

Photosynthetic acclimation of overstory *Populus tremuloides* and understory *Acer saccharum* to elevated atmospheric CO₂ concentration: interactions with shade and soil nitrogen

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Summary We exposed *Populus tremuloides* Michx. and *Acer saccharum* Marsh. to a factorial combination of ambient and elevated atmospheric CO₂ concentrations ([CO₂]) and high-nitrogen (N) and low-N soil treatments in open-top chambers for 3 years. Our objective was to compare photosynthetic acclimation to elevated [CO₂] between species of contrasting shade tolerance, and to determine if soil N or shading modify the acclimation response. Sun and shade leaf responses to elevated [CO₂] and soil N were compared between upper and lower canopy leaves of *P. tremuloides* and between *A. saccharum* seedlings grown with and without shading by *P. tremuloides*. Both species had higher leaf N concentrations and photosynthetic rates in high-N soil than in low-N soil, and these characteristics were higher for *P. tremuloides* than for *A. saccharum*. Electron transport capacity (J_{\max}) and carboxylation capacity (V_{\max}) generally decreased with atmospheric CO₂ enrichment in all 3 years of the experiment, but there was no evidence that elevated [CO₂] altered the relationship between them. On a leaf area basis, both J_{\max} and V_{\max} acclimated to elevated [CO₂] more strongly in shade leaves than in sun leaves of *P. tremuloides*. However, the apparent [CO₂] × shade interaction was largely driven by differences in specific leaf area (m² g⁻¹) between sun and shade leaves. In *A. saccharum*, photosynthesis acclimated more strongly to elevated [CO₂] in sun leaves than in shade leaves on both leaf area and mass bases. We conclude that trees rooted freely in the ground can exhibit photosynthetic acclimation to elevated [CO₂], and the response may be modified by light environment. The hypothesis that photosynthesis acclimates more completely to elevated [CO₂] in shade-tolerant species than in shade-intolerant species was not supported.

Keywords: A/C_i analysis, global change, leaf nitrogen, sugar maple, trembling aspen.

Introduction

The effects of increasing atmospheric CO₂ concentration ([CO₂]) on leaf photosynthesis (A) are fairly well understood for light-saturated conditions (see reviews by Gunderson and Wullschlegler 1994, Sage 1994, Curtis 1996, Drake et al. 1997, Saxe et al. 1998, Norby et al. 1999). Comparing light-saturated A among leaves produced in ambient and elevated [CO₂] at a common internal [CO₂] (C_i) often results in lower A for the leaves grown with atmospheric CO₂ enrichment. This decline in photosynthetic capacity is associated with changes in protein and pigment pool sizes in elevated [CO₂] that have been referred to as acclimation responses (Gunderson and Wullschlegler 1994). From a theoretical standpoint, photosynthetic acclimation to elevated [CO₂] has received considerable attention with respect to optimizing nitrogen (N) use and regulating the co-limitation of photosynthesis by light and dark reactions (Sage 1990, 1994, Woodrow 1994, Hikosaka and Terashima 1995, Medlyn 1996). These considerations are important because they affect photosynthetic N-use efficiency, the ability of leaves to photosynthesize when subject to light and N limitations, and the sustainability of elevated [CO₂] stimulation of photosynthesis.

According to N-use optimization models, three functional components of the photosynthetic apparatus can change in response to changes in resource (i.e., N, light, CO₂) availability to maintain stoichiometric balance (cf. Hikosaka and Terashima 1995). One component is the pool of the carboxylating enzyme, Rubisco, the activity of which is functionally represented by the maximum carboxylation rate (V_{\max}). A second component comprises the Calvin cycle proteins, which are associated with electron transport and photophosphorylation for the production of NADPH and ATP, and the utilization of these reducing factors in RuBP regeneration. This component is functionally represented by the maximum rate of electron transport (J_{\max}). The third component, which ultimately supplies energy to the others, comprises the light harvesting com-

plexes (LHCs) and can be represented by leaf chlorophyll concentration.

Woodrow (1994) calculated that, as leaf internal CO₂ concentration increases from 350 to 700 ppm, the amount of Rubisco needed to maintain a constant carboxylation rate would decrease by about 41% because the competitive effects of O₂ would decrease. This prediction has been a common theme in several photosynthetic CO₂-response models (Sage 1990, Medlyn 1996). The theoretical decrease in Rubisco content would not necessarily be coupled with a decline in the rate of electron transport or RuBP cycling because the need for ATP and NADPH production (from electron transport) and utilization (in RuBP cycling) would also remain constant. Consequently, Medlyn (1996) estimated that J_{\max}/V_{\max} would increase by an average of 38% in response to a doubling of atmospheric [CO₂]. This should be the case whether the carboxylation rate remained constant, as in the Woodrow calculations, or increased and thus required a greater throughput of energy.

Experimental evidence in support of these model predictions is variable, perhaps reflecting variation in the abundance of other resources, such as N and light (Curtis 1996, Curtis and Wang 1998). For example, there is strong experimental evidence that low irradiance increases investment in chlorophyll-protein complexes to facilitate the capture of scarce photons at the expense of Rubisco and electron carriers (Evans 1989, Pons and Pearcy 1994). Similarly, light attenuation through a *Populus* canopy was directly related to progressively decreasing V_{\max} and J_{\max} , and increasing chlorophyll per leaf N content, from upper to lower canopy leaves (Kull and Niinemets 1998). Because both [CO₂] and light environment induce changes in the stoichiometric balance among reactions that limit photosynthesis, the CO₂-acclimation response has been compared qualitatively to the light-acclimation response (Sage 1994, Kubiske and Pregitzer 1996, Kubiske et al. 1997, DeLucia and Thomas 2000). This has led some investigators to hypothesize that (1) shading and elevated [CO₂] should interact to drive a more complete acclimation response than either factor alone (Kubiske and Pregitzer 1996, Herrick and Thomas 1999, 2001, DeLucia and Thomas 2000) and (2) species that are particularly plastic in their photosynthetic response to shade should also be more responsive to elevated [CO₂] (Kubiske and Pregitzer 1996, Kerstiens 1998).

The purpose of our study was to compare photosynthetic acclimation to elevated [CO₂] between two species with contrasting shade tolerance, and to determine if soil N availability or shading modifies the acclimation response. A shade-tolerant, late successional tree species, *Acer saccharum* Marsh., was grown alone and in combination with a shade-intolerant, early successional species, *Populus tremuloides* Michx., in open-top chambers in a factorial design of [CO₂] and soil N availability for nearly 3 years. The experimental design was such that *P. tremuloides* quickly out grew, and provided the shade treatment for, the much slower growing *A. saccharum* seedlings. Consequently, sun and shade treatment effects were investigated among different *A. saccharum* seedlings alone

and in competition with larger *P. tremuloides* trees, respectively, and within the *P. tremuloides* canopies.

Methods

Experimental design

In March 1997, we propagated ramets of *P. tremuloides* genotypes that occurred naturally at the University of Michigan Biological Station (UMBS) near Pellston, Michigan (45°33'30" N, 84°4'28" W). Ramets were rooted in blocks of compressed peat. The genotypes were previously compared for their responses to elevated [CO₂] and soil N availability (Kubiske et al. 1998, Curtis et al. 2000). We also propagated seedlings of *A. saccharum* from an unknown number of parent trees naturally occurring in northern Michigan. Seeds were sown on March 31, 1997 in 500-ml pots containing a peat:vermiculite:sand (2:1:1; v/v) medium and 0.5 g of slow-release fertilizer (N:P:K, 9:6:6).

We constructed a randomized complete block array of 20 open-bottom root boxes and open-top chambers at UMBS. The array consisted of five blocks with one replicate of a 2 × 2 factor (soil N treatment and [CO₂]) experiment in each block. The root boxes (3.4 m square × 0.5 m deep) were constructed of plywood and rested on the C horizon of a native Entic Haplorthod (Rubicon sand) with no restriction for root penetration into the native soil. A plywood partition divided each box, and one half was further divided into two quarter sections. The boxes were then filled with either a mixture of one part A horizon (Kalkaska series, Typic Haplorthod) and four parts C horizon (Rubicon sand, Entic Haplorthod), referred to as low-N soil, or pure Kalkaska A horizon, referred to as high-N soil. Total N concentrations of the low-N and high-N soils at the beginning of the study were 310 and 1370 mg kg⁻¹, respectively.

On June 9, 1997, six *A. saccharum* and six *P. tremuloides* were planted in separate quarter-box sections (referred to as the *Acer*-only and *Populus*-only chamber sections, respectively). In addition, we planted a mixture of six *P. tremuloides* and six *A. saccharum* in the remaining half-box section. Plantings were consistent in terms of spacing (41 cm) and juxtaposition of species and genotypes within each box. Over the top of each root box we placed a 3-m diameter by 3.6-m tall open-top chamber. The main experimental unit was the box or chamber; box section was treated as the subunit in a split-plot design. Beginning at the time of planting, we injected pure CO₂ into the blower of half the chambers to elevate [CO₂] within the chambers to normal ambient concentration plus 200 μl l⁻¹ (referred to as the elevated [CO₂] treatment). Carbon dioxide concentration was monitored continuously in all of the chambers assigned to the elevated [CO₂] treatment and in one chamber in the ambient [CO₂] treatment with an infrared gas analyzer (Model 6252, Li-Cor, Lincoln, NE) connected to a computer.

Following planting, the *P. tremuloides* ramets quickly overtopped the *A. saccharum* seedlings (except for those in the *Acer*-only section). Thus, our chambers contained a *P. tremu-*

loides canopy with an *A. saccharum* understory. Although we realize that open-top chamber mesocosms do not closely resemble true forest canopy and understory conditions, there was a strong light gradient imposed by *P. tremuloides* and we will refer to these experimental conditions as a “canopy” and “understory” for simplicity.

Light and photosynthesis measurements

During 1997 and 1998, we monitored photosynthetic photon flux density (PPFD) under the aspen foliage in the mixed-species half of the chamber. A Li-Cor quantum sensor was positioned at the apex of an *A. saccharum* seedling that was centrally located within the chamber half. Instantaneous PPFD was measured every 60 s and the 15-min mean was logged continuously from 0500 to 2100 h. A single sensor was positioned outside the chambers to record ambient unshaded PPFD. Daily integrated PPFD was compared among CO₂ × N treatments by a series of paired *t*-tests and the 1998 season-integrated PPFD was compared by ANOVA in a randomized complete block design.

Because there was considerable shading within the chambers, we were able to study the interactive effects of N, [CO₂] and light on photosynthesis. In July of 1997 and 1998 and in late May–early June of 1999, we measured photosynthetic responses to leaf internal [CO₂] (*A/C_i* curves) with a portable photosynthesis system (Model CIRAS-I, PP Systems, Hertfordshire, England). The *A/C_i* curves were measured on a fully expanded leaf from an *A. saccharum* seedling in the *Acer*-only section (i.e., *A. saccharum* sun plants) and in the *Populus–Acer* mixed section (i.e., *A. saccharum* shade plants). Response curves were also measured on a fully expanded, upper canopy sun leaf and a lower canopy shade leaf of a *P. tremuloides* tree in the *Populus–Acer* mixed section (i.e., sun and shade leaves, respectively). Cuvette [CO₂] was increased from about 50 to 1800 μl l⁻¹ in six or seven uneven increments allowing approximately 5 min between measurements for leaf equilibration. At the end of each equilibration period, three measures of *A* and *C_i* (calculated by the system software according to von Caemmerer and Farquhar (1981)) were recorded in rapid succession. Constant photosynthetically active radiation (PAR) of 1300 μmol m⁻² s⁻¹ was supplied by a bank of quartz-halogen lamps. Cuvette temperature and relative humidity were maintained within 4 °C and 50% of ambient, respectively, by a Peltier block and desiccant column. Leaf temperature was measured with an infra-red sensor. Photosynthetic parameters (carboxylation and electron transport maxima, *V_{cmax}* and *J_{max}*, respectively) were derived from the *A/C_i* curves with PHOTOSYN software (Dundee Scientific, Dundee, Scotland). PHOTOSYN employs the Farquhar biochemical models (von Caemmerer and Farquhar 1981) as modified by Harley et al. (1992).

Immediately following the measurement of each *A/C_i* curve in 1998 and 1999 (no destructive sampling was done in 1997), the leaves were collected and stored frozen for leaf N and chlorophyll analyses. Total chlorophyll concentration was determined colorimetrically according to Leegood (1993). Chloro-

phyll was extracted from 0.5–1.0 g fresh weight of ground leaf tissue in 80% acetone in the dark for about 12 h, filtered in a Buchner funnel, and washed until clear. Chlorophyll concentration in the supernatant was calculated from absorbance at 664 and 647 nm measured with a spectrophotometer (Spectronic 20D+, Rochester, NY) and corrected to leaf oven dry weight (70 °C for 48 h). Total leaf N concentration was measured on ground, air-dried leaf samples with a CN combustion analyzer (Carlo Erba NA1500, Milan, Italy).

All leaf chemistry, morphology and photosynthesis data were analyzed with a randomized complete block split-plot ANOVA for each species. Atmospheric [CO₂] and soil N were the main effects and leaf light environment (upper and lower canopy leaves for *P. tremuloides*, open and understory seedlings for *A. saccharum*) was the split plot effect. When necessary, data were log-transformed to satisfy ANOVA assumptions. Means were compared by Fisher's protected least significant difference test.

Results

Light environment

Daily PPFD monitored below the *P. tremuloides* canopy represents the predominant light environment for shade leaves of both *P. tremuloides* and *A. saccharum* (Figure 1). According to paired *t*-tests (based on the mean daily integrated value of all chambers in each treatment combination), daily integrated PPFD was significantly lower in the high-N soil treatment than in the low-N soil treatment, and lower in the elevated [CO₂] treatment than in the ambient [CO₂] treatment in both soil types. These differences reflected treatment differences in total *P. tremuloides* leaf area per chamber (data not shown). Integrated PPFD for the entire 1998 growing season was significantly lower in the high-N chambers than in the low-N chambers (soil N main effect *P* < 0.05), but there was no CO₂ effect.

Leaf morphology and chemistry

Main effects of soil N availability and elevated [CO₂] Neither soil N availability nor elevated [CO₂] significantly affected specific leaf area (SLA) of *P. tremuloides* in 1998 or 1999 (Table 1). The high-N soil treatment significantly increased the leaf N concentration of *P. tremuloides* on both leaf mass (*N_{mass}*) and area (*N_{area}*) bases in both years. Elevated [CO₂] decreased *N_{mass}* in 1998, but not in 1999. In 1999, the high-N soil treatment increased chlorophyll concentration per leaf area, but not per leaf mass. The proportion of *P. tremuloides* leaf N partitioned to chlorophyll–protein complexes was not significantly affected by soil N or [CO₂].

For *Acer saccharum*, elevated [CO₂] significantly reduced SLA in 1999, but not in 1998 (Table 2). As with *P. tremuloides*, leaf N concentration of *A. saccharum* increased in response to the high-N soil treatment. Elevated [CO₂] significantly decreased mass-based N and chlorophyll concentrations. Across both years, elevated [CO₂] decreased *N_{mass}* by 20% in the

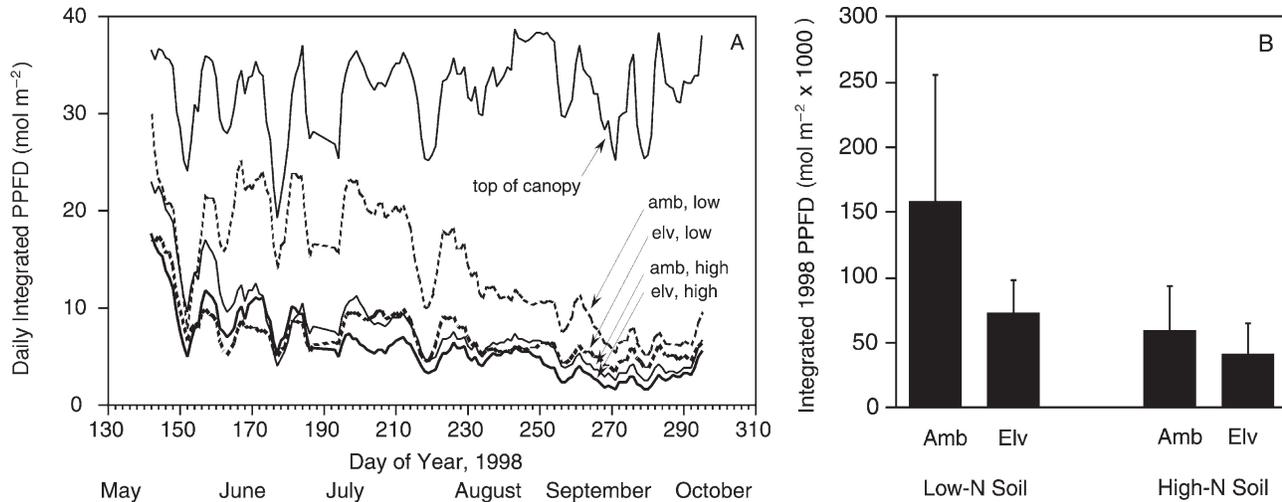


Figure 1. Photosynthetic photon flux density (PPFD) in 1998 measured under *P. tremuloides* trees that were grown in two soil treatments (low N and high N) and in current ambient ($360 \mu\text{l l}^{-1}$) and elevated ($560 \mu\text{l l}^{-1}$) $[\text{CO}_2]$. (A) The 5-day running mean of daily integrated PPFD ($n = 5$). Heavy lines are from elevated $[\text{CO}_2]$ chambers, and broken lines are from low-N soil chambers. (B) Integrated PPFD for the 1998 growing season (mean \pm SE, $n = 5$). Abbreviations: Amb = ambient; and Elv = elevated.

low-N soil treatment and 13% in the high-N soil treatment. In 1999, elevated $[\text{CO}_2]$ decreased chlorophyll concentration by 40% in the low-N soil treatment and by 20% in the high-N soil treatment. In 1999, the partitioning of N to chlorophyll–protein complexes generally declined with elevated $[\text{CO}_2]$, but the effect was not statistically significant ($P = 0.12$).

Shade effects and interactions with $[\text{CO}_2]$ and soil N Shading had several significant effects on the leaf parameters of both species, but the species differed in their responses. Compared with sun leaves, shade leaves in the *P. tremuloides* canopies had significantly greater SLA and less N_{mass} and N_{area} in both years. In 1998, the shade-induced decrease in N_{mass} was

Table 1. Characteristics (mean \pm SE, $n = 5$) of *Populus tremuloides* leaves from upper-canopy (sun) and lower-canopy (shade) positions of trees that were grown for nearly 3 years in two soil treatments (low N and high N) and in current ambient and elevated $[\text{CO}_2]$ (ambient + 200 ppm).

Parameter ¹	Leaf type	Low-N soil		High-N soil	
		Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$	Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$
<i>1998</i>					
Specific leaf area ($\text{cm}^2 \text{g}^{-1}$) ^Q	Sun	135 \pm 6	126 \pm 6	150 \pm 6	129 \pm 5
	Shade	162 \pm 6	159 \pm 9	209 \pm 15	170 \pm 17
N concentration (mg g^{-1}) ^{C,N,Q,N\timesQ}	Sun	23.8 \pm 1.1	20.4 \pm 0.9	38.6 \pm 2.1	32.9 \pm 1.8
	Shade	22.8 \pm 0.7	18.9 \pm 0.9	32.8 \pm 2.3	28.3 \pm 1.9
N concentration (mg cm^{-2}) ^{N,Q}	Sun	0.18 \pm 0.02	0.17 \pm 0.01	0.26 \pm 0.02	0.26 \pm 0.02
	Shade	0.14 \pm 0.01	0.12 \pm 0.01	0.16 \pm 0.01	0.17 \pm 0.01
<i>1999</i>					
Specific leaf area ($\text{cm}^2 \text{g}^{-1}$) ^Q	Sun	144 \pm 9	127 \pm 9	137 \pm 7	139 \pm 9
	Shade	174 \pm 11	159 \pm 4	189 \pm 13	178 \pm 13
N concentration (mg g^{-1}) ^{N,Q}	Sun	28.3 \pm 1.0	25.6 \pm 1.5	33.4 \pm 1.6	30.6 \pm 1.1
	Shade	25.5 \pm 0.5	25.2 \pm 0.9	29.0 \pm 0.9	30.1 \pm 1.7
N concentration (mg cm^{-2}) ^{N,Q}	Sun	0.20 \pm 0.02	0.20 \pm 0.01	0.25 \pm 0.02	0.23 \pm 0.02
	Shade	0.15 \pm 0.01	0.15 \pm 0.01	0.16 \pm 0.01	0.18 \pm 0.02
Total chlorophyll ($\mu\text{mol g}^{-1}$) ^{N\timesQ}	Sun	183 \pm 12	220 \pm 36	282 \pm 30	199 \pm 18
	Shade	207 \pm 9	282 \pm 19	238 \pm 20	189 \pm 33
Total chlorophyll ($\mu\text{mol cm}^{-2}$) ^N	Sun	1.40 \pm 0.08	1.37 \pm 0.11	1.84 \pm 0.27	1.55 \pm 0.12
	Shade	1.32 \pm 0.10	1.24 \pm 0.01	1.54 \pm 0.14	1.51 \pm 0.08
Total chlorophyll N ⁻¹ ($\mu\text{mol mg}^{-1} \text{N}$) ^Q	Sun	7.5 \pm 0.9	6.8 \pm 0.7	7.5 \pm 1.1	6.6 \pm 0.3
	Shade	8.6 \pm 0.2	8.1 \pm 0.3	9.8 \pm 0.9	7.4 \pm 1.2

¹ Significant ($P < 0.05$) treatment effects are indicated by: C = CO_2 main effect, N = soil N main effect, Q = light main effect and N \times Q = N by light interaction.

Table 2. Characteristics (mean \pm SE, $n = 5$) of *Acer saccharum* leaves from unshaded (sun) and shaded understory (shade) seedlings that were grown for nearly 3 years in two soil treatments (low N and high N) and in current ambient and elevated [CO₂] (ambient + 200 ppm).

Parameter ¹	Leaf type	Low-N soil		High-N soil	
		Ambient [CO ₂]	Elevated [CO ₂]	Ambient [CO ₂]	Elevated [CO ₂]
<i>1998</i>					
Specific leaf area (cm ² g ⁻¹) ^{ns}	Sun	165 \pm 12	151 \pm 14	151 \pm 8	163 \pm 18
	Shade	162 \pm 9	149 \pm 9	174 \pm 13	173 \pm 9
N concentration (mg g ⁻¹) ^{C,N}	Sun	18.4 \pm 1.5	16.0 \pm 1.4	28.5 \pm 2.3	22.6 \pm 1.2
	Shade	16.9 \pm 0.9	15.4 \pm 1.0	23.6 \pm 2.7	23.8 \pm 2.3
N concentration (mg m ⁻²) ^N	Sun	0.11 \pm 0.01	0.11 \pm 0.02	0.19 \pm 0.02	0.14 \pm 0.02
	Shade	0.10 \pm 0.01	0.10 \pm 0.01	0.14 \pm 0.02	0.14 \pm 0.02
<i>1999</i>					
Specific leaf area (cm ² g ⁻¹) ^C	Sun	172 \pm 4	145 \pm 12	174 \pm 7	151 \pm 9
	Shade	187 \pm 15	144 \pm 14	187 \pm 11	160 \pm 6
N concentration (mg g ⁻¹) ^{C,N}	Sun	21.0 \pm 1.2	15.6 \pm 0.8	25.0 \pm 1.9	19.5 \pm 2.2
	Shade	21.9 \pm 1.8	15.4 \pm 0.6	20.7 \pm 2.4	19.3 \pm 1.6
N concentration (mg cm ⁻²) ^N	Sun	0.13 \pm 0.01	0.11 \pm 0.02	0.14 \pm 0.01	0.13 \pm 0.02
	Shade	0.12 \pm 0.02	0.11 \pm 0.01	0.11 \pm 0.02	0.12 \pm 0.01
Total chlorophyll (μ mol g ⁻¹) ^{C,Q}	Sun	220 \pm 32	122 \pm 15	214 \pm 19	150 \pm 10
	Shade	344 \pm 31	215 \pm 50	312 \pm 26	272 \pm 20
Total chlorophyll (μ mol cm ⁻²) ^Q	Sun	1.27 \pm 0.48	0.85 \pm 0.06	1.23 \pm 0.11	1.00 \pm 0.06
	Shade	2.00 \pm 0.19	1.60 \pm 0.60	1.74 \pm 0.26	1.68 \pm 0.09
Total chlorophyll N ⁻¹ (μ mol mg ⁻¹ N) ^Q	Sun	9.9 \pm 3.9	7.8 \pm 1.2	8.6 \pm 0.7	8.1 \pm 1.2
	Shade	15.8 \pm 1.1	14.4 \pm 4.7	14.9 \pm 2.0	13.5 \pm 1.1

¹ Significant ($P < 0.05$) treatment effects are indicated by: ns = no significant treatment effects, C = CO₂ main effect, N = soil N main effect and Q = light main effect.

greater in the high-N soil treatment than in the low-N soil treatment resulting in a significant light by soil N interaction. Chlorophyll concentration of *P. tremuloides* was not affected by shading; consequently, the amount of N partitioned to light-harvesting complexes increased.

Shading had no significant effect on SLA or leaf N concentration in *A. saccharum* seedlings (Table 2). Shading had greater effects on the light-harvesting systems in *A. saccharum* than in *P. tremuloides*. Compared with unshaded seedlings, shaded *A. saccharum* seedlings had significantly greater chlorophyll concentrations on both leaf mass and area bases, and N partitioning to chlorophyll increased by 70% across all [CO₂] and N treatment combinations.

A/C_i responses

Main effects of soil N availability and elevated CO₂ In 1997, little shading developed in the chambers and, with few exceptions, the *A. saccharum* seedlings did not produce new leaves following planting. Nonetheless, in both *P. tremuloides* and *A. saccharum*, elevated [CO₂] decreased J_{\max} and V_{\max} in both soil treatments (Tables 3 and 4). In 1998 and 1999, J_{\max} and V_{\max} of both species were generally decreased by elevated [CO₂] and increased by the high-N soil treatment. These changes were seen on both leaf area and leaf mass bases, indicating that they were not driven by treatment differences in SLA. Thus, both species exhibited biochemical changes in photosynthetic capacity in response to elevated [CO₂] and high-N soil. The relationship between J_{\max} and V_{\max} was not al-

tered by elevated [CO₂] across all species and treatment combinations (Figure 2).

Shade effects and interactions with [CO₂] and soil N In 1998 and 1999, shade leaves in the lower *P. tremuloides* canopies had significantly lower V_{\max} but similar J_{\max} compared with sun leaves. Although the light by CO₂ interactions were not significant, the shade-induced decreases in V_{\max} were more than twice as large in the elevated [CO₂] treatment (28% decrease across both soils and years) as in the ambient [CO₂] treatment (13% decrease across both soils and years). This CO₂ effect was not an artifact of increased shading in response to elevated [CO₂] because a plot of below-canopy PPFD versus the change in V_{\max} from sun to shade leaves in 1998 (PPFD was not monitored in 1999) indicated that the two CO₂ treatments were on different trajectories (Figure 3). Therefore, the difference in magnitude of the CO₂-related changes in V_{\max} between sun and shade leaves of *P. tremuloides* was the result of an interactive effect of [CO₂] and light conditions. However, shading did not decrease V_{\max} of *P. tremuloides* on a leaf mass basis, suggesting that SLA played an important role.

Similar to that in *P. tremuloides*, the CO₂ by light interaction was not significant for *A. saccharum*. However, there was a pattern in the CO₂-related changes in J_{\max} and V_{\max} of sun and shade leaves in high-N soil. In high-N soil, elevated [CO₂] decreased J_{\max} and V_{\max} by 12 to 28% in sun leaves, but caused a 19% decrease to a 5% increase in these parameters in shade leaves. This pattern occurred for both area- and mass-based parameters, and was stronger in 1998 than in 1999.

Table 3. Maximum rates of electron transport (J_{\max}) and carboxylation (V_{\max}) (mean \pm SE, $n = 5$) calculated from photosynthetic response curves to internal $[\text{CO}_2]$ (A/C_i curves) for *Populus tremuloides*. Trees were grown for nearly 3 years in two soil treatments (low N and high N) and in current ambient and elevated $[\text{CO}_2]$ (ambient + 200 ppm).

Year	Parameter ¹	Leaf type	Low-N soil		High-N soil	
			Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$	Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$
<i>Leaf area basis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)</i>						
1997	J_{\max}^{C}		217.5 \pm 17.8	165.5 \pm 9.6	195.8 \pm 17.8	186.3 \pm 13.5
	V_{\max}^{C}		73.8 \pm 6.9	53.4 \pm 5.9	73.8 \pm 7.9	67.8 \pm 6.1
1998	$J_{\max}^{\text{C,N}}$	Sun	105.1 \pm 8.5	95.4 \pm 7.9	176.8 \pm 35.8	124.8 \pm 15.9
		Shade	99.4 \pm 5.6	87.2 \pm 7.5	173.5 \pm 32.2	82.9 \pm 13.1
1999	$V_{\max}^{\text{C,N,Q}}$	Sun	48.1 \pm 3.7	45.5 \pm 2.4	71.0 \pm 7.9	56.8 \pm 9.2
		Shade	45.2 \pm 1.8	28.8 \pm 2.0	60.7 \pm 4.9	38.3 \pm 7.3
	$J_{\max}^{\text{C,N}}$	Sun	75.9 \pm 6.5	65.2 \pm 7.8	93.6 \pm 7.8	68.4 \pm 2.7
		Shade	65.4 \pm 8.4	47.5 \pm 5.5	74.2 \pm 7.4	57.2 \pm 4.9
$V_{\max}^{\text{C,N,Q}}$	Sun	20.0 \pm 1.4	17.8 \pm 3.2	31.2 \pm 3.8	19.0 \pm 2.1	
	Shade	16.4 \pm 1.1	13.0 \pm 1.0	26.2 \pm 4.4	16.4 \pm 1.0	
<i>Leaf mass basis ($\mu\text{mol g}^{-1} \text{s}^{-1}$)</i>						
1998	$J_{\max}^{\text{C,N,Q}}$	Sun	1.45 \pm 0.12	1.33 \pm 0.09	2.28 \pm 0.35	1.66 \pm 0.29
		Shade	1.91 \pm 0.10	1.49 \pm 0.13	2.71 \pm 0.42	1.41 \pm 0.19
	V_{\max}^{C}	Sun	0.67 \pm 0.06	0.64 \pm 0.03	0.94 \pm 0.13	0.76 \pm 0.16
		Shade	0.88 \pm 0.07	0.49 \pm 0.03	1.04 \pm 0.29	0.65 \pm 0.11
1999	$J_{\max}^{\text{C,N,Q}}$	Sun	1.01 \pm 0.11	0.84 \pm 0.13	1.17 \pm 0.19	0.94 \pm 0.03
		Shade	1.09 \pm 0.20	0.82 \pm 0.11	1.73 \pm 0.26	1.22 \pm 0.23
	$V_{\max}^{\text{C,N}}$	Sun	0.29 \pm 0.02	0.23 \pm 0.05	0.38 \pm 0.06	0.23 \pm 0.02
		Shade	0.25 \pm 0.02	0.22 \pm 0.02	0.50 \pm 0.09	0.34 \pm 0.05

¹ Significant ($P < 0.05$) treatment effects are indicated by: C = CO_2 main effect, N = soil N main effect and Q = light main effect.

Table 4. Maximum rates of electron transport (J_{\max}) and carboxylation (V_{\max}) (mean \pm SE, $n = 5$) calculated from photosynthesis response curves to internal $[\text{CO}_2]$ (A/C_i curves) for *Acer saccharum*. Trees were grown for nearly 3 years in two soil treatments (low N and high N) and in current ambient and elevated $[\text{CO}_2]$ (ambient + 200 ppm).

Year	Parameter ¹	Leaf type	Low-N soil		High-N soil	
			Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$	Ambient $[\text{CO}_2]$	Elevated $[\text{CO}_2]$
<i>Leaf area basis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)</i>						
1997	J_{\max}^{C}		92.4 \pm 6.4	63.8 \pm 5.6	91.8 \pm 6.8	71.9 \pm 4.9
	V_{\max}^{C}		29.6 \pm 2.1	20.3 \pm 1.9	32.2 \pm 3.5	24.9 \pm 2.9
1998	$J_{\max}^{\text{C,N}}$	Sun	49.3 \pm 4.4	31.9 \pm 4.1	61.3 \pm 2.3	49.1 \pm 2.9
		Shade	49.7 \pm 9.3	38.4 \pm 7.4	47.1 \pm 4.7	47.6 \pm 5.8
1999	$V_{\max}^{\text{C,N}}$	Sun	18.0 \pm 1.9	11.5 \pm 2.0	26.4 \pm 1.7	19.0 \pm 0.9
		Shade	20.6 \pm 4.7	15.1 \pm 3.8	19.7 \pm 1.6	19.5 \pm 2.0
	$J_{\max}^{\text{C,N}}$	Sun	56.3 \pm 10.3	28.3 \pm 5.0	60.9 \pm 5.2	53.5 \pm 2.6
		Shade	58.0 \pm 13.1	27.6 \pm 4.3	41.0 \pm 7.6	43.4 \pm 2.9
$V_{\max}^{\text{C,N}}$	Sun	15.6 \pm 10.3	6.4 \pm 1.8	16.8 \pm 1.7	14.4 \pm 2.6	
	Shade	17.4 \pm 4.4	8.0 \pm 1.5	13.5 \pm 2.7	13.1 \pm 1.5	
<i>Leaf mass basis ($\mu\text{mol g}^{-1} \text{s}^{-1}$)</i>						
1998	J_{\max}^{C}	Sun	0.80 \pm 0.16	0.51 \pm 0.19	0.96 \pm 0.06	0.80 \pm 0.11
		Shade	0.81 \pm 0.19	0.69 \pm 0.13	0.78 \pm 0.05	0.72 \pm 0.09
	$V_{\max}^{\text{C,N}}$	Sun	0.29 \pm 0.05	0.19 \pm 0.05	0.42 \pm 0.03	0.31 \pm 0.04
		Shade	0.34 \pm 0.10	0.27 \pm 0.07	0.31 \pm 0.02	0.30 \pm 0.03
1999	J_{\max}^{C}	Sun	1.01 \pm 0.16	0.40 \pm 0.07	1.05 \pm 0.07	0.79 \pm 0.17
		Shade	0.99 \pm 0.22	0.38 \pm 0.05	0.78 \pm 0.17	0.69 \pm 0.04
	V_{\max}^{C}	Sun	0.28 \pm 0.05	0.09 \pm 0.03	0.29 \pm 0.03	0.22 \pm 0.05
		Shade	0.24 \pm 0.08	0.11 \pm 0.02	0.26 \pm 0.06	0.21 \pm 0.03

¹ Significant ($P < 0.05$) treatment effects are indicated by: C = CO_2 main effect, N = soil N main effect and Q = light main effect.

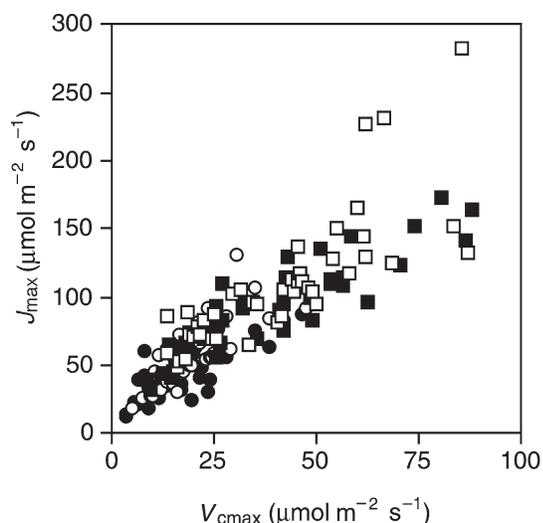


Figure 2. Scatter plot of carboxylation capacity (V_{cmax}) versus electron transport capacity (J_{max}) of *Populus tremuloides* (□, ■) and *Acer saccharum* (○, ●) trees grown in current ambient ($360 \mu\text{l l}^{-1}$; □, ○) and elevated ($560 \mu\text{l l}^{-1}$; ■, ●) $[\text{CO}_2]$ in open-top chambers for 3 years.

Discussion

We attempted to determine if photosynthetic acclimation to elevated $[\text{CO}_2]$ interacts with shade conditions in a manner consistent with a species' shade tolerance. In general, *P. tremuloides* acclimated more strongly to elevated $[\text{CO}_2]$, whereas *A. saccharum* acclimated more strongly to shade. We note that

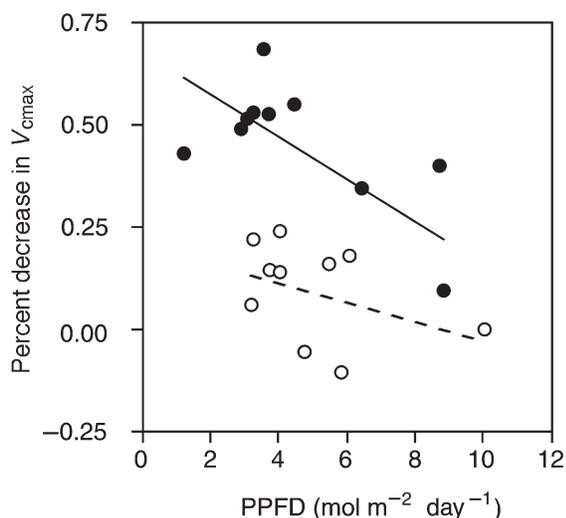


Figure 3. Percent decrease in carboxylation capacity (V_{cmax}) from sun to shade leaves in the crowns of *Populus tremuloides* trees versus mean daily integrated light environment (photosynthetic photon flux density, PPFD) of the shade leaves for the 10-day period preceding V_{cmax} measurements in July 1998. Trees were grown in ambient ($360 \mu\text{l l}^{-1}$, ○) and elevated $[\text{CO}_2]$ ($560 \mu\text{l l}^{-1}$, ●). Each value represents data from one open-top chamber. Least-squares regression lines are: ambient $[\text{CO}_2]$ $y = 0.21 - 0.02x$, $r^2 = 0.13$; elevated $[\text{CO}_2]$ $y = 0.65 - 0.04x$, $r^2 = 0.46$.

interpretation of these results must be made with caution because the shade and sun effects on *P. tremuloides* occurred within the same trees, whereas the shade and sun effects on *A. saccharum* occurred between seedlings that were and were not, respectively, under competition from much larger *P. tremuloides* saplings. For *P. tremuloides*, leaf N concentrations were generally lower in shade leaves than in sun leaves, particularly on a leaf area basis, indicating internal competition for N among leaves in different light environments (Hollinger 1996, Kull and Niinemets 1998, Schoettle and Smith 1999, Takeuchi et al. 2001). In contrast, leaf N concentration did not differ between sun and shade plants of *A. saccharum*, indicating that neither shaded conditions nor competition with *P. tremuloides* affected leaf N concentration.

Decreases in SLA and leaf N concentrations in response to elevated $[\text{CO}_2]$ have been widely reported (Gunderson and Wullschlegel 1994, Curtis 1996, Drake et al. 1997, Saxe et al. 1998). In both of our study species, elevated $[\text{CO}_2]$ had a stronger effect on leaf N concentration than on SLA. Decreased leaf N concentrations are often associated with increases in non-structural biomass or decreases in protein concentrations (Cotrufo et al. 1998). Values of SLA may decrease in elevated $[\text{CO}_2]$ as a result of an increase in structural leaf tissue per unit area, or simply an accumulation of starch and other non-structural carbohydrates (Curtis et al. 2000). In a recent study of *P. tremuloides*, the decrease in leaf N concentration in response to elevated $[\text{CO}_2]$ was attributed largely to a decrease in Rubisco concentration (Takeuchi et al. 2001). In our study, the result of the modest changes in SLA of *A. saccharum* in response to elevated $[\text{CO}_2]$ was that amounts of N and chlorophyll per unit leaf area remained constant despite significant CO_2 -related decreases in their mass-based concentrations. Implicit in our hypothesis was that elevated $[\text{CO}_2]$ would affect N partitioning to light-harvesting complexes (LHCs), similar to the shade response of shade-tolerant species, as part of a general mechanism that increases energy flow through the photosynthetic system. Photosynthetic N-optimization models indicate redistribution of N away from Rubisco toward light harvesting in shade (Sage 1990, Hikosaka and Terashima 1995) and toward electron transport capacity in elevated $[\text{CO}_2]$ (Sage 1990, Medlyn 1996). There is strong experimental evidence for the shade effect (Evans 1989, Pons and Pearcy 1994), whereas evidence for the $[\text{CO}_2]$ effect has been found in some species (Tissue et al. 1999, Liozon et al. 2000), but not in others (DeLucia and Thomas 2000, Herrick and Thomas 2001). Both of our study species exhibited increases in the chlorophyll/N ratio in response to shading (more so for *A. saccharum* than for *P. tremuloides*), indicating increased investment of N in LHCs. However, elevated $[\text{CO}_2]$ had no effect on the chlorophyll/N ratio in either species. These results, and the N optimization models, do not support the idea that elevated $[\text{CO}_2]$ affects N partitioning to LHCs in parallel with the degree of shade tolerance of the species.

We generally found greater photosynthetic capacity for *P. tremuloides* than for *A. saccharum* in all treatment combinations, and greater photosynthetic capacity for both species grown in high-N soil compared with low-N soil. We also

found significant decreases in photosynthetic capacity in both species in all 3 years in the elevated $[\text{CO}_2]$ treatment. Photosynthetic acclimation to elevated $[\text{CO}_2]$ is often considered to be a negative feedback response to the accumulation of starch in photosynthetic tissues caused by reduced export to sinks. However, significant acclimation was reported for several species freely rooted in the ground (Rey and Jarvis 1998, Turnbull et al. 1998, Li et al. 1999, Tissue et al. 1999, Griffin et al. 2000). In their meta-analysis of over 500 studies, Curtis and 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 about evenly split among those with downward acclimation, no effect and upward acclimation. Reduced photosynthate export may result from physical limitations to carbohydrate transport (Thomas and Strain 1991, Myers et al. 1999), phenological changes in sink activity (Kubiske et al. 1998, Lewis et al. 1999), or nutrient limitations (Kubiske et al. 1997, Curtis et al. 2000). In our study, the CO_2 by N interaction was not significant for J_{max} or V_{cmax} in any year or species. Nonetheless, elevated- $[\text{CO}_2]$ -induced decreases in both area and mass-based J_{max} and V_{cmax} were generally larger for *A. saccharum* seedlings growing in low-N soil (38–44%) than in high-N soil (9–18%), supporting the notion that photosynthetic acclimation to $[\text{CO}_2]$ varies directly with nutrient limitations.

We found significant effects of light environment on photosynthetic parameters of *P. tremuloides*. Leaf area-based V_{cmax} of *P. tremuloides* decreased from sun leaves to shade leaves, and the decrease was generally greater in elevated $[\text{CO}_2]$. That is, shade leaves of *P. tremuloides* had greater photosynthetic acclimation to elevated $[\text{CO}_2]$ than sun leaves. A similar pattern was observed in a *P. tremuloides* canopy grown in free-air CO_2 enrichment (FACE) (Takeuchi et al. 2001), supporting the hypothesis that shade leaves acclimate to elevated $[\text{CO}_2]$ to a greater extent than sun leaves. However, changes in SLA have the potential to affect photosynthetic measurements when they are expressed on leaf area bases simply because of variation in the amount of photosynthetic mass per unit area. When expressed on a leaf mass basis, the light effect on V_{cmax} was eliminated. Thus, taking into account sun–shade differences in SLA, V_{cmax} of *P. tremuloides* shade leaves in elevated $[\text{CO}_2]$ reflected the combined influence of increased SLA in response to shade and decreased photosynthetic biochemistry in response to elevated $[\text{CO}_2]$.

Compared with *P. tremuloides*, *A. saccharum* generally had greater elevated $[\text{CO}_2]$ -induced acclimation in sun leaves compared with shade leaves but only in high-N soil and there were no CO_2 or light effects on SLA. The sun and shade effects on *A. saccharum* were confounded by competition with *P. tremuloides* and a greater amount of shading was imposed by *P. tremuloides* in high-N soil than in low-N soil. Thus, we would expect to see greater shade effects on *A. saccharum* in the high-N soil. The photosynthetic acclimation of *A. saccharum* sun leaves in elevated $[\text{CO}_2]$ may have been a result of either carbohydrate feedback inhibition or some form of photo-damage in this determinate growth shade-tolerant species.

The elevated $[\text{CO}_2]$ treatment caused significant decreases in the photosynthetic capacities of the two sympatric tree species with contrasting life-history traits. As has been reported for other species (Rey and Jarvis 1998, Turnbull et al. 1998, Li et al. 1999, Tissue et al. 1999, Griffin et al. 2000), our results demonstrate that trees rooted freely in the ground can exhibit photosynthetic acclimation to elevated $[\text{CO}_2]$. Our results further demonstrate that such acclimation can interact with light environment. There was no evidence that elevated $[\text{CO}_2]$ altered the $J_{\text{max}}/V_{\text{cmax}}$ relationship as mechanistic N-optimization models have predicted. Furthermore, there was no evidence that shade-tolerance characteristics, such as the response of *A. saccharum* to increase N partitioning to LHCs, modify photosynthetic acclimation to elevated $[\text{CO}_2]$. Nonetheless, leaf-area based measures of photosynthetic parameters may be affected by interactions between elevated $[\text{CO}_2]$ and leaf light environment that are driven by changes in SLA. The interaction between photosynthetic acclimation to elevated $[\text{CO}_2]$ and light environment is potentially important in the competitive interactions between species. In addition, greater acclimation of shade leaves than sun leaves in forest canopies may limit the response to elevated $[\text{CO}_2]$ at the whole-canopy level in terms of both C assimilation and leaf area development.

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References

- Cotrufo, M.F., P. Ineson and A. Scott. 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biol.* 4: 43–54.
- Curtis, P.S. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell Environ.* 19:127–137.
- Curtis, P.S. and X. Wang. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* 113:299–313.
- Curtis, P.S., C.S. Vogel, X. Wang, K.S. Pregitzer, D.R. Zak, J. Lussenhop, M.E. Kubiske and J.A. Teeri. 2000. Gas exchange, leaf nitrogen, and growth efficiency of *Populus tremuloides* in a CO_2 -enriched atmosphere. *Ecol. Appl.* 10:3–17.
- DeLucia, E.H. and R.B. Thomas. 2000. Photosynthetic responses to CO_2 enrichment of four hardwood species in a forest understory. *Oecologia* 122:11–19.
- Drake, B.G., M.A. Gonzalez-Meier and S.P. Long. 1997. More efficient plants: a consequence of rising CO_2 ? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:608–639.
- Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78:9–19.

- Griffin, K.L., D.T. Tissue, M.H. Turnbull and D. Whitehead. 2000. The onset of photosynthetic acclimation to elevated CO₂ partial pressure in field-grown *Pinus radiata* D. Don after 4 years. *Plant Cell Environ.* 23:1089–1098.
- Gunderson, C.A. and S.D. Wullschleger. 1994. Photosynthetic acclimation in trees to rising atmospheric CO₂: A broader perspective. *Photosynth. Res.* 39:369–388.
- Harley, P.C., R.B. Thomas, J.F. Reynolds and B.R. Strain. 1992. Modeling photosynthesis of cotton grown in elevated CO₂. *Plant Cell Environ.* 15:271–282.
- Herrick, J.D. and R.B. Thomas. 1999. Effects of CO₂ enrichment on the photosynthetic light response of sun and shade leaves of canopy sweetgum trees (*Liquidambar styraciflua*) in a forest ecosystem. *Tree Physiol.* 19:779–786.
- Herrick, J.D. and R.B. Thomas. 2001. No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO₂ enrichment at the Duke Forest FACE experiment. *Plant Cell Environ.* 24:53–64.
- Hikosaka, K. and I. Terashima. 1995. A model of the acclimation of photosynthesis in the leaves of C₃ plants to sun and shade with respect to nitrogen use. *Plant Cell Environ.* 18:605–618.
- Hollinger, D.Y. 1996. Optimality and nitrogen allocation in a tree canopy. *Tree Physiol.* 16:627–634.
- Kerstiens, G. 1998. Shade-tolerance as a predictor of responses to elevated CO₂ in trees. *Physiol. Plant.* 102:472–480.
- Kubiske, M.E. and K.S. Pregitzer. 1996. Effects of elevated CO₂ and light availability on the photosynthetic light response of trees of contrasting shade tolerance. *Tree Physiol.* 16:351–358.
- Kubiske, M.E., K.S. Pregitzer, C.J. Mikan, D.R. Zak, J.L. Maziasz and J.A. Teeri. 1997. *Populus tremuloides* photosynthesis and crown architecture in response to elevated CO₂ and soil N availability. *Oecologia* 110:328–336.
- Kubiske, M.E., K.S. Pregitzer, C.J. Mikan and D.R. Zak. 1998. Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO₂ and soil N availability. *New Phytol.* 140:251–260.
- Kull, O. and Ü. Niinemets. 1998. Distribution of leaf photosynthetic properties in tree canopies: comparison of species with different shade tolerance. *Funct. Ecol.* 12:472–479.
- Leegood, R.C. 1993. Carbon metabolism. In *Photosynthesis and Production in a Changing Environment*. Eds. D.O. Hall et al. Chapman and Hall, New York, 464 p.
- Lewis, J.D., D. Olszyk and D.T. Tingey. 1999. Seasonal patterns of photosynthetic light response in Douglas-fir seedlings subjected to elevated atmospheric CO₂ and temperature. *Tree Physiol.* 19:243–252.
- Li, J.-H., P. Dijkstra, C.R. Hinkle, R.M. Wheeler and B.G. Drake. 1999. Photosynthetic acclimation to elevated atmospheric CO₂ concentration in the Florida scrub-oak *Quercus geminata* and *Quercus myrtifolia* growing in their native environment. *Tree Physiol.* 19:229–234.
- Liozon, R., F.-W. Badeck, G. Bernard, S. Meyer and B. Saugier. 2000. Leaf photosynthetic characteristics of beech (*Fagus sylvatica*) saplings during three years of exposure to elevated CO₂ concentration. *Tree Physiol.* 20:239–247.
- Medlyn, B.E. 1996. The optimal allocation of nitrogen within the C₃ photosynthetic system at elevated CO₂. *Aust. J. Plant Physiol.* 23:593–603.
- Myers, D.A., R.B. Thomas and E.H. DeLucia. 1999. Photosynthetic responses of loblolly pine (*Pinus taeda*) needles to experimental reduction in sink demand. *Tree Physiol.* 19:235–242.
- Norby, R.J., S.D. Wullschleger, C.A. Gunderson, D.W. Johnson and R. Ceulemans. 1999. Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell Environ.* 22:683–714.
- Pons, T.L. and R.W. Pearcy. 1994. Nitrogen reallocation and photosynthetic acclimation in response to partial shading in soybean plants. *Physiol. Plant.* 92:636–644.
- Rey, A. and P.G. Jarvis. 1998. Long-term photosynthetic acclimation to increased atmospheric CO₂ concentration in young birch (*Betula pendula*) trees. *Tree Physiol.* 18:441–450.
- Sage, R.F. 1990. A model describing the regulation of ribulose-1,5-bisphosphate carboxylase, electron transport, and triose phosphate use in response to light intensity and CO₂ in C₃ plants. *Plant Physiol.* 94:1728–1734.
- Sage, R.F. 1994. Acclimation of photosynthesis to increasing atmospheric CO₂: the gas exchange perspective. *Photosynth. Res.* 39:351–368.
- Saxe, H., D.S. Ellsworth and J. Heath. 1998. Tree and forest functioning in an enriched CO₂ atmosphere. *Tansley Review No. 98*. *New Phytol.* 139:395–436.
- Schoettle, A.W. and W.K. Smith. 1999. Interrelationships among light, photosynthesis and nitrogen in the crown of mature *Pinus contorta* spp. *latifolia*. *Tree Physiol.* 19:13–22.
- Takeuchi, Y., M.E. Kubiske, J.G. Isebrands, K.S. Pregitzer, G.R. Hendrey and D.F. Karnosky. 2001. Photosynthesis, light and nitrogen interrelationships throughout a young *Populus tremuloides* canopy under open air CO₂ enrichment. *Plant Cell Environ.* 24:1257–1268.
- Thomas, R.B. and B.R. Strain. 1991. Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. *Plant Physiol.* 96:627–634.
- Tissue, D.T., K.L. Griffin and T. Ball. 1999. Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO₂. *Tree Physiol.* 19:221–228.
- Turnbull, M.H., D.T. Tissue, K.L. Griffin, G.N.D. Rogers and D. Whitehead. 1998. Photosynthetic acclimation to long-term exposure to elevated CO₂ concentration in *Pinus radiata* D. Don is related to age of needles. *Plant Cell Environ.* 21:1019–1028.
- von Caemmerer, S. and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376–387.
- Woodrow, I.E. 1994. Optimal acclimation of the C₃ photosynthetic system under enhanced CO₂. *Photosynth. Res.* 39:401–412.