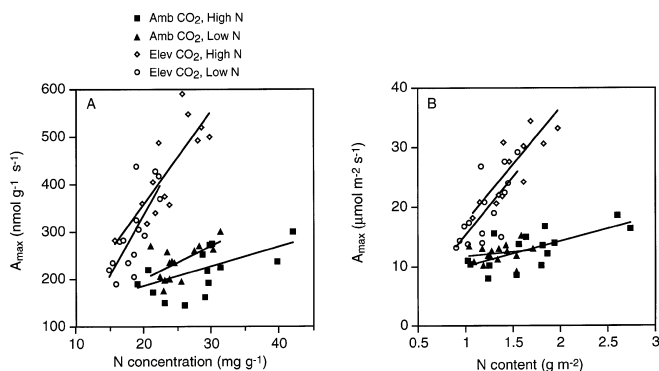
At the end of the growing season, the stems were harvested for allometric analysis. Number of nodes, internode length, and internode diameter at the midpoint were measured. Starting with the apex, stems were cut immediately above every third node, such that each "stem segment" consisted of three nodes and three internodes. The dry mass of these three-node/internode stem segments was statistically compared to the total leaf area for each segment, and the mean  $A_{\text{area}}$  for each segment averaged across all sample dates, using multiple regression.

The relative position of the stem segments was determined as described above. Two-way ANOVA was used to test for treatment differences in dry mass, length and diameter of stem segments with relative crown position used as a co-variate.

## Results

### Photosynthesis

At ambient  $\text{CO}_2$ ,  $A_{\text{max}}$  of each tree was similar at both high and low soil N concentrations (low N  $A_{\text{max}} = 12.92 \pm 0.47 \mu\text{mol m}^{-2} \text{s}^{-1}$ , high N  $A_{\text{max}} = 13.18 \pm 0.76 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Elevated  $\text{CO}_2$  resulted in significantly higher  $A_{\text{max}}$  at both N levels, with trees in high-N soil



**Fig. 1** Maximum leaf photosynthesis ( $A_{\text{max}}$ ) versus leaf N on a leaf mass (A) and leaf area basis (B) for *Populus tremuloides* trees grown in ambient and elevated  $\text{CO}_2$  and in low and high soil N availability. Corresponding regression equations are given in Table 1

**Table 1** Regression relationships between maximum leaf photosynthesis ( $A_{\text{max}}$ ) and leaf N concentration in *Populus tremuloides* trees grown in ambient and twice ambient (elevated)  $\text{CO}_2$ , and in high and low soil N availability. Corresponding regression lines and data are illustrated in Fig. 1

Leaf mass basis ( $A_{\text{max}}$ , $\text{nmol g}^{-1} \text{s}^{-1}$ ; N $\text{mg g}^{-1}$ )		$r^2$	$P$
Ambient $\text{CO}_2$ , Low N	$A_{\text{max}} = 70.9(\text{N}) + 55.9$	0.346	0.027
Ambient $\text{CO}_2$ , High N	$A_{\text{max}} = 41.2(\text{N}) + 102.9$	0.253	0.017
Elevated $\text{CO}_2$ , Low N	$A_{\text{max}} = 259.9(\text{N}) - 185.1$	0.568	0.001
Elevated $\text{CO}_2$ , High N	$A_{\text{max}} = 195.4(\text{N}) - 34.5$	0.641	0.001
Leaf area basis ( $A_{\text{max}}$ , $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; N, $\text{g m}^{-2}$ )		$r^2$	$P$
Ambient $\text{CO}_2$ , Low N	$A_{\text{max}} = 4.4(\text{N}) + 6.3$	0.359	0.030
Ambient $\text{CO}_2$ , High N	$A_{\text{max}} = 5.6(\text{N}) + 4.3$	0.452	0.006
Elevated $\text{CO}_2$ , Low N	$A_{\text{max}} = 19.3(\text{N}) - 4.0$	0.513	0.003
Elevated $\text{CO}_2$ , High N	$A_{\text{max}} = 19.1(\text{N}) - 1.6$	0.620	0.001

having higher ( $P < 0.05$ )  $A_{\text{max}}$  than those in low-N soil (low N  $A_{\text{max}} = 19.99 \pm 1.12 \mu\text{mol m}^{-2} \text{s}^{-1}$ , high N  $A_{\text{max}} = 26.14 \pm 1.35 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Trees grown in elevated  $\text{CO}_2$  had significantly higher  $A_{\text{max}}$  per leaf N concentration, expressed on either a leaf mass or leaf area basis, than those in ambient  $\text{CO}_2$  (Fig. 1, Table 1). These regression relationships were not significantly affected by soil N availability, although soil N availability had an effect on instantaneous NUE throughout the tree crowns. Lower crown leaves in low N soil had NUE that was less ( $P < 0.05$ ) than those of the mid and upper crown, whereas lower crown leaves in high N soil had NUE that was similar to that of the mid and upper crown (Table 2).

The relative distribution of  $A_{\text{leaf}}$  within the *P. tremuloides* crowns was strongly affected by elevated  $\text{CO}_2$  in both high and low-N soil (Fig. 2). Leaves throughout the crowns had significantly higher  $A_{\text{leaf}}$  ( $P < 0.05$ ) in elevated than in ambient  $\text{CO}_2$ . The ratio of elevated  $A_{\text{leaf}}$  to ambient  $A_{\text{leaf}}$  tended to increase from top to bottom of the crown, indicating that the  $\text{CO}_2$  stimulation of  $A_{\text{leaf}}$  was greater toward the bottom of the crown. Crown distribution of leaf N content ( $\text{mg N/leaf}$ ) and leaf mass, both which may greatly affect  $A_{\text{leaf}}$ , were significantly greater in high-N than low-N soil, but there was no significant effect due to elevated  $\text{CO}_2$  (Fig. 3). Thus, the  $\text{CO}_2$ -related changes evident in the distribution of crown  $A_{\text{leaf}}$  were not due to large changes in the distribution of leaf tissue or N content.

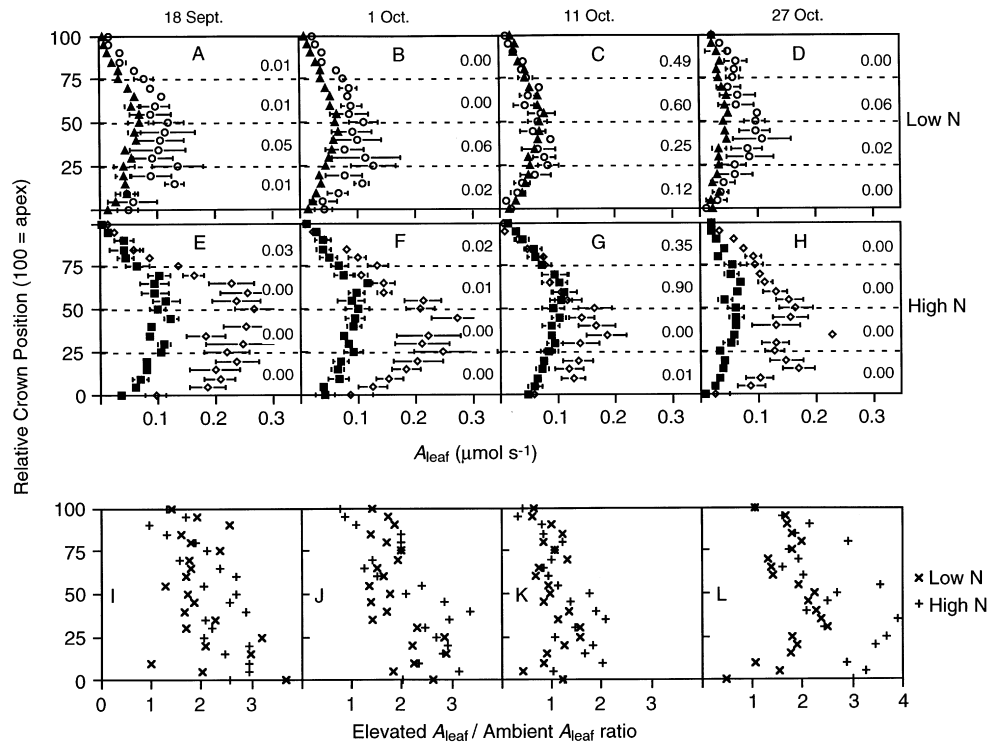
Soil N availability and elevated  $\text{CO}_2$  both had significant ( $P < 0.05$ ) effects on  $A/C_i$  relationships, and interacted with crown position (Table 2). Leaves in the mid-crown position had significantly lower  $V_{\text{cmax}}$  and  $J_{\text{max}}$  in elevated compared to those produced in ambient  $\text{CO}_2$ . Thus, only leaves in the mid-section of the crowns exhibited down-regulation of photosynthesis in elevated  $\text{CO}_2$ . In high-N soil, the ratio of  $V_{\text{cmax}}/J_{\text{max}}$  tended to decrease from top to bottom of the crown in elevated  $\text{CO}_2$ , indicating that lower leaves maintained a proportionally high capacity for electron transport (relative to carboxylation capacity) compared to lower crown leaves in ambient  $\text{CO}_2$ .

**Table 2** Parameters from photosynthesis versus internal CO<sub>2</sub> relationships ( $A/C_i$ ) for *Populus tremuloides* trees grown in ambient or elevated atmospheric CO<sub>2</sub> (373 ± 1 ml l<sup>-1</sup> and 713 ± 3 ml l<sup>-1</sup>, respectively) and low or high soil N availability (89 ± 7 and 333 ± 16 ng N g<sup>-1</sup> day<sup>-1</sup> net mineralization, respectively). Parameters are instantaneous photosynthetic N use efficiency (NUE,  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$ ), maximum carboxylation capacity ( $V_{c_{\text{max}}}$ ,

$\mu\text{mol m}^{-2} \text{ s}^{-1}$ ), the maximum rate of electron transport ( $J_{\text{max}}$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ), and the ratio of  $V_{c_{\text{max}}}/J_{\text{max}}$ . NUE was calculated from instantaneous measurement of leaf  $A$ , whereas  $V_{c_{\text{max}}}$  and  $J_{\text{max}}$  were derived from  $A/C_i$  relationships. Means (±SE) of a parameter within a row or column followed by the same letter are not statistically different according to Fisher's protected LSD ( $P > 0.05$ )

	Crown position	Low N		High N	
		Amb CO <sub>2</sub>	Elev CO <sub>2</sub>	Amb CO <sub>2</sub>	Elev CO <sub>2</sub>
NUE	Top	107.8 ± 9.8 a	257.6 ± 47.6 c	82.6 ± 8.4 b	176.4 ± 30.8 d
	Middle	120.4 ± 4.2 a	190.4 ± 18.2 c	93.8 ± 4.2 b	190.4 ± 9.8 cd
	Bottom	84.0 ± 7.0 b	152.6 ± 14.0 a	79.8 ± 4.2 b	170.8 ± 14.0 d
$V_{c_{\text{max}}}$	Top	37.5 ± 4.1 a	61.8 ± 9.1 ab	94.1 ± 11.3 bc	106.0 ± 9.8 c
	Middle	109.0 ± 8.1 b	50.7 ± 2.3 a	107.9 ± 8.8 b	84.6 ± 10.3 d
	Bottom	66.9 ± 13.6 b	45.7 ± 0.6 ab	75.5 ± 9.8 b	62.8 ± 11.7 bd
$J_{\text{max}}$	Top	96.1 ± 8.2 a	139.2 ± 18.7 a	218.1 ± 18.8 b	227.2 ± 37.2 b
	Middle	223.2 ± 3.2 b	117.0 ± 5.8 a	251.9 ± 29.0 b	195.6 ± 23.7 b
	Bottom	138.5 ± 25.2 ab	119.2 ± 4.4 ab	185.1 ± 19.5 b	160.1 ± 22.4 b
$V_{c_{\text{max}}}/J_{\text{max}}$	Top	0.39 ± 0.02 a	0.44 ± 0.03 ac	0.43 ± 0.02 ac	0.48 ± 0.03 c
	Middle	0.49 ± 0.04 c	0.43 ± 0.01 ac	0.43 ± 0.02 ac	0.43 ± 0.02 bc
	Bottom	0.48 ± 0.02 c	0.38 ± 0.01 a	0.41 ± 0.03 a	0.39 ± 0.02 ab

**Fig. 2A–H** whole leaf photosynthesis ( $A_{\text{leaf}}$ ) by relative crown position for *Populus tremuloides* trees grown in ambient and elevated CO<sub>2</sub> and in low N (A–D) or high N (E–H) soil. Dark symbols are for trees grown in ambient CO<sub>2</sub> and open symbols are for trees grown in elevated CO<sub>2</sub>. Means are from four individuals in each treatment, error bars are one se. Values given for each 25% zone are P values from a paired, two-tail t-test. Panels I–L: the ratio of mean  $A_{\text{leaf}}$  in elevated CO<sub>2</sub> over mean  $A_{\text{leaf}}$  in ambient CO<sub>2</sub> for both low N (x) and high N (+) soil (from means in A–H)



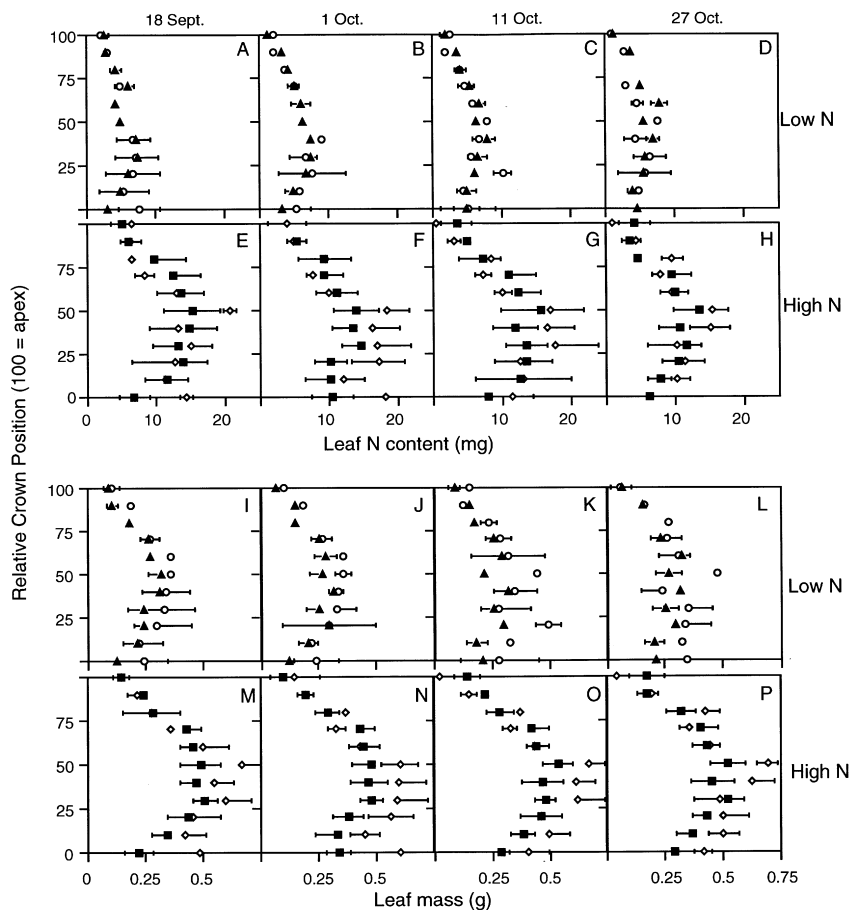
### Crown architecture and allocation

High soil N availability, but not elevated CO<sub>2</sub>, resulted in significantly higher leaf area, total stem height, number of leaves and internodal distances than low N availability (Table 3). Elevated atmospheric CO<sub>2</sub> did not significantly affect the distribution of mass, length or mean diameter in the stems in either low or high N soil, but these characteristics all increased ( $P < 0.05$ ) in high soil N availability (Fig. 4). Stem segment mass and

length of the upper 50% of the stems, and segment mean diameter of the lower 75% of the stems, were significantly higher ( $P < 0.05$ ) in high-N than in low-N soil.

Across all treatments, stem segment mass was significantly related to the vertical crown position of the segment and to the total leaf area of the segment, but not to the mean  $A_{\text{area}}$  of the segment (Table 4). Analysis of variance of these predictor variables indicated that stem mass and leaf area were significantly affected by soil N availability, whereas  $A_{\text{area}}$  was affected by elevated CO<sub>2</sub>

**Fig. 3** Leaf N content (A–H) and leaf mass (I–P) versus relative crown position for *Populus tremuloides* trees grown in either low N (A–D, I–L) or high N (E–H, M–P) soil and ambient (closed symbols) and twice ambient CO<sub>2</sub> (open symbols). Means are from four individuals in each treatment, error bars are one se.



**Table 3** Mean ( $\pm$  se) total leaf area (cm<sup>2</sup>), stem height (cm), number of leaves, and internodal distance (cm) for *Populus tremuloides* trees grown in ambient and twice ambient CO<sub>2</sub> and in low and high soil fertility. Means of a parameter followed by the same letter are not significantly different ( $P > 0.05$ )

	Ambient CO <sub>2</sub>		Elevated CO <sub>2</sub>	
	Low N	High N	Low N	High N
Total leaf area	836 $\pm$ 177 a	2316 $\pm$ 280 b	816 $\pm$ 236 a	2190 $\pm$ 473 b
Stem height	56.0 $\pm$ 14.8 a	112.4 $\pm$ 5.4 b	48.0 $\pm$ 12.0 a	112.2 $\pm$ 10.5 b
Number of leaves	32.7 $\pm$ 4.1 a	44.5 $\pm$ 1.9 b	27.0 $\pm$ 2.1 a	44.7 $\pm$ 2.7 b
Internodal dist	1.7 $\pm$ 0.1 a	2.5 $\pm$ 0.1 b	1.8 $\pm$ 0.1 a	2.5 $\pm$ 0.1 b

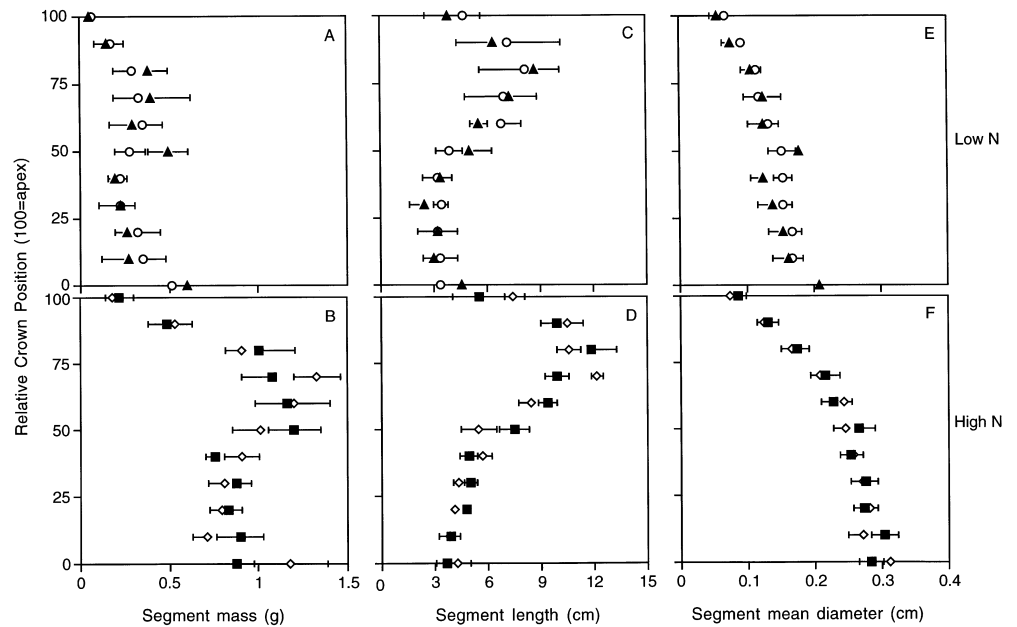
primarily in high N soil (Table 5). Thus, despite a significant increase in photosynthesis due to elevated CO<sub>2</sub>, there was not a proportional increase in the local stem segment mass.

**Discussion**

Our original hypothesis was partially supported by the observations of increased photosynthesis, increased N use efficiency, and a redistribution of *A* toward the lower crown in elevated CO<sub>2</sub>. Several species and hybrids of *Populus* have been shown to respond to increased CO<sub>2</sub> availability with increased instantaneous *A* (Ceulemans et al. 1995a; Curtis and Teeri 1992; Curtis et al. 1994, 1995). The mode of increased *A* in *Populus* trees grown in elevated CO<sub>2</sub> generally results from increased C<sub>i</sub>

stimulating greater carboxylation activity of rubisco, which may be accompanied by an increase in photochemical reactions (Ceulemans et al. 1995a). While the instantaneous activity of rubisco almost always increases in elevated CO<sub>2</sub>, the maximum carboxylation capacity of rubisco ( $V_{c_{max}}$ ) may decrease (Curtis and Teeri 1992; Curtis et al. 1995), even in field-rooted trees (cf. Tissue et al. 1996). This instantaneous increase in rubisco activity often results in higher NUE and light use efficiency (Percy and Björkman 1983; Tolbert and Zelitch 1983; Kubiske and Pregitzer 1996a). Therefore, we expected to see a large increase in NUE in the lower crown leaves. Values and CO<sub>2</sub>-induced increases of NUE in this study were similar to those of *Betula* and *Acer* seedlings grown in similar soil (Kubiske and Pregitzer 1996b). Moreover, it is interesting to note that as the photosynthetic C limitation was reduced by growing trees at elevated CO<sub>2</sub>

**Fig. 4** Mass (A–B), length (C–D) and mean diameter (E–F) of stem segments consisting of 3 nodes and internodes from *Populus tremuloides* trees grown in low N (A, C, E) or high N (B, D, F) soil and in ambient (closed symbols) or twice ambient (open symbols) atmospheric CO<sub>2</sub>. Segment position on the stem was relativized by crown position according to 10% increments



**Table 4** Least squares regression for mass (g) of stem segments of *Populus tremuloides* cuttings consisting of 3 nodes and internodes versus the vertical position along the stem of each segment (segment number), the total leaf area per stem segment (cm<sup>2</sup>), and the mean net photosynthesis rate per unit leaf area ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) late in the growing season. Data consisted of trees grown in ambient and twice-ambient CO<sub>2</sub> and in low and high soil N availability ( $n = 4$ )

Dependent Variable = stem segment mass (g)				
Independent variable	Coefficient	SE	<i>t</i>	<i>P</i>
Constant	0.155	0.112	1.384	0.169
Segment number	0.040	0.011	3.647	0.000
Leaf area	0.002	0.000	5.543	0.000
$A_{\text{area}}$	-0.002	0.008	-0.212	0.832

Source	<i>df</i>	MS	<i>P</i>
Regression	3	2.972	0.000
Residual	126	0.132	

in this study, the linear relationship between  $A_{\text{max}}$  and N had a higher  $r^2$ , indicating a closer coupling between photosynthetic capacity and leaf N status.

Contrary to our hypothesis, however, the shift in crown distribution of  $A$  was apparently not due to a redistribution of crown N. Rather, lower crown leaves in elevated CO<sub>2</sub> exhibited a decrease in the proportion of maximum rubisco activity compared to electron transport capacity ( $V_{\text{cmax}}/J_{\text{max}}$  ratio) in high-N soil, which indicated an increase in capacity of ribulose biphosphate regeneration relative to consumption. In contrast, elevated CO<sub>2</sub> increased maximum photochemical capacity in upper and mid-crown, but not lower-crown, leaves in two *Populus* clones (Ceulemans et al. 1995a) similar to our results for low-N soil.

**Table 5** Analysis of variance for stem mass per 3-node and internode stem segment (g), total leaf area per segment (cm<sup>2</sup>), and mean photosynthesis per unit leaf area ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in each stem segment for *Populus tremuloides* cuttings grown in ambient and elevated CO<sub>2</sub>, and in low and high soil fertility. Segment number was used as a covariate

Parameter	Source	<i>df</i>	MS	<i>P</i>
Stem mass	Segment	1	0.884	0.001
	Block	3	0.140	0.128
	CO <sub>2</sub>	1	0.005	0.914
	Error 1	3	0.397	
	N	1	9.006	0.015
	Error 2	3	0.349	
	CO <sub>2</sub> × N	1	0.184	0.114
Leaf area	Segment	1	21770	0.047
	Block	3	8729	0.790
	CO <sub>2</sub>	1	503	0.892
	Error 1	3	22897	
	N	1	97851	0.050
	Error 2	3	9673	
	CO <sub>2</sub> × N	1	5760	0.305
$A_{\text{area}}$	Segment	1	4.6	0.432
	Block	3	45.5	0.001
	CO <sub>2</sub>	1	623.6	0.061
	Error 1	3	72.6	
	N	1	5.32	0.556
	Error 2	3	12.2	
	CO <sub>2</sub> × N	1	97.45	0.000
Error 3	117	7.4		

Photosynthetic down-regulation in elevated CO<sub>2</sub> has been related to growing plants in a limited rooting volume that restricts root sink strength and results in negative feedback inhibition of photosynthesis (Thomas and Strain 1991; Curtis 1996). Negative feedback inhibition of photosynthesis may also be related to the

growth activity of the plant, with down-regulation occurring after bud set (Tissue et al. 1996), and to assimilate export capacity from the mesophyll into vascular tissue (Körner et al. 1995). In this study, the photosynthetic CO<sub>2</sub> response during active growth was highly dependent on crown position of the leaves, with the mid-crown leaves, i.e., the most productive leaves, exhibiting photosynthetic down-regulation. One important conclusion here is that much of the conflicting information regarding photosynthetic down-regulation in elevated CO<sub>2</sub> (cf. Gunderson and Wullschlegel 1994) may also be related to the relative crown position of the leaves measured. A second conclusion is that down-regulation may be least prevalent in leaves that are closest to actively growing sinks where the phloem transport gradient for photoassimilate is generally highest (Wardlaw 1990). The strongest sinks are likely to be actively growing shoot tips for the upper crown leaves and roots for the lower crown leaves (Dickson 1991), whereas highly productive mid-crown leaves that are farthest from sinks may be most susceptible to excess carbohydrate feedback inhibition of *A* irrespective of rooting condition. It is important to note that the trees in our study had not developed lateral branches which would serve as an additional sink for mid-crown leaves (Fisher et al. 1983), and that acclimation of photosynthesis to elevated CO<sub>2</sub> may represent an optimization of resources (specifically N) rather than negative feedback inhibition.

Increased *A* in elevated CO<sub>2</sub> ultimately leads to a 30–60% stimulation of growth in nearly every C3 species studied (Poorter 1993; Ceulemans and Mousseau 1994), although reports of changes in stem growth and allometry in response to elevated CO<sub>2</sub> have been variable. Whereas some authors have reported no change in shoot allometry (Curtis and Teeri 1992; Norby et al. 1992), others have reported genotypic (Ceulemans and Impens 1983; Ceulemans et al. 1995b, 1996) and interspecific (Reekie and Bazzaz 1989) variation in allometric responses. In *Populus*, most studies report an increase in stem height with elevated CO<sub>2</sub>, resulting from either an increase in the number of nodes produced (Bosac et al. 1995; Curtis et al. 1995), or from an increase in internodal distance (Ceulemans et al. 1995a). These responses did not occur in our study, but the trees exhibited large increases in growth with high N availability.

The specific pattern of C allocation within a tree is largely dependent upon sink strength, source strength, and the vascular connections and distances between sources and sinks (Dickson 1986). In young *Populus*, the absolute position along the stem at which developing leaves convert from C sinks to C sources shifts downward as stem height and number of leaves increases (Dickson and Shive 1982). Vascular tissues develop such that each maturing leaf is directly connected to the stem segment immediately subtending the leaf (Isebrands and Larson 1977; Dickson 1986). Carbon may be exported from source leaves in several directions depending upon crown position of the leaves: acropetally for developing

leaves and stem elongation from leaves in the upper crown, bi-directionally from leaves in the middle of the crown, and basipetally for stem secondary growth and roots from leaves in the lower crown (Vogelmann et al. 1982; Dickson 1986, 1989; Dickson and Isebrands 1991). These developmental and anatomical features tend to be similar among species with indeterminate growth patterns (Dickson and Isebrands 1991) and among different age classes of *Populus* trees (Dickson 1989). Thus, an increase in the relative proportion of whole-tree C assimilation contributed by lower crown leaves would likely result in a similar increase in C allocation to roots.

During active growth, C assimilated in *Populus* trees is largely allocated to shoots (up to 80% in hybrid poplar) with a steeply increasing proportion allocated to roots as shoot growth ceases (Horwath et al. 1994). Nonetheless, much of the growth stimulation in elevated CO<sub>2</sub> tends to occur below ground with a significant and disproportionate increase in C allocation to fine roots even during periods of active shoot growth (Zak et al. 1993; Pregitzer et al. 1995). The data presented here suggest a change in the proportion of C allocated to stem growth, evidenced by the lack of a CO<sub>2</sub> effect on shoot mass despite an increase in photosynthetic capacity. We suggest that increased assimilated C, represented by higher photosynthetic capacity of the lower crown, was allocated to roots. Minirhizotron data from these study trees indicated that fine root production significantly increased in elevated CO<sub>2</sub>, particularly in high-N soil (M. Kubiske, K. Pregitzer, D. Zak and C. Mikan, unpublished work) consistent with our previous findings (Zak et al. 1993; Pregitzer et al. 1995) and with the mechanics of C allocation within young *Populus* trees.

Photosynthetic and carbon allocation responses to elevated CO<sub>2</sub> are quite variable and may be directly related to several life-history characteristics of genotypes or species, and to the availability of other resources such as N and light. In addition to such variability, our understanding of C allocation processes and source-sink relations limits our ability to predict changes in forest and ecosystem function with increasing CO<sub>2</sub> (Eamus 1996). In this study, a single clone of shade-intolerant *P. tremuloides* was grown in unshaded conditions which likely facilitated the downward redistribution of crown *A* in elevated CO<sub>2</sub>. This redistribution of assimilation capacity likely influenced source-sink relations at the tree level resulting in a shift in C allocation that is consistent with observed increases in root production in elevated CO<sub>2</sub> (Zak et al. 1993; Pregitzer et al. 1995). These results suggest that increased C allocation to roots in elevated CO<sub>2</sub> may be related to a fundamental response in the photosynthetic apparatus of lower crown leaves in trees with an indeterminate growth pattern. In turn, carbon allocation to tree roots plays an important role in numerous ecosystem and forest community processes, such as stimulation of microbial mineralization of N (Norby et al. 1987; Zak et al. 1993) and plant water balance (Kubiske and Pregitzer 1996b). If C allocation at the tree level depends upon source-sink

relations, it is at least as important to address the modular behavior of C sources as it is the behavior of C sinks to elucidate the fundamental mechanisms driving ecosystem processes. These long-term effects are difficult to quantify using open-top chamber technology and the refinement of large-scale CO<sub>2</sub> fumigation protocols is essential in predicting the future of forest ecosystems.

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