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Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment

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ABSTRACT

Light-saturated photosynthetic and stomatal responses to elevated CO_2 were measured in upper and mid-canopy foliage of a sweetgum (*Liquidambar styraciflua* L) plantation exposed to free-air CO_2 enrichment (FACE) for 3 years, to characterize environmental interactions with the sustained CO_2 effects in an intact deciduous forest stand. Responses were evaluated in relation to one another, and to seasonal patterns and natural environmental stresses, including high temperatures, vapour pressure deficits (*VPD*), and drought. Photosynthetic CO_2 assimilation (*A*) averaged 46% higher in the +200 μmol mol^{-1} CO_2 treatment, in mid- and upper canopy foliage. Stomatal conductance (g_s) averaged 14% (mid-canopy) and 24% (upper canopy) lower under CO_2 enrichment. Variations in the *relative* responses of *A* and g_s were linked, such that greater relative stimulation of *A* was observed on dates when relative reductions in g_s were slight. Dry soils and high *VPD* reduced g_s and *A* in both treatments, and tended to diminish treatment differences. The *absolute* effects of CO_2 on *A* and g_s were minimized whenever g_s was low (<0·15 mol m^{-2} s^{-1}), but *relative* effects, as the ratio of elevated to ambient rates, varied greatly under those conditions. Both stomatal and non-stomatal limitations of *A* were involved during late season droughts. Leaf temperature had a limited influence on *A* and g_s, and there was no detectable relationship between prevailing temperature and CO_2 effects on *A* or g_s. The responsiveness of *A* and g_s to elevated CO_2, both absolute and relative, was maintained through time and within the canopy of this forest stand, subject to seasonal constraints and variability associated with limiting air and soil moisture.

Key-words: *Liquidambar styraciflua* (sweetgum); drought; elevated CO_2; free-air CO_2 enrichment (FACE); photosynthesis; stomatal conductance; temperature; trees; vapour pressure deficit.

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INTRODUCTION

Understanding leaf-level responses to CO_2 enrichment, and the impact of environmental variation on those responses, remains essential for projecting long-term changes in forest ecosystem function and structure. Considerable uncertainty surrounds such potential secondary and tertiary effects of increasing atmospheric CO_2 as altered productivity and water use, feedbacks to the carbon, nitrogen and water cycles, and patterns of forest succession (Eamus & Jarvis 1989; Ceulemans & Mousseau 1994; Field, Jackson & Mooney 1995; Johnson & Ball 1996; Drake, Gonzàlez-Meler & Long 1997; Saxe, Ellsworth & Heath 1998; Norby *et al.* 1999), yet the question of higher scale consequences is moot without sustained foliar responses.

The CO_2 enrichment of seedlings and saplings has repeatedly been shown to increase light-saturated photosynthesis (*A*), but the degree of stimulation has varied widely, not only among studies (e.g. from none to +180%), but on different dates within each study (reviewed in Ceulemans & Mousseau 1994; Gunderson & Wullschleger 1994; Curtis 1996; Curtis & Wang 1998; Saxe *et al.* 1998; Medlyn *et al.* 1999; Norby *et al.* 1999). Taxonomic differences, duration of the experiments, developmental stage of the trees, temperature differences, and environmental stresses such as drought have all been proposed as significant sources of variability (Gunderson & Wullschleger 1994; Curtis 1996; Saxe *et al.* 1998). The latter two are most likely to explain within-experiment variability, notwithstanding differences related to leaf age (Rey & Jarvis 1998; Turnbull *et al.* 1998). It has been hypothesized that the relative effect of CO_2 enrichment on *A* will be greater under water-limited conditions (Idso & Idso 1994), and at higher temperatures (Long 1991; Idso & Idso 1994), yet the evidence for such patterns in mature tree canopies remains quite limited. Indeed, when considering the ecosystem-scale impacts of elevated CO_2, the *absolute* differences in assimilation under stressful conditions may be more meaningful than the *relative* responses.

Despite the well-known responsiveness of stomatal aperture to [CO_2], documenting a sustained stomatal response to CO_2 enrichment in woody plants has not been straightforward, and reported impacts range from none (even increases) to reductions of 60% (reviewed in Morison 1985; Gunderson & Wullschleger 1994; Field *et al.* 1995;

Drake *et al*. 1997; Curtis & Wang 1998; Saxe *et al*. 1998; Norby *et al*. 1999; Medlyn *et al*. 2001). Taxonomic, ontogenetic, and environmental factors have been proposed to account for some of the differences. The hypotheses most pertinent to within-experiment variability involve interactions between CO_2 exposure and water limitations (Morison 1993; Beerling *et al*. 1996). It has been hypothesized that CO_2 enrichment reduces *relative* stomatal sensitivity to drought, vapour pressure deficit (Heath 1998), or to CO_2 itself (discussed in Sage 1994; Assmann 1999), but again, *absolute* differences may be more important for predicting stand-level water balance. Because photosynthesis and stomatal conductance (g_s) are tightly coupled, environmental influences on one cannot be considered in isolation from the other. Environmentally induced variation in stomatal sensitivity to CO_2 could thus affect photosynthetic responses, in the same way that species differences in stomatal sensitivity have been proposed to explain differences in photosynthetic response to CO_2 (Idso 1991).

Because the ultimate goal of CO_2 enrichment studies is to be able to predict and model changes in intact forest ecosystems, the focus of research has moved toward multi-year CO_2 exposures of large-stature trees, using methods such as free-air CO_2 enrichment (FACE) (Hendrey *et al*. 1999). The challenge of current research is to relate variation in leaf-level CO_2 responses to variations in atmospheric and soil conditions, and to integrate this information to the canopy scale, providing a link to forest growth and ecosystem processes.

In this article we report on 3 years of foliar gas exchange in a closed-canopy stand of deciduous trees (*Liquidambar styraciflua* L., or sweetgum) exposed to CO_2 enrichment in a FACE facility (Norby *et al*. 2001; Wullschleger & Norby 2001). The objectives included quantifying both the sustained responses to elevated CO_2 and the environmental sources of variation in photosynthetic and stomatal responses, focusing on temperature, vapour pressure deficit, and soil moisture availability. We also examined the interdependence of stomatal and photosynthetic responses to CO_2 enrichment, and compared responses in upper and mid-canopy foliage. The following hypotheses were addressed: (1) that photosynthetic enhancement and stomatal sensitivity would persist, and not decrease through acclimation to CO_2 enrichment; (2) that the relative effect of CO_2 on photosynthesis would be greater under environmental stress such as low soil moisture, high vapour pressure deficit, and high temperatures; and (3) that moisture stress would limit the impact of CO_2-induced stomatal closure.

MATERIALS AND METHODS

Site description

The experimental site is in a 1·7 ha plantation of sweetgum trees (*Liquidambar styraciflua* L) established in 1988 from 1 year bare-root seedlings on the Oak Ridge National Environmental Research Park in Roane County, Tennessee (35°54′ N, 84°20′ W). The soil is classified as an Aquic Hapludult with a silty clay loam texture, pH approximately 5·5–6·0, and is moderately well drained (van Miegroet, Norby & Tschaplinski 1994; Norby *et al*. 2001). Seedling spacing in the 185 m by 70 m section of the plantation used for the FACE study was 2·3 m × 1·2 m. Herbicide application was used during establishment (1989 and 1990), but no fertilizer was applied. The canopy has been closed since at least 1996, and the trees are in a linear growth phase (for additional details, see Norby *et al*. 2001). When the CO_2 exposure began in the spring of 1998, the trees were 12–13 m in height, with live crown beginning at 8–9 m above the ground. Each year the trees have added approximately 1 m in height, and by the next spring have lost lower branches so that the live crown remains approximately the same depth (4–5 m). In the spring of 2000, the trees were approximately 15–16 m tall.

FACE facility and treatments

The FACE facility at Oak Ridge National Laboratory consists of five circular plots, 25 m in diameter, 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. The target CO_2 concentration within the plot is regulated based on the design, equipment and software of Hendrey *et al*. (1999). Details of the Oak Ridge FACE facility and operation may be found in Norby *et al*. (2001). 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 CO_2 plot without towers or blowers. No differences in gas exchange (data not shown) or other response variables have been detected between blower and non-blower controls (Norby *et al*. 2001).

The CO_2 treatment began in April 1998, prior to leaf-out, and CO_2 regulation was fully operational by 11 May 1998. Fumigation was terminated after leaf senescence each autumn and re-initiated each spring before new leaves emerged. The treatment set-point for 1998 was a constant 560 μmol mol^{-1} CO_2, approximately 200 μmol mol^{-1} above global average [CO_2]. This was modified in 1999 and 2000 to incorporate a natural pattern of diurnal variation, with day-time set-point of 565 μmol mol^{-1} and a higher night-time set point of 645 μmol mol^{-1}. Actual mid-day CO_2 concentrations coincident with gas exchange sampling (0900 to 1600 h) averaged 548, 556 and 555 μmol mol^{-1} in 1998, 1999, and 2000, respectively, at the centre of the plots. Means include periods when fumigation was suspended for experimental purposes, or because of high winds, equipment failure, or delayed CO_2 delivery. Mid-day [CO_2] in the control plots averaged 362, 365, and 366 μmol mol^{-1} in 1998, 1999, and 2000, respectively.

Canopy access and leaf selection

Canopy access was achieved using hydraulic personnel lifts (Model UL48; UpRight, Inc., Selma, CA, USA) positioned at the centre of each plot. The aerial work platforms extended up to 15·5 m, providing access to multiple crown positions. Lifts became operational in July 1998, and gas exchange measurements began at that point, ending in mid-September. Measurements for 1999 and 2000 were conducted between May and October.

Leaves selected for measurements were representative of general canopy conditions, fully expanded, and near the tips of the branches in either the upper or mid-canopy. Foliage from four to six trees in each plot was accessible from the lift, and data from all leaves measured at a given canopy position (four to eight leaves per plot on each date) were used to derive mean values for the plot.

Upper canopy leaves were chosen from branches exposed to mid-day full sunlight, in the top 2–3 m of the canopy, i.e. from the upper 20–30% of canopy foliage. Upper canopy measurements were taken on five to seven dates each season.

Mid-canopy (i.e. mid-height) measurements were taken approximately 3–4 m down into the canopy, on fully expanded leaves near the branch tips. Leaves within this portion of the canopy received 20–50% of full PPFD (estimated each summer after full canopy development, using a 1 m line quantum sensor (model LI-191SA; Li-Cor, Inc., Lincoln, NE, USA). Mid-canopy leaves were measured in July of 1998 and 1999, and four times in 2000.

Gas exchange methods

Gas exchange responses were evaluated at the nominal treatment [CO_2], under saturating light conditions. Measurements were conducted between 0900 and 1600 h, on a single date in some cases, or over 2 to 4 d in other cases, when light or CO_2 response curves were being conducted in the same sampling period.

Data from 21 July 1998 onward were obtained using the LI-6400 steady state photosynthesis system (Li-Cor, Inc.) using the 6 cm^2 cuvette. Measurements were taken at saturating irradiance (1800–2000 μmol m^{-2} s^{-1} PAR) provided by red/blue LED light source (model LI 6400–02B; Li-Cor, Inc.).

Cuvette temperatures were set based on mid-afternoon temperatures forecast for each measurement period. Resulting cuvette air temperatures were highly correlated with prevailing air temperatures, and means were ±1–2 °C of mid-day site air temperatures during the measurements (Fig. 1a; R^2 = 0·83 for the correlation). Heat generated by the light source increased leaf temperatures by 1–2·8 °C, but leaf temperatures (Fig. 1a) were still correlated with prevailing air temperatures (R^2 = 0·82). Cuvette humidity was not controlled, except as needed to avoid condensation on rare occasions when the relative humidity of the cuvette exceeded 80%. Atmospheric vapour pressure deficits (*VPD*) at the site varied considerably among measurement dates (Fig. 1b), but also changed markedly within a day, mostly as a function of increasing air temperatures throughout the day. For example, between 0900 and 1600 h, *VPD* increased from 0·9 to 2·3 kPa on day 208, 1999, and from 1·4 to 2·8 kPa on day 258, 1999. Nevertheless, mean leaf-to-air vapour pressure deficits in the cuvette tracked mid-day atmospheric *VPD* within 0·7 kPa (Fig. 1b), notwithstanding the counteracting influences of elevated leaf temperatures and higher humidity of the cuvette environment (R^2 = 0·52 for the correlation). There were no significant differences in *VPD* and leaf temperature between treatments.

The CO_2 concentrations in the cuvette were regulated using a CO_2 mixer and injector system (Model LI-6400–01; Li-Cor, Inc.) and cartridges of compressed CO_2. Inlet air CO_2 concentrations were set to 360–365 μmol mol^{-1} for measurements in the ambient CO_2 plots, and 560–565 μmol mol^{-1} in the elevated CO_2 plots, resulting in mean cuvette CO_2 concentrations (C_a) of 346 and 541 μmol mol^{-1} in the ambient and elevated CO_2 treatments, respectively. Some of the gas exchange data were obtained while developing CO_2 response curves, using 10 inlet CO_2 concentrations between 0 and 1500 μmol mol^{-1} (Sholtis *et al.* MS in preparation). The relationships between *A* and the intercellular CO_2 concentration (C_i) in these curves were also used to assess stomatal limitation.

In addition to the LI-6400 data, on 7–9 July 1998, measurements were obtained using the LI-6200 photosynthesis system (Li-Cor, Inc.), using prevailing light, temperatures, humidity, and [CO_2], in both upper and mid-canopy positions. Mean cuvette conditions for the upper canopy measurements were 1213 μmol m^{-2} s^{-1} PAR, 37 °C, and CO_2 concentrations of 336 ± 9 and 550 ± 25 μmol mol^{-1} in ambient and elevated CO_2 treatments, respectively. Mean mid-canopy PAR was 687 ± 210 μmol mol^{-1}, at or near saturating for both *A* and g_s in mid-canopy sweetgum leaves (data not shown).

Data obtained as part of the gas exchange measurements included area-based light-saturated net photosynthetic CO_2 assimilation (*A*), stomatal conductance to water vapour (g_s), leaf and air temperature, relative humidity, leaf-to-air vapour pressure deficit (*VPD*), intercellular CO_2 concentration (C_i), and leaf-level photosynthetic water use efficiency, or instantaneous transpiration efficiency (*ITE*), which was calculated as assimilation/transpiration.

Soil moisture measurements

Soil water content (%, v/v, integrated from 0 to 20 cm soil depth) was measured periodically with a time domain reflectometer (TDR; 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, to a depth of 20 cm, 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 (*SWP*) 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 for each date that measurements were taken. Treatment *SWP* values (Fig. 1c) were calculated from the plot means (n = 2 elevated CO_2 plots and n = 3

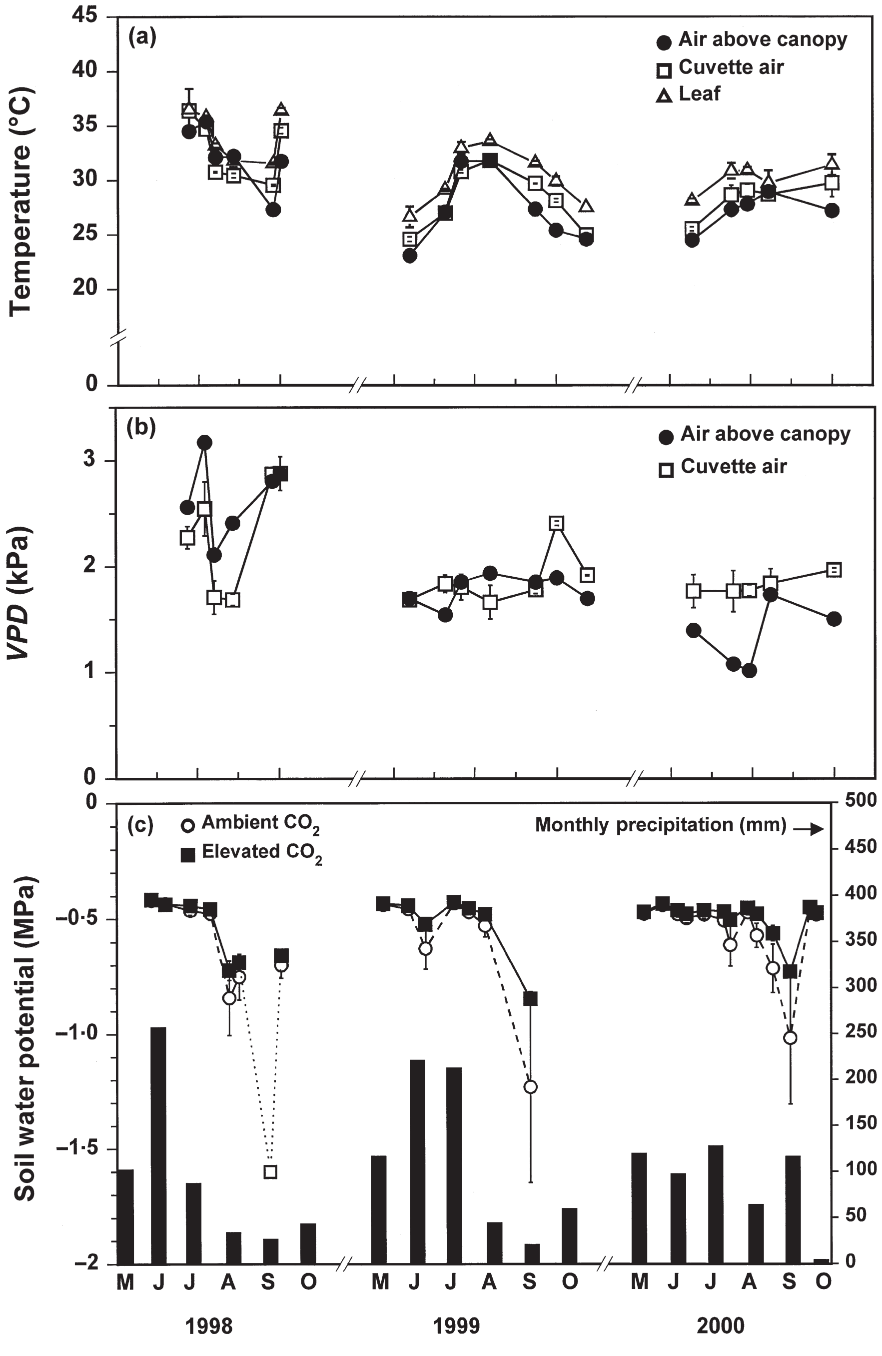

Figure 1. Environmental variation over the three growing seasons in (a) air and leaf temperatures during the measurements; (b) vapour pressure deficits at the site (mid-day) and in the cuvette; (c) soil water potential in the two treatments, and total precipitation for each month. Means (from n = 2 or 3 plots) are shown; bars indicate standard error of the mean. Symbols are as indicated on the figure, and in (c), the open square connected by a dotted line indicates *SWP* measured between plots.

ambient CO_2 plots). *SWP* data paired with the September 1998 gas exchange measurements were obtained by treatment-specific interpolation during the extended drought period, as only limited measurements were taken during that period. A trend toward slightly higher *SWP* in the elevated CO_2 plots was apparent during dry periods, particularly in 2000, but differences were not statistically significant.

Statistical analysis

Treatment effects were evaluated using the mean values of gas exchange and atmospheric and soil conditions from each plot for each measurement period. Two-way analysis of variance (CO_2 treatment × date) with plot as the experimental unit (n = 2 and n = 3) was used to assess treatment effects on gas exchange.

Environmental influences

Light-saturated upper canopy gas exchange relationships were evaluated with respect to variation in individual environmental variables, using both linear and non-linear models. Least-squares regression techniques were applied to A and g_s, and to treatment differences and ratios of rates (elevated/ambient) as a function of *VPD*, *SWP*, and temperature. Mid-canopy data were insufficient for analysis in relation to environmental variation.

Differences between significant regression lines were evaluated with an *F*-test, based on the principle of conditional error, as described by Neter & Wasserman (1974). Where differences were apparent, tests for homogeneity of slopes were also performed (Cody & Smith 1997).

Multiple linear regression analysis using the stepwise selection technique was applied to upper canopy A, g_s and *ITE* (and to the ratios and differences between CO_2 treatments for each of these) as a function of *VPD*, *SWP*, and temperature (Cody & Smith 1997).

Relationships between photosynthetic and stomatal responses

The relationships between photosynthetic and stomatal responses in each treatment were evaluated by linear and non-linear least-squares regression using three empirical approaches. First, mean A in each treatment group was described as a logarithmic function of mean g_s. Second, the relative enhancement of A on each date was described as a linear function of the relative change in g_s (Idso 1991). In the third approach, using the steady-state (LI-6400) data, A and g_s within each treatment were related using the Ball *et al.* model (Ball, Woodrow & Berry 1987) which describes g_s as a linear function of the index ($A \times h_s/C_a$), where h_s is the relative humidity (as a fraction) at the leaf surface. This model describes the interdependent covariance of A and g_s in response to humidity and [CO_2] (Aphalo & Jarvis 1993); it is used to relate A and g_s in models of CO_2 and water vapour exchange at canopy and ecosystem scales (e.g. Harley & Baldocchi 1995; Dang, Margolis & Collatz 1998). Differences between regressions were evaluated as described above.

Stomatal limitation of A in relation to moisture stress

Stomatal limitation and its influence on photosynthetic response to CO_2 were evaluated by comparing the C_i/C_a ratios for each treatment and by estimating relative stomatal limitation of A (*RSL*) from upper canopy A-C_i curves generated on 10 dates. Because there were no significant treatment differences in model parameters for the A-C_i curves (Sholtis *et al.* MS in preparation), a composite curve was constructed for each date. Composite curves were constructed using means of the parameters generated by least-squares regressions for individual leaves using the empirical function describing A as a rectangular hyperbolic function of C_i (Olsson & Leverenz 1994; Photosyn Assistant software; Dundee Scientific, Dundee Scotland, UK). *RSL* was then calculated as $(A_0 - A)/A_0$, where A was the mean assimilation rate for the treatment, and A_0 the assimilation rate that would occur if resistance to CO_2 diffusion were zero, that is, A at C_i equal to the treatment C_a (Farquhar & Sharkey 1982). A paired comparisons *t*-test (pairing by sample date) was used to evaluate treatment differences.

RSL, C_i/C_a ratios, and relative photosynthetic enhancement by CO_2 enrichment (as A_{560}/A_{360}) were also evaluated as functions of total moisture stress, using g_s as an indicator of combined atmospheric and soil moisture stress.

RESULTS

CO_2 effects on photosynthesis

Light-saturated photosynthetic rates (A) in the sweetgum upper canopy were highest in mid-summer and declined late in the growing season. The decline was especially pronounced in 1998 (Fig. 2a) during a prolonged drought, when the July to mid-September rainfall totalled only 124 mm (Fig. 1c). Seasonal patterns were similar in both treatments, however, and A remained higher in elevated CO_2 over the 3 year period. Differences between treatments were significant, averaging 5·0 μmol m^{-2} s^{-1} higher in elevated CO_2 (Table 1). The relative effect of CO_2 enrichment on A, calculated as a ratio of elevated/ambient (A_{560}/A_{360}), was remarkably stable during most of the growing season (mean 1·46, or 46% higher, Table 1), but varied late in the 1998 and 1999 growing seasons, when rates in ambient CO_2 were low. For example, on 10 September 1998, when g_s and A were sharply reduced by drought ($A \leq$ 3 μmol m^{-2} s^{-1} in either treatment, Fig. 2a), the treatment differences were necessarily small (e.g. $A_{560} - A_{360} = -1·1$ μmol m^{-2} s^{-1}), and A_{560}/A_{360} dropped to 0·65. Five days later, the rates remained low and differences small, but positive (+1·8 μmol m^{-2} s^{-1}). The result was a rebound in the *relative* enhancement to 1·33. This suggests that the apparent loss of enhancement on 10 September was not an indication of acclimation to the CO_2 treatment, but the result of variability obscuring small treatment differences. In September and October of 1999, by contrast, A_{560} was more than double A_{360} (Fig. 2a). As in 1998, this was associated with low rainfall and a fairly small denominator ($A_{360} < 8$ μmol m^{-2} s^{-1}), which tends to magnify relative differences.

Mean light-saturated A in mid-canopy foliage (Fig. 3a) was 15–20% lower than A in upper canopy foliage, but the effect of CO_2 enrichment was almost identical, and A_{560}/A_{360} averaged 47% higher in elevated CO_2 (Table 1). There was no decline in enhancement over the 3 year period, nor within the 2000 growing season.

CO_2 effects on stomatal conductance

Upper canopy g_s (Fig. 2b) followed the same seasonal patterns as did A, with highest g_s in July, and reductions in late summer. Rates were almost always lower in elevated CO_2 foliage than in ambient CO_2 (mean difference –0·06 mol m^{-2} s^{-1}, Table 1), and overall, treatment differences were highly significant (Table 1). The relative reductions in g_s averaged 24% over the three growing seasons (Table 1). As was true for A, the ratio ($g_{s\text{-}560}/g_{s\text{-}360}$) was most variable late in 1998 and 1999 when g_s was reduced by drought and differences were small, varying from 0·48 (September 1998) to 1·06 (October 1999).

Mid-canopy g_s (Fig. 3b) tended to be lower than upper-

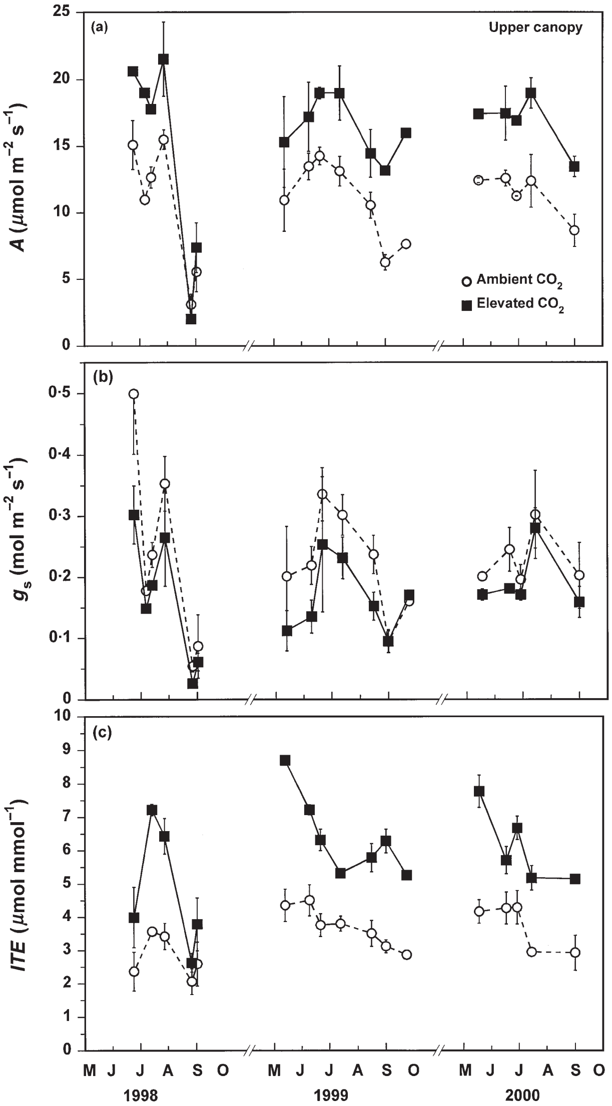

Figure 2. Light-saturated gas exchange of upper canopy sweetgum foliage, measured at the nominal treatment [CO_2], during 3 years of CO_2 enrichment: (a) net assimilation; (b) stomatal conductance to water vapour; and (c) instantaneous transpiration efficiency. Means (from $n = 2$ or 3 plots); bars indicate standard error of the mean.

canopy g_s (Fig. 2b) for comparable time periods, and the reduction with elevated CO_2 averaged only 14% ($P = 0{\cdot}06$, Table 1). As in the upper canopy, differences were minimal on the dates when g_s was low, i.e. near 0·1 mol m^{-2} s^{-1}.

CO_2 effects on water use efficiency

Leaf-level photosynthetic water use efficiency, or instantaneous transpiration efficiency, *ITE* (*A*/transpiration) was always higher in elevated CO_2, in both canopy positions (Figs 2c & 3c). *ITE* declined in both treatments late in the season, and in association with high *VPD (*when transpiration was higher). The CO_2-induced difference in *ITE* averaged 2·41 and 2·76 μmol CO_2/mmol H_2O in upper and mid-canopy foliage, respectively (Table 1) with differences smaller during the drought of September 1998 (Fig. 2c). The mean relative increases were 68% in the upper canopy, and 78% in mid-canopy (Table 1), larger than relative effects on either *A* or transpiration alone. The ratio of *A* to g_s can also be used to assess the contrasting impacts of elevated CO_2 on carbon gain and water loss. This index, which removes the influence of *VPD* on transpiration, still varied by a factor of two over time (data not shown). As with *ITE*, A/g_s was always higher in elevated CO_2, by a mean of 92% (SE, 6) in the upper canopy, and 75% (SE, 9) in mid-canopy foliage.

Effects of environmental variation on gas exchange responses

The range of natural environmental variation observed in this experiment (Fig. 1) is potentially sufficient to alter both *A* and g_s through a combination of biochemical and stomatal mechanisms. In order to quantify the impacts, we first evaluated the individual relationships between upper canopy gas exchange and *VPD*, *SWP*, and leaf temperature, and then used multiple linear regression to assess the relative impacts of each variable.

Photosynthesis and g_s both decreased at high *VPD* (Fig. 4), which occurred at various times during the experiment (Fig. 1b). Of three equations commonly used to describe the response of g_s to *VPD*, the best fit was achieved using an exponential function (Fig. 4a). The curves were significantly different in an *F*-test ($P = 0{\cdot}04$), although the differences were less pronounced at high *VPD*. Fits of linear and logarithmic regressions were also acceptable over this *VPD* range ($R^2 = 0{\cdot}46$–$0{\cdot}64$).

The best fit for *A* was as a linear function of *VPD* (Fig. 4b). The lines were significantly different ($P < 0{\cdot}01$), but the slopes were not, indicating that the increase in *A* with elevated CO_2 was similar across all levels of *VPD*. Similar decreases in *ITE* were observed with increasing *VPD (*data not shown), and variation in *VPD* explained 62 and 51% of the variation in *ITE* in ambient and elevated CO_2, respectively.

Reduced gas exchange was also associated with drought and drying soils (Fig. 5), which occurred late in the growing season, particularly in 1998 and 1999, when July, August and September rainfall was unusually low (Fig. 1c). The variation in *SWP* in the upper 20 cm of soil explained 41% (ambient) and 34% (elevated) of the total variation in g_s using linear regression (Fig. 5a). Across the range of soil moisture contents, g_s was lower in elevated CO_2, and the regressions for the two treatments were significantly different ($P = 0{\cdot}01$), although the slopes were not. Variation in *SWP* explained more of the total variation in *A* (Fig. 5b), approximately 60%. The two regressions were significantly different ($P < 0{\cdot}01$), as were the slopes ($P < 0{\cdot}01$), so that the lines converged at low soil water potentials. The relationships between *ITE* and *SWP* in the treatments (not shown) were similar to those observed between *A* and

Table 1. Light-saturated gas exchange, ratios, and differences in upper and mid-canopy foliage, measured at the treatment [CO_2]. Values are 3 year means and standard errors (in parentheses) for each treatment (*cf* Fig. 2)

	A	g_s	ITE	C_i/C_a
Upper canopy (18 dates)				
Elevated	15·9 (1·2)	0·17 (0·02)	5·85 (0·37)	0·64 (0·01)
Ambient	10·9 (0·8)	0·23 (0·03)	3·44 (0·18)	0·67 (0·01)
Elevated/ambient	1·46 (0·31)	0·76 (0·03)	1·69 (0·06)	0·94 (0·01)
Elevated–ambient	5·0 (0·5)	–0·06 (0·01)	2·41 (0·23)	–0·04 (0·01)
Significance probabilities				
CO_2 treatment	<0·01	<0·01	<0·01	0·03
Date	<0·01	<0·01	<0·01	<0·01
Treatment × date	NS	NS	<0·01	NS
Mid-canopy (six dates)				
Elevated	13·1 (2·0)	0·16 (0·02)	6·33 (0·58)	0·65 (0·03)
Ambient	9·0 (1·6)	0·19 (0·03)	3·56 (0·33)	0·69 (0·03)
Elevated/ambient	1·47 (0·10)	0·86 (0·09)	1·78 (0·32)	0·94 (0·02)
Elevated–ambient	4·1 (0·6)	–0·04 (0·02)	2·76 (0·31)	–0·04 (0·02)
Significance probabilities				
CO_2 treatment	<0·01	0·06	<0·01	0·04
Date	0·01	<0·01	<0·01	<0·01
Treatment × date	NS	NS	NS	NS

Significance probabilities are given for ANOVA associated with CO_2 treatment and time, using plot as the unit (n = 2 elevated CO_2 plots, n = 3 ambient plots). Variables are photosynthesis (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), instantaneous transpiration efficiency (ITE = A/transpiration, μmol CO_2 mmol^{-1} H_2O), and ratios of intercellular to ambient [CO_2] (C_i/C_a).

SWP (R^2 = 0·41 and 0·36 in ambient and elevated CO_2, respectively).

Variation in leaf temperature alone explained very little of the total variation in A or g_s with either a linear or parabolic fit (R^2 = 0·01–0·12, data not shown). There was a slightly stronger relationship between *ITE* and temperature (R^2 = 0·12 and 0·17), because higher temperatures tend to increase transpiration, thus decreasing *ITE*.

Multiple linear regression was also used to assess the interacting effects of environmental variation on steady-state A, g_s, and *ITE*, and on CO_2 effects, as treatment differences and ratios. Variables were selected using the stepwise method (Cody & Smith 1997) to account for correlation among variables. For example, high *VPD* tended to occur more often during drought, or at high temperatures. In most cases, after accounting for variation in the most significant individual environmental factor, adding the others did not result in an improvement in the explained variance (Table 2). Variation in *VPD* was the best single regressor for many of the responses, and higher *VPD* tended to decrease both rates and treatment differences. After accounting for the effects of *VPD* on g_s and A_{360}, leaf temperature explained a small but significant additional portion of the variation (Table 2), and indicated that A and g_s tended to increase with temperature. Reductions in $A_{560,}$ and diminished treatment differences in A, were best explained by decreasing *SWP*. Leaf temperature explained only 20–30% of the variability in CO_2 treatment effects on *ITE,* and no other factors were significant. None of the environmental factors explained a significant portion of the variation in *relative* CO_2 effects on A or g_s, i.e. the variation in A_{560}/A_{360}, or $g_{s\text{-}560}/g_{s\text{-}360}$.

Relationships between stomatal and photosynthetic effects

Rates of A and g_s were tightly coupled, and A at elevated CO_2 was always higher than at ambient CO_2 for an equivalent g_s (Fig. 6a). Within each treatment, A could be characterized as a logarithmic function of g_s (Fig. 6a). These curvilinear relationships are consistent with restriction of C_i by partial stomatal closure in response to the environment, rather than with a stomatal response to variation in photosynthetic capacity (Wong, Cowan & Farquhar 1979). Logarithmic relationships similar to those in Fig. 6a were also observed among A and g_s of individual leaves within a sampling date (data not shown), indicating a robust association between the two variables and suggesting that variability in g_s is responsible for much of the within-treatment variability in A.

The *relative* effect of [CO_2] on g_s on any given date was also tightly coupled with the *relative* enhancement of A on that date. Photosynthetic enhancement by elevated CO_2 (A_{560}/A_{360}) was greater on dates when high CO_2 caused the least relative reduction in g_s (Fig. 6b), and this was true in both upper and mid-canopy foliage.

The relationships between A and g_s were also compared using the Ball *et al.* (1987) model which incorporates stomatal sensitivity to humidity and C_a. In this analysis, mid-canopy relationships were not significantly different from those in the upper canopy, and data from both positions were used in the regressions (Fig. 7). Regressions for the two CO_2 treatments were significantly different ($P < 0{\cdot}01$) because g_s was lower in elevated CO_2 for an equivalent index $A \times h_s/C_a$. The slopes were not different, suggesting

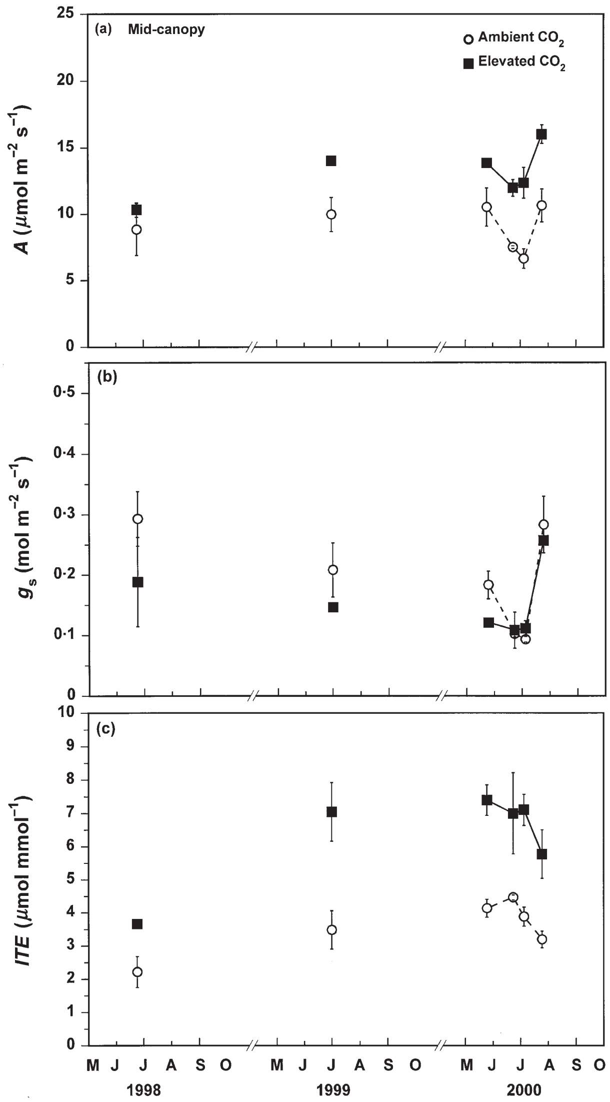

Figure 3. Light-saturated gas exchange of mid-canopy sweetgum foliage, measured at the nominal treatment [CO_2], during 3 years of CO_2 enrichment: (a) net assimilation; (b) stomatal conductance to water vapour; and (c) instantaneous transpiration efficiency. Bars indicate standard error of the mean.

that stomata were not significantly more or less sensitive to variation in C_a or h_s. This was confirmed by individual regressions of g_s on h_s, A, and A/C_a. The pairs of regressions ($0{\cdot}55 < R^2 < 0{\cdot}85$) remained significantly different overall for the two treatments ($P < 0{\cdot}05$), and the slopes (apparent sensitivity of g_s to each factor) similar.

Stomatal limitation of photosynthesis

As CO_2-mediated decreases in g_s appeared to proportionally reduce photosynthetic enhancement (Fig. 6b), we examined the CO_2 effects on stomatal control of photosynthesis by two methods. First, we evaluated the effects of reduced g_s on the C_i/C_a ratio (Sage 1994). Although elevated CO_2 reduced g_s by approximately 24%, the effect on the C_i/C_a ratio was small but significant (Table 1). The C_i/C_a ratio at elevated CO_2 averaged 94% of that in ambient CO_2 leaves. The effect was fairly consistent, and almost identical in the upper canopy and mid-canopy foliage. A CO_2-mediated reduction in the C_i/C_a ratio is described by Sage (1994) as a conservative pattern of stomatal acclimation, beneficial if water limits growth more than does carbon supply. If the lower C_i/C_a ratio in these leaves is the result of conservative stomatal acclimation, however, the degree of adjustment did not respond to *variation* in the water limitations that reduced g_s. That is, although the C_i/C_a ratio tended to decrease in response to integrated moisture stress, as reflected by decreasing g_s (Fig. 8a), this was true in both treatments, and the relative reduction in C_i/C_a by elevated CO_2 did not change. During the most severe drought, in September 1998, direct limitations to A appeared to outweigh stomatal constraints such that the C_i/C_a ratio *increased* in both treatments (points inside the dotted ellipse in Fig. 8a), and remained lower in elevated CO_2.

The relative stomatal limitation to photosynthesis (*RSL*) was also assessed by the method of Farquhar & Sharkey (1982), using data from A–C_i curves. The mean *RSL* in ambient CO_2 was 26·9% (SD, 5); mean *RSL* was actually lower in elevated CO_2, by 3–7%, with a mean of 22·8% (SD, 6). The differences, although small, were significant in a paired comparisons *t*-test ($P < 0{\cdot}01$, pairing by sample date). *RSL* in both treatments tended to increase when g_s was low (Fig. 8b), again with the exception of the September 1998 data (inside ellipse), when both g_s and A were strongly limited by drought, and mesophyll limitations were relatively greater than stomatal limitations. The treatment difference in *RSL* on that date was maintained, however.

As the environmental factors controlling gas exchange interact in a complex fashion, patterns in the responses of C_i/C_a (Fig. 8a), *RSL* (Fig. 8b), and A (Fig. 6) were clearer when expressed relative to the concurrent g_s than when expressed as a function of the underlying environmental variation (*cf*, for A, Figs 4b & 5b versus Fig. 6). Accordingly, A_{560}/A_{360} was evaluated using $g_{s\text{-}360}$ to integrate atmospheric and soil moisture stress of the site (Fig. 8c). Overlaid on the data is the theoretical prediction of A_{560}/A_{360} generated using ratios of the logarithmic regressions from Fig. 6a, using the assumption that $g_{s\text{-}560}$ is 24% lower than $g_{s\text{-}360}$. The responses of upper and mid-canopy foliage were similar, and fell close to the predicted curve, except at low g_s, when CO_2-induced stomatal closure was most variable (Fig. 2b). The point substantially below the prediction line, at g_s = 0·05 mol m^{-2} s^{-1} and A_{560}/A_{360} = 0·65, is one of two points from the September 1998 drought, which, as noted previously, induced additional non-stomatal limitations of A.

DISCUSSION

Overall responses of A and g_s

Relative photosynthetic enhancement in the elevated CO_2 treatment was remarkably stable throughout the first 3 years of this study, varying primarily under conditions of

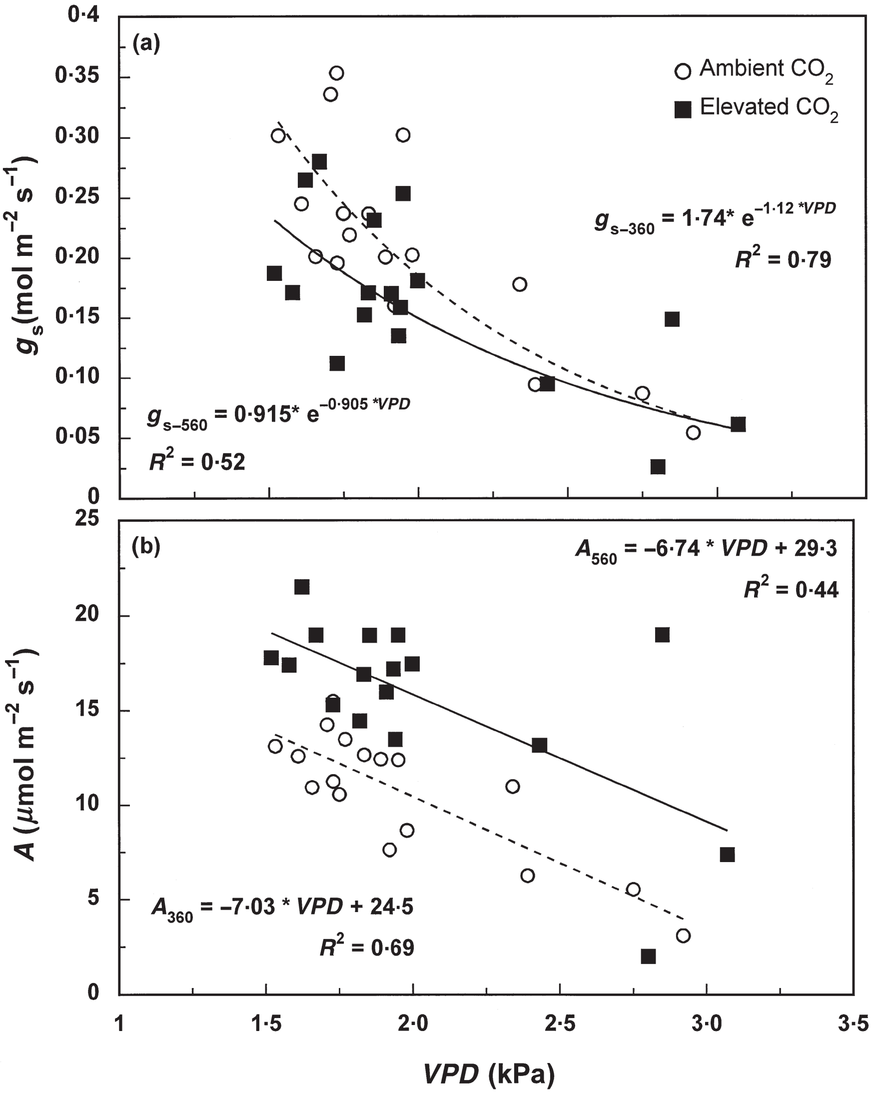

Figure 4. Upper canopy gas exchange as a function of leaf-to-air vapour pressure deficit: (a) stomatal conductance; (b) photosynthetic CO_2 assimilation. Curves (dashed lines for ambient CO_2; solid lines for elevated CO_2) represent the least-squares regressions using (a) exponential and (b) linear models.

moisture stress, either high *VPD* or low *SWP*, which caused partial stomatal closure and, during the driest period, additional biochemical limitations to *A*. The mean value of A_{560}/A_{360} was 1·48 in the summer of 2000, higher than either the 1998 mean, or the 3 year mean, indicating that the CO_2 effect on *A* was not lost over time. The 1·46 mean value of A_{560}/A_{360} was comparable with the results from CO_2 enrichment studies in other woody species; for example, the mean responses ranging from approximately 1·45 in early pot studies (Gunderson & Wullschleger 1994; Curtis 1996), to 1·66 and 1·51 in seedlings and saplings growing directly in the ground (Norby *et al.* 1999; Medlyn *et al.* 1999). The slightly lower enhancement in the current study is consistent with the smaller CO_2 enrichment (+200 versus +300 to +350 μmol mol^{-1} CO_2 in many of the earlier studies).

The mean relative enhancement of *A* in this study thus supports trends observed in younger plants, but is of note because it has been sustained for 3 years in a stand of large trees that has attained a constant leaf area index, and no longer has the additional sink capacity provided by exponential growth (Norby *et al.* 2001). Moreover, the relative enhancement of light-saturated *A* was equivalent in mid-canopy and upper canopy foliage, regardless of differences in leaf chemistry and anatomy associated with the development of shade leaves (Gunderson *et al.* 1999). This is in contrast to the findings of Herrick & Thomas (1999), who reported comparable enhancements in shade leaves, but a surprising 92–166% enhancement in sun leaves.

The effect of elevated CO_2 on g_s was a sustained reduction, indicating that stomatal sensitivity to CO_2 was not lost over time. The 24% mean relative decrease in g_s was smaller than the increase in *A*, and comparable to mean declines of 11–30% reported across multiple woody species, albeit with appreciable variation within and among studies (Field *et al.* 1995; Curtis 1996; Drake *et al.* 1997; Curtis & Wang 1998; Medlyn *et al.* 2001). Treatment differences in this study, if evaluated for individual dates, would not always have been statistically significant, which is a frequent observation in CO_2 experiments with trees (Gunderson, Norby & Wullschleger 1993; Beerling *et al.* 1996; Heath 1998; Rey &

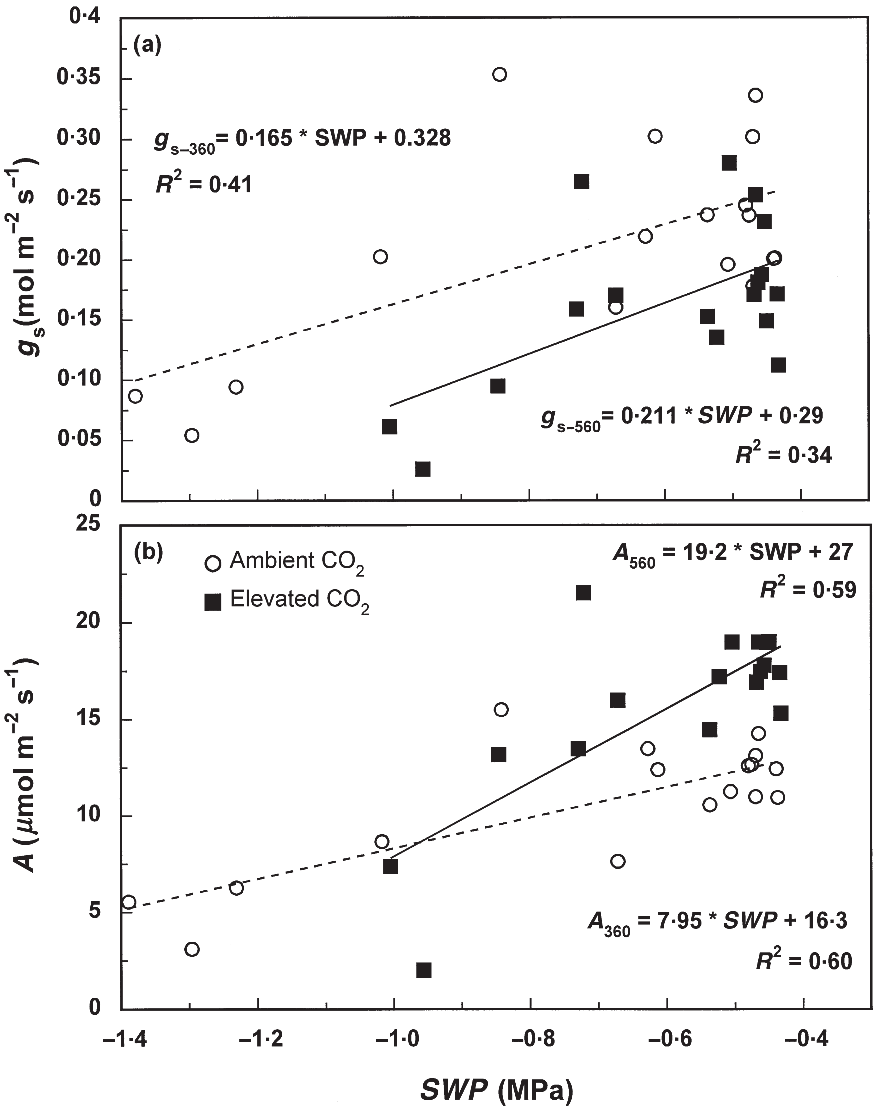

Figure 5. Upper canopy gas-exchange as a function of soil water potential from 0 to 20 cm: (a) stomatal conductance; (b) photosynthetic CO_2 assimilation. Lines (dashed lines for ambient CO_2; solid lines for elevated CO_2) represent the least-squares linear regressions.

Table 2. Coefficients, intercepts, and significance probabilities in the stepwise multiple linear regressions of steady-state light- saturated upper canopy gas exchange (and CO_2 effects on gas exchange) as functions of vapour pressure deficit (*VPD*), soil water potential (*SWP*) and leaf temperature (*T-leaf*)

	VPD	*P*	*SWP*	*P*	*T-leaf*	*P*	Intercept	*P*
Assimilation								
Ambient CO_2	–7·84	<0·01	–	NS	0·343	0·07	15·4	0·01
Elevated CO_2	–	NS	19·2	<0·01	–	NS	27·0	<0·01
Elevated–ambient	–	NS	3·2	0·06	–	NS	7·2	<0·01
Stomatal conductance								
Ambient CO_2	–0·192	<0·01	–	NS	0·010	0·05	0·277	0·06
Elevated CO_2	–0·133	<0·01	–	NS	0·013	0·03	0·031	0·83
Ambient – elevated	–0·045	0·02	–	NS	–	NS	0·137	<0·01
Instantaneous transpiration efficiency								
Ambient CO_2	–1·40	<0·01	–	NS	–	NS	6·2	<0·01
Elevated CO_2	–2·45	<0·01	–	NS	–	NS	10·8	<0·01
Elevated – ambient	–	NS	–	NS	–0·210	0·03	9·0	<0·01
Elevated/ambient	–	NS	–	NS	–0·042	0·09	3·0	<0·01

NS indicates that the remaining variables did not explain a significant portion of the residual variability (i.e. $P > 0{\cdot}15$) after accounting for the most significant factors (those listed). None of the factors explained a significant portion of the variability in the relative CO_2 effects on A or g_s (as elevated/ambient).

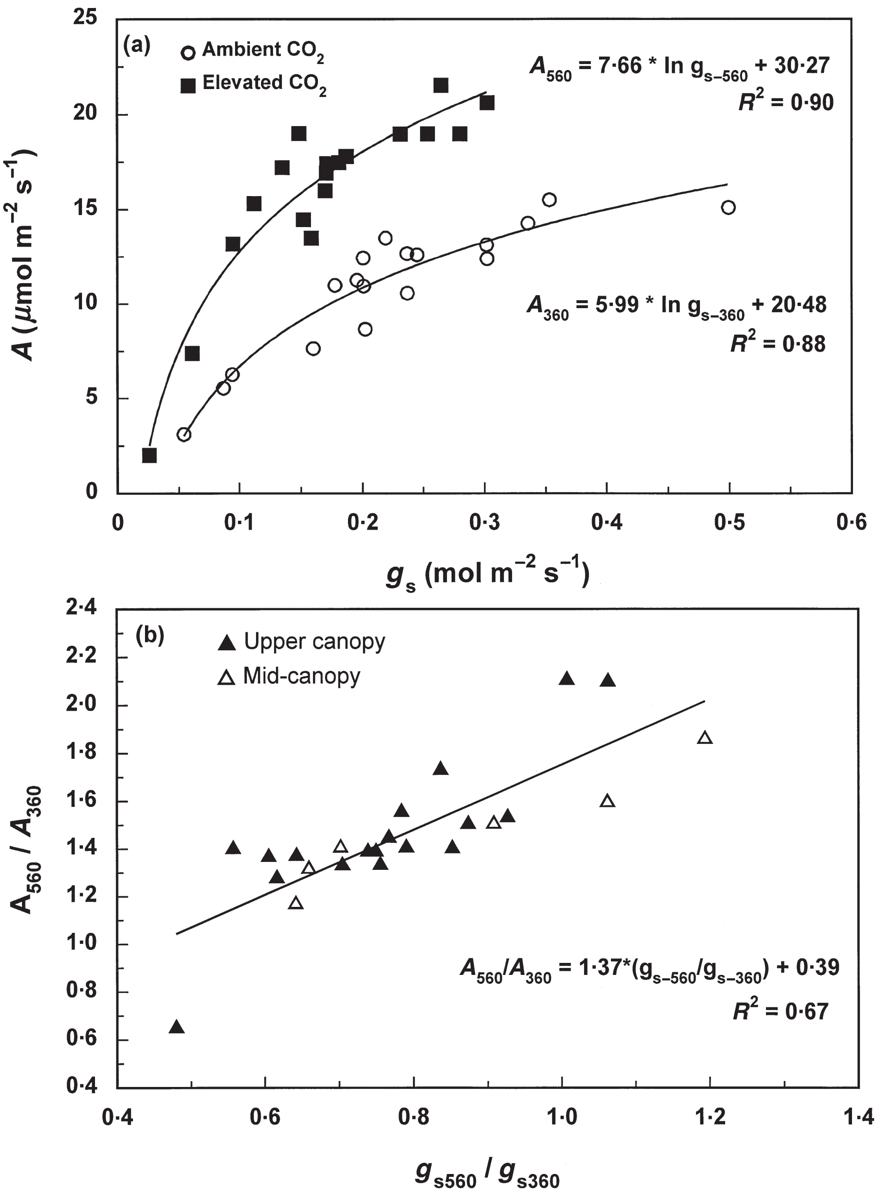

Figure 6. (a) Upper canopy assimilation as a function of stomatal conductance to water vapour in ambient and elevated CO_2 foliage, measured on 18 dates over three growing seasons. Curves are logarithmic regressions of photosynthesis on conductance. (b) Relative effect of elevated CO_2 on photosynthesis (as the ratio of elevated rate/ambient rate), plotted as a function of relative stomatal effect, shown with the linear regression combining upper canopy (solid triangles) and mid-canopy (open triangles) data.

Jarvis 1998; Saxe *et al.* 1998; Ellsworth 1999; Norby *et al.* 1999). Differences were always significant when evaluated over time, in the context of the relationships between g_s and each environmental variable, or between g_s and combinations of variables, e.g. the Ball *et al.* index.

Interactions with environmental stress

In this experiment, as in most field studies, some degree of correlation was observed among environmental variables. Nevertheless, over three seasons, patterns appeared with respect to both *VPD* and *SWP*, and multiple regression confirmed the relationships. Overall, *VPD* remained the most significant environmental influence, explaining more of the variation than either temperature or soil water potential. On the other hand, *SWP* had a strong effect on *A*, especially A_{560}, and decreased treatment differences during severe drought, because of limitations to photosynthetic function in addition to the stomatal effects of drought. Leaf temperature alone (within the range of 26–36 °C observed here), explained very little of the within-treatment variation in either *A* or g_s, (cf. Fig. 5, Gurderson, Norby & Wullschleger, 2000) although it was responsible for some of the residual variation after accounting for *VPD*.

Absolute reductions in g_s with elevated CO_2 were smaller in magnitude when *VPD* was high, and in general, whenever g_s was low, although the *relative* decreases, as $g_{s\text{-}560}/g_{s\text{-}360}$, were highly variable at low g_s. Small stomatal effects of CO_2 have previously been noted in connection with inherently low g_s, for example, during the dry season, when *VPD* was high and g_s was low (Goodfellow, Eamus & Duff 1997), on warm sunny days with high *VPD* (Beerling *et al.* 1996; Heath 1998), and in species having intrinsically

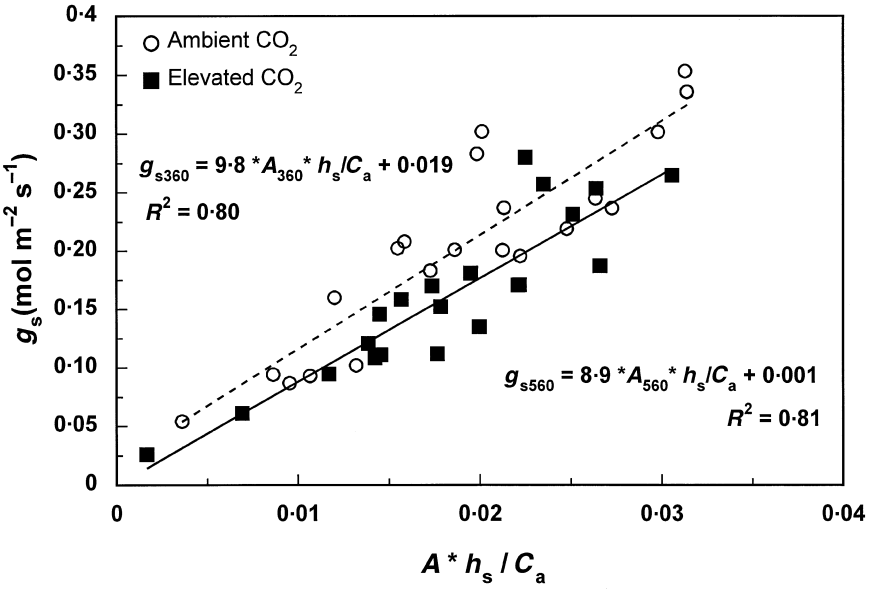

Figure 7. Stomatal conductance as a function of the Ball *et al.* (1987) index. Lines represent the best-fit linear regressions for each CO_2 treatment, combining upper and mid-canopy data (dashed lines for ambient CO_2; solid lines for elevated CO_2).

lower g_s (Morison 1985; Saxe *et al.* 1998). These observations are all consistent with the Curtis (1996) meta-analysis, which indicated that *absolute* reductions in g_s tended to be less significant in stressed plants. Similar trends with respect to *VPD* and *SWP* were apparent in a few of the long-term European tree studies recently reviewed (Medlyn *et al.* 2001), but a meta-analysis of those data actually detected a greater *relative* effect size ($g_{s\text{-elevated}}/g_{s\text{-ambient}}$) in the studies ($n = 5$) involving water-stressed trees.

Results from large trees in CO_2 enrichment studies have been mixed with respect to photosynthetic enhancement during naturally occurring dry periods involving high *VPD* or low *SWP*. Several reported *greater* relative enhancement during drought (Dixon, Le Thiec & Garrec 1995, in *Picea abies* only; Scarascia-Mugnozza *et al.* 1996; Kellomäki & Wang 1996, in the elevated temperature treatment; Goodfellow *et al.* 1997; Heath 1998, in *Fagus sylvatica*; Myers, Thomas & DeLucia 1999, in the July data), but others reported *reduced* enhancement during drought (Dixon *et al.* 1995, in *Quercus rubra*; Ellsworth 1999, during peak drought). In the present study, *A* declined in response to late season drought in both 1998 and 1999, as a result of both stomatal and non-stomatal limitations, and the absolute *difference* between CO_2 treatments was lower under dry conditions. Calculating *relative* effects, however, can magnify any noise in the signal whenever the denominator is small, as during drought. In many of the studies cited, rates were quite low and treatment differences small during dry periods, and it is likely that much of the variability in drought–CO_2 interactions results from the use of relative response ratios.

Stomatal sensitivity

It has been suggested that CO_2 enrichment reduces stomatal 'sensitivity' to *VPD* and soil water potential, potentially reducing drought tolerance and survival under stress (Heath 1998), but it is important to evaluate stomatal sensitivity, for example, to *VPD* (or the resulting water vapour flux; Mott & Parkhurst 1991), in an appropriate context. In a survey of species differences, Oren *et al.* (1999) evaluated the slope and both intercepts of the line describing g_s as a function of the natural logarithm of *VPD*. The authors defined *sensitivity* as the slope of that line, that is, change in g_s with change in ln *VPD*. They also described responses in terms of the *y*-intercept, which is the maximum g_s under favourable conditions, that is, when $VPD = 1{\cdot}0$ kPa (ln $VPD = 0$). Lastly, they evaluated the *x*-intercept, the level of *VPD* at which the extrapolated g_s equals zero. By their definition of sensitivity, the high CO_2 sweetgum in the present study could be considered marginally less sensitive to *VPD* ($P = 0{\cdot}1$ for the difference in slopes). However, because g_s under favourable conditions was already lower in elevated CO_2 (i.e. the *y*-intercept would be lower), the decrease from a lower maximum to zero (or to a common minimum g_s) would necessarily be smaller in magnitude. In fact, across species, Oren *et al.* (1999) noted a strong correlation between high maximum g_s and high sensitivity to *VPD* (steep slope). Although change in g_s with increase in *VPD* certainly defines stomatal sensitivity in one sense, the inability of partially closed stomata to close *in parallel with* those in ambient foliage, as observed here and in other species (Goodfellow *et al.* 1997; Heath 1998) does not indicate a susceptibility to low humidity stress. Parallel slopes, in fact, would require full stomatal closure at a lower *VPD* than the controls. Using this reasoning, Medlyn *et al.* (2001) chose to evaluate the effects of CO_2 treatment on the *x*-intercept, or the *VPD* at which g_s would reach zero. Using either a linear fit (as in Medlyn *et al.* 2001) or a logarithmic fit (Oren *et al.* 1999) to our data, the extrapolated g_s versus *VPD* lines crossed and thus predicted that a slightly (but not significantly) higher *VPD* would be required to com-

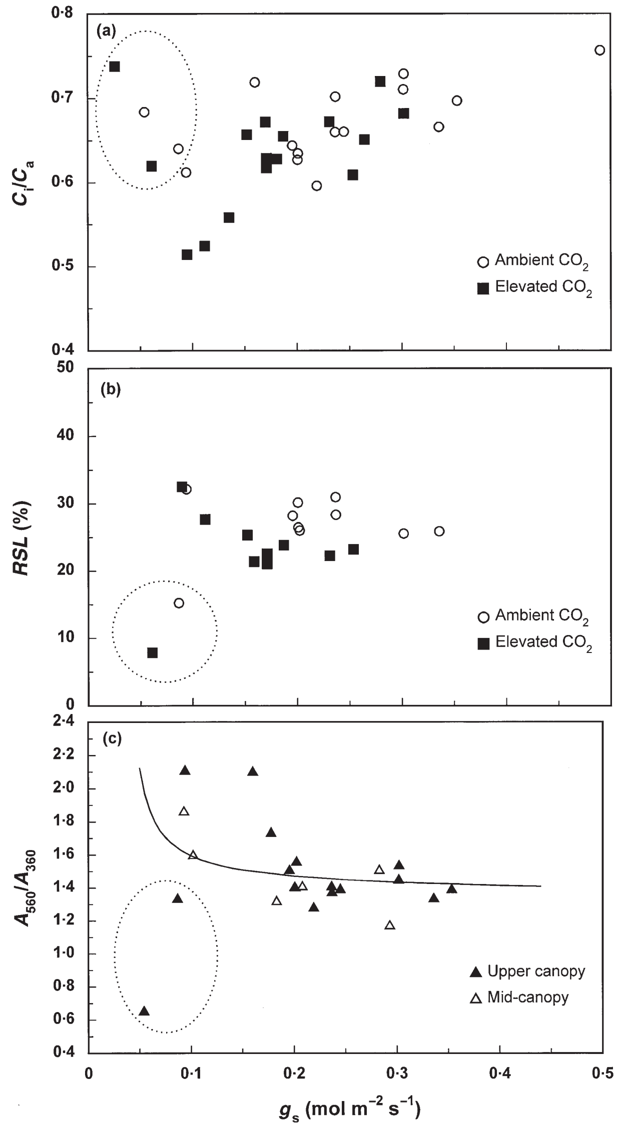

Figure 8. Gas exchange responses expressed using stomatal conductance to water vapour to integrate the effects of interacting environmental stresses. Stomatal control of A as indicated by (a) the ratio of intercellular to ambient [CO_2] and (b) relative stomatal limitation of photosynthesis (see text). (c) Relative photosynthetic enhancement (elevated/ambient) in upper and mid-canopy foliage as a function of g_s in the ambient treatment on that date. The solid line is the predicted enhancement, calculated from ratios of the logarithmic relationships of Fig. 6a, assuming that g_s is 24% lower in elevated CO_2. Dotted ellipses enclose data from September 1998, during the most severe drought, when non-stomatal factors predominated.

pletely close stomata at elevated CO_2. Because g_s never reached zero in this study and treatment differences were not distinguishable at high *VPD*, we conclude, as did Medlyn *et al.* (2001), that there was not a significant treatment difference in the *VPD* required to close stomata, and that CO_2 enrichment did not result in greater susceptibility to water loss at high *VPD*. The conclusion that stomatal sensitivity was not altered by elevated CO_2 is also consistent with an unchanged slope in the Ball *et al.* (1987) model (Medlyn *et al.* 2001)

Stomatal and photosynthetic interactions

In this experiment, environmental factors that limited stomatal conductance, e.g. low rainfall and high *VPD*, tended to reduce the *differences* in g_s between the two CO_2 treatments ($g_{s\text{-}360} - g_{s\text{-}560}$), and consequently also the *differences* in A. Under stressful conditions, however, gas exchange rates were low in both treatments (as a result of stomatal and non-stomatal limitations in the case of A). Under these conditions, the *relative* effects of CO_2, as the ratio of elevated/ambient, on any given date, could be highly variable, yet those relative effects on A and g_s were strongly coupled. Idso (1991) described a general inverse relationship between the relative CO_2-induced photosynthetic enhancement and the relative reduction in g_s. That relationship was proposed to characterize species differences in CO_2 responses, but, because of the tight coupling between A and g_s, the relationship is equally valid to describe temporal variation in CO_2 responses within a species. Whenever the relative stomatal reduction in this study was small, relative photosynthetic enhancement by CO_2 was larger. The inter-relationships between A and g_s in the two treatments predict a greater *relative* enhancement of A by CO_2 at very low g_s (at or below 0·15 mol m^{-2} s^{-1}), but again, when gas exchange was low, *relative* effects on both A and g_s were most variable, partly because of potential sampling error (low signal-to-noise ratio), and partly because photosynthesis at extremely low *SWP* is subject to additional non-stomatal limitations, as evidenced by the altered relationships between C_i/C_a and *RSL* at the depth of the drought in 1998.

SUMMARY

A mean 46% photosynthetic enhancement was sustained in this experiment, without attenuation, through three seasons of CO_2 enrichment, as was a 24% reduction in g_s. Thus our results support Hypothesis 1, that photosynthetic and stomatal sensitivity to CO_2 would persist, subject to transitory impacts of environmental variation. Hypothesis 2, that CO_2 would have a greater relative impact on photosynthesis in plants exposed to stress, is theoretically supported, at least for stresses that reduce g_s below a threshold, based on the tight logarithmic relationships between A and g_s. In practice, small absolute treatment differences, proportionally higher variability, and additional biochemical limitations may reduce the importance of relative enhancement under dry conditions. High temperatures likewise should increase photosynthetic stimulation by CO_2, but the stronger influences of *VPD* and *SWP* appeared to mute any effects of seasonal temperature variation in sweetgum. As for hypothesis 3, *relative* stomatal closure did proportionately limit *relative* photosynthetic enhancement, and that portion of the hypothesis was supported. However, it was the CO_2-associated *difference* in g_s that decreased under moisture stress (as when stomata were essentially closed in both treatments), at which time the *relative* effects were highly variable.

The foliar gas exchange responses of this closed-canopy stand of established trees were generally consistent with trends observed in earlier chamber studies with young seedlings, but contradicted some assumptions regarding environmental interactions. These findings are an important step toward the ultimate use of leaf-level responses to predict forest growth and function in a changing environment. They indicate that CO_2 responsiveness is not limited to early seedling growth nor to upper canopy foliage, and they may help explain previous conflicting observations about altered responsiveness late in the growing season, or during stress conditions. Relative CO_2 responses have proved valuable in summarizing the long-term responses of a species and in making comparisons across species where long-term records are available. Absolute responses, however, may be more pertinent than relative responses when assessing environmental interactions with CO_2, and for scaling up to predict stand-level carbon gain or water use. Only by observing the long-term record of CO_2 responses under a sufficient range of environmental conditions, as in this study, is it possible to separate and identify the sources of variability in the CO_2 responses of A and g_s.

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