Sensitivity of stomatal and canopy conductance to elevated CO_2 concentration – interacting variables and perspectives of scale

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Summary

• The hydrological response of forests to rising CO_2 is a critical biotic feedback in the study of global climate change. Few studies, however, have investigated this highly dynamic response at relevant temporal and spatial scales.

• A combination of leaf and whole-tree measurements and stand-level extrapolations were used to assess how stomatal conductance, canopy transpiration and conductance, and evapotranspiration might be affected by future, higher CO_2 concentrations.

• Midday measurements of stomatal conductance for leaves sampled in a 12-yr-old sweetgum (*Liquidambar styraciflua*) stand exposed to free-air CO_2 enrichment were up to 44% lower at elevated than at ambient CO_2 concentrations, whereas canopy conductance, averaged over the growing season, was only 14% lower in stands exposed to CO_2 enrichment. The magnitude of this response was dependent on vapor pressure deficit and soil water potential. Annual estimates of evapotranspiration showed relatively small reductions due to atmospheric CO_2 enrichment.

• These data illustrate that the hydrological response of a closed-canopy plantation to elevated CO_2 depends on the temporal and spatial scale of observation. They emphasize the importance of interacting variables and confirm that integration of measurements over space and time reduce what, at the leaf level, might otherwise appear to be a large and significant response.

Key words: canopy transpiration, evapotranspiration, forest water use, global change, *Liquidambar styraciflua* (sweetgum), sap velocity, transpiration.

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Introduction

Stomata orchestrate one of plant biology's greatest concerts; microscopic pores on the leaf surface through which, each year, pass 122 Gt of carbon and enough water vapor to more than equal the annual flow of all rivers on earth (Baumgartner & Reichel, 1975; Post *et al.*, 1990). Given the magnitude of these fluxes, it is understandable that stomata have historically occupied a critical position in the study of plant responses to global environmental change, including many studies designed to quantify the responses of plants and ecosystems to elevated CO_2 (Baker & Allen, 1994; Saxe *et al.*, 1998; Norby *et al.*, 1999). To the extent that stomatal responses are involved in determining CO_2-induced enhancements of photosynthesis (Rey & Jarvis, 1998; Gunderson *et al.*, 2002; Noormets *et al.*, 2001), differences in stomatal conductance caused by CO_2 have been of interest in the study of terrestrial carbon dynamics. Stomata and their response to elevated CO_2 have also been the focus of multiscale investigations related to transpiration, water-use efficiency and ecosystem water use (Jackson *et al.*, 1994; Tognetti *et al.*, 1998; Eamus, 1999; Wullschleger *et al.*, 2002). There was so much interest in the response of stomata to elevated CO_2 in the mid-1990s that experimental observations that stomatal resistance might

increase 50% with a doubling of atmospheric CO_2 were quickly incorporated into land-surface models for addressing potential CO_2-induced interactions between terrestrial ecosystems and climate (Henderson-Sellers *et al.*, 1995; Pollard & Thompson, 1995; Sellers *et al.*, 1996).

As the spatial and temporal scale of our measurements and experiments has increased, so too has our perception of how stomata are involved in regulating the carbon and water cycles at large spatial scales and over long periods of time (Field *et al.*, 1995; Wilson *et al.*, 1999). It is now generally recognized that while certain ecosystems are especially responsive to elevated CO_2 concentrations (Bremer *et al.*, 1996; Field *et al.*, 1997), other systems are not (Ellsworth *et al.*, 1995). Interestingly, this difference may not be directly dependent on whether elevated CO_2 elicits a major response in stomatal conductance (Niklaus *et al.*, 1998), and conclusions must inevitably account for higher-order changes in leaf area index and boundary layer considerations (Wilson *et al.*, 1999). Physiological feedbacks are also important (Sellers *et al.*, 1996; Wilson *et al.*, 1999), as are interactions between climate (e.g. vapor pressure deficit) and stomatal conductance. Furthermore, the scale at which the relationships between CO_2 concentration, stomatal conductance and ecosystem water use are observed may also shape our perspective of how one process affects the others.

We have previously reported for a 12-year-old sweetgum (*Liquidambar styraciflua* L.) plantation that canopy transpiration measured by sap-flow techniques is reduced at elevated CO_2 concentration (Wullschleger & Norby, 2001). This response was less, however, than might be anticipated based solely on leaf-level measurements of stomatal conductance (Gunderson *et al.*, 2002). Here, we expand these analyses to include processes that take place at spatial scales larger than individual leaves, and integrate insights from leaf and canopy measurements to assess how stomatal conductance, canopy transpiration and conductance, and evapotranspiration may be affected in a future, higher CO_2 world. In keeping with previous theoretical and/or modeling discussions (Field *et al.*, 1995; Raupach, 1998; Wilson *et al.*, 1999), we explore the possibility that the hydrological response of a closed-canopy forest to elevated CO_2 may depend on the scale of observation, and that interacting variables (e.g. radiation, vapor pressure deficit and soil water potential) and integration of processes over time and space will collectively act to reduce what at the leaf level might otherwise appear to be a large and significant response.

Materials and Methods

Study site

The study site is in a 1.7 ha plantation of sweetgum trees established in 1988 from 1-yr-old bare-root seedlings on the Oak Ridge National Environmental Research Park in Roane County, Tennessee, USA (35°54′ N, 84°20′ W). Seedling spacing at the time of planting was 2.3×1.2 m or 3625 trees ha^{-1} (van Miegroet *et al.*, 1994). The canopy has been closed since 1996 and the trees are in a linear growth phase (Norby *et al.*, 2001). A survey of the site in 1998 indicated that the 10-yr-old plantation had a basal area of about 29 m^2 ha^{-1}, with an average height of 12 m and a leaf area index of 5.5 m^2 m^{-2} (data not shown). Mean annual temperature (1962–93) at the study site is 13.9°C and annual precipitation averages 1371 mm. Soils are classified Aquic Hapludult with a silty clay loam texture (Soil Conservation Service, 1967).

Free-air CO_2 enrichment (FACE) facility and treatments

The FACE facility consists of five 25 m diameter circular plots within the sweetgum plantation. In each of two elevated CO_2 plots, the air is enriched with CO_2 dispensed from surrounding vent pipes, according to wind direction, and is regulated to maintain the target CO_2 concentration near the top of the canopy, based on the design, equipment, and software of Hendrey *et al.* (1999). Three ambient CO_2 plots serve as controls for the experiment, two surrounded by the same towers, vent pipes and blowers as the elevated CO_2 plots, but receiving only ambient air, and a third ambient plot without towers or blowers. The CO_2 concentration in the two elevated rings is maintained near a target concentration of 565 ppm (day) and 645 ppm (night) by a computer employing an algorithm that allowed CO_2 to be dispensed at a rate determined by wind speed. During the 1999 season, the CO_2 concentration of the elevated rings averaged 538 ppm during the day, whereas the CO_2 concentration of the ambient rings averaged 394 ppm (Norby *et al.*, 2001).

Environmental and soil moisture monitoring

Instruments for measuring air temperature, relative humidity, global radiation, and wind speed were located at the top of one of the 18-m aluminum towers from which vent pipes were suspended. A capacitance-type sensor was used to measure relative humidity (MP101A-C5, Rotronics Instrument Corp., Huntington, NY, USA). Global radiation was measured with a pyranometer (LI-200SA, Li-Cor Inc., Lincoln, NE, USA). Radiation, temperature, relative humidity and wind speed were measured every minute and data averaged each hour. Rainfall was measured above the canopy with a tipping bucket rain gauge. Estimates of daily mean air temperature (Ta), vapor pressure deficit (δe), and global radiation (Rg) were derived from hourly averages.

Soil water content (%, v : v, integrated from 0 to 20 cm soil depth) was measured with a time domain reflectometer (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) following the procedure of Topp & Davis (1985). Six pairs of stainless steel rods were installed in each plot, providing a total

of 12 and 18 soil water content observations for the elevated and ambient CO_2 treatments, respectively. Volumetric soil water content was converted to soil water potential using bulk density determinations and a moisture release curve constructed using thermocouple psychrometry (True Psi, Decagon, Pullman, WA, USA). Soil water potentials from all rod positions in a plot were averaged to produce plot means. Treatment soil water potentials were calculated from the plot means (n = 2 elevated CO_2 plots and n = 3 ambient CO_2 plots).

Gas exchange methods

Gas exchange responses were evaluated in fully expanded leaves selected from the tips of branches. Leaves sampled were representative of general canopy conditions, and in September and October were chosen from leaves exhibiting the least amount of senescence. Foliage from four to six trees in each plot was accessible from a central location, and data from all leaves measured at a given canopy position were used to derive plot means.

Gas-exchange data were obtained with either the LI-6400 steady-state photosynthesis system, the LI-6200 portable photosynthesis system or the LI-1600 steady-state porometer (Li-Cor, Inc.). In the case of the LI-6400, cuvette temperatures were set based on mid-afternoon temperatures forecast for each measurement period. Cuvette humidity was not controlled, except as needed to avoid condensation on occasions when cuvette relative humidity exceeded 80%. All measurements (four to eight leaves per plot on each date) were taken at saturating irradiance and at the treatment CO_2 concentration (Gunderson *et al.*, 2002). Measurements were conducted between 09 : 00 h and 16 : 00 h. Some gas exchange data were obtained while developing CO_2 response curves, using 10 inlet CO_2 concentrations between 0 and 1500 ppm. In the case of the LI-1600 steady-state porometer, data were collected on leaves from four to six trees per treatment plot. Measurements were made at prevailing light and climatic conditions, and were conducted between 10 : 00 h and 13 : 00 h. All data were collected in 1999, except for measurements taken in 1998 with the LI-6200 to characterize differences in stomatal conductance with depth in the canopy. These measurements were also taken at prevailing light and climatic conditions.

Canopy access for measurements of stomatal conductance was achieved using hydraulic lifts (Model UL48, UpRight, Inc., Selma, CA, USA) positioned at the center of each plot. The aerial work platforms extended to 15.5 m and provided easy access to multiple canopy positions.

Stand characteristics and measurements of total sapwood area

Trees within the ambient and elevated rings were fitted with stainless-steel dendrometer bands and stem circumference was measured monthly on 84–95 trees per ring (Norby *et al.*, 2001). Leaf area index was estimated for each ring from a series of seven 0.19-m^2 litter collection baskets placed above the plantation understory. A canopy-averaged value of leaf mass per unit leaf area (LMA) was determined from leaves collected throughout the canopy. Leaf area index was calculated from the mass of leaves collected over the season and LMA (Norby *et al.*, 2001).

An allometric equation that related calculated sapwood area to measured stem diameter was established using 58 trees outside the study plots (Wullschleger & Norby, 2001). Stem diameter at breast height (1.3 m) was measured with a diameter tape. Bark thickness for each tree was determined with a digital caliper. Sapwood thickness was determined by removing 5-mm diameter cores of wood with an increment bore. Sapwood area for each tree was calculated from sapwood depth and stem diameter after subtracting bark thickness. Total stand sapwood area was estimated by applying the allometric equation to all trees within the ambient and elevated CO_2 rings on which stem diameters were measured.

Sap velocity and estimates of stand transpiration

The heat-pulse technique (SF-300, Greenspan Technology Pty. Ltd, Warwick, Queensland, Australia) was used to measure sap velocity for four trees in two of the ambient and two of the elevated CO_2 rings (16 trees total). One heat-pulse probe was positioned in each tree so that the sensing thermistor was located at a sapwood depth of 19 mm. The data logger was programmed to provide a heat pulse for 1.8 s and measurements were recorded every 60 min. All estimates of sap velocity were corrected for probe implantation effects (Swanson & Whitfield, 1981).

The heat-pulse technique also was used to estimate the fraction of sapwood functional in water transport for six trees adjacent to the study plots (Wullschleger & Norby, 2001). Radial variation in sap velocity was determined in each tree using two heat-pulse probes: one that served as a fixed reference and a second that, once inserted into the sapwood, was used to measure heat-pulse velocity at defined intervals as it was withdrawn from the stem. An overall ratio was calculated using an area-weighted average of the point estimates.

Hourly rates of stand transpiration (mm h^{-1}) for each of the two ambient and two elevated CO_2 rings were estimated as a function of measured sap velocity, total stand sapwood area, and the fraction of sapwood functional in water transport. Daily rates of stand transpiration (mm d^{-1}) were calculated using a simple summation of hourly rates.

Calculation of canopy conductance and the decoupling coefficient

Daily estimates of canopy conductance (g_c) were calculated from daily rates of canopy transpiration (Wullschleger &

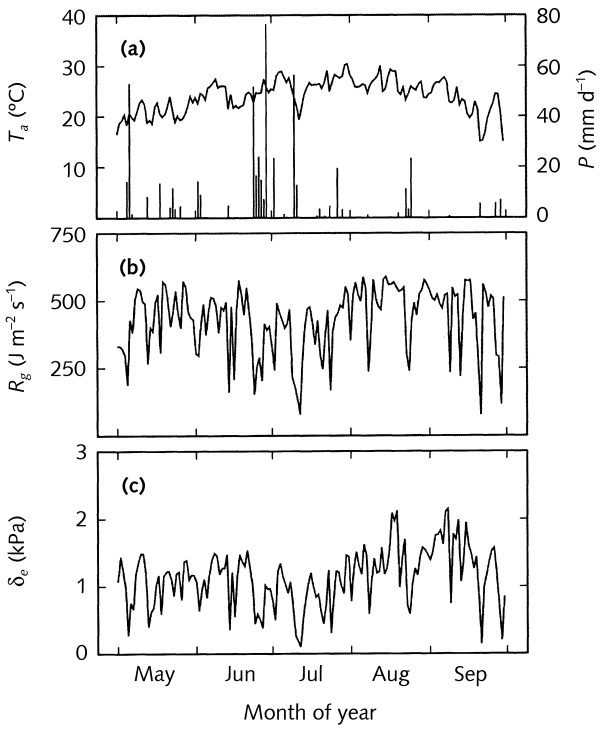

Fig. 1 Average daily (a) air temperature and precipitation (b) above-canopy global radiation and (c) vapor pressure deficit for the months during which daily canopy conductance was calculated for sweetgum trees exposed to ambient and free-air CO_2 enrichment.

Norby, 2001) and measured leaf area index for the ambient and elevated CO_2 rings (Norby *et al.*, 2001). All calculations of g_c (m s^{-1}) were derived by inverting the Penman–Monteith equation (Stewart, 1988),

$$\lambda E_c = \frac{s(Rn - G) + \rho C_p \delta e g_a}{s + \gamma[1(g_a / g_c)]} \qquad \text{Eqn 1}$$

where E_c is canopy transpiration, λ is the latent heat of vaporization of water (J kg^{-1}), Rn is net radiation above the stand (J m^{-2} s^{-1}), G is heat flux to soils (J m^{-2} s^{-1}), ρ is density of dry air (kg m^{-3}), C_p is specific heat of air at constant pressure (J kg^{-1} K^{-1}), δe is atmospheric humidity deficit (kPa), s is rate of change of saturation water vapor pressure with temperature (kPa K^{-1}), γ is the psychrometric constant (kPa K^{-1}), and g_a is the acrodynamic conductance (m s^{-1}). Net radiation and G were measured at a nearby site, as described by Wilson *et al.* (2000). Aerodynamic conductance was estimated from wind speed (Granier *et al.*, 2000). Equation 1 was solved for g_c using daily averages of all quantities (Phillips & Oren, 1998). Thermodynamic variables were calculated based on air temperature averaged over daylight hours (Fig. 1). Daily δe and Rn were obtained by averaging hourly values throughout the day. Temperature dependencies for the parameters ρ, C_p, λ and γ were as shown in Phillips & Oren (1998).

The decoupling coefficient (Ω) was calculated according to Jarvis & McNaughton (1986),

$$\Omega = (1 + \varepsilon)/(1 + \varepsilon + g_a/g_c) \qquad \text{Eqn 2}$$

where ε is the change of latent heat relative to the change in sensible heat of saturated air.

Sensitivity of stomatal and canopy conductance to vapor pressure deficit

The response of stomatal and canopy conductance to δe was quantified using the approach outlined in Oren *et al.* (1999). Non-linear regression techniques were used to fit coefficients to the equation,

$$g = -m \ln(\delta e) + b \qquad \text{Eqn 3}$$

where g is either stomatal or canopy conductance (mmol m^{-2} s^{-1}); b is the reference conductance at δe = 1 kPa (mmol m^{-2} s^{-1}); m describes the stomatal sensitivity to δe (mmol m^{-2} s^{-1} ln(kPa)$^{-1}$); and $e^{b/m}$ (kPa) is the extrapolated δe where stomata are closed. The parameter m refers to the magnitude by which g_s or g_c are reduced with increasing δe (Oren *et al.*, 1999). Conversion of canopy conductance (m s^{-1}), expressed originally on a ground area basis, to leaf-area based molar units (mmol m^{-2} s^{-1}) was done according to Pearcy *et al.* (1989) using estimates of leaf area index for each of the CO_2 treatment rings (Wullschleger & Norby, 2001).

Statistical analysis

A repeated measures analysis of variance (ANOVA) model was used to test for CO_2 effects on seasonal trends in canopy conductance. Individual rings were the experimental unit (n = 2) and a probability level of P = 0.05 was considered significant. Statistical tests and regressions were performed with the SYSTAT 8.0 statistical package (SPSS Inc., Chicago, IL, USA). Treatment effects for all leaf-level measurements were evaluated using the mean values of gas exchange and atmospheric and soil conditions from each plot for each date that measurements were taken. Two-tailed t-tests for each date, with plot as the experimental unit (n = 2 elevated CO_2 plots and n = 3 ambient CO_2 plots), were used to compare rates and environmental conditions in the elevated CO_2 plots with those in the ambient CO_2 plots. Differences between regression lines describing the response of g_s and g_c to vapor pressure deficit for the two CO_2 treatments were evaluated with an F-test, based on the principle of conditional error, as described by Neter & Wasserman (1974). Analysis and regressions of stomatal conductance data were performed with SAS statistical software (SAS Institute, Cary, NC, USA).

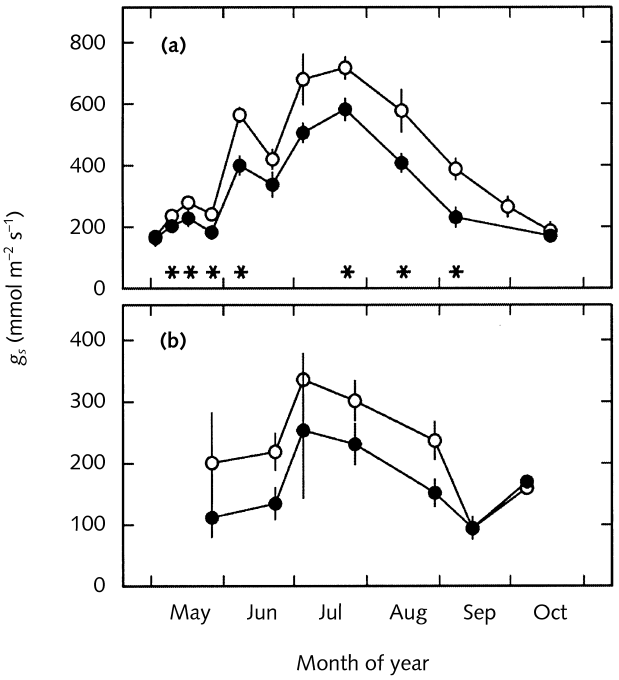

Fig. 2 Seasonal patterns of stomatal conductance measured (a) with a LI-1600 steady-state porometer or (b) with a LI-6400 steady-state photosynthesis system. Data (mean ± SE) for leaves from both the ambient (open circles) and elevated (closed circles) CO_2 treatments are shown. Asterisks indicate significant differences between CO_2 treatments. Measurements of stomatal conductance with the LI-1600 were taken between 10 : 00 and 13 : 00 h, whereas those with the LI-6400 were made between 09 : 00 and 16 : 00 h.

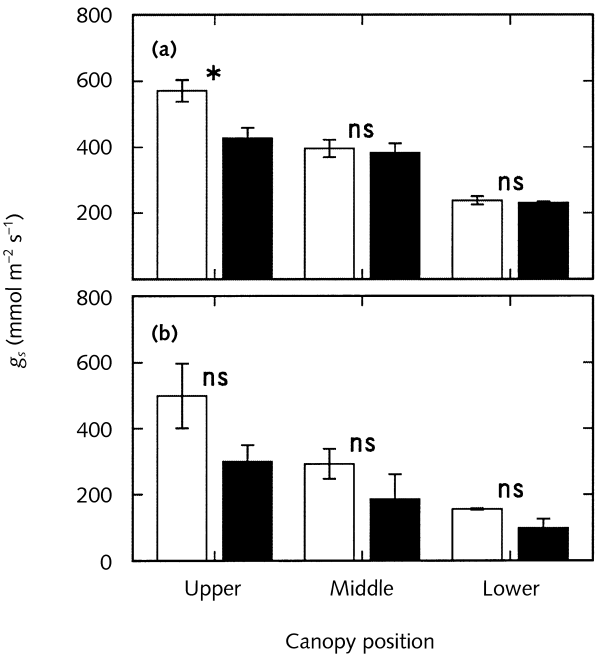

Fig. 3 Stomatal conductance (mean ± SE) measured at ambient (open squares) and elevated (closed squares) CO_2 for leaves selected from each of three canopy positions. Measurements were made either (a) with a LI-1600 steady-state porometer or (b) with a LI-6200 photosynthesis system. Asterisks indicate significant differences between ambient and elevated CO_2 treatments and ns indicates no significant treatment differences.

Results

CO_2 effects on stomatal conductance

A strong seasonal pattern was observed for stomatal conductance measured on leaves in the upper canopy (Fig. 2). Estimates of g_s for leaves from both the ambient and elevated CO_2 treatments were at or below 200 mmol m^{-2} s^{-1} early in the season, increased to considerably higher values in July and then exhibited gradual reductions throughout late summer. This seasonal variation in g_s was observed regardless of whether measurements were made using a steady-state porometer (Fig. 2a) or an open-path photosynthesis system (Fig. 2b). Differences in the absolute estimates of g_s obtained using the two instruments are related to the timing of the measurements. Data from the steady-state porometer were collected early in the day (10 : 00–13 : 00 h) when vapor pressure deficits were generally low, whereas data from the open-path photosynthesis system were collected throughout the day (up to 16 : 00 h). As a result, mean g_s values from the open-path system were influenced by the high vapor pressure deficits during the afternoon hours.

Stomatal conductance was almost always lower in leaves measured at elevated compared with ambient CO_2 concentration, although differences tended to be small late in the growing season. In the two data sets, treatment-induced reductions in g_s ranged from 14 to 40% (mean difference of 85 mmol m^{-2} s^{-1}; Fig. 2a) and from 24 to 44% (mean difference of 60 mmol m^{-2} s^{-1}; Fig. 2b). Significant effects of elevated CO_2 on g_s were observed on seven out of 11 days for measurements collected before 13 : 00 h (based on individual t-tests; Fig. 2a) and repeated measures analysis of variance showed that seasonal differences between CO_2 treatments were highly significant. Data collected over the full course of the day were more variable, although the same trends were observed and differences between CO_2 treatments as indicated by a 2-way analysis of variance were statistically significant (Fig. 2b). The relative reduction of stomatal conductance by elevated CO_2 averaged 22% (Fig. 2a) and 23% (Fig. 2b) across the season.

Maximum estimates of g_s for leaves sampled in ambient CO_2 plots ranged from 335 to 715 mmol m^{-2} s^{-1} (Fig. 2a,b). By comparison, mid-canopy measurements of g_s were typically lower than those measured in the upper canopy (Fig. 3a,b). Stomatal conductance measured on mid-canopy leaves at prevailing environmental conditions was, on average, 30–40% lower than that measured on upper-canopy leaves. No significant differences were observed for g_s between ambient and elevated CO_2 at the middle canopy position (Fig. 3a,b). Estimates of g_s for leaves located in the bottom one-third of the canopy were also lower than those measured at the higher canopy positions. Stomatal conductance

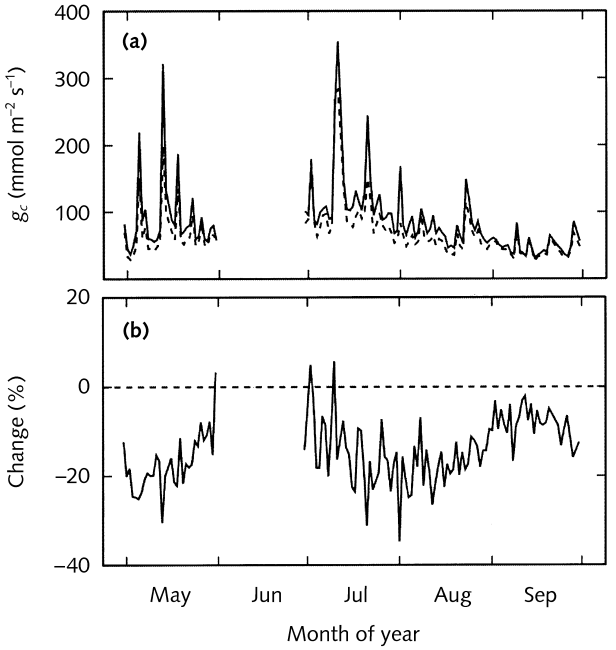

Fig. 4 Seasonal patterns of (a) mean daily canopy conductance derived from sap velocity measurements for sweetgum stands exposed to ambient (solid line) and elevated (dashed line) CO_2 and (b) the per cent difference between CO_2 treatments. Data during June were lost due to operator error.

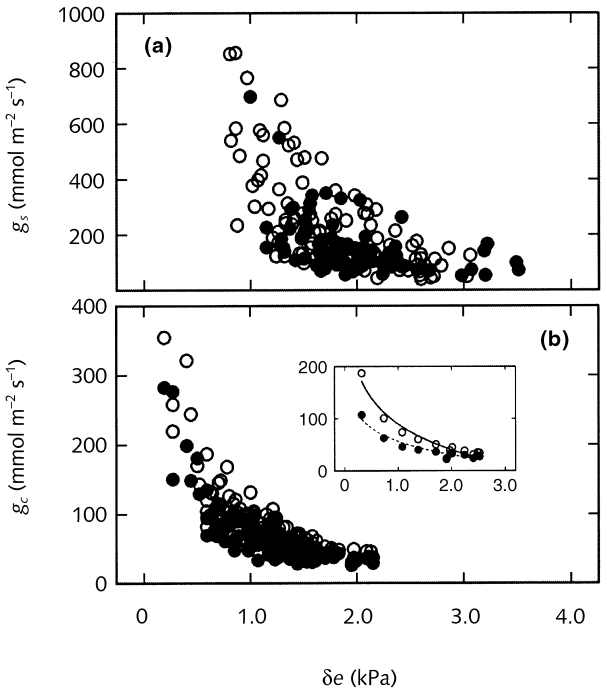

Fig. 5 The response of (a) stomatal conductance and (b) mean daily canopy conductance to vapor pressure deficit. Data are shown for both the ambient (open circles) and elevated (close circles) CO_2 treatments. The insert in (b) shows a representative relationship between hourly canopy conductance and vapor pressure deficit for sweetgum stands exposed to either ambient and elevated CO_2 concentration. Hourly data are from 10 May, 1999.

measured on lower-canopy leaves was typically 60–70% lower than that of upper-canopy leaves and, on average, 45% lower than that of mid-canopy leaves. No significant treatment differences were observed for g_s between ambient and elevated CO_2 in the lower canopy (Fig. 3a,b).

CO_2 effects on canopy conductance

Compared with stomatal conductance, daily mean canopy conductance followed a less distinct seasonal pattern with monthly estimates highest in July (mean 125 mmol m^{-2} s^{-1}) and lowest in September (mean 45 mmol m^{-2} s^{-1}). There were modest reductions in g_c with atmospheric CO_2 enrichment, averaging 14% over the growing season (Fig. 4a). Relative changes in g_c due to elevated CO_2 varied throughout the year from +6% in early July to –34% in late July (Fig. 4b). The greatest reductions in g_c due to elevated CO_2 were generally observed during early May, late July and early August, with more modest reductions during early July and much of September (Fig. 4b).

Effects of environmental variation on stomatal and canopy conductance

Multiple regressions of stomatal conductance and mean daily canopy conductance against radiation and δe revealed that much of the variation in g_s and g_c was explained by day-to-day changes in δe (data not shown). Both g_s and g_c showed marked declines in response to increases in vapor pressure deficit (Fig. 5a,b). The shape of the relationship between g_s or g_c and δe could be adequately described using the equation suggested by Oren *et al.* (1999). That equation quantified the stomatal sensitivity to δe (parameter m in Eqn 3), the reference conductance at δe = 1 kPa (parameter b in Eqn 3) and the extrapolated δe where stomata are completely closed (parameter $e^{b/m}$ in Eqn 3) for both g_s of leaves and g_c of canopies at ambient and elevated CO_2 (Table 1). Numeric reductions in both the reference g_s at δe = 1 kPa and stomatal sensitivity to δe at elevated CO_2 were observed and treatment differences for these two parameters ranged from 21 to 23%. Although the parameters b and m were reduced at elevated CO_2 concentration, the equations that described the response of g_s to δe at ambient and elevated CO_2 were not significantly different in an F-test (P = 0.07).

Similar regressions were used to describe the response of g_c to δe and, in this case, the two curves were significantly different in an F-test (P = 0.03). Individual equation parameters were significantly different ($\alpha < 0.10$) and were, on average, 21% lower at elevated compared with ambient CO_2 (Table 1). As was observed for stomatal conductance, differences in g_c between ambient and elevated CO_2 tended to be less pronounced at high δe. Stratification of the data to

Table 1 Regression coefficients (mean ± SD) used to describe the dependency of stomatal and canopy conductance on vapor pressure deficit

Measure/CO_2 treatment	b (mmol m^{-2} s^{-1})	m (mmol m^{-2} s^{-1} ln(kPa)$^{-1}$)	$e^{b/m}$ (kPa)
Stomatal conductance			
Ambient	439 ± 35	373 ± 54	3.28 ± 0.36
Elevated	345 ± 134	289 ± 191	3.94 ± 1.59
$P > T$	0.494	0.301	0.508
Canopy conductance			
Ambient	95 ± 2	105 ± 2	2.46 ± 0.01
Elevated	76 ± 11	82 ± 12	2.52 ± 0.02
$P > T$	0.057	0.076	0.046

Nonlinear regression techniques were used to fit coefficients to the equation g_s or $g_c = -m \ln(\delta e) + b$ (Oren *et al.*, 1999) where b is the reference conductance at $\delta e = 1$ kPa, m describes stomatal sensitivity to δe, and $e^{b/m}$ is the extrapolated δe where stomata are completely closed. Stomatal and canopy conductance are both expressed on a leaf area basis and statistically significant differences between CO_2 treatments at $\alpha < 0.05$ as indicated by t-tests are shown.

Table 2 Mean daily canopy conductance (mean ± SD) for trees in the ambient and elevated CO_2 rings stratified according to mean daily vapor pressure deficit

		Canopy conductance (mmol m^{-2} s^{-1})			
Vapor pressure deficit (kPa)	n[1]	Ambient	Elevated	Change (%)	$P > T$
0–0.5	6	243 ± 63	207 ± 60	–15.1	ns
0.5–1.0	32	100 ± 29	86 ± 18	–14.5	*
1.0–1.5	59	66 ± 17	57 ± 14	–13.3	*
> 1.5	24	44 ± 10	40 ± 8	–9.2	ns

*Significant ($\alpha < 0.05$) reductions in mean daily canopy conductance due to elevated CO_2 concentration; ns, treatment differences were nonsignificant. [1]The number of days included in each stratification level.

discrete ranges of δe showed that CO_2-induced reductions in g_c were 9–15% across all δe, but absolute differences were smaller and nonsignificant at higher δe (Table 2). A similar analysis applied to g_s also indicated a general trend toward a less pronounced effect of elevated CO_2 on g_s as δe increased. The per cent reduction in g_s for leaves exposed to elevated CO_2 was 25% at low δe (< 1.5 kPa) and only 14% at medium (1.5–2.0 kPa) or high (> 2.0 kPa) δe (data not shown).

In addition to vapor pressure deficit, stomatal and canopy conductances were also affected by soil water potential (Fig. 6). Although data on soil water availability over this time period are limited to only four dates, both g_s and g_c exhibited similar patterns of decline as soil water potentials fell from field capacity (–0.4 MPa) to below –1.0 MPa. Stomatal conductance measured on leaves from the ambient and elevated CO_2 treatments decreased to roughly 25% and 30%, respectively, of their maximum values as soil water potentials approached –1.0 to –1.2 MPa (Fig. 6a). Canopy conductance was similarly responsive to soil drying, with g_c decreasing to 45–50% of their maximum values over the same period (Fig. 6b). Reductions in g_s and g_c due to elevated CO_2 ranged from 22% to 28%, respectively, under conditions of ample soil water. Only small differences (< 5%), however, were observed between ambient and elevated CO_2 treatments for either g_s or g_c under drier soil conditions.

CO_2 effects on the decoupling coefficient

Estimates of the decoupling coefficient varied throughout the year, ranging from 0.08 to 0.15 early and late in the season to 0.61 during mid-July (Fig. 7). Differences in the decoupling coefficient between ambient and elevated CO_2 were marginal, with reductions due to atmospheric CO_2 enrichment approaching 14%. Averaged across the season, the decoupling coefficient was 0.28 for trees exposed to ambient CO_2 and 0.24 for trees exposed to elevated CO_2 concentrations.

Discussion

A common expectation from many, albeit not all, studies that address the physiological response of plants to elevated CO_2 is that stomatal conductance will be reduced. In herbaceous species, these reductions can approach 27–40% (Morison, 1985; Field *et al.*, 1995), whereas in some coniferous species the response may be considerably less (Ellsworth, 1999; Teskey, 1995; Tissue *et al.*, 1997). In addition, stomata are also highly responsive to other environmental variables, such that high vapor pressure deficits, drying soils and low light may all act to reduce g_s from its theoretical maximum. Within this matrix of interactions, the complex response of stomatal conductance to CO_2 enrichment must be considered and

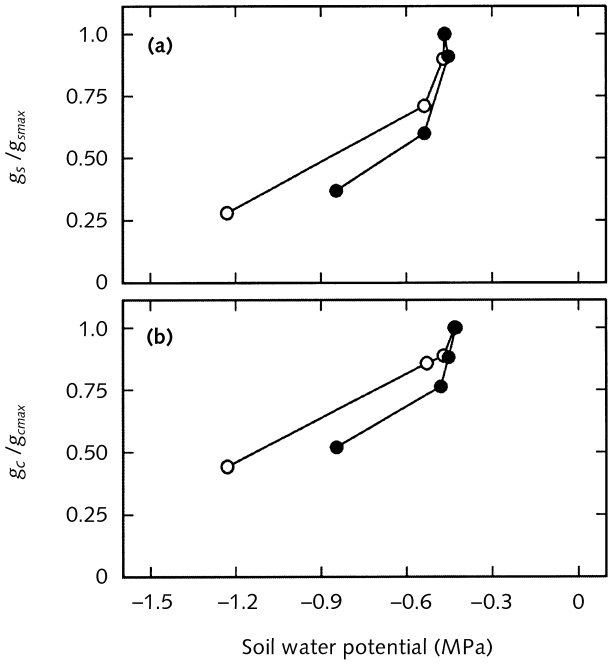

Fig. 6 Relationship of (a) stomatal conductance and (b) daily canopy conductance to soil water potential as measured on leaves and trees at ambient (open circles) and elevated (closed circles) CO_2 concentration. Stomatal and canopy conductance are expressed relative to maximum values of each variable measured at field capacity.

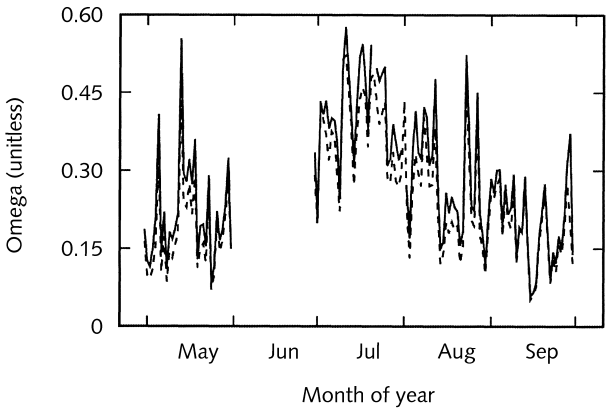

Fig. 7 Seasonal variation in daily estimates of the decoupling coefficient (Ω) for sweetgum stands exposed to ambient and elevated CO_2 concentration.

integrated to understand the impacts of environmental change at higher scales. In this study, the mean 22–24% decrease in g_s by elevated CO_2 was comparable to means reported across multiple woody species, including appreciable variation within and among studies (e.g. 20–23%; Field *et al.*, 1995; Drake *et al.*, 1997; Medlyn *et al.*, 2001). Although reductions in g_s were smaller in magnitude and less statistically robust than concurrent increases in photosynthesis, they were similarly sustained over three growing seasons (Gunderson *et al.*, 2002), indicating that stomatal sensitivity to elevated CO_2 was not lost over time. Stomatal interactions with environmental variation over the course of the day and the growing season such as those we observed for sweetgum trees doubtlessly contributed to the lack of statistical significance on individual dates, and may contribute to the often ambiguous effects on g_s observed in woody species (Curtis & Wang, 1998), where stomatal responses tend to be smaller than in herbaceous plants (Gunderson *et al.*, 1993; Beerling *et al.*, 1996; Heath, 1998; Rey & Jarvis, 1998; Saxe *et al.*, 1998; Ellsworth, 1999; Norby *et al.*, 1999).

A decrease in g_s with depth in the canopy is a common observation in trees growing in a closed-canopy forest or plantation (Whitehead, 1998). It reflects the fact that leaves in the lower canopy are older, often possess lower nitrogen concentrations and are, as a result, less physiologically active than are upper-canopy leaves (Warren & Adams, 2001). In addition, lower-canopy leaves are exposed to vastly different environmental conditions, including radiation, than are upper-canopy leaves and this too can preclude maximal stomatal function (Leuning *et al.*, 1995). In the case of the closed-canopy sweetgum plantation studied here, our observation that g_s decreases with depth in the canopy primarily reflects lower light availability (Gunderson *et al.*, 2002). More interesting perhaps is the observation that not only was the absolute magnitude of g_s dependent on canopy position, but so too was the relative difference in g_s between CO_2 treatments. Although variability was admittedly high and differences not always significant, marked reductions in g_s observed in upper-canopy leaves at elevated CO_2 were not typically seen at middle or lower canopy positions. Since so few studies have been conducted on closed-canopy stands exposed to atmospheric CO_2 enrichment, the observation that the effect of elevated CO_2 on g_s is dependent on radiation regime is unique. Herrick & Thomas (1999, 2001) examined the effects of elevated CO_2 on photosynthesis of sun and shade leaves in sweetgum trees at the Duke University FACE facility, and argued that conclusions about the response of plants to elevated CO_2 must take into account the complex nature of the light environment within a canopy and how light interacts with CO_2 to affect photosynthesis. We extend this argument to stomatal conductance and suggest that CO_2-induced reductions in g_s may also be dependent on canopy light environment. If this is shown to be true, such a response would have important implications for how effects of elevated CO_2 on stomatal conductance measured on only upper-canopy leaves should be scaled throughout plant canopies. It would also have relevance to the sensitivity of understory vegetation to elevated CO_2 (DeLucia & Thomas, 2000) where low light may limit some physiological responses of plants to CO_2 enrichment.

Since stomatal conductance of upper-canopy leaves was sensitive to atmospheric CO_2 enrichment, whereas mid- and lower-canopy leaves were less so, it is expected that only

moderate effects of elevated CO_2 would be observed on canopy conductance. Treatment differences in g_c ranged from +6 to –34% and averaged –14% over the growing season. This agrees, as it should, with our previous findings that sap velocity and stand transpiration were only moderately responsive to elevated CO_2 (Wullschleger & Norby, 2001). Ellsworth *et al.* (1995) reported no effect of elevated CO_2 on sap velocity in loblolly pine (*Pinus taeda* L.) and others have reported that treatment differences in sap velocity or whole-tree water use are difficult to detect (Senock *et al.*, 1996; Kellomäki & Wang, 1998). Such detection difficulties were attributed in our earlier paper to the dependency of stand transpiration on prevailing weather conditions, particularly vapor pressure deficit and radiation (Wullschleger & Norby, 2001). Others have drawn similar conclusions about whole-tree and canopy transpiration, but few studies have been conducted such that the response of canopy conductance to ambient and elevated CO_2 could be explicitly compared. Pataki *et al.* (1998) reported an 8% reduction in sap velocity for 4-year-old loblolly pines exposed in open-top chambers to a +300 ppm increase in atmospheric CO_2 and a similar, albeit highly variable, decrease in mean daily canopy conductance. Estimates of daily g_c in the study of Pataki *et al.* (1998) varied between 9 and 16 mmol m^{-2} s^{-1} (all-sided leaf area) and all measurements were taken during periods of the year when mean daily air temperatures did not exceed 10°C. A combination of low temperatures and inherently low g_c for small pine saplings, compared with the environmental and growth conditions of our study, may have limited a response to elevated CO_2 in their study. Nonetheless, our results agree with the general conclusions drawn by Pataki *et al.* (1998) that canopy conductance in loblolly pine, and now in sweetgum, was only marginally affected by the CO_2 treatments imposed.

Vapor pressure deficit was the most significant environmental influence on leaf gas exchange and g_s in both CO_2 treatments of this study, explaining more variation than either temperature, radiation, or soil water potential (Gunderson *et al.*, 2002). A similar observation was made for canopy conductance, as daily variability in δe explained approximately 75% of total variation in measured canopy conductance. Our observation that g_s and g_c decline exponentially with increasing δe is consistent with relationships found in a variety of leaf, whole-tree and stand-level studies (Köstner *et al.*, 1992; Granier *et al.*, 1996; Köstner *et al.*, 1996). It is interesting that based on our data, the equations and associated parameters used to describe the dependency of both g_s and g_c on δe (i.e. Oren *et al.*, 1999) were different between leaves and trees measured in the two CO_2 treatments. Application of Eqn 3 to available data sets indicated that the sensitivity of both g_s and g_c to increasing δe was, on average, 20% lower at elevated than it was at ambient CO_2 concentration, as were estimates of g_s and g_c at a reference δe of 1.0 kPa. Tognetti *et al.* (1999) and Heath (1998) both reported reduced sensitivity of stomata to δe for trees growing in CO_2-enriched atmospheres, whereas Kellomäki & Wang (1998) suggested that a decrease in sap flow at elevated CO_2 was largely due to a CO_2-induced increase in stomatal sensitivity to high vapor pressure deficit. Morison (1998) points out that the response of stomata to elevated CO_2 is important in understanding not only stomatal physiology, but also in understanding plant–atmosphere interactions at scales from the individual plant to global vegetation. Unfortunately, few studies have addressed stomatal acclimation to elevated CO_2 or examined the specific sensitivity of either g_s or g_c to δe (Drake *et al.*, 1997). Despite this lack of information, our data suggest that the sensitivity of g_s and g_c to δe, at least as defined in Oren *et al.* (1999), does decline with atmospheric CO_2 enrichment. Such leaf- and canopy-scale observations are in general agreement with conclusions drawn from a variety of studies (Hollinger, 1987; Will & Teskey, 1997; Heath, 1998; Tognetti *et al.*, 1998).

Estimates of g_s and g_c obtained across a range of vapor pressure deficits and soil water potentials indicated that the effects of elevated CO_2 on these parameters becomes less as g_s and g_c are reduced in absolute magnitude. Differences between treatments that were 25–35% at low δe and ample soil moisture, were more typically less than 10% under conditions of high δe and drought. Comparatively small CO_2-induced reductions in g_s have elsewhere been associated with species having intrinsically lower g_s (Morison, 1985; Saxe *et al.*, 1998), with dry season conditions, when δe was high and g_s was low (Goodfellow *et al.*, 1997), and with warm sunny days with high δe (Beerling *et al.*, 1996; Heath, 1998). All of these results are consistent with the observation by Curtis (1996) that reductions in g_s due to CO_2 tend to be less in stressed plants. Pataki *et al.* (2000) observed that stomatal conductance in plants at a free-air CO_2 enrichment experiment in an undisturbed Mojave Desert ecosystem was reduced in the high CO_2 treatment, although the effect was apparent only under conditions of ample soil moisture. Similarly, Ellsworth *et al.* (1995) suggested that stomatal closure in *Pinus taeda* under high CO_2 concentration, which was found to be minimal under drought conditions, may be more pronounced when soil moisture is abundant. Although this conclusion was refuted by Pataki *et al.* (1998) our results suggest that as g_s and g_c become less, so too does the magnitude of the CO_2 affect. Stratification of available data indicated that the per cent change in g_c due to elevated CO_2 for conditions where g_c was > 150 mmol m^{-2} s^{-1} was –28%, whereas within the range of 50–150 mmol m^{-2} s^{-1} it was roughly –15% and at < 50 mmol m^{-2} s^{-1} it was only –10%. We conclude that any condition that decreases the absolute magnitude of stomatal or canopy conductance, including vapor pressure deficit, soil water availability, or canopy position, will also reduce the effect that elevated CO_2 has on these exchange processes.

Changes in whole-plant water use under high CO_2 are of interest for predictions of large-scale water vapor fluxes, as well as stand growth and composition under future elevated concentrations of atmospheric CO_2 (Pataki *et al.*, 1998). In

Table 3 Estimated rates of annual evapotranspiration for a closed-canopy sweetgum stand exposed to ambient and elevated CO_2 concentration

Treatment	Transpiration (mm)	Interception (mm)	Evaporation (mm)	Evapotranspiration (mm)
Ambient	540	95	110	745
Elevated	484	95	110	689
Change (%)	–10	–	–	–7

Annual transpiration rates were derived from measurements of sap velocity, stand sapwood area, and fraction of sapwood functional in water transport (Wullschleger & Norby, 2001). Individual components of interception and soil evaporation were calculated as described by Shuttleworth & Wallace (1985).

canopies with high leaf area index, boundary layer and aerodynamic conductance may exert a stronger control on water vapor exchange than stomatal conductance, so that any change in g_s induced by elevated CO_2 may only marginally affect transpiration and hence, stand water use. Niklaus *et al.* (1998) reported that ecosystem-level controls of the water balance can, in responsive systems such as grasslands, far outweigh the physiological effects of elevated CO_2 observed at the leaf level. Our observation that the daily decoupling coefficient or Ω was high during mid-summer suggests that changes in g_s due to elevated CO_2 may lead to only marginal reductions in transpiration. For example, for a closed-canopy forest with an Ω of 0.5, a 24% change in stomatal conductance for leaves exposed to CO_2 enrichment would result in only a 12% change in transpiration. Such a partial uncoupling of CO_2-induced effects on g_s at the level of individual leaves from associated impacts on transpiration at the scale of the canopy were, in our study, the result of low wind speeds that contributed to relatively low estimates of aerodynamic conductance.

In speculating about the effects of elevated CO_2 on forest hydrology and evapotranspiration (ET), it is important to consider that not all components of ET will be affected in a CO_2-enriched atmosphere. Interception losses and soil evaporation might not change with rising CO_2 concentration, particularly in a closed-canopy plantation such as the one we studied where leaf area index was not different between ambient and elevated CO_2 (Norby *et al.*, 2001). Using estimates of annual transpiration from Wullschleger & Norby (2001) and modeled estimates of interception losses and soil evaporation, we calculate that annual ET would be 745 mm and 689 mm for the ambient and elevated CO_2 treatments, respectively (Table 3): a difference of only 7% for the year. Wilson *et al.* (1999) emphasized in a modeling study that feedbacks associated with changing leaf area and soil moisture due to elevated CO_2 were important considerations in understanding effects of CO_2 enrichment on ET and showed that the impact of these factors on ET for agricultural crops could be significant. There has been a trend for slightly higher soil water potential (and content) in the elevated CO_2 plots of our study (Gunderson *et al.*, 2002), but differences between treatments have not been significant. Although our calculations, as presented in Table 3 are speculative, we suspect that feedbacks associated with leaf area and soil moisture will play only minor roles in determining annual rates of ET for closed-canopy forests exposed to elevated CO_2 concentration. Nonetheless, future studies should (as best they can) include efforts to monitor all components of ET (Field *et al.*, 1995).

Decreases in estimated ET at elevated CO_2 are theoretically less than decreases in single-leaf g_s not only because of canopy decoupling, but also because of negative feedbacks associated with in-canopy vapor pressure deficit (Jarvis & McNaughton, 1986). As the spatial scale increases from stomata to canopy, and atmospheric transport processes become more limiting, thermodynamic considerations suggest that the lower g_s at elevated CO_2 will result in higher leaf temperatures and lower humidity in the canopy. This feedback acts to increase the driving force for transpiration (δe) and partly counteracts decreases in stomatal conductance. Midseason values of the decoupling coefficient of 0.5, which are an indication of the magnitude of this feedback, suggest that canopy transpiration rates may only be 50% as large as a change in stomatal conductance. Although the use of the 'decoupling coefficient' is not strictly valid for small plots, such as the FACE rings, feedbacks associated with canopy temperature and humidity are nonetheless likely. It is also likely that the feedback associated with Ω is smaller in the FACE plots than it would be in more extensive canopy. As a result, CO_2-induced decreases in ET would be even less in more natural settings. Similar feedbacks at even larger scales, such as in a region with a diameter of several kilometers that is mostly forested, would further diminish the CO_2 effect on ET (Jacobs & De Bruin, 1997; Wilson *et al.*, 1999).

Finally, natural ecosystems provide a critical biotic feedback between the Earth's terrestrial vegetation and our ever-changing climatic system. One is very much dependent on the other, and large-scale studies that examine the dynamic and often complex interaction between vegetative surfaces and the atmosphere are needed. A feedback of critical importance to the study of climate change is the hydrological response of forests to rising CO_2 concentration. Few studies, however, have investigated this response at relevant temporal and spatial scales. Our results show that the response of g_s to elevated CO_2 in a fluctuating environment is indeed complex, and that simple reductions in g_s with rising CO_2 are dampened or accentuated depending on canopy location and

interactions with vapor pressure deficit, soil water potential and canopy position. It is also clear that as the scale of observation increased, there was a general decline in the relative magnitude to which elevated CO_2 impacts processes related to forest water use. Thus, we conclude that despite large effects of elevated CO_2 on stomatal conductance, the influence of these effects on ET and larger-scale patterns of water use are likely to be minimal in forests that approximate conditions of the sweetgum plantation studied in this investigation.

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