

Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient carbon dioxide concentrations

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Summary We examined the effects of elevated CO₂ concentration ([CO₂]) on leaf demography, late-season photosynthesis and leaf N resorption of overstory sweetgum (*Liquidambar styraciflua* L.) trees in the Duke Forest Free Air CO₂ Enrichment (FACE) experiment. Sun and shade leaves were subdivided into early leaves (formed in the overwintering bud) and late leaves (formed during the growing season). Overall, we found that leaf-level net photosynthetic rates were enhanced by atmospheric CO₂ enrichment throughout the season until early November; however, sun leaves showed a greater response to atmospheric CO₂ enrichment than shade leaves. Elevated [CO₂] did not affect leaf longevity, emergence date or abscission date of sun leaves or shade leaves. Leaf number and leaf area per shoot were unaffected by CO₂ treatment. A simple shoot photosynthesis model indicated that elevated [CO₂] stimulated photosynthesis by 60% in sun shoots, but by only 3% in shade shoots. Whole-shoot photosynthetic rate was more than 12 times greater in sun shoots than in shade shoots. In senescent leaves, elevated [CO₂] did not affect residual leaf nitrogen, and nitrogen resorption was largely unaffected by atmospheric CO₂ enrichment, except for a small decrease in shade leaves. Overall, elevated [CO₂] had little effect on the number of leaves per shoot at any time during the season and, therefore, did not change seasonal carbon gain by extending or shortening the growing season. Stimulation of carbon gain by atmospheric CO₂ enrichment in sweetgum trees growing in the Duke Forest FACE experiment was the result of a strong stimulation of photosynthesis throughout the growing season.

Keywords: leaf demography, nitrogen resorption, FACE, carbon gain, leaf phenology, carbon balance, ontogeny.

Introduction

Elevated atmospheric CO₂ concentration ([CO₂]) stimulates net photosynthesis and growth of woody species under a variety of environmental conditions (Drake et al. 1996, Curtis and Wang 1998, Norby et al. 1999). Long-term experiments have

shown sustained photosynthetic enhancement in response to several years of exposure to elevated [CO₂] (Gunderson et al. 1993, Norby et al. 1999, Herrick and Thomas 2001). An aspect of this stimulation that has been given little attention is whether elevated [CO₂] changes leaf phenology during the growing season, especially in natural systems where nutrients, light and water may be limiting. Two factors that should be considered when investigating patterns of carbon uptake during the growing season are the number of leaves maintained on the plant at different times during the season and the photosynthetic rates of these leaves, especially in the late season. The response of photosynthesis to elevated [CO₂] as a function of leaf age and CO₂-induced changes in leaf longevity could affect seasonal integrated carbon gain (Nelson et al. 1982). Changes in leaf senescence of canopy trees could affect nutrient availability by altering residual nutrients in leaf litter (Norby et al. 2000) and could indirectly affect understory plants by altering light availability (McConnaughay et al. 1996). These effects cannot be determined by studies that focus on instantaneous measurements taken only during mid-growth season.

Timing of leaf senescence can have a significant impact on ecosystem productivity. For example, Goulden et al. (1996) found that the annual gross production of a temperate deciduous forest was increased by about 500 kg C ha⁻¹ when the canopy senesced 5–10 days later in the year. Studies of leaf senescence in response to atmospheric CO₂ enrichment in a variety of herbaceous and woody species have produced conflicting results. Increased growth in elevated [CO₂] has been accompanied by earlier leaf and whole-plant senescence in some herbaceous species (St. Omer and Hovarth 1983, Paez et al. 1983) and later senescence in other species (Curtis et al. 1989). Li et al. (2000) found that leaf senescence of *Quercus myrtifolia* W. in an oak scrubland was delayed by atmospheric CO₂ enrichment. Gunderson et al. (1993) found that elevated [CO₂] had no significant effect on the rate of leaf fall in white oak and tulip poplar saplings. These experiments were per-

formed with a variety of methods ranging from controlled indoor growth chambers to open top chambers in the field. There are no reports of the effects of atmospheric CO₂ enrichment on leaf senescence of sun and shade leaves of trees growing in a forest ecosystem.

Leaf senescence is generally controlled by environmental cues such as day length and air temperature, and it has been hypothesized that the nutrient status of the plant can also alter rates of senescence (Thomas and Stoddart 1980). Resorption of foliar nitrogen (N) is an important process by which plants enhance nitrogen-use efficiency. Nutrient resorption is defined as the process by which nutrients are mobilized from senescing leaves and transported to other plant parts (Killingbeck 1986). When N is resorbed and stored in permanent tissues, it can be used for early growth in the subsequent year when N availability and uptake from the soil may be insufficient to meet the demands of developing foliage (Kang et al. 1982). In response to atmospheric CO₂ enrichment, N resorption may become an increasingly important N source as soil nutrients become depleted under rapidly growing vegetation.

Elevated [CO₂] may also affect the ontogeny of leaf photosynthesis. In general, leaves of dicotyledonous plants go through two distinct phases of ontogeny with respect to photosynthesis (Gepstein 1988). In the first phase, leaf photosynthesis increases as the leaf expands and reaches a peak following leaf maturation. In the second phase, photosynthetic rate slowly declines during a prolonged period of senescence. Several studies on herb and crop plants have reported an interaction between the relative stimulatory effects of elevated [CO₂] on photosynthesis and leaf age: there is a time-dependent down-regulation of photosynthesis as leaves age in elevated [CO₂] (Nie et al. 1995, Pearson and Brooks 1995, van Oosten and Besford 1995). Thus, it is important to understand the ontogeny of leaf photosynthesis in order to integrate the response to elevated [CO₂] across an entire growth season.

Our objective was to determine if elevated [CO₂] affects leaf senescence and late-season photosynthesis of overstory sweetgum (*Liquidambar styraciflua* L.) trees at the Duke Forest Free Air CO₂ Enrichment (FACE) experiment. Sweetgum is the most common deciduous tree species in the Duke Forest FACE experiment and is interspersed naturally among the dominant loblolly pine (*Pinus taeda* L.) trees. Differential light environments at the top and bottom of the loblolly pine forest canopy produce distinct sun and shade leaf types in sweetgum trees. Generally, sun leaves of sweetgum trees are thicker, have more N per unit leaf area and have greater photosynthetic capacity than shade leaves (Herrick and Thomas 1999, Herrick and Thomas 2001). In previous studies, we found that elevated [CO₂] stimulates net photosynthesis of sweetgum by 63% in sun leaves and 48% in shade leaves, and this enhancement has been sustained during more than 3 years of atmospheric CO₂ enrichment (Herrick and Thomas 2001). However, little is known about the effects of elevated [CO₂] on late-season photosynthesis and leaf senescence of sun or shade leaves of sweetgum trees. We hypothesized that atmospheric CO₂ enrichment would delay senescence of sun and

shade leaves, because leaves exposed to elevated [CO₂] would retain a positive carbon balance later in the season. Sweetgum trees have shoots that exhibit indeterminate monopodial growth and leaves that are produced continuously across the growth season. Thus, we followed demography and late-season gas exchange of leaves produced at the beginning of the season as well as leaves produced later in the season.

Materials and methods

Duke Forest Free-Air CO₂ Enrichment (FACE) experiment

The Duke Forest FACE experiment is located in a *Pinus taeda* plantation in the Blackwood division of the Duke Forest (35°97' N, 79°09' W). No management measures have been taken to prevent the growth of other tree species since the current plantation was established in 1983 after a clear-cut in 1979. As a result, the forest is dominated by loblolly pine (1733 stems ha⁻¹), but there are significant numbers of sweetgum (620 stems ha⁻¹) and yellow poplar (*Liriodendron tulipifera* L., 68 stems ha⁻¹), as well as other hardwood species in the canopy and the understory. Sweetgum is quite common in the southeastern USA and in piedmont North Carolina, and it invades early succession broomsedge (*Anthropogon virginicus* L.) fields during secondary succession (Oosting 1942). The Duke Forest FACE experiment is located on a nutrient-poor, clay-rich loam soil that is typical of many upland areas in southeastern USA.

Within this forest, six 30-m-diameter experimental circular plots were established. Three of these FACE rings are replicate CO₂ treatments and the remaining three plots are ambient experimental controls. Each FACE ring consists of 32 vertical pipes that extend from the forest floor through the forest canopy. In the treatment FACE rings, pipes deliver a controlled amount of CO₂ throughout the entire forest volume with a target [CO₂] of ambient + 200 µl l⁻¹. Three control rings receive the same volume of air to replicate any micrometeorological effects on the forest that occur during the operation of the FACE facility. The Duke Forest FACE experiment began full operation in August 1996, and since then the elevated [CO₂] treatment has been applied continuously, except when the air temperature is below 5 °C for more than an hour. During the first 2 years of the experiment (1997 and 1998), the mean daytime [CO₂] was 574 µl l⁻¹ in the elevated [CO₂] rings and 379 µl l⁻¹ in the ambient control rings. To control for topographic variation (~5 m) and potential gradients in site fertility between rings, the three control and three elevated [CO₂] rings were arranged in a complete block design (three pairs).

Mean minimum and maximum air temperatures during September were 21.6 and 34.9 °C, respectively. In October, mean minimum and maximum air temperatures were 19.2 and 34.0 °C, respectively. In November, mean minimum and maximum air temperatures were 14.5 and 27.1 °C, respectively. A moderate drought occurred during late July, August and early September 1998 (Figure 1). Soil water content averaged about 17% throughout September and October and about 25% in November (K. Schäfer, Duke University, unpublished data).

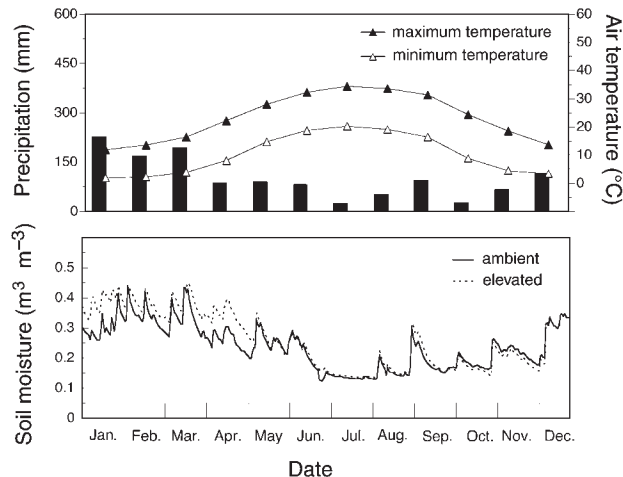


Figure 1. Environmental conditions at the Duke Forest FACE experiment during the second year of CO₂ treatment (1998). Parameters include total monthly precipitation, mean monthly maximum and minimum temperatures and soil water content. Measurements began in mid-April and ended in mid-November.

There were no differences in soil water content between the elevated and ambient [CO₂] treatments.

Leaf demography

Two overstory sweetgum trees (8–12 m tall in 1998) were selected in each FACE ring based on the proximity of trees to areas accessible from portable hydraulic lifts. All of these trees had leaves exposed to full sunlight at the top of the crown and leaves in deep shade at the bottom of the crown. The same trees and leaf positions were used by Herrick and Thomas (1999, 2001). Diffuse irradiance for the shade leaves was about 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, punctuated by intermittent sunflecks. Sun leaf irradiance was typically saturating and varied between 1100 and 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during midday on sunny days (Herrick and Thomas 1999).

Shoots of sweetgum trees exhibit indeterminate monopodial growth and produce leaves continuously from late April until early July of each year. We define a shoot as the current season's growth from a single apical bud after bud break. To examine the leaf dynamics of the entire 1998 growing season, leaf counts were taken every 2 weeks between May and mid-September, and then weekly from mid-September through November during rapid leaf senescence. For each count, we monitored four sun shoots and four shade shoots on each tree. New leaves were marked with lightweight colored phone wire attached to the petiole. The abscission date was considered to be the sample date on which the leaf disappeared or when the leaf could be knocked off the shoot by gentle shaking. Using this technique, we calculated the emergence date, abscission date and leaf longevity for each leaf cohort of sun and shade shoots. Based on the emergence date, the leaves on these shoots were divided into two categories: early leaves and late leaves. Early leaves were those formed in the overwintering bud and were the oldest leaves on each shoot. Late

leaves were those formed during the growth season after the initial early flush.

Gas exchange and estimates of leaf chlorophyll

Measurements of gas exchange began on September 23, 1998, about 55 days before complete leaf senescence, on attached leaves from two sun shoots and two shade shoots from the shoots used for the assessment of leaf demography. Measurements were made on one early leaf and one late leaf from each shoot. Measurements were repeated on the same leaves every other week from September 23 until complete senescence in late November. When a leaf abscised from the tree, it was excluded from further analysis. Some leaves remained on the tree even after they ceased to function. These leaves were measured and included in our analysis until they abscised from the tree.

In situ measurements of light-saturated net photosynthesis (A_{net}) were taken with an open-flow infrared gas analyzer with an attached red/blue LED light source (LI-6400, Li-Cor, Lincoln, NE). Measurements of A_{net} were made at saturating irradiance (1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the sun and 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the shade) after the leaves were allowed to equilibrate for at least 10 min in the cuvette. Preliminary trials indicated that photosynthetic rates reached steady state within 5 min following attachment of the cuvette. Gas exchange measurements were made between 1000 and 1500 h on sunny days to minimize diurnal effects on photosynthesis. Leaf temperatures were not controlled during measurements, but were not significantly different between the CO₂ treatments. Mean leaf temperatures were 27.3 \pm 1.0 $^{\circ}\text{C}$ on September 23, 25.2 \pm 0.6 $^{\circ}\text{C}$ on October 5, 26.0 \pm 0.5 $^{\circ}\text{C}$ on October 19 and 21.8 \pm 0.9 $^{\circ}\text{C}$ on November 3. Trees in one blocked pair of rings were measured each day so that slight differences in daily weather conditions could be included in the block effect in the analysis of variance.

To better assess the combined effects of leaf senescence and photosynthesis on late-season carbon gain, we calculated whole-shoot photosynthesis. First we calculated mean leaf area for individual sun and shade leaves by sampling leaves from all rings. Whole-shoot leaf area was estimated by multiplying the number of leaves per shoot by the mean individual sun and shade leaf area. Whole-shoot leaf area was divided into early and late leaf area. Whole-shoot photosynthesis was determined by calculating the mean early and late leaf-level photosynthetic rates from each shoot, and multiplying that rate by the respective leaf areas per shoot. The carbon gain from the early and late leaves was summed to give whole-shoot carbon gain for five sample periods from late September to early November.

To estimate chlorophyll content, relative light absorbance (α_r) was measured weekly with a nondestructive Minolta SPAD-502 meter (Spectrum Technologies, Plainfield, IL). The SPAD meter has two LEDs that emit red (~650 nm) and infrared (~940 nm) wavelengths through an intact leaf sample. At the red wavelength, absorbance by chlorophyll is high and unaffected by carotene. The Minolta SPAD meter uses a scale

from -9.9 to 99.9 (from least to greatest absorbance) and estimates the amount of chlorophyll present by measuring the amount of light that is transmitted through a leaf. The SPAD meter reliably estimates differences in chlorophyll content (Yadava 1986, Marquard and Tipton 1987, Richardson et al. 2002) and has been used in other atmospheric CO₂ enrichment studies (Kubiske and Pregitzer 1996) to estimate leaf chlorophyll contents nondestructively. Measurements were made on the same leaves used for gas exchange weekly from September 23 until complete senescence.

Senescent leaf properties

Senescent leaves, defined as leaves that abscised from or fell off the shoot following gentle shaking of the branch, were collected during each census. Once leaves had been measured for gas exchange, as many as possible were collected. Because leaves disappeared between censuses, however, we were unable to collect all gas exchange leaves. Therefore, comparable leaf samples from nearby shoots were also collected in order to have at least two (one early leaf and one late leaf) sun and shade leaves from each tree, for a total of four sun and four shade leaves from each FACE ring. Leaf mass per unit area (LM_a), leaf N on a mass basis (N_m) and leaf N on a leaf area basis (N_a) were measured for each leaf. Leaf mass per unit area was calculated by measuring the dry mass of leaf disks of a known area. Leaf tissue for N analyses was frozen in liquid N₂, stored at -80 °C and later dried at 65 °C to a constant mass. Leaf N was assayed following Dumas combustion with a Carlo Erba CNS autoanalyzer (Fisons Instruments, Milan, Italy). In addition, we calculated relative N resorption on a mass and leaf area basis as:

$$\%N \text{ resorption} = \left(\frac{\text{green leaf N} - \text{senescent leaf N}}{\text{green leaf N}} \right) 100. \quad (1)$$

Green leaves were collected in mid-June for the early leaves and early September for the late leaves. We were unable to collect enough senescent leaf material to analyze senescent N and N resorption for differences between early and late leaves. Therefore, we pooled those two groups to analyze for differences between the CO₂ treatments and positions in the canopy.

Data analysis

Treatment effects on leaf population numbers, demography parameters, senescent leaf properties and whole-shoot photosynthesis were evaluated by an analysis of variance (ANOVA) model with CO₂ treatment, leaf position and blocked ring pair as main effects (Data Desk, 1997, Data Description, Ithaca, NY). Gas exchange and leaf absorbance measured with the SPAD meter (September 23–November 16) were analyzed by an ANOVA model with CO₂ treatment, leaf position, leaf age and blocked ring pair as main effects and tree nested within the [CO₂] by ring pair interaction. Post hoc comparison of parameter means was performed with the Bonferroni test (Data Desk, 1997). The probability level was set to 0.1 because of

the low number of replicate rings (Ellsworth 1999, Herrick and Thomas 2001).

Results

Leaf demography

Leaf number per shoot was not significantly affected by [CO₂] at any time during the 1998 growing season (Figure 2). Sun shoots had more leaves than shade shoots at every sample period during the 1998 growing season ($P = 0.03$). In the middle of the growing season (July 14), sun shoots averaged 139% more leaves per shoot than shade shoots.

Demographic parameters of early leaves, late leaves and all sun and shade leaves are shown in Table 1. When early and late leaf types were pooled, elevated [CO₂] did not significantly affect emergence date, abscission date or leaf longevity of sun leaves or shade leaves. Early leaves were formed in the overwintering bud and emerged at about the same date regardless of CO₂ treatment. In addition, abscission date of early leaves and leaf longevity were unaffected by CO₂ treatment. Likewise, atmospheric CO₂ enrichment did not significantly affect emergence date, abscission date or leaf longevity of late leaves. Shade leaves had a greater longevity than sun leaves when early and late leaves were pooled ($P = 0.07$, Table 1). This was because the mean emergence date of late leaves was earlier in the season for shade shoots than for sun shoots ($P = 0.066$). Emergence date, abscission date and longevity of early leaves did not differ between sun shoots and shade shoots. There were no significant interactions between CO₂ treatment and leaf position (sun/shade) on any of the leaf demographic parameters.

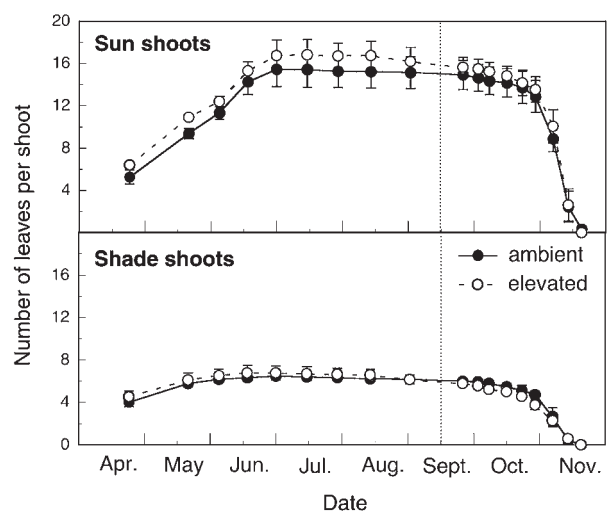


Figure 2. Total number of leaves per shoot on sun and shade shoots from overstory *Liquidambar styraciflua* trees growing in the Duke Forest FACE experiment in ambient (●) or elevated (○) [CO₂]. The vertical dashed line indicates the time when biweekly measurements of leaf photosynthesis and weekly measurements of leaf absorbance began.

Table 1. Leaf emergence date, abscission date and life span estimated for sun and shade branches from overstory *Liquidambar styraciflua* in a forest ecosystem exposed to ambient and elevated [CO₂]. Each value is the mean of three rings (\pm SE) from each CO₂ treatment, ignoring block effects. Means within a row followed by the same letter are not statistically different according to Bonferroni's post-hoc test ($P < 0.1$). Abbreviation: DOY = day of year.

	Sun shoots		Shade shoots	
	Elevated [CO ₂]	Ambient [CO ₂]	Elevated [CO ₂]	Ambient [CO ₂]
<i>Emergence date (DOY)</i>				
Early leaves ¹	105	105	105	105
Late leaves	141 \pm 2 ab	145 \pm 4 a	128 \pm 2 b	134 \pm 1 ab
All leaves	132 \pm 3 a	131 \pm 4 a	116 \pm 1 b	117 \pm 2 b
<i>Abscission date (DOY)</i>				
Early leaves	292 \pm 5 a	291 \pm 1 a	288 \pm 5 a	292 \pm 3 a
Late leaves	307 \pm 1 a	302 \pm 7 a	305 \pm 2 a	306 \pm 1 a
All leaves	302 \pm 2 a	296 \pm 9 a	299 \pm 2 a	301 \pm 1 a
<i>Life span (DOY)</i>				
Early leaves	187 \pm 5 a	186 \pm 1 a	181 \pm 5 a	187 \pm 3 a
Late leaves	162 \pm 2 a	162 \pm 1 a	169 \pm 5 ab	173 \pm 1 b
All leaves	172 \pm 4 a	170 \pm 5 a	180 \pm 4 ab	185 \pm 1 b

¹ Early leaves emerged from the overwintering buds on about the same date and were counted on the same date.

Leaf area per shoot, gas exchange and leaf absorbance

Elevated [CO₂] increased A_{net} of early and late leaves on sun shoots throughout September, October and early November ($P = 0.062$; Figure 3). The A_{net} of sun leaves was stimulated by atmospheric CO₂ enrichment to a greater extent than that of shade leaves ([CO₂] \times leaf position, $P = 0.053$; Figures 3 and 4). There was a significant stimulation of A_{net} by elevated [CO₂] in shade leaves only during the sample period in early October (Figure 4).

During late September and late October, leaves produced later in the season had significantly greater A_{net} than the older leaves produced earlier in the season ($P = 0.075$; Figures 3 and 4). In early October and November, A_{net} of early and late leaves was not significantly different. In sun and shade shoots, all early leaves had ceased photosynthesis by November 3, whereas many late leaves were photosynthetically active on that date.

There were no significant effects of atmospheric CO₂ enrichment on stomatal conductance (g_s) of sweetgum leaves during the late-season period (Figures 3 and 4). Sun leaves had 133% greater g_s than shade leaves ($P = 0.088$) during September and October. By early November, differences in g_s between sun and shade leaves disappeared. Stomatal conductance did not differ between early and late leaves. A blocked ring pair effect on g_s was found during October and September ($P = 0.074$), but there was no block effect during November.

The area of individual sun leaves on a shoot averaged 105.7 \pm 0.8 cm² in the elevated [CO₂] treatment and 116.2 \pm 6.8 cm² in the ambient [CO₂] treatment. Area of individual shade leaves averaged 66.7 \pm 2.1 cm² in elevated [CO₂] and 81.8 \pm 4.2 cm² in ambient [CO₂]. Individual leaves were slightly smaller when grown in elevated [CO₂] than in ambient

[CO₂] ($P = 0.023$) and shade leaves were smaller than sun leaves ($P < 0.001$). Elevated [CO₂], however, had no effect on overall leaf area per shoot (Figures 3 and 4), which was estimated by multiplying mean individual leaf area by the number of leaves per shoot. We used leaf area per shoot and light-saturated photosynthetic rates per unit leaf area (Figures 3 and 4) to estimate whole-shoot photosynthesis (Figure 5). Whole-shoot photosynthesis was stimulated by atmospheric CO₂ enrichment on September 23 and October 5 ($P = 0.049$, Figure 5), but not on later sample dates. This effect was dramatic in the sun shoots, but not evident in the shade shoots ([CO₂] \times leaf position, $P = 0.054$). On September 23 and October 5, elevated [CO₂] stimulated whole-shoot photosynthesis by 60% in the sun, but only by 3% in the shade. After October 5, elevated [CO₂] did not significantly affect whole-shoot photosynthesis. During the entire senescence period, net photosynthetic rates were more than 12 times greater in sun shoots than in shade shoots ($P = 0.018$).

Relative light absorbance (α_r), a surrogate of leaf chlorophyll content, was measured on leaves used for the gas exchange determinations. Elevated [CO₂] did not significantly affect α_r of any leaf type during the sampling period from September 23 to November 16 (Figure 6). Sun leaves had greater α_r than shade leaves during this sampling period ($P = 0.044$). After October 23, however, α_r of sun leaves and shade leaves did not differ significantly. Additionally, late leaves had a greater α_r than early leaves through late October ($P = 0.043$, Figure 6). By November 3, most early leaves had completely senesced.

Senescent leaf properties

Elevated [CO₂] did not have a strong effect on N resorption

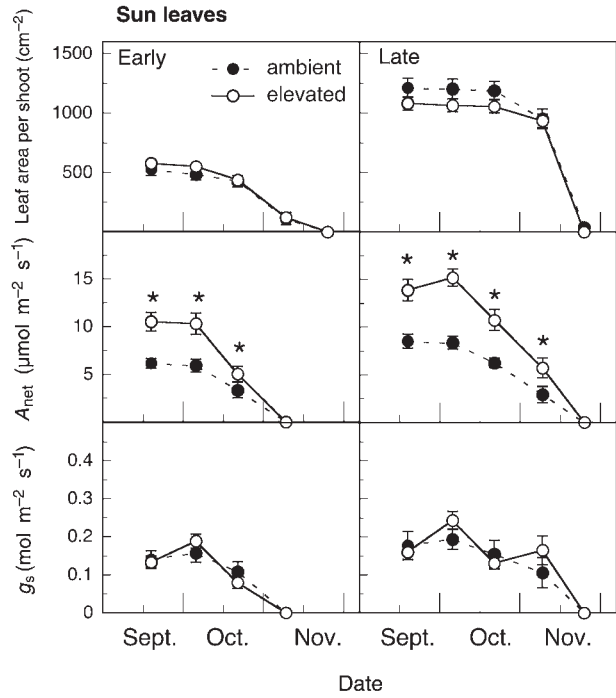


Figure 3. Estimated leaf area per shoot, light-saturated net photosynthesis (A_{net}) and stomatal conductance (g_s) of early and late sun leaves of overstory *Liquidambar styraciflua* trees growing at the Duke Forest FACE experiment in ambient (●) or elevated (○) [CO₂]. Measurements were made at saturating irradiance between 1000 and 1500 h on sunny days. Leaves were measured until they completely senesced. After senescence, leaves were no longer included in this graph or in the statistical analysis. An asterisk (*) indicates a significant effect ($P < 0.1$) of [CO₂] according to the Bonferroni post-hoc test.

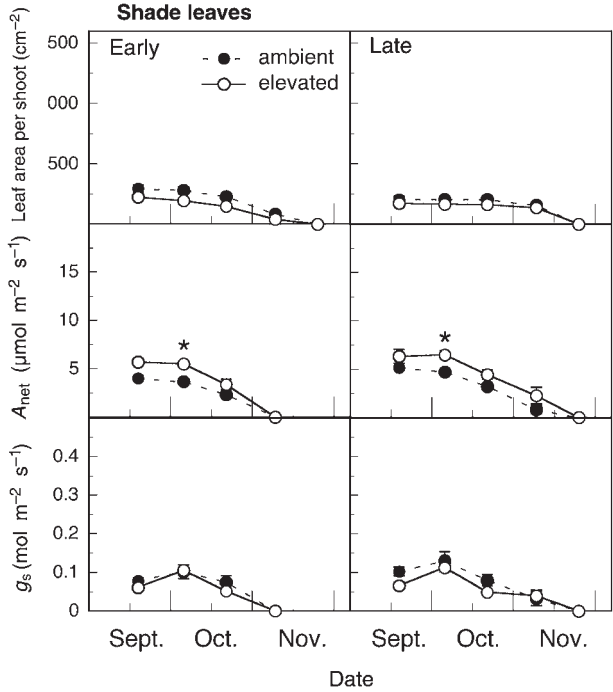


Figure 4. Estimated leaf area per shoot, light-saturated net photosynthesis (A_{net}) and stomatal conductance (g_s) of early and late shade leaves of overstory *Liquidambar styraciflua* trees growing at the Duke Forest FACE experiment in ambient (●) or elevated (○) [CO₂]. Measurements were made at saturating irradiance between 1000 and 1500 h on sunny days. Leaves were measured until they completely senesced. After senescence, leaves were no longer included in this graph or in the statistical analysis. An asterisk (*) indicates a significant effect ($P < 0.1$) of CO₂ according to the Bonferroni post-hoc test.

proficiency, the residual leaf N_m and N_a of completely senesced sweetgum leaves (Table 2). There was a [CO₂] × leaf position interaction ($P = 0.099$), wherein senescent sun leaves had 14% greater N_a in elevated [CO₂] than in ambient [CO₂],

but there was no effect of CO₂ treatment on N_a of senescent shade leaves (Table 2). This was a result of a large increase in LM_a of the senescent sun leaves grown in elevated [CO₂] ($P = 0.066$). Senescent sun and shade leaves did not significantly

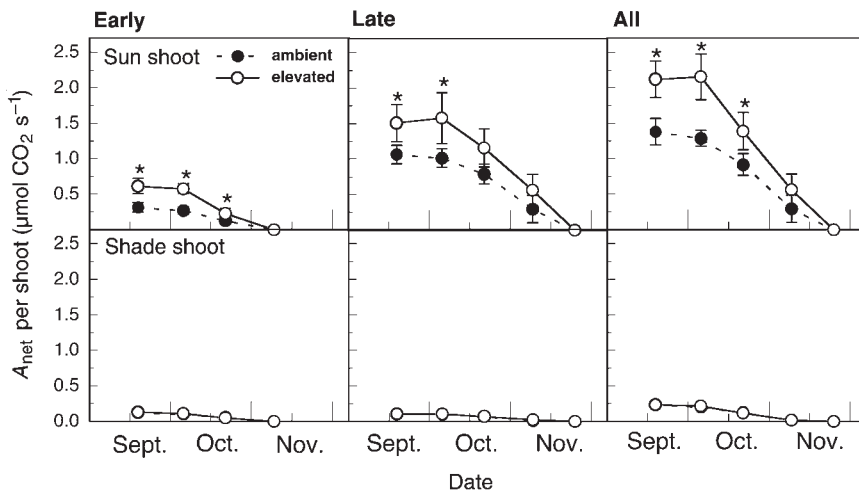


Figure 5. Modeled whole-shoot light-saturated net photosynthesis (A_{net}) of sun and shade leaves *Liquidambar styraciflua* trees growing at the Duke Forest FACE experiment in ambient (●) or elevated (○) [CO₂]. Early and late graphs show the contributions of early and late leaves to whole-shoot photosynthesis. Whole-shoot photosynthesis was modeled by calculating leaf area per shoot, measuring light-saturated photosynthetic rates per unit leaf area (Figures 3 and 4), and multiplying them together. An asterisk (*) indicates a significant effect ($P < 0.1$) of [CO₂] according to the Bonferroni post-hoc test.

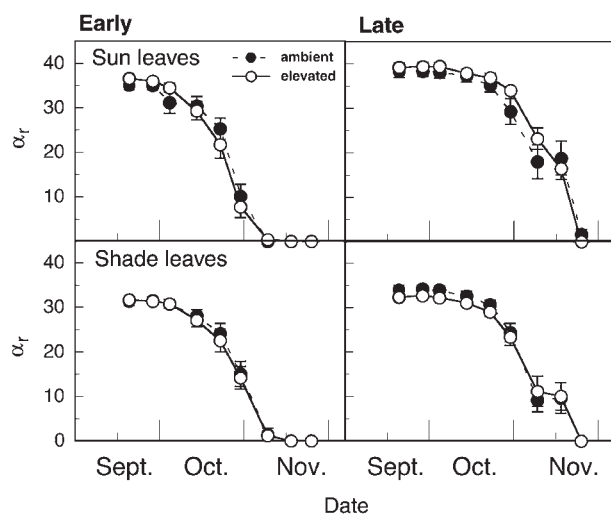


Figure 6. Relative light absorbance (α_r) of early and late sun and shade leaves of *Liquidambar styraciflua* trees growing at the Duke Forest FACE experiment in ambient (●) or elevated (○) [CO₂]. Measurements were made with a nondestructive Minolta SPAD-502 meter (Spectrum Technologies, Plainfield, IL) on the same leaves and at the same time as gas exchange measurements. Leaves were measured until they completely senesced. After senescence, leaves were no longer included in this graph or in the statistical analysis.

differ in N_m , but differed in N_a ($P = 0.006$) and LM_a ($P = 0.007$). Senescent sun leaves had 56% more N_a and 71% greater LM_a than shade leaves. The N_a also varied significantly between the blocked ring pairs ($P = 0.088$).

Relative N resorption, expressed as a percentage of pre-senescent amounts, is presented on a mass basis and a leaf area basis (Table 2). There was a [CO₂] × leaf position interaction for N resorption on a mass basis ($P = 0.065$), wherein N resorption in elevated [CO₂] was 9% lower in shade leaves than in sun leaves (Table 2). Nitrogen resorption calculated on a leaf area basis was not significantly affected by elevated [CO₂], but it was slightly lower in shade leaves than in sun leaves ($P = 0.074$, Table 2).

Discussion

An acceleration or delay in leaf senescence of forest trees could lead to a significant change in seasonally integrated carbon gain (Nelson et al. 1982). There is no consensus on whether an increase in [CO₂] will promote, retard or have no effect on senescence (Gunderson et al. 1993, McConnaughay et al. 1996, Li et al. 2000). We hypothesized that atmospheric CO₂ enrichment would delay senescence of sun and shade leaves because leaves in elevated [CO₂] would retain a positive carbon balance later in the season. However, we found no evidence that atmospheric CO₂ enrichment affected leaf phenology of overstory sweetgum trees growing in the Duke Forest FACE experiment (Figure 2, Table 1). Elevated [CO₂] did not affect emergence date, abscission date or leaf longevity of sun or shade leaves of sweetgum, although subtle differences may have been missed because of the 7-day duration of the leaf census. During the period that leaf senescence occurred, atmospheric CO₂ enrichment stimulated photosynthesis in the sweetgum leaves (Figures 3 and 4), but the magnitude of the CO₂ response was variable as the leaves senesced. Our data indicate that atmospheric CO₂ enrichment stimulates seasonally integrated C gain of sweetgum trees by increasing photosynthesis, but not by changing leaf phenology.

Elevated [CO₂] stimulates photosynthesis of many plant species when rates are measured during the middle portion of the growing season (Gunderson et al. 1993, Norby et al. 1999, Herrick and Thomas 2001). Less is known, however, about the late-season gas exchange of these species while growing in elevated [CO₂]. In a previous study, we found that elevated [CO₂] increased photosynthetic rates of sweetgum trees by 48% in the shade and 63% in the sun during the middle portion of the growing season (Herrick and Thomas 2001). In the current study, as leaves were senescing, elevated [CO₂] also stimulated photosynthesis in the sweetgum leaves from late September until early November (Figures 3 and 4), but the magnitude of the stimulation varied between 23 and 51% in shade leaves and between 51 and 96% in sun leaves. We found no evidence that atmospheric CO₂ enrichment induced accel-

Table 2. Morphological and chemical properties of senesced sun and shade leaves from overstory *Liquidambar styraciflua* (L.) in a forest ecosystem with ambient and elevated [CO₂]. Leaves were collected in November 1998 after abscission. Each value is the mean of three rings (\pm SE) from each CO₂ treatment, ignoring block effects. Means within a row followed by the same letter are not statistically different according to Bonferroni's post-hoc test ($P > 0.1$).

	Sun leaves		Shade leaves	
	Elevated [CO ₂]	Ambient [CO ₂]	Elevated [CO ₂]	Ambient [CO ₂]
LM_a (g m ⁻²)	72.38 \pm 4.16 a	58.28 \pm 3.10 b	39.70 \pm 3.00 c	36.58 \pm 1.75 c
N_m (mg g ⁻¹)	6.71 \pm 0.11 a	7.33 \pm 0.19 ab	7.57 \pm 0.41 ab	7.79 \pm 0.53 b
N_a (g m ⁻²)	0.49 \pm 0.02 a	0.43 \pm 0.02 b	0.30 \pm 0.02 c	0.29 \pm 0.03 c
% N Resorption (mass basis)	60.7 \pm 0.8 a	61.0 \pm 1.6 a	55.4 \pm 2.1 b	60.1 \pm 1.2 a
% N Resorption (area basis)	66.3 \pm 1.3 a	67.2 \pm 1.5 a	60.4 \pm 3.5 a	61.4 \pm 4.1 a

erated ontogeny or early decline in leaf photosynthesis, as has been found in previous studies (Nie et al. 1995, Pearson and Brooks 1995, van Oosten and Besford 1995, Miller et al. 1997). Sun and shade leaves remained photosynthetically functional until about November 3 in early leaves and November 10 in late leaves (Figures 3 and 4).

In many studies, leaf area is stimulated by elevated $[\text{CO}_2]$ when adequate amounts of nutrients and water are supplied (Norby et al. 1992, 1995, Ceulemans et al. 1995). Elevated $[\text{CO}_2]$ may enhance the amount of leaf area on a plant by stimulating the number of leaves produced or by increasing the size of individual leaves (Radoglou and Jarvis 1990, Wong et al. 1992, Ceulemans et al. 1995). We found that the number of leaves on individual sweetgum shoots was unaffected by elevated $[\text{CO}_2]$ (Figure 2). In a growth chamber study, sweetgum seedlings produced a greater number of leaves in elevated $[\text{CO}_2]$ than in ambient $[\text{CO}_2]$ (Sionit et al. 1985). However, this was a result of an increased number of branches produced by these seedlings rather than an increase in the number of leaves per branch. We found that the area of individual leaves was 16% less in elevated $[\text{CO}_2]$ than in ambient $[\text{CO}_2]$ (data not shown), although this difference did not result in a significant reduction of whole shoot leaf area (Figures 3 and 4).

Individual leaf photosynthesis and shoot leaf area were combined using a simple shoot photosynthesis model. Elevated $[\text{CO}_2]$ stimulated net photosynthesis of sun shoots, but not shade shoots (Figure 5). This effect was caused by a combination of a large enhancement of leaf-level photosynthesis in sun leaves and the development of leaves late into the season (Figure 3). In the sun, 71% of the leaves on a shoot were produced after the initial flush and these leaves accounted for 78% of the carbon fixed by sun shoots in September and October. In contrast, fewer late leaves developed in the shade (Figure 2) and the enhancement of leaf-level photosynthesis by elevated $[\text{CO}_2]$ was much lower in the shade (Figure 4). In the shade, 45% of the leaves on a shoot were produced after the initial flush and these leaves accounted for 51% of the carbon fixed by shade shoots in September and October (Figure 5). With a greater photosynthetic capacity and many more late leaves, sun shoots had photosynthetic carbon uptake rates more than 12 times those of the shade shoots. Most of the sweetgum shoots in the Duke Forest canopy develop in the shade and have an important role in whole-tree carbon balance. These data indicate that, in the latter part of the season, elevated $[\text{CO}_2]$ primarily stimulates carbon gain in the sun portion of the canopy and particularly in late-formed leaves.

Leaf senescence is an orderly loss of leaf function, ultimately leading to recovery of some of the nutrients in leaves. Senescence is generally controlled by climatic factors such as day length and temperature, but can be modified by nutrient and water availability (Thomas and Stoddart 1980). For example, drought can cause early senescence in some species such as tulip poplar (Burns and Honkala 1990). During our experiment, trees in the Duke Forest FACE experiment were exposed to a moderate drought from early July through early September (Figure 1). Sweetgum leaves, however, did not ap-

pear to be senescing early in either CO_2 treatment (Figure 2). Relative light absorbance (α_r), a surrogate for chlorophyll concentration, was not significantly affected by elevated $[\text{CO}_2]$ at any time during the late season (Figure 6). These data are consistent with mid-season measurements, where neither chlorophyll nor leaf N was affected by CO_2 treatment (Herrick and Thomas 2001). The N_m of senesced leaves, defined as resorption proficiency (Killingbeck 1996), was also unaffected by CO_2 treatment. There was higher N_a in senescent sun leaves grown in elevated $[\text{CO}_2]$ and this probably reflected leaf structural changes as indicated by increased leaf mass per area (Table 2, Herrick and Thomas 2001). Resorption efficiency of N on a mass basis was slightly lower in shade leaves of sweetgum trees grown in elevated $[\text{CO}_2]$. Values of N resorption from sweetgum leaves ranged between 55 and 61% and were similar to those reported by Finzi et al. (2001) for sweetgum trees in the Duke FACE experiment. In our study, small differences between CO_2 treatments disappeared when resorption efficiency was expressed on a leaf area basis (Table 2). Overall, atmospheric CO_2 enrichment had little effect on the N status of sun or shade leaves of sweetgum trees or the retranslocation of N out of these leaves.

In herbaceous species, increased $[\text{CO}_2]$ often reduces stomatal conductance (Field et al. 1995, Knapp et al. 1996) and therefore increases water-use efficiency (Drake et al. 1996). In a review of 48 tree studies, however, Curtis and Wang (1998) found that the average response to elevated $[\text{CO}_2]$ was highly variable with a nonsignificant reduction in g_s of 11% (Curtis and Wang 1998). We found no effect of atmospheric CO_2 enrichment on g_s in the sun and shade leaves of sweetgum trees as the leaves senesced (Figures 3 and 4). This contrasts with other sweetgum seedling studies that have shown reduced g_s in response to elevated $[\text{CO}_2]$ (Fetcher et al. 1988, Tschaplinski et al. 1995) as well as measurements made at the Duke FACE experiment during the midseason (J.D. Herrick, unpublished data). The lack of response of g_s to atmospheric CO_2 enrichment in this study might be explained by a combination of the time of year (late season) as well as the dry soil conditions. Stomatal responses to many environmental factors are reduced as leaves mature (Field 1987). In addition, Tschaplinski et al. (1995) found that the reduction in g_s of sweetgum seedlings as a result of atmospheric CO_2 enrichment disappeared when combined with drought, even when sweetgum seedlings were watered after drought.

In summary, leaf phenology and photosynthetic function define how much carbon a plant fixes during the season. A change in either of these factors will substantially affect carbon gain as $[\text{CO}_2]$ continues to increase. We found that elevated $[\text{CO}_2]$ stimulated leaf photosynthesis throughout the later part of the growth season but did not significantly affect the amount of leaf area per shoot. Elevated $[\text{CO}_2]$ did not alter the length of the growing season. Thus, enhanced carbon gain in response to atmospheric CO_2 enrichment in these trees will be the result of a strong stimulation of photosynthesis throughout the growing season, including the portion of the season during which leaves senesce.

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