

# Reduced stomatal conductance in sweetgum (*Liquidambar styraciflua*) sustained over long-term CO<sub>2</sub> enrichment

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## Summary

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- Over 4 yr (1998–2001) we examined the effects of CO<sub>2</sub> enrichment on stomatal conductance ( $g_s$ ) of sun and shade leaves of overstorey sweetgum (*Liquidambar styraciflua*) grown at the Duke Forest Free Air Carbon CO<sub>2</sub> Enrichment (FACE) experiment.
- Gas-exchange measurements were taken in June and September of each year and relationships between water stress and stomatal conductance were examined. Stomatal density was measured in June 2000. Relative stomatal limitation ( $l_g$ ) was calculated from gas-exchange measurements.
- We found a 28% reduction of  $g_s$  in elevated CO<sub>2</sub> that was sustained over the study period and was similar in sun and shade leaves. Elevated CO<sub>2</sub> reduced  $l_g$  by 26%. Stomatal density was not affected by CO<sub>2</sub> enrichment. Elevated CO<sub>2</sub> did not change the sensitivity of  $g_s$  to soil moisture or vapor pressure deficit.
- The data illustrate that decreased  $g_s$  of sweetgum leaves in CO<sub>2</sub> enrichment is consistent over long periods of time and under varying environmental conditions.

**Key words:** Free air CO<sub>2</sub> enrichment (FACE), stomatal conductance, *Liquidambar styraciflua* (sweetgum), sun, shade, stomatal density.

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## Introduction

Numerous studies have shown that elevated atmospheric CO<sub>2</sub> reduces stomatal conductance ( $g_s$ ) in many woody plants (Morison, 1987; Drake *et al.*, 1997; Medlyn *et al.*, 2001). At the leaf, whole-plant and ecosystem levels, this decrease could lead to reduced evapotranspiration and result in increased soil moisture and run-off production (Field *et al.*, 1995; Sellers *et al.*, 1996; Bounuoa *et al.*, 1999). Stomatal responses to elevated CO<sub>2</sub> are quite variable, with literature reviews indicating average reductions ranging from 11 to 40% (Morison, 1987; Drake *et al.*, 1997; Curtis & Wang, 1998; Medlyn *et al.*, 2001). In some species  $g_s$  may not change, or may even increase with elevated CO<sub>2</sub> (Dixon *et al.*, 1995; Ellsworth, 1999).

Variation in stomatal response to elevated CO<sub>2</sub> may be caused by several factors, indicating a need for measurements over extended periods. For example, plants may be less sensitive to CO<sub>2</sub> in the short term (< 1 yr) relative to the long term (> 1 yr; Medlyn *et al.*, 2001). Plant and leaf age may also be

factors, as stomata of mature trees are typically less responsive to CO<sub>2</sub> than are tree seedlings and saplings (Medlyn *et al.*, 2001). In addition, environmental variations in light, temperature, humidity and soil moisture conditions can also interact with elevated CO<sub>2</sub> to influence  $g_s$  (Sage, 1994; Santrucek & Sage, 1996; Curtis & Wang, 1998; Wullschlegel *et al.*, 2002; Maherali *et al.*, 2003).

The development of leaf structure and function is tightly coupled to variation in the abiotic environment. For example, leaves within plant canopies are often exposed to different light, humidity and temperature environments, leading to distinct sun and shade morphologies (Givnish, 1988). Sun leaves are typically thicker, and have more nitrogen per unit leaf area and greater photosynthetic capacity than shade leaves. In addition, sun leaves usually have a higher stomatal density than shade leaves. These observations suggest that structural differences in leaf tissue caused by the environment, in addition to temporal environmental variation, may lead to differential responses of  $g_s$  to elevated CO<sub>2</sub>.

In this study we report the effects of elevated CO<sub>2</sub> on  $g_s$  of overstory sweetgum trees (*Liquidambar styraciflua* L.) over 4 yr at the Duke Forest Free Air CO<sub>2</sub> Enrichment (FACE) experiment. Sweetgum is the most common deciduous tree species in the overstory in the Duke Forest FACE experiment, and differential light environments at the top and the bottom of the forest canopy cause distinct sun and shade leaf types. The long-term nature of this study and the physiological differences among sun and shade leaves allowed us to examine whether stomatal responses to CO<sub>2</sub> could be modified by variation in the light environment, as well as year-to-year changes in atmospheric and soil moisture deficits. We predicted that responses of  $g_s$  to CO<sub>2</sub> enrichment would be greater in sun leaves than in shade leaves because photosynthesis responds to CO<sub>2</sub> to a greater extent in sun leaves (Herrick & Thomas, 1999, 2001). We were specifically interested in the relative response of stomatal conductance to elevated CO<sub>2</sub> in sun and shade leaves, and how changes in stomatal conductance resulting from growth under elevated CO<sub>2</sub> limit photosynthesis.

## Materials and Methods

### Duke Forest Free-Air CO<sub>2</sub> Enrichment (FACE) experiment

The Duke Forest FACE experiment is located in a *Pinus taeda* L. (loblolly pine) 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<sup>-1</sup>), but there are significant numbers of sweetgum (620 stems ha<sup>-1</sup>) and yellow poplar trees (*Liriodendron tulipifera* L., 68 stems ha<sup>-1</sup>) as well as other hardwood species in the canopy and the understory. Sweetgum is common in the south-eastern USA and in piedmont North Carolina and it invades early succession broomsedge (*Anthropogon virginicus* L.) fields during the course of secondary succession (Oosting, 1942). The experimental forest occurs on a nutrient-poor, clay-rich loam soil that is typical of many upland areas in the south-eastern USA.

Within this forest, six 30 m diameter experimental circular plots (FACE rings) were established in 1995. Three of these FACE rings are replicate CO<sub>2</sub> 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 elevated treatment FACE rings, these pipes deliver a controlled amount of CO<sub>2</sub> throughout the entire forest volume with a target CO<sub>2</sub> concentration of ambient plus 200 µl l<sup>-1</sup>. Three control rings receive the same volume of air to replicate any micrometeorological effects on the forest that occurs during the operation of the FACE facility. Beginning in August 1996, the CO<sub>2</sub> treatment has been applied continuously 24 h d<sup>-1</sup> except when

the air temperature was < 5°C for more than an hour. During the first 5 yr of the experiment (1997–2001) the daytime average CO<sub>2</sub> concentration was 572 µl l<sup>-1</sup> in the elevated treatment rings and 376 µl l<sup>-1</sup> 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-CO<sub>2</sub> rings were arranged in a complete block design (three pairs, two rings in each block).

**Steady-state gas exchange** To determine  $g_s$ , steady-state gas exchange was measured during 7 d twice a year around June 25 and September 1 1998, 1999, 2000 and 2001. June measurements were made ≈ 68 d after leaf initiation, and September measurements ≈ 50 d before leaves began to senesce. Minimum air temperatures during June and September sample periods ranged from 14 to 19°C and maximum temperatures from 30 to 34°C. A drought occurred from late July to early September 1998. Soil moisture averaged 24.4% in late June 1998 and 15.0% during early September 1998 (Schäfer *et al.*, 2002). During the 1999, 2000 and 2001 sample periods, soil moisture averaged ≈ 21%, except in late June 2001 where soil moisture was nearly 30% (Schäfer *et al.*, 2002; H. Kim, unpublished results). Volumetric soil moisture was calculated from measurement of the soil dielectric constant in the upper 30 cm of the soil profile using modified time-domain reflectometry techniques with waveguides (CS615, Campbell Scientific, Ogden UT, USA). There were four sensors within each ring and the signals were recorded every half hour. Average soil moisture for each measurement period was calculated by averaging soil moisture from all the rings for the week when gas exchange was measured (Schäfer *et al.*, 2002; H. Kim, unpublished results).

Two overstory sweetgum trees (7–11 m high in 1997) were selected in each FACE ring, based on the proximity of trees to areas accessible from static towers and portable hydraulic lifts. All these trees had leaves exposed to full sunlight at the top of the crown and deep shade at the bottom of the crown. The same trees and leaf positions were used by Herrick & Thomas (1999, 2001). Sun leaf irradiance was typically saturating and varied between 1100 and 1400 µmol m<sup>-2</sup> s<sup>-1</sup> during midday on sunny days. Diffuse irradiance for the shade leaves was ≈ 50 µmol m<sup>-2</sup> s<sup>-1</sup> punctuated by intermittent sunflecks (Herrick & Thomas, 1999).

Steady-state leaf-gas exchange was measured *in situ* on one sun leaf and one shade leaf from each tree using an open flow infrared gas analyzer with an attached red–blue LED light source (LI-6400, Li-Cor, Lincoln, NE). Measurements were made with a constant irradiance of 1400 µmol m<sup>-2</sup> s<sup>-1</sup> photon flux density after the leaves were allowed to equilibrate for at least 10 min in the leaf cuvette. Preliminary trials indicated that stomatal conductance reached steady state within 7 min. Steady-state stomatal conductance ( $g_s$ ) was determined when the coefficient of variation of change in water vapor was < 0.05% as indicated by the LI-6400. Fully expanded leaves at least 2–3 wk old were measured between 1000 and 1500 EST

on sunny days to minimize diurnal effects on gas exchange. Leaf temperatures were not controlled during measurements and did not differ between CO<sub>2</sub> treatments. Leaf temperatures averaged 31.3 ± 1.2°C in June and 30.6 ± 0.9°C in September. Vapor pressure deficit averaged 1.7 ± 0.1 kPa and did not differ between CO<sub>2</sub> treatments or canopy position. 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 ANOVA.

The relative stomatal limitation of photosynthesis ( $l_g$ ) for each sample period was based on the response of net photosynthesis ( $A_{\text{net}}$ ) to variation in calculated intercellular CO<sub>2</sub> ( $A_{\text{net}}-C_i$  curves) measured concurrently with the steady-state gas-exchange measurements. Some of these data were originally taken to assess photosynthetic acclimation (Herrick & Thomas, 2001).  $A_{\text{net}}-C_i$  curves were measured over a range of 10 external CO<sub>2</sub> partial pressures ( $C_a$ ) from ≈ 50–1200 μl l<sup>-1</sup>. A steady-state measurement at growth CO<sub>2</sub> was made first, then  $C_a$  was dropped down to 50 μl l<sup>-1</sup> and increased in nine steps up to 1200 μl l<sup>-1</sup> (Herrick & Thomas, 2001). Measurements were made with a constant saturating irradiance of 1400 μmol m<sup>-2</sup> s<sup>-1</sup> photon flux density. Preliminary trials indicated that photosynthetic rates reached steady state within 3 min following an increase in  $C_a$ . The curves were fitted using nonlinear least squares with the equation:

$$A_{\text{net}} = A_{\text{max}} [1 - (1 - \alpha/A_{\text{max}})^{(1-C_i/\Gamma)}] \quad \text{Eqn 1}$$

where  $A_{\text{max}} = A_{\text{net}}$  at CO<sub>2</sub> saturation,  $\alpha = y$  intercept and  $\Gamma = \text{CO}_2$  compensation point. This model provided a good fit to our data ( $r^2 = 0.99$ ) and has been used by Gunderson *et al.* (1993) for other tree species. Relative stomatal limitation was calculated using the differential method (V) of Jones (1985), which is specifically intended for nonlinear  $A_{\text{net}}-C_i$  curves:

$$l_g = r_g / (r_g + r^*) \quad \text{Eqn 2}$$

where  $r_g$  is the gas-phase resistance to CO<sub>2</sub> uptake and  $r^*$  is the slope of the  $A_{\text{net}}-C_i$  curve. We calculated  $r_g$  as  $(C_a - C_i)/A_{\text{net}}$  at the operating  $C_i$  and  $r^*$  as the first derivative of equation 1 at the operating  $C_i$  (Jones, 1985).

**Stomatal density** Stomatal density ( $s$ ) was measured by counting the number of stomata per unit area after measuring gas exchange in June 2000. Two sun and two shade leaves were collected from two trees in each ring. Clear nail polish was used to make impressions of areas on the leaf that were not heavily veined (Ceulemans *et al.*, 1995). Sweetgum leaves are hypostomatous, therefore impressions of only the abaxial side of each leaf were made. Each impression was analyzed at 400 × with a light microscope interfaced with a solid-state TV camera (Model CCD-72-SX; DAGE-MTI Inc., Michigan City, IN) using NIH Image 1.58 (US National Institute of Health). Three fields of view per slide were sampled

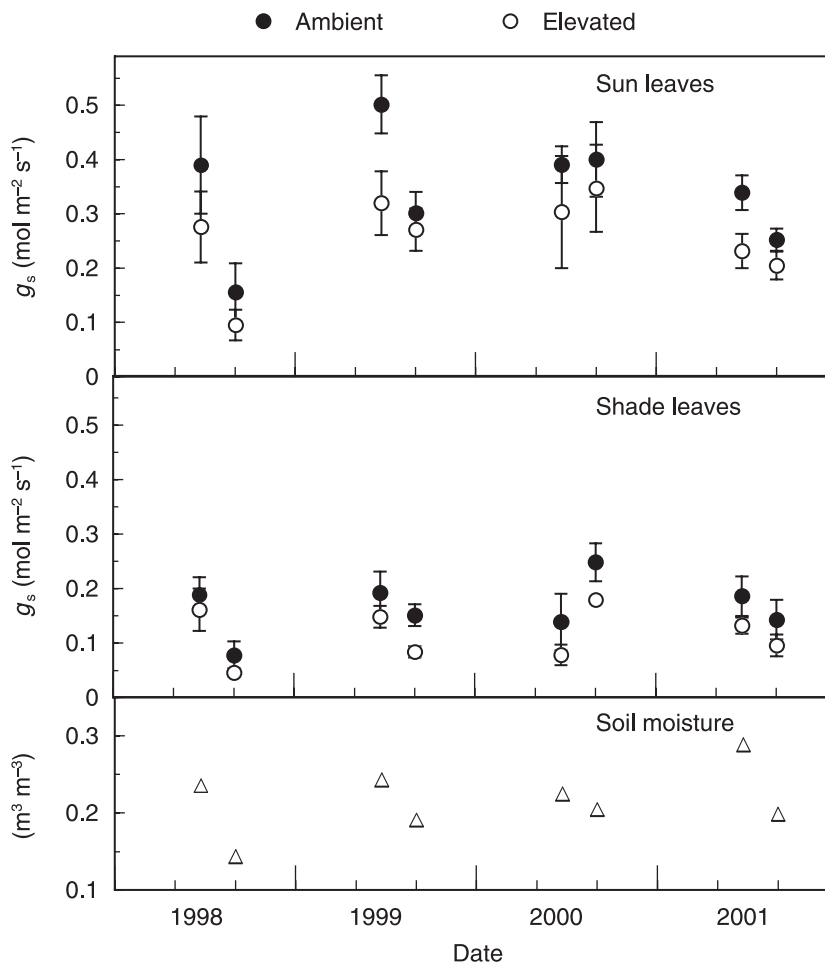
and averaged for each leaf. In addition we measured epidermal cell density ( $e$ , number of epidermal cells mm<sup>-2</sup>) and stomatal index. Stomatal index was calculated as  $[s/(e + s)] \times 100$  (Salisbury, 1927). Guard cells were included in the epidermal cell density.

## Data analysis

Gas-exchange parameters ( $g_s$ ,  $l_g$ ,  $C_i/C_a$ ) were analyzed using a repeated-measures analysis of variance (ANOVAR) model with CO<sub>2</sub> treatment, leaf position, month, year and blocked ring pair as main effects (Data Desk, 1997). Measurements from each ring were averaged, and individual rings ( $n = 3$ ) were considered replicates for the purposes of statistical analysis. Stomatal density, epidermal cell density and stomatal index were analyzed with an ANOVA with CO<sub>2</sub> treatment, leaf position and blocked ring pair as main effects (Data Desk, 1997). *Post hoc* comparisons of parameter means were determined using Bonferroni corrected multiple-comparison tests (Data Desk, 1997). Least-squares linear regression was used to analyze the relationship of  $g_s$  to soil moisture and leaf-to-air vapor pressure deficit. Analysis of covariance (ANCOVA) was used to determine if the CO<sub>2</sub> treatment influenced these relationships. A significant interaction would indicate that the slopes were different between CO<sub>2</sub> treatments. If the slopes were the same, then the  $y$  intercepts were compared by examining the main effect of CO<sub>2</sub> treatment (Sokal & Rolf, 1995). Data were log-transformed, when appropriate, to meet the assumptions of parametric statistics. The probability level was set *a priori* to 0.1 because of the low number of replicate rings in this study (Ellsworth, 1999; Herrick & Thomas, 2001).

## Results

Elevated CO<sub>2</sub> reduced steady-state stomatal conductance ( $g_s$ ) by an average of 28% in sun and shade sweetgum leaves during 1998–2001 ( $P = 0.026$ ). CO<sub>2</sub> enrichment decreased  $g_s$  by 31% in sun leaves and by 25% in shade leaves, but the relative response to CO<sub>2</sub> was not affected by leaf position ( $P = 0.398$ ; Fig. 1) or time of year (June and September,  $P = 0.166$ ). Steady-state  $g_s$  was more than twice (122%) as high for sun leaves than for shade leaves ( $P = 0.005$ ; Fig. 1). Variation in  $g_s$  was influenced by time of year ( $P = 0.050$ ) and block ( $P = 0.001$ ). Stomatal conductance decreased with decreasing soil moisture content ( $r^2 = 0.53$ ,  $P = 0.040$ ), but the slopes of the relationships did not differ significantly between CO<sub>2</sub> treatments or leaf position (Fig. 2). In general,  $g_s$  decreased with increasing leaf-to-air vapor pressure deficit (VPD) ( $r^2 = 0.49$ ,  $P = 0.059$ ; Fig. 3), but high VPD data influence this relationship with  $g_s$ . Nonetheless, elevated CO<sub>2</sub> did not affect the slope of the response of  $g_s$  to VPD in the sun leaves and there was not a strong relationship between  $g_s$  and VPD in shade leaves grown at elevated CO<sub>2</sub> ( $r^2 = 0.32$ ,



**Fig. 1** Steady-state stomatal conductance ( $g_s$ ) of sun and shade leaves of overstory *Liquidambar styraciflua* trees growing at the Duke Forest FACE experiment in ambient (closed symbols) and elevated (open symbols) CO<sub>2</sub>. Gas exchange was measured around 25 June and 1 September of each year. Measurements were made at saturating irradiance and were restricted to the hours between 10 : 00 and 15 : 00 h on sunny days. Each point is the mean of three rings ( $\pm$  SE) for each CO<sub>2</sub> treatment. Soil moisture during the measurement period is added for reference.

$P = 0.144$ ). Despite decreased  $g_s$  of sweetgum leaves under CO<sub>2</sub> enrichment, relative stomatal limitation of photosynthesis ( $I_g$ ) was lower at elevated than at ambient CO<sub>2</sub> ( $P = 0.029$ ; Fig. 4). The CO<sub>2</sub> response was not modified by canopy position ( $P = 0.265$ ) or time of year ( $P = 0.375$ ). Relative stomatal limitation of photosynthesis was 24% greater in sun leaves than in shade leaves ( $P = 0.002$ ). Relative stomatal limitation of photosynthesis also varied significantly between blocks ( $P = 0.001$ ) and between years ( $P = 0.006$ ).

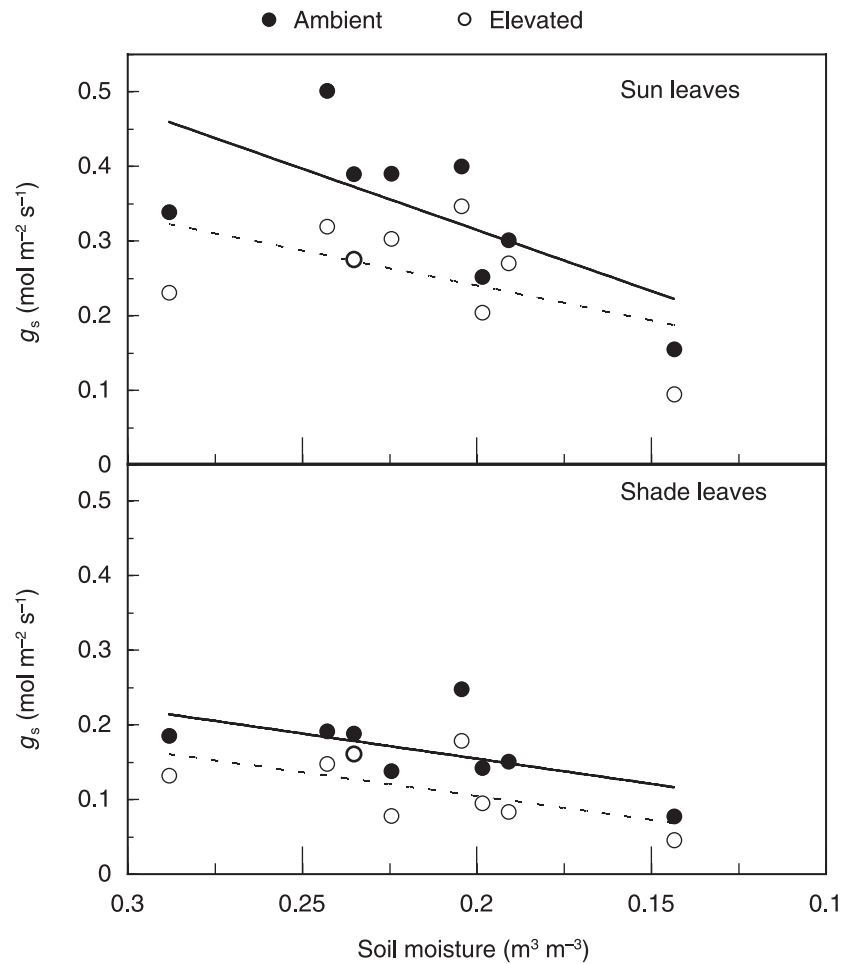
Elevated CO<sub>2</sub> reduced the  $C_i/C_a$  ratio by an average 5% in sun and shade sweetgum leaves over the 4 yr of this study ( $P = 0.064$ ; Fig. 5).  $C_i/C_a$  ratio was not affected by leaf position or time of the year (June and September). However,  $C_i/C_a$  ratio was affected by year ( $P = 0.007$ ) and block ( $P > 0.001$ ).

During the fourth year of CO<sub>2</sub> treatment stomatal densities of sun and shade leaves were unaffected by elevated CO<sub>2</sub> (Table 1). Sun leaves had 32% higher stomatal densities than shade leaves ( $P = 0.005$ ). Epidermal cell density was not affected by CO<sub>2</sub> enrichment, nor was it different between sun and shade leaves. Stomatal index was not affected by CO<sub>2</sub> treatments, but was higher in sun leaves than in shade leaves ( $P = 0.034$ ).

## Discussion

Stomatal conductance ( $g_s$ ) of overstory sweetgum trees exposed to elevated CO<sub>2</sub> was reduced by an average of 28% in sun and shade leaves throughout the 4 yr study period. The decrease in  $g_s$  of sweetgum leaves was sustained over 4 yr of CO<sub>2</sub> treatment, indicating that stomatal sensitivity to CO<sub>2</sub> did not subside over time. This is consistent with the 21% reduction in  $g_s$  for 13 long-term field-based studies of woody plants (Medlyn *et al.*, 2001), but greatly different from the dominant species of the Duke FACE experiment, loblolly pine, which shows no effect of elevated CO<sub>2</sub> on  $g_s$  (Ellsworth, 1999). This underscores the importance of species composition when predicting CO<sub>2</sub> effects on stand-level processes.

The elevated CO<sub>2</sub>-induced reduction in  $g_s$  was similar for both sun (31%) and shade (25%) leaves. Wullschleger *et al.* (2002) observed a 23% decrease in  $g_s$  with elevated CO<sub>2</sub> for sun leaves of sweetgum trees at the Oak Ridge, TN FACE experiment, but no effect of CO<sub>2</sub> on  $g_s$  for leaves in the middle and lower portions of the canopy. The difference between our observations on shade leaves at the Duke Forest FACE experiment and those at the Oak Ridge FACE experiment may



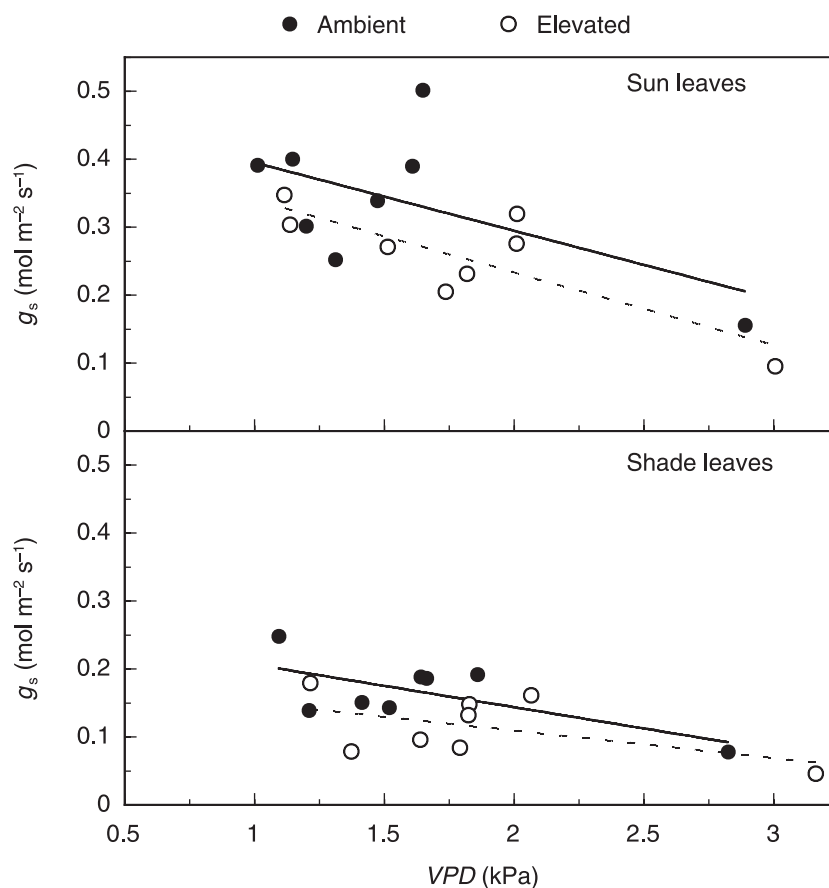
**Fig. 2** Relationship between steady-state stomatal conductance ( $g_s$ ) from overstory *Liquidambar styraciflua* trees and soil moisture at the Duke Forest FACE experiment. Sun leaves and shade leaves were grown and measured in ambient (closed symbols, solid line) and elevated (open symbols, dashed line) CO<sub>2</sub>.

reflect differences in canopy light or temperature regimes. The Oak Ridge FACE experiment is a closed-canopy sweetgum plantation, whereas in our study sweetgum trees are growing among a more open loblolly pine plantation. Our shade leaf  $g_s$  measurements were taken at the very bottom of the sweetgum canopy where the light regimes are relatively stable and diffuse (Herrick & Thomas, 1999), resulting in relatively uniform shade leaf morphology (Table 1; Herrick & Thomas, 2001). By contrast, shade leaves in the broadleaved canopy of sweetgum trees at the Oak Ridge FACE experiment are probably more deeply shaded and cooler than in our study.

Our leaf-level results were similar to the 25% reduction in sap-flux density ( $\text{gH}_2\text{O m}^{-2} \text{sapwood s}^{-1}$ ) at elevated CO<sub>2</sub> of the same sweetgum trees from 1999–2000 (Schäfer *et al.*, 2002). There has been no evidence that leaf-area index (LAI) has changed between the CO<sub>2</sub> treatments at the Duke FACE site (Schäfer *et al.*, 2002), but LAI of sweetgum alone has not been directly measured. Nevertheless, the overall similarity of LAI across CO<sub>2</sub> treatments suggests that our leaf-level results are representative of whole-tree responses in this system. Wullschleger *et al.* (2002) found only a 14% reduction in mean canopy conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) of sweetgum at

the Oak Ridge FACE experiment, and concluded that weaker responses in the lower canopy moderated the overall influence of CO<sub>2</sub> on canopy conductance. Results from the Oak Ridge FACE site also indicate no differences in LAI between the CO<sub>2</sub> treatments (Norby *et al.*, 2003). The differences between the Oak Ridge and Duke Forest FACE experiments among overstory sweetgum trees demonstrate the utility of experiments at multiple sites to quantify the effects of elevated CO<sub>2</sub> on  $g_s$  of a given species.

Stomatal acclimation to CO<sub>2</sub> enrichment can enhance the reduction of  $g_s$  by elevated CO<sub>2</sub> (Bunce, 2001; Lodge *et al.*, 2001; Maherali *et al.*, 2002), but has rarely been examined in plants growing in forest ecosystems (Medlyn *et al.*, 2001). Sage (1994) suggested the use of the  $C_i/C_a$  ratio as an index for stomatal acclimation because it reflects any changes in the relationship between stomatal conductance and photosynthetic capacity. Stomatal acclimation would occur if stomata close relative to photosynthetic activity and reduce  $C_i/C_a$  (Sage, 1994). We found a modest 5% ( $P = 0.064$ ) reduction overall for sun and shade leaves during all measurement periods (Fig. 5). However, the overall effect of elevated CO<sub>2</sub> on  $C_i/C_a$  varied based on month, year and canopy position. For



**Fig. 3** Relationship between steady-state stomatal conductance ( $g_s$ ) from overstory *Liquidambar styraciflua* trees and leaf-to-air vapor pressure deficit (VPD) at the Duke Forest FACE experiment. Sun leaves and shade leaves were grown and measured in ambient (closed symbols, solid line) and elevated (open symbols, dashed line)  $\text{CO}_2$ .

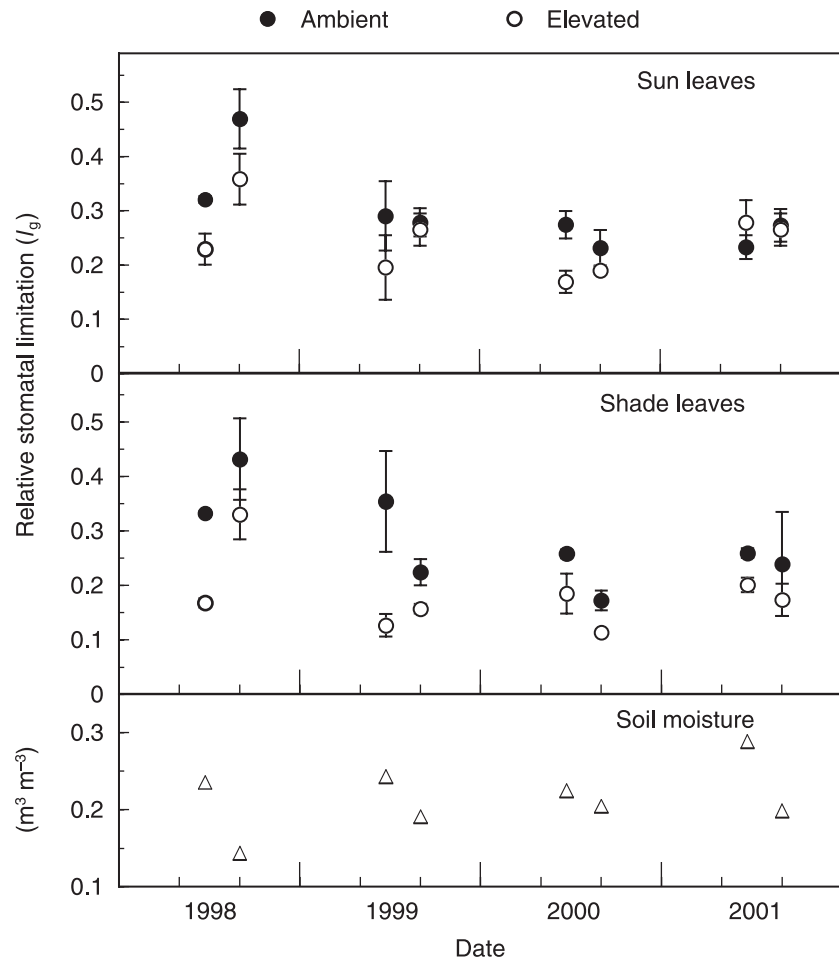
example, in September 1998 and 1999, elevated  $\text{CO}_2$  reduced  $C_i/C_a$  by 12% for both sun and shade leaves, whereas there was no effect of  $\text{CO}_2$  on  $C_i/C_a$  in 2000. In 2001 elevated  $\text{CO}_2$  reduced  $C_i/C_a$  by 8% in sun leaves, but only by 2% in shade leaves. These results suggest that at some points, stomatal acclimation probably occurred for sweetgum. Our results also indicate that stomatal acclimation during these times happened independently from the regulation of photosynthesis, because no photosynthetic acclimation occurred in these same trees (Herrick & Thomas, 2001). Although  $C_i/C_a$  responses were variable, we note that the strongest effect of  $\text{CO}_2$  on  $C_i/C_a$  was in the driest year (1998), suggesting that water stress is associated with stomatal acclimation. This interpretation is consistent with Sage's (1994) observation that stomatal acclimation to elevated  $\text{CO}_2$  is more common in cotton during leaf water stress.

Several studies suggest that a reduction in  $g_s$  in long-term  $\text{CO}_2$  enrichment may be related to a reduction in stomatal density or stomatal index (Woodward & Bazzaz, 1987; Kurschner, 1997; Beerling *et al.*, 1998). We found no evidence to support this in sweetgum trees at the Duke Forest FACE experiment. Stomatal density, epidermal cell density and stomatal index were all unaffected by  $\text{CO}_2$  treatment in June 2000, despite a 33% reduction in  $g_s$  during that period

(Table 1). These results indicate that  $g_s$  responses to  $\text{CO}_2$  need not correlate with the magnitude or direction of stomatal density responses to  $\text{CO}_2$ .

To determine whether the reduction in  $g_s$  at elevated  $\text{CO}_2$  could be limiting carbon assimilation, we calculated relative stomatal limitation of photosynthesis ( $l_g$ ; Jones, 1985) from  $A-C_i$  curves measured concurrently with steady-state gas-exchange measurements. Elevated  $\text{CO}_2$  decreased  $l_g$  (26%, Fig. 4) despite reducing  $g_s$  (Fig. 1). Similarly, Gunderson *et al.* (2002) found a reduction in relative stomatal limitation in elevated  $\text{CO}_2$  in sweetgum trees at the Oak Ridge FACE experiment. These results indicate that the enhancement of photosynthesis by elevated  $\text{CO}_2$  more than compensated for the diffusional limitation imposed by stomatal closure at elevated  $\text{CO}_2$  (Tissue *et al.*, 1995).

There were effects of canopy position on stomatal characteristics that were independent of  $\text{CO}_2$  treatment. Steady-state stomatal conductance was more than twice as high (+122%) in sun leaves as in shade leaves (Fig. 1), which is a common observation in forest trees (Whitehead, 1998). This might be partially caused by a 32% higher stomatal density in sun leaves than in shade leaves (Table 1), or related to a greater photosynthetic capacity in sun leaves (Herrick & Thomas, 2001). Epidermal cell density was not different between sun



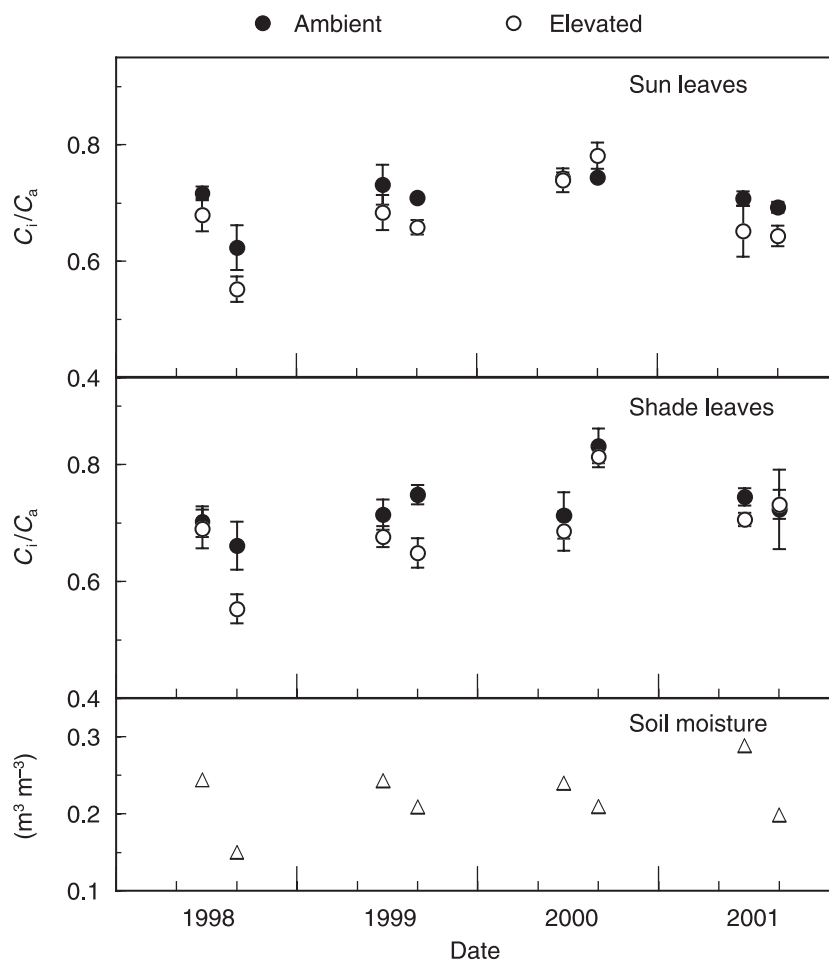
**Fig. 4** Relative stomatal limitation of photosynthesis ( $l_g$ ; Jones, 1985) calculated from  $A-C_i$  curves measured on sun and shade leaves of overstory *Liquidambar styraciflua* trees growing at the Duke Forest FACE experiment in ambient (closed symbols) and elevated (open symbols)  $\text{CO}_2$ . Gas exchange was measured around 25 June and 1 September of each year. Measurements were made at saturating irradiance and were restricted to the hours between 10 : 00 and 15 : 00 h on sunny days. Each point is the mean of three rings ( $\pm$  SE) for each  $\text{CO}_2$  treatment. Soil moisture during the measurement period is added for reference.

and shade leaves, but stomatal index was lower in the shade, indicating there were effects of leaf position on the initiation of stomata. Relative stomatal limitation ( $l_g$ ) of photosynthesis was 24% greater in sun leaves than shade leaves, and was probably a result of lower photosynthetic capacity in the shade leaves (Herrick & Thomas, 2001). These results demonstrate that the complex nature of within-canopy light environments is important in considering larger-scale canopy water use.

The response of  $g_s$  to water stress in  $\text{CO}_2$  enrichment is particularly important because global climate change may increase the potential for drought conditions in some areas (Rind *et al.*, 1990). Stomatal conductance decreased with decreasing soil moisture content, but elevated  $\text{CO}_2$  did not change the drought sensitivity of  $g_s$  in sun or shade leaves (Fig. 2). We also observed that  $g_s$  generally decreased with increasing leaf-to-air VPD, yet elevated  $\text{CO}_2$  did not affect stomatal sensitivity to VPD (Fig. 3). This is consistent with results in sweetgum at the Oak Ridge FACE experiment (Gunderson *et al.*, 2002). In other species, the relative reductions in  $g_s$  at elevated  $\text{CO}_2$  have been reported to be larger in low light and at low vapor pressure deficits than in high light and high vapor pressure deficits (Heath & Kerstiens, 1997;

Bunce, 2001). We note, however, that these relationships with sweetgum leaves were pooled from different measurement dates with different VPDs, and observed responses would probably change if VPD was experimentally manipulated over individual leaves (Maherali *et al.*, 2003).

Medlyn *et al.* (2001) and Sage (1994) observed that elevated  $\text{CO}_2$  most strongly reduced  $g_s$  in drought stress. Our results provide some evidence for this pattern, as elevated  $\text{CO}_2$  reduced  $g_s$  by 40% during a moderate drought in September 1998, while  $g_s$  was reduced by only an average of 26% during the other measurement periods in this study (Fig. 1). However, the low magnitude of  $g_s$  during the drought caused the absolute reduction of  $g_s$  to be less during the drought of September 1998 than during other measurement periods (Fig. 1). In a previous study we found that elevated  $\text{CO}_2$  had no effect on  $g_s$  when measured during the late season (23 September–16 November 1998) after the 1998 drought, as sweetgum leaves senesced (Herrick & Thomas, 2003). In that study it was not possible to separate the effects of leaf age from drought. Wullschleger *et al.* (2002) also found that the  $\text{CO}_2$ -induced reduction in  $g_s$  disappeared after mid-September, and argued that any condition that reduces the absolute



**Fig. 5** Steady-state  $C_i/C_a$  ratio of sun and shade leaves of overstory *Liquidambar styraciflua* trees growing at the Duke Forest FACE experiment in ambient (closed symbols) and elevated (open symbols)  $CO_2$ . Gas exchange was measured around 25 June and 1 September of each year. Measurements were made at saturating irradiance and were restricted to the hours between 10 : 00 and 15 : 00 h on sunny days. Each point is the mean of three rings ( $\pm$  SE) for each  $CO_2$  treatment. Soil moisture during the measurement period is added for reference.

**Table 1** Stomatal density, epidermal cell density and stomatal index of sun and shade leaves from canopy *Liquidambar styraciflua* trees grown in a forest ecosystem in ambient or elevated  $CO_2$

Parameter	Sun leaves		Shade leaves	
	Elevated $CO_2$	Ambient $CO_2$	Elevated $CO_2$	Ambient $CO_2$
Stomatal density ( $mm^{-2}$ )	$385 \pm 39^a$	$395 \pm 19^a$	$288 \pm 28^b$	$303 \pm 21^b$
Epidermal cell density ( $mm^{-2}$ )	$2795 \pm 159^a$	$2665 \pm 80^a$	$2406 \pm 140^a$	$2513 \pm 182^a$
Stomatal index	$12.00 \pm 0.45^a$	$13.12 \pm 0.56^a$	$10.66 \pm 0.32^b$	$10.75 \pm 0.06^b$

Leaves were collected after gas-exchange measurements in June 2000. Each value is the mean of three rings ( $\pm$  SE) from both  $CO_2$  treatments ignoring block effects. Within a measurement period values designated by the same letter are not different at the 0.1 level of significance.

magnitude of  $g_s$  will also reduce the effect that elevated  $CO_2$  has on these gas-exchange processes. Our results support this conclusion with respect to the effects of leaf age and the absolute difference under water stress.

In summary, we found that the reductions in stomatal conductance in elevated  $CO_2$  in our study were sustained over four growing seasons with varying soil moisture conditions, a result consistent with other long-term studies of trees (Medlyn *et al.*, 2001). The observed decrease in leaf-level stomatal conductance at elevated  $CO_2$  was similar in magnitude to the

reduction in sap flux density of sweetgum at the Duke Forest FACE experiment (Schäfer *et al.*, 2002). We also found consistent  $CO_2$  effects between sun and shade leaves. Our results demonstrate the value of long-term studies on large trees under variable environmental conditions as a tool to understand the effects of rising  $CO_2$  on the biosphere. In particular, long-term studies in natural systems are essential to confirm or refute previous studies done on smaller trees under more artificial conditions. Our results confirm that decreases in leaf-level stomatal conductance caused by  $CO_2$  enrichment

are relatively consistent across a variety of environmental conditions, and do not abate over time.

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## References

- Berling DJ, McElwain JC, Osborne CP. 1998. Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO<sub>2</sub> concentrations. *Journal of Experimental Botany* 49: 1603–1607.
- Bounoua L, Collatz GJ, Sellers PJ, Randall DA, Dazlich DA, Los SO, Berry JA, Fung I, Tucker CJ, Field CB, Jensen TG. 1999. Interactions between vegetation and climate: radiative and physiological effects of doubled atmospheric CO<sub>2</sub>. *Journal of Climate* 12: 309–324.
- Bunce JA. 2001. Direct and acclamatory responses of stomatal conductance to elevated carbon dioxide in four herbaceous crop species in the field. *Global Change Biology* 7: 323–331.
- Ceulemans R, Vanpraet L, Jiang XN. 1995. Effects of CO<sub>2</sub> enrichment, leaf position and clone on stomatal index and epidermal-cell density in Poplar (*Populus*). *New Phytologist* 131: 99–107.
- Curtis PS, Wang X. 1998. A meta-analysis of elevated CO<sub>2</sub> on woody plant mass, form and physiology. *Oecologia* 113: 299–313.
- Data Desk. 1997. *Data Desk*, Version 5.0. Ithaca, NY, USA: Data Description, Inc.
- Dixon M, le Thiec D, Garrec JP. 1995. The growth and gas exchange response of soil-planted Norway spruce (*Picea abies* L. Karst.) and red oak (*Quercus rubra* L.) exposed to elevated CO<sub>2</sub> and to naturally occurring drought. *New Phytologist* 129: 265–273.
- Drake BG, Gonzalez-Meler MA, Long SP. 1997. More efficient plants: a consequence of rising atmospheric CO<sub>2</sub>. *Annual Review Plant Physiology Plant Molecular Biology* 48: 609–639.
- Ellsworth DS. 1999. CO<sub>2</sub> enrichment in a maturing pine forest: are CO<sub>2</sub> exchange and water status in the canopy affected? *Plant, Cell & Environment* 22: 461–472.
- Field CB, Jackson RB, Mooney HA. 1995. Stomatal responses to increased CO<sub>2</sub>: implications from the plant to global scale. *Plant, Cell & Environment* 18: 1214–1225.
- Givnish TJ. 1988. Adaptation to sun and shade: a whole-plant perspective. *Australian Journal of Plant Physiology* 15: 63–92.
- Gunderson CA, Norby RJ, Wullschlegel SD. 1993. Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO<sub>2</sub>: no loss of photosynthetic enhancement. *Plant, Cell & Environment* 16: 797–807.
- Gunderson CA, Sholtis JD, Wullschlegel SD, Tissue DT, Hanson PJ, Norby RJ. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during three years of CO<sub>2</sub> enrichment. *Plant, Cell & Environment* 25: 379–393.
- Heath J, Kerstiens G. 1997. Effects of elevated CO<sub>2</sub> on leaf gas exchange in beech and oak at two levels of nutrient supply: consequences for the sensitivity to drought in beech. *Plant, Cell & Environment* 20: 57–67.
- Herrick JD, Thomas RB. 1999. Effects of enrichment on the photosynthetic light response of sun and shade leaves of canopy sweetgum trees (*Liquidambar styraciflua* L.) in a forest ecosystem. *Tree Physiology* 19: 779–786.
- Herrick JD, Thomas RB. 2001. No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO<sub>2</sub> enrichment at the Duke Forest FACE experiment. *Plant, Cell & Environment* 24: 53–64.
- Herrick JD, Thomas RB. 2003. Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstorey sweetgum (*Liquidambar styraciflua*) grown in elevated carbon dioxide concentration. *Tree Physiology* 23: 108–118.
- Jones HG. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant, Cell & Environment* 8: 95–104.
- Kurschner WM. 1997. The anatomical diversity of recent and fossil leaves of the durmast oak (*Quercus petraea* Lieblein/ *Q. pseudocastanea* Goepfert): implications for their use as biosensors of palaeoatmospheric CO<sub>2</sub> levels. *Review of Palaeobotany and Palynology* 96: 1–30.
- Lodge RJ, Dijkstra P, Drake BG, Morison JIL. 2001. Stomatal acclimation to increased CO<sub>2</sub> concentration in a Florida scrub oak species, *Quercus myrtifolia* Willd. *Plant, Cell & Environment* 24: 77–88.
- Maherali H, Reid CD, Polley HW, Johnson HB, Jackson RB. 2002. Stomatal acclimation over a subambient to elevated CO<sub>2</sub> gradient in a C<sub>3</sub>/C<sub>4</sub> grassland. *Plant, Cell & Environment* 25: 557–566.
- Maherali H, Johnson HB, Jackson RB. 2003. Stomatal sensitivity to vapor pressure difference over a subambient to elevated CO<sub>2</sub> gradient in a C<sub>3</sub>/C<sub>4</sub> grassland. *Plant, Cell & Environment* 26: 1297–1306.
- Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomaki S, Laita E, Rey A, Robertz P, Sigurdsson BD, Strassmeyer J, Wang K, Curtis PS, Jarvis PG. 2001. Stomatal conductance of forest species after long-term exposure to elevated to CO<sub>2</sub> concentration: a synthesis. *New Phytologist* 149: 247–264.
- Morison JIL. 1987. Intercellular CO<sub>2</sub> concentration and stomatal response to CO<sub>2</sub>. In: Zeiger E, Cowan IR, Farquhar GD, eds. *Stomatal Function*. Stanford, CA, USA: Stanford University Press, 229–251.
- Norby RJ, Sholtis JD, Gunderson CA, Jawdy SS. 2003. Leaf dynamics of a deciduous forest canopy: no response to elevated CO<sub>2</sub>. *Oecologia* 136: 574–584.
- Oosting HJ. 1942. An ecological analysis of the plant communities of piedmont, North Carolina. *American Midland Naturalist* 28: 1–126.
- Rind D, Suozzo R, Balachandran NK. 1990. Climate change and the middle atmosphere: the doubled CO<sub>2</sub> climate. *Journal of the Atmospheric Sciences* 47: 475–494.
- Sage RF. 1994. Acclimation of photosynthesis to increasing atmospheric CO<sub>2</sub>: the gas exchange perspective. *Photosynthesis Research* 39: 351–368.
- Salisbury EJ. 1927. On the causes and ecological significance of stomatal frequency, with special reference to woodland flora. *Philosophical Transactions of the Royal Society of London B* 216: 1–65.
- Santrucek J, Sage RF. 1996. Acclimation of stomatal conductance to a CO<sub>2</sub>-enriched atmosphere and elevated temperature in *Chenopodium album*. *Australian Journal of Plant Physiology* 23: 467–478.
- Schäfer KVR, Oren R, Lai C, Katul GG. 2002. Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO<sub>2</sub> concentration. *Global Change Biology* 8: 895–911.
- Sellers PJ, Bounoua L, Collatz GJ, Randall DA, Dazlich DA, Los SO, Berry JA, Fung I, Tucker CJ, Field CB, Jensen TG. 1996. Comparison of radiative and physiological effects of doubled atmospheric CO<sub>2</sub> on climate. *Science* 271: 1402–1406.
- Sokal AK, Rolf FJ. 1995. *Biometry: The principles and practice of statistics in biology research*. New York, NY, USA: W. H. Freeman.

Tissue DT, Griffin KL, Thomas RB, Strain BR. 1995. Effects of low and elevated CO<sub>2</sub> on C<sub>3</sub> and C<sub>4</sub> annuals. II. Photosynthesis and leaf biochemistry. *Oecologia* 101: 21–28.

Whitehead D. 1998. Regulation of stomatal conductance and transpiration in forest canopies. *Tree Physiology* 18: 633–644.

Woodward FI, Bazzaz FA. 1987. The responses of stomatal density to

CO<sub>2</sub> partial-pressure. *Journal of Experimental Botany* 39: 1771–1781.

Wullschlegel SD, Gunderson CA, Hanson PJ, Wilson KB, Norby RJ. 2002. Sensitivity of stomatal and canopy conductance to elevated CO<sub>2</sub> concentration – interacting variables and perspectives of scale. *New Phytologist* 153: 485–496.



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