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Sap velocity and canopy transpiration in a sweetgum stand exposed to free-air CO_2 enrichment (FACE)

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Summary

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• Canopy transpiration rates, as a major component of forest hydrologic budgets, are reported for 12-yr-old sweetgum (*Liquidambar styraciflua*) trees growing in a free-air CO_2 enrichment (FACE) study in eastern Tennessee, USA.

• The compensated heat-pulse technique was used to measure rates of sap velocity, and stand transpiration was estimated as a function of measured sap velocity, total stand sapwood area and the fraction of sapwood functional in water transport.

• Sap velocity averaged 13% less for trees in elevated compared with ambient CO_2 concentration. Stand transpiration reached 5.6 and 4.4 mm d^{-1} for the ambient and elevated CO_2 treatments, respectively. Stratification of the data showed that significant differences in stand transpiration were observed between ambient and elevated CO_2 only at mean daily radiation levels > 400 J m^{-2} s^{-1} and at vapor pressure deficits > 1.0 kPa.

• These data illustrate that while elevated CO_2 may reduce canopy transpiration, the apparent dependency of such an effect on prevailing weather makes detecting a CO_2-induced impact on forest water use difficult.

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

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Introduction

There is a widespread belief that CO_2-induced reductions in stomatal conductance will have important consequences for forest water use and, in turn, for ecosystem-scale processes that depend on soil water availability. Although measurements of stomatal conductance have been exhaustively made on plants from across a range of terrestrial ecosystems, few studies have documented the effect of elevated CO_2 on whole-plant water use or canopy transpiration for plants growing under realistic field conditions. Transpiration or evapotranspiration has been shown to decrease in tallgrass prairie (Bremer *et al.*, 1996; Owensby *et al.*, 1997), grasslands (Jackson *et al.*, 1994; Field *et al.*, 1997) and wheat (Senock *et al.*, 1996) exposed to elevated CO_2 concentrations, but differences between treatments in cotton (Dugas *et al.*, 1994; Hunsaker *et al.*, 1994; Kimball *et al.*, 1994) and rice (Baker *et al.*, 1997) have been highly variable and/or otherwise too small to measure. Studies of sap flow for trees growing at

elevated CO_2 in closed chambers (Kellomäki & Wang, 1998), natural CO_2 springs (Tognetti *et al.*, 1999), and free-air CO_2 enrichment (FACE) facilities (Ellsworth *et al.*, 1995) have also shown that a significant whole-tree response to elevated CO_2 can be difficult to detect.

Various arguments have been put forth to explain why ecosystems potentially differ in their water-use response to elevated CO_2 (grasslands vs forests, for example) or differ from expectations based solely on measurements of stomatal conductance (Field *et al.*, 1995; Wilson *et al.*, 1999). These explanations include differences in aerodynamic conductance between vegetation of differing heights, potential increases in leaf area for plants grown at elevated compared with ambient CO_2 that offset reductions in water use due to partial stomatal closure, energy balance considerations that promote increased canopy temperatures that compensate for lower stomatal conductance at elevated CO_2 concentrations, and other feedbacks that result from mixed layer and soil evaporation considerations (Wilson *et al.*, 1999). Unfortunately, few

experimental data sets are available from which to test these hypotheses. As a result, there remains a critical need to better understand the water-use characteristics of terrestrial ecosystems exposed to elevated CO_2 concentrations so as to describe and model their response to a changing climate properly (Sellers *et al.*, 1997; Raupach, 1998; Lockwood, 1999).

Studying the water-use characteristics of forests, however, is problematic and simple measurements of water use on seedlings or saplings growing in isolation are insufficient to capture the complex temporal and spatial control of transpiration that inevitably takes place in closed-canopy stands. How then should these processes be studied? Senock *et al.* (1996) proposed that the effects of elevated CO_2 concentration on water use are best evaluated on plants growing under field conditions and with measurement techniques that do not unnecessarily disturb the natural function of the plant. Sap-flow probes offer one such approach to measuring water use in trees and, through the use of up-scaling techniques that require information about the radial profile of sap velocity and total sapwood area, one can derive estimates of whole-tree and stand water use (Smith & Allen, 1996; Wullschleger *et al.*, 1998). Therefore, the compensated heat-pulse technique was used to measure rates of sap velocity for 12-yr-old sweetgum trees (*Liquidambar styraciflua*) growing at ambient and elevated CO_2 concentrations in a FACE study in eastern Tennessee, USA. Our objectives were to quantify seasonal patterns of sap velocity between ambient and elevated CO_2 treatments, and to assess CO_2-induced differences in stand transpiration. It was also of interest to evaluate the response of daily transpiration to mean daily radiation and vapor pressure deficit, and to identify whether CO_2-induced differences in stand transpiration (if any) were dependent on prevailing environmental conditions.

Materials and Methods

Study site and facility design

The site for this project was a 1.7-ha plantation of sweetgum (*Liquidambar styraciflua* L.) trees located on the Oak Ridge National Environmental Research Park in Roane County, TN, USA (35°54′ N and 84°20′ W). The plantation was originally established in early 1988 for the purpose of evaluating sweetgum as a short-rotation bioenergy crop. One-yr-old, bare-rooted seedlings were planted at a 2.3-m × 1.2 m spacing (van Miegroet *et al.*, 1994). 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 as Aquic Hapludult (Soil Conservation Service, 1967).

Development of the FACE facility began in late 1997 with the establishment of six 25-m circular rings within the plantation (Norby *et al.*, 2001 – see pp. 477–487 in this issue). Four of these rings had complete FACE infrastructure (towers, blowers, plenum, etc.) with two designated as 'ambient' and two designated as 'elevated' CO_2 rings. All four rings had similar infrastructure, and associated monitoring and control systems (Hendrey *et al.*, 1999). The CO_2 concentration in the two elevated rings was maintained near a target concentration of 565 p.p.m. (day) and 645 p.p.m. (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 (April 6 to November 2) the CO_2 concentration of the elevated rings averaged 538 p.p.m. during the day, whereas the CO_2 concentration of the ambient rings averaged 394 ppm (Norby *et al.*, 2001). Ambient CO_2 concentrations are higher than globally averaged CO_2 (368 ppm in 1999) because of low dispersion of CO_2 released in soil/plant respiration during generally low-wind conditions at night and early morning hours. Periodic modifications to hardware/software, CO_2 delivery problems and inclement weather (e.g. lightning) contributed to some interruption of CO_2 exposure during the year.

Environmental monitoring

Instruments for measuring air temperature, relative humidity, global radiation and wind speed were located on 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, 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. Soil water contents (0–35 cm) were measured at least twice a month at two locations within each ring using a time-domain reflectometer (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Estimates of daily mean air temperature (Ta), vapor pressure deficit (δ_e), and global radiation (Rg) were derived from hourly averages (Fig. 1). Only data for the daylight period were used to characterize environmental variables.

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 throughout the growing season on 84–95 trees per ring (Norby *et al.*, 2001). Tree heights were measured on 25 trees per ring with an ultrasonic hypsometer (Haglöf Sweden, Långele, Sweden). Leaf area index was estimated for each ring from a series of seven 0.19-m^2 litter collection baskets placed just above the plantation understory.
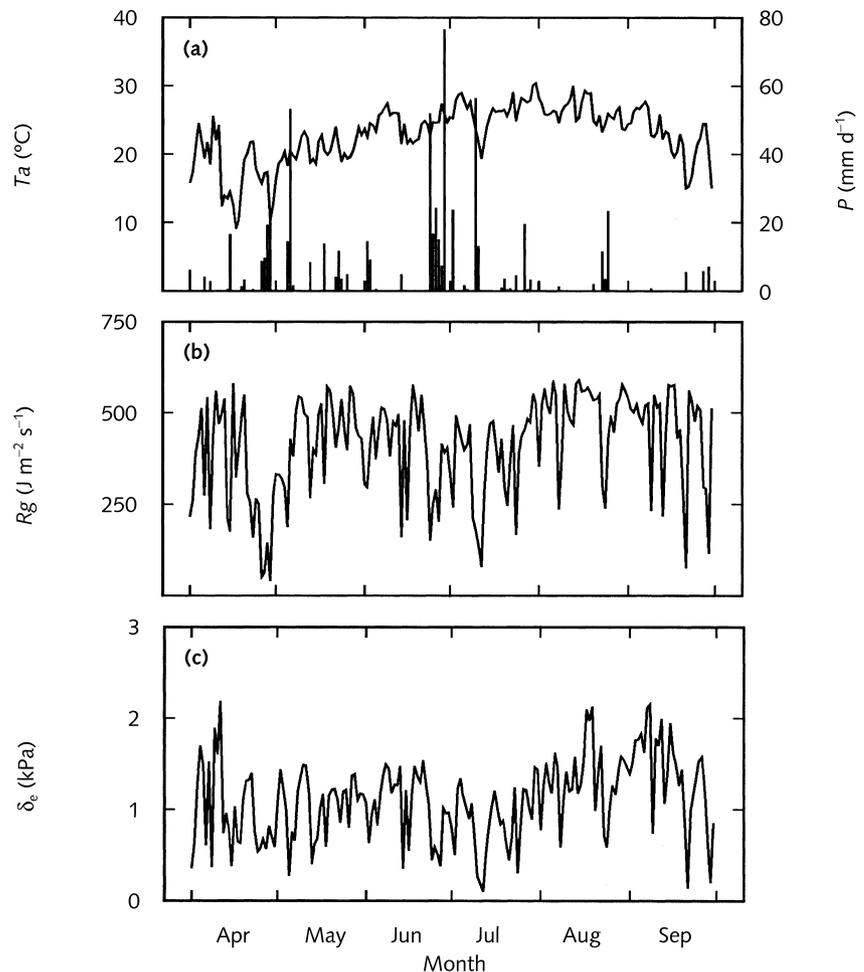
Fig. 1 Average daily estimates of (a) air temperature and precipitation (b) above-canopy global radiation and (c) vapor pressure deficit for the months during which whole-tree sap velocity was measured on sweetgum tress exposed to elevated and ambient CO_2 treatments.

Litter was collected throughout the growing season, but most leaves fell between mid-September and early November. 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 trees outside the study plots. Stem diameter at breast height (1.3 m) for 58 trees ranging in size from 3.1 to 16.3 cm d.b.h. was measured with a diameter tape. Bark thickness was determined with a digital caliper by drilling a 1.3-cm hole through the bark and stopping just at the bark–sapwood interface. Sapwood thickness was determined at the same location as bark thickness by removing a 5-mm diameter core of wood with an increment borer. Sapwood area for each tree was calculated from sapwood depth and stem diameter after subtracting bark thickness. Sapwood areas were fitted to the equation (Vertessy *et al.*, 1995):

$$As = kD^B \qquad \qquad \text{Eqn 1}$$

(As, the sapwood area (cm^2); D, measured tree diameter at breast height (cm); and k and B, coefficients as determined with nonlinear regression techniques.) Total stand sapwood area was estimated by applying Eqn 1 to all trees within the ambient and elevated CO_2 rings on which stem diameters were measured.

Sap velocity and calculations of canopy transpiration

The compensated heat-pulse technique (SF-300, Greenspan Technology Pty. Ltd, Warwick, Queensland, Australia) was used to measure the sap velocity for four trees in each of two ambient and elevated CO_2 rings (16 trees in total). These trees were located near the center of each ring and ranged from 12.4 to 14.7 cm in diameter. A single heat-pulse probe was positioned in each tree so that the sensing thermistor was located at a sapwood depth of 19 mm. The control module/data logger was programmed to provide a heat pulse for 1.8 s, and measurements were recorded every 60 min. Sap velocity was calculated from corrected heat pulse velocity (V_h) based on Eqn 3 of Barrett *et al.* (1995):

Table 1 Stand characteristics (mean ± SD) and estimates of cross-sectional basal area, sapwood area and leaf area index (LAI) for 12-yr-old trees growing in the ambient and elevated CO_2 treatments. Tree heights were measured on 25 individuals per ring, whereas stem diameters were measured on 84–95 trees in each of the rings

Treatment	Tree height (m)	Stem diameter (cm)	Basal area (m² ha⁻¹)	Sapwood area (m² ha⁻¹)	LAI (m² m⁻²)
Ambient	14.1 ± 2.0	12.6 ± 3.0	32.3	23.5	6.2
Ambient	14.7 ± 2.4	12.2 ± 3.4	34.5	25.1	6.4
Elevated	15.1 ± 1.7	12.4 ± 3.2	31.5	22.9	5.8
Elevated	14.7 ± 1.5	12.6 ± 3.0	32.0	23.3	6.5
$P > F$	ns	ns	ns	ns	ns
Average	14.6	12.5	32.6	23.7	6.2

ns designates treatment differences as nonsignificant.

$$V_s = \frac{V_h \rho_b (C_w + m C_s)}{(\rho_s C_s)} \qquad \text{Eqn 2}$$

(C_w, the specific heat capacity of wood (J g⁻¹ °C⁻¹); C_s, the specific heat capacity of water (J g⁻¹ °C⁻¹); ρ_b, the density of sapwood (kg m⁻³); ρ_s, the density of sap (kg m⁻³); and m, the moisture content of sapwood (kg water kg⁻¹ dry wood). An increment borer was used to extract a sample of sapwood from trees outside the treatment rings for determination of sapwood density and moisture content. Cores were taken at random from six trees each month throughout the growing season. Sapwood density averaged (± SD) across sampling dates was 508 ± 32 kg m⁻³ and sapwood moisture content was 1.074 ± 0.014 kg kg⁻¹. All estimates of sap velocity were corrected for probe implantation effects based on a measured wound diameter of 2.7 mm (Swanson & Whitfield, 1981). Wound diameter was measured directly on several trees outside the study plots by applying a dilute solution of Indigo Carmine dye to a hole drilled below the heat-pulse probe, and then 4 h later measuring the difference in sapwood color above the probe with a digital caliper. Earlier studies with sweetgum trees somewhat smaller than those in the FACE experiment indicated good agreement between estimated (heat pulse) and measured (gravimetric) rates of sap velocity. Sap velocity nomenclature is consistent with that recommended by Edwards *et al.* (1996).

The heat-pulse technique was also used to estimate the fraction of sapwood functional in water transport for six trees adjacent to the study plots. These trees varied in stem diameter from 13.2 to 14.9 cm. 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. The fixed reference probe was inserted to a sapwood depth of 19 mm. Sap velocity ratios were calculated at multiple depths as the velocity given by the moving probe was divided by that given by the fixed probe (Zang *et al.*, 1996) and an overall ratio was calculated using an area-weighted average of the point estimates. All measurements of radial variation in sap velocity were completed during a 1-wk period in late August. Following

each tree-specific measurement, a 60-mm sample of sapwood was extracted near to where the probes were implanted for determining radial variation in sapwood density and moisture content. The samples were taken to the laboratory, sectioned into smaller segments (4–6 mm each) and the fresh mass, volume and dry mass were determined.

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 (Table 1) and the fraction of sapwood functional in water transport. Since the heat-pulse technique does not distinguish low-flow from no-flow conditions (Becker, 1998), all night-time measurements of sap velocity < 0.0197 mm s⁻¹ were set to zero. This lower limit of detection corresponds to a 165-s return to thermal equilibrium time ($t_2 = t_e$) between the upstream and downstream sensor probes (Smith & Allen, 1996). Daily rates of stand transpiration (mm d⁻¹) were calculated via a simple summation of hourly rates.

Sap velocity data for the month of June were inadvertently lost for each of the ambient and elevated rings due to operator error. In order to estimate annual water use for the two CO_2 treatments, it was necessary to devise gap-filling procedures for stand transpiration during this month. A multiple regression model was developed for the ambient and elevated treatments by regressing daily stand transpiration against daily de and *Rg* using data from 3 wk before (beginning May 10) to 8 wk after (ending August 28) the period of missing data. Canopy leaf area index was close to maximum during this time and there was no evidence of soil water deficits. These equations were used to estimate daily and then monthly transpiration rates for June, and these data were, along with known estimates for the other months, used to calculate annual water use for the two treatments.

Statistical analysis

A repeated measures ANOVA model was used to test for CO_2 effects on seasonal patterns of maximum mid-day sap velocity and daily stand transpiration. A probability level of 0.05 was considered significant. Treatment differences in stand transpiration
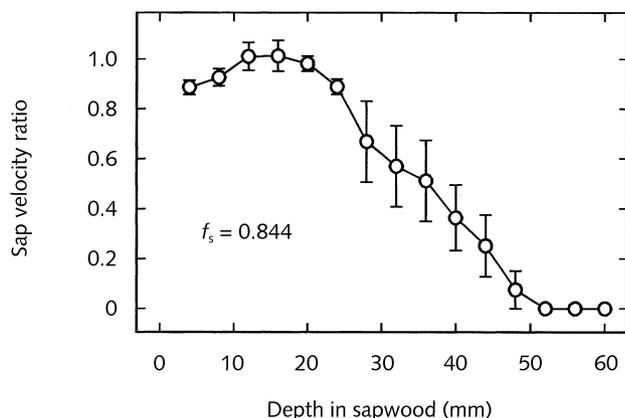
Fig. 2 Depth-dependent variation in relative sap velocity (mean ± SE) for six 12-yr-old sweetgum trees measured near the study site. Relative sap velocity was calculated at each depth as the velocity given by the moving probe divided by that given by the fixed probe, and an overall ratio (f_s) was calculated using an area-weighted average of point estimates. Data were collected between August 23 and 27, 1999.

during April, May, July, August and September were determined using a Student's *t*-test. Individual rings were the experimental unit ($n = 2$). Differences between multiple regression equations were determined according Neter & Wasserman (1974). All statistical tests and regressions were preformed with the SYSTAT 8.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Rates of sap velocity were highly dependent on the depth of probe insertion into the sapwood (Fig. 2). Sap velocity ratios in the outer 0–10 mm of sapwood were slightly lower than those for the 10–20 mm region, and the 10–20 mm region

tended to have the greatest rates of sap velocity for all the trees measured. Beyond this zone of maximum sap velocity, there was a gradual decline in the sap velocity ratio with increasing sapwood depth and, on average, sap velocity ratios were 0.78 for the 20–30 mm region, 0.48 for the 30–40 mm region and 0.23 for the 40–50 mm region (Fig. 2). Rates of sap velocity at depths closer to the heartwood (> 50 mm) were generally below the lower limit of detection for the heat-pulse probes. There were no significant differences in either sapwood density or moisture content with sapwood depth for the six trees measured. Area weighted sap velocity ratios varied between 0.756 and 0.962 for the six trees, and across all trees averaged 0.844 (Fig. 2).

Daily maximum rates of sap velocity for trees measured in both the ambient and elevated CO_2 rings showed a strong seasonal pattern, with rates increasing in mid- to late April as canopy leaf area developed, reaching a broad plateau between mid-May and mid-August, and then declining in late August and September (Fig. 3). Sap velocity rates tended to be less for trees in the elevated compared with ambient CO_2 rings (an average 13% reduction), but a repeated measures ANOVA indicated that differences between CO_2 treatments across the season were not significant ($P = 0.32$). Significant differences between ambient and elevated treatments were observed, however, during a 2-wk period in early May ($P = 0.03$).

The diurnal patterns of sap velocity for representative days in late-May and early August showed that while maximum midday rates were greater for trees from the ambient compared with the elevated CO_2 treatments (Fig. 4), differences in sap velocity between treatments were also observed during other times of the day. Averaged across the season, CO_2-induced reductions in sap velocity were generally observed between 1100 and 1800 hours with reductions in hourly sap velocity ranging from 5 to 20% (Fig. 5).
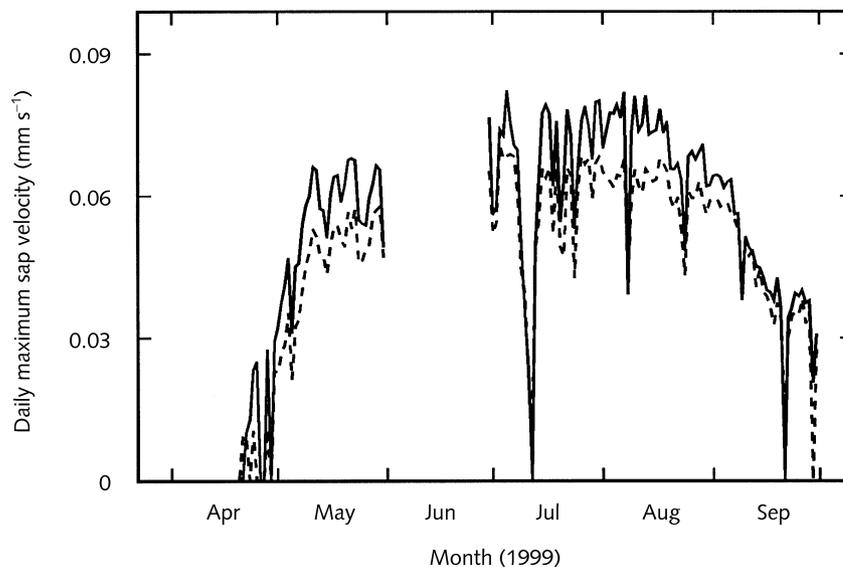

Fig. 3 Seasonal pattern of daily maximum sap velocity rates for trees measured in both the ambient (solid line) and elevated (dashed line) CO_2 rings. Data for the month of June were lost due to operator error. Ambient, solid line; elevated, dashed line.
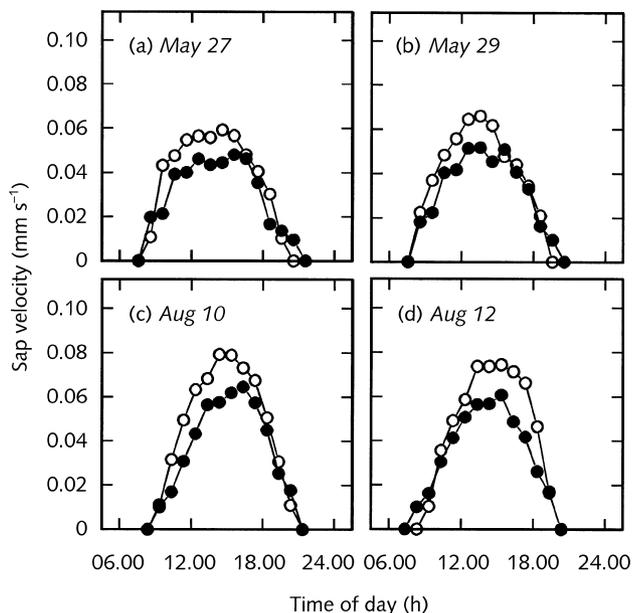
Fig. 4 Hourly rates of sap velocity for trees from the ambient (open circles) and elevated (closed circles) CO_2 treatments during two representative days in late May and two during mid-August.
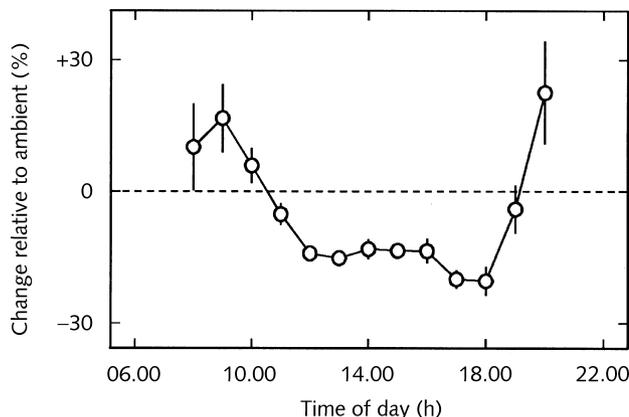

Fig. 5 Relative CO_2-induced change (mean ± SE) in rates of sap velocity throughout an average diurnal cycle for trees from elevated compared with ambient CO_2 concentration. The graph represents an 82-d ensemble (May 10 to August 28, 1999).

Daily rates of stand transpiration, as expected, showed a strong seasonal pattern with rates reaching a maximum of 5.6 and 4.4 mm d^{-1} (a 21% difference) for trees from the ambient and elevated CO_2 rings, respectively (Fig. 6a). As was observed
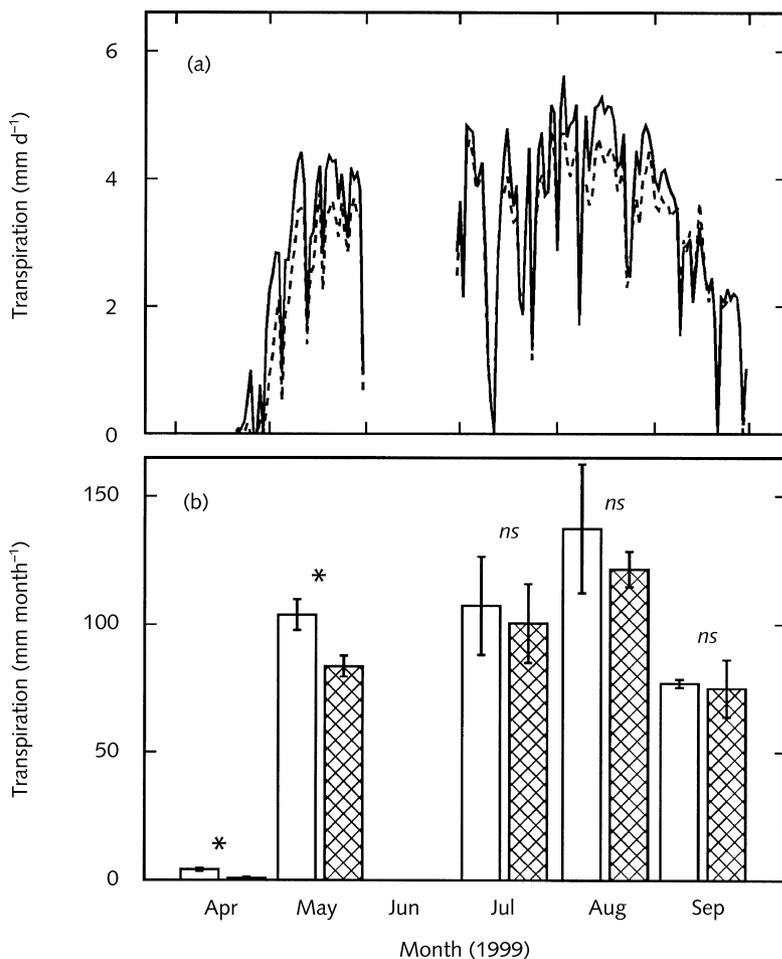

Fig. 6 Seasonal pattern of (a) stand transpiration and (b) monthly rates of water use (± SD) for trees measured in both the ambient (solid line or open bar) and elevated (dashed line or hatched bar) CO_2 rings. Asterisks indicate significant differences between CO_2 treatments. ns designates no significant differences.
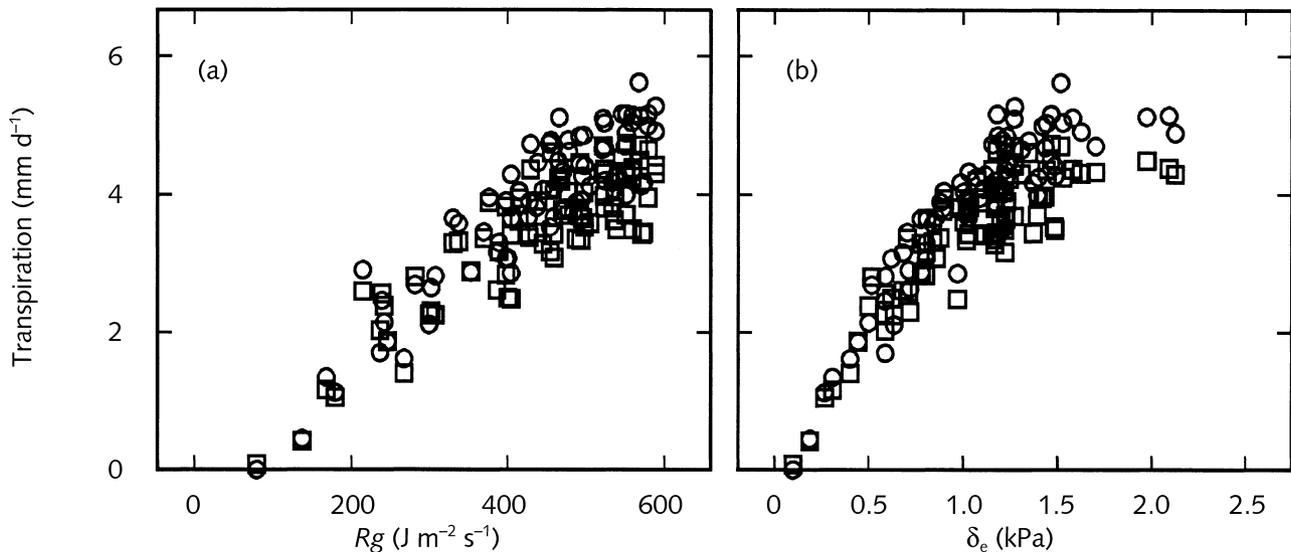
Fig. 7 Relationship of daily stand transpiration to (a) mean daily radiation and (b) mean daily vapor pressure deficit. Data analysis was restricted to a 82-d period of the season (May 10 to August 28) during which leaf area index was maximum and avoids late-season complications during canopy senescence. Ambient, open circles; elevated, open squares.

for daily maximum sap velocity, stand transpiration also tended to be less for trees in the elevated compared with ambient rings and across the season averaged 2.8 and 3.1 mm d^{-1} in the two treatments, respectively (a 10% reduction). A repeated measures ANOVA indicated that treatment effects across the season were not significant ($P = 0.43$), although differences were significant during a 2-wk period in early May ($P = 0.02$). An analysis of stand transpiration on a monthly basis also indicated significant differences between treatments early in the growing season (Fig. 6b). These differences were evident during April and especially during May when stand transpiration was 104 mm for the ambient treatment, but only 84 mm for the elevated CO_2 treatment (a 19% reduction). Transpiration during the other months were also lower for trees in the elevated CO_2 rings, although none were significant. Cumulative rates of stand transpiration, not including June, for trees in the ambient treatment was 430 mm compared with 381 mm for the elevated treatment. These two estimates of stand transpiration were not significantly different ($P = 0.37$).

Daily transpiration rates for both the ambient and elevated stands were strongly correlated with mean daily radiation and vapor pressure deficit (Fig. 7). Transpiration increased with increases in each environmental variable. This dependency of stand transpiration on mean daily radiation and vapor pressure deficit for the ambient CO_2 treatment was described by:

$$E_c = 0.205 + 1.034\text{de} + 0.006Rg \quad r^2 = 0.86 \qquad \text{Eqn 3}$$

and for the elevated CO_2 treatment by:

$$E_c = 0.527 + 0.860\text{de} + 0.005Rg \quad r^2 = 0.78 \qquad \text{Eqn 4}$$

(E_c, daily stand transpiration (mm d^{-1}); de, daily mean vapor pressure deficit (kPa); and Rg, daily mean global radiation ($J m^{-2} s^{-1}$).) These equations were significantly different from one another. Although there was considerable scatter in the data, treatment differences in stand transpiration were greatest at higher levels of radiation and vapor pressure deficit. Stratification of the data according to Rg and de showed that significant differences were observed at radiation levels above 400 J $m^{-2} s^{-1}$ and at vapor pressure deficits above 1.0 kPa (Table 2). Differences in daily E_c were not, however, significant at lower levels of radiation or vapor pressure.

Discussion

Our study indicated that treatment differences ranged from a 3–24% reduction in sap velocity at elevated compared with ambient CO_2 and that these differences averaged 13% over a 5-month growing season. Hourly differences in sap velocity and stand transpiration were also observed between treatments, and these were often of a magnitude greater than those observed on a daily basis. There are, unfortunately, only a few field-scale CO_2 enrichment studies against which we can compare our results. Tognetti *et al.* (1999) measured sap flow in *Querscus pubescens* Willd. trees for two consecutive summers under Mediterranean field conditions and reported that while mean and diurnal sap fluxes were consistently lower in trees at a natural CO_2 spring than they were for trees growing at a near-by control site, the mean sap flux per unit foliage area did not differ between trees at the two sites. Similarly, Kellomäki & Wang (1998) reported that elevated CO_2 reduced sap flow in 30-yr-old Scots pine (*Pinus sylvestris* L.) trees by 4–14% compared with controls, but noted that these differences

Climatic variable	n (#)	Daily Stand Transpiration		Change (%)	P > F
		Ambient (mm d⁻¹)	Elevated (mm d⁻¹)		
Radiation (J m⁻² s⁻¹)					
0–200	4	0.7 ± 0.6	0.7 ± 0.5	0	ns
200–400	19	2.8 ± 0.7	2.7 ± 0.7	–3.6	ns
400–500	33	4.1 ± 0.8	3.6 ± 0.7	–12.2	*
> 500	26	4.7 ± 0.5	4.1 ± 0.4	–12.7	*
Vapor Pressure Deficit (kPa)					
0–0.5	7	1.2 ± 0.8	1.2 ± 0.8	0	ns
0.5–1.0	22	3.2 ± 0.6	3.0 ± 0.6	–6.3	ns
1.0–1.5	45	4.3 ± 0.7	3.8 ± 0.6	–11.6	*
> 1.5	8	5.1 ± 0.3	4.4 ± 0.1	–13.7	*

Table 2 Daily rates of stand transpiration (mean ± SD) for trees in the ambient and elevated CO_2 rings stratified according to mean daily radiation and vapor pressure deficit. In order to avoid complications due to canopy leaf area development and/or senescence, analyses were restricted to an 82-d subset of data collected between May 10 and Aug 28 when LAI was at a maximum. The number of days (*n*) included in each stratification level is shown

*indicate significant reductions in stand transpiration due to elevated CO_2 concentration.
ns designates treatment differences as nonsignificant.

were significant for only a few days out of a 32-d measurement period.

In addition to field studies that have used closed-top chamber technology (Kellomäki & Wang, 1998) and natural springs (Tognetti *et al.*, 1999) to expose trees to elevated CO_2 concentrations, sap flow rates have also been measured in one other FACE experiment (Ellsworth *et al.*, 1995). The results of that short-term study showed that over an 8-d exposure period there were small (6–7%), but significant, reductions in sap flow for loblolly pine (*Pinus taeda* L.) exposed to 550 p.p.m. CO_2 concentration. Small differences such as these led Ellsworth *et al.* (1995) to suggest that leaf and whole-tree water-use responses to elevated CO_2 might be mediated (and thus muted or amplified) by interactions with other environmental factors such as vapor pressure deficit and soil water availability. Senock *et al.* (1996) found few CO_2-induced differences in sap flow between ambient and elevated CO_2 treatments in a FACE wheat experiment, noting that CO_2 effects were somewhat obscured on cloudy days, and concluded that separating the sap flow data based on daily solar radiation levels might be appropriate as a means of observing treatment differences across environmental conditions. Kellomäki & Wang (1998) similarly noted that the magnitude of a CO_2 effect on sap flow in Scots pine was dependent on weather. More specifically, Kellomäki & Wang (1998) speculated that a CO_2-induced decrease in sap flow was associated with a high demand for transpiration, because the largest decrease in their study occured during the afternoon. We too observed that treatment differences in stand transpiration were greatest in the afternoon and that differences were most pronounced on days when mean vapor pressure deficit was high (greater than 1.0 kPa). Such days are not, however, characteristic of the generally humid conditions that typify our local climate. During the 1999 growing season, for example, 29 out of 183 d at our site had mean vapor pressure deficits above 1.5 kPa and only 19 of those days occurred during the time we were making our sap velocity measurements. More importantly, only 8 of

those 19 days fell within the 82-d period during which much of our analyzes were focused (Table 2). If indeed the relative magnitude of a CO_2-induced effect on sap velocity is dependent on prevailing weather, it clearly complicates our ability to detect treatment differences in sap velocity and canopy transpiration. Add to this the fact that precipitation occurred on 43 d during the season (further complicating our analysis) and it becomes exceedingly difficult to separate day-to-day and weather dependent 'noise' from what might otherwise be significant differences in stand transpiration between ambient and elevated CO_2 treatments.

Although the apparent dependency of sap velocity and canopy transpiration on prevailing climate does hinder our ability to identify consistent and significant differences between ambient and elevated CO_2 treatments, it is important to evaluate the results from our studies in the light of potential changes in future climate, particularly temperature. The Intergovernmental Panel on Climate Change (IPCC) has concluded that continued increases in CO_2 and other greenhouse gases in the atmosphere are expected to induce an additional 1–3.5°C increase in average global surface temperatures by the year 2100 (Kattenberg *et al.*, 1996). All else being the same, a 3.5°C rise in air temperature at our site during 1999 would have increased the number of days characterized by a mean vapor pressure deficit above 1.5 kPa from 29 to 73 days. Under conditions such as these, reductions in sap velocity and canopy transpiration due to the potential interaction of elevated CO_2 and enhanced temperature (mediated through vapor pressure deficit) might have been easier to observe than under conditions of elevated CO_2 alone.

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. This is in apparent contrast to the findings of Tognetti *et al.* (1999) and Heath (1998) who both reported that stomata of trees growing in CO_2-enriched atmospheres showed reduced sensitivity to vapor pressure deficit. Although we have

collected sufficient leaf-level measurements to conclude that mid-season stomatal conductance is reduced by 18–29% for sweetgum trees growing at elevated CO_2 concentration (S. D. Wullschleger, unpublished), whether these trees demonstrate increased or decreased stomatal sensitivity to vapor pressure deficit has not yet been resolved (C. A. Gunderson, unpublished). We have, however, used estimates of stand transpiration and leaf area index obtained in this study to calculate daily mean canopy conductance (mmol m^{-2} s^{-1}) for stands exposed to ambient and elevated CO_2 concentration. While the analyzes are not yet complete, it is our preliminary conclusion that at elevated CO_2 there is an approximate 15% decrease in the *sensitivity* (*sensu* Oren *et al.*, 1999) of canopy conductance to vapor pressure deficit. Such a canopy-scale observation would agree with leaf-level conclusions drawn from a variety of studies (Hollinger, 1987; Will & Teskey, 1997; Tognetti *et al.*, 1998).

One of the more obvious differences between ambient and elevated CO_2 treatments was in the response of sap velocity and stand transpiration to elevated CO_2 concentrations during the early portion of the growing season. For both April and May these CO_2-induced differences were significant, although the causes of such differences are not clear. There were measurable reductions in stomatal conductance during the early weeks of exposure, but such differences were small (S. D. Wullschleger, unpublished). We suspect that a more likely explanation is that canopy leaf area development was initiated earlier and proceeded more rapidly for the ambient compared with the elevated CO_2 treatment. Additional evidence in support of a difference in canopy development between treatments was a more rapid decline in radiation beneath the canopy in the ambient CO_2 plots during 1999 and an earlier onset of basal area growth in trees growing at ambient CO_2 (Norby *et al.*, 2001 – see pp. 477–487 in this issue). However, although such a response is potentially intriguing, caution is advised in that a CO_2-induced difference in the timing of leaf-out between treatments was not observed in the Spring of 2000 (data not shown).

Increases in instantaneous water-use efficiency (WUE) at the scale of a leaf or canopy are often reported to be a rather consistent response of plants to elevated CO_2 (Eamus, 1991). It is frequently implied, although seldom shown, that such an increase in WUE should be a benefit to CO_2-grown plants exposed to water-limited conditions. Ellsworth (1999) reported that water use efficiency (*Anet/E*) for loblolly pine exposed to elevated CO_2 in the Duke FACE experiment was approximately twice that of trees exposed to ambient CO_2 concentrations, while *Anet/E* for the same sweetgum trees that we examined in this study was up to 75% higher at elevated compared with ambient CO_2 concentration (C. A. Gunderson, unpublished). Although these leaf-level estimates of WUE may have some utility, seldom has it been possible to express WUE in terms of biomass produced per unit water transpired and to assess the magnitude of this effect for trees exposed to CO_2 enrichment.

However, our estimates of seasonal water use combined with the growth data from Norby *et al.* (2001) allow us to make such a comparison. Annual water use from transpiration (following gap-filling for missing June data) was estimated to be 540 mm (540 kg m^{-2}) for the ambient plots and 484 mm (484 kg m^{-2}) for the elevated plots. Above-ground dry matter increment during roughly this same period was 0.80 and 0.92 kg m^{-2} for the two CO_2 treatments, respectively. Therefore, dividing dry matter increment by annual water use results in a calculated WUE of 1.48 g kg^{-1} for the ambient and 1.90 g kg^{-1} for the elevated CO_2 stand. This translates into a 28% increase in stand-level WUE, which is more than either the CO_2-induced increase in dry matter increment (15%) or decrease in water use (12%) alone, but considerably less than the 50–75% increase in WUE calculated at the leaf-level (C. A. Gunderson, unpublished).

There is always the temptation in CO_2 enrichment studies to equate reductions in stomatal conductance with potential reductions in transpiration at scales ranging from that of single leaves to that of entire ecosystems (Field *et al.*, 1995). There are, of course, reasons why a large response in stomatal conductance and transpiration at the leaf-level might be muted or otherwise dampened at that of larger scales (Sellers *et al.*, 1997; Raupach, 1998; Wilson *et al.*, 1999). The results of our study show that large reductions in stomatal conductance at elevated CO_2 do not necessarily translate to reductions in rates of canopy transpiration, particularly if CO_2-induced reductions in stomatal conductance are limited to upper-canopy leaves and are not observed at other layers within the canopy (S. D. Wullschleger, unpublished). Such a canopy-dependent response to elevated CO_2 acts to produce a smaller effect at the whole-plant level than what might otherwise be predicted from leaf-level measurements of stomatal conductance and transpiration alone. And finally, over longer periods of time, the dependency of a CO_2 effect on prevailing weather means that day-to-day variability in radiation and vapor pressure deficit will potentially mask what otherwise might seem like a large response given clear sky and dry atmospheric conditions. Such conclusions are not intuitively obvious. Future studies should attempt to further evaluate the magnitude by which weather modifies the response of stand transpiration to elevated CO_2 and to quantify the potential interaction between CO_2 and temperature as it relates to ecosystem water balance.

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