

# Leaf- and stand-level responses of a forested mesocosm to independent manipulations of temperature and vapor pressure deficit

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## Summary

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- Alterations in temperature (T) and vapor pressure deficit (VPD) strongly influence gas exchange, but because VPD is highly influenced by T, the effects of these two factors are difficult to separate.
- Here, the concomitant effects of T and VPD on CO<sub>2</sub> uptake, stomatal conductance, and transpiration at leaf- and canopy-levels were examined for a stand of trees (*Populus deltoides*) enclosed within large mesocosms. T and VPD were independently altered to yield a factorial combination of treatments of low (24°C) or high (30°C) T and low (0.75) or high (1.75 kPa) VPD. Traditional leaf-level gas exchange measurements were compared with whole-canopy exchange to verify typical scaling methods.
- Elevated T resulted in an average 40% and 14% increase in midday leaf-level and canopy-level net CO<sub>2</sub> uptake, respectively. Other physiological responses to elevated T and VPD were similar at both scales, but the magnitude of change was usually less pronounced at the canopy-level.
- Surprisingly, only minimal interactions between T and VPD were found to influence responses of CO<sub>2</sub> uptake and stomatal conductance at either level.

**Key words:** Biosphere 2 Laboratory, conductance, net ecosystem carbon exchange, photosynthesis, *Populus deltoides*, sap flux, scaling.

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## Introduction

The manner and degree to which a plant is affected by water stress is determined by its combined response to contributing abiotic (soil moisture, vapor pressure deficit, temperature, wind, etc.) and biotic factors (plant architecture, root-soil water relationships, water capacitance and conductance and stomatal properties), all of which effect net CO<sub>2</sub> uptake and water loss at the leaf and canopy levels. Variations in temperature (T) and vapor pressure deficit (VPD) can strongly affect rates of gas exchange, but because VPD is highly influenced by temperature, the effects of the two factors are often difficult to separate. Responses of leaf gas exchange to changes in T

typically follow a nonlinear response curve, with increased rates of net CO<sub>2</sub> assimilation (Bassman & Zwier, 1991; Horton *et al.*, 2001) and stomatal conductance (Horton *et al.*, 2001) with increasing temperatures up to an optimum after which they decrease. Net CO<sub>2</sub> assimilation (Day, 2000; Horton *et al.*, 2001) and conductance (Will & Teskey, 1997; Day, 2000; Horton *et al.*, 2001; Lu *et al.*, 2003), however, decrease exponentially with increasing VPD.

Whereas leaf level measurements provide valuable information pertinent to underlying physiological mechanisms, the response of an entire canopy exposed to concurrent changes in T and VPD is needed to appreciate gas exchange dynamics at the stand and community levels. Considering the relevance of

predicted increases in global temperatures over the next century (Rosenzweig & Hillel, 1993; IPCC, 2001) and the predictions of reduced precipitation and more extreme or extended drought, which would alter atmospheric VPD (Rosenzweig & Hillel, 1993; Gregory *et al.*, 1997), experiments examining tree responses to these factors at leaf and whole-canopy levels are needed. Saxe *et al.* (2001) note that, despite the likely increase of land surface temperatures at mid latitudes owing to global warming, few experiments have isolated the effects of T and VPD on trees and forest stands. Researchers often do not take into account whole-plant feedbacks because of the concurrent effects of the many variations in T, VPD or light possible within a canopy at any single point in time when interpreting leaf-level responses and scaling them to an entire canopy (Griffin *et al.*, 2002a). The influences of T and VPD are often merged together and thought of as a combined effect because of the strong correlation between the two parameters and the difficulty in maintaining a constant VPD across temperatures in experimental studies (Medlyn *et al.*, 2002). This assumption is unfortunate since T and VPD mechanistically affect plant processes in different manners. As such, T *directly* influences both net CO<sub>2</sub> assimilation and stomatal conductance, but VPD only *directly* influences stomatal conductance and *indirectly* influences CO<sub>2</sub> assimilation via those changes in stomatal conductance. In this experiment, we were able to independently maintain settings of both T and VPD so that we could study of each of these variables separately, while maintaining the ability to examine interactions.

Carbon gain and water loss in trees and their responses to T and VPD are clearly factors that will determine the productivity, species composition, and survival of a forest in response to changing climates (Chen *et al.*, 2002). While Eamus (2003) discussed the relative influence of ecosystem water balance on net primary productivity in woody ecosystems throughout the world, the effects of VPD and T on carbon uptake in stands of trees have usually been based on field observational studies that lack experimental control over the climatic variables involved (Lindroth & Cienciala, 1996; Hogg & Hurdle, 1997; Bassow & Bazzaz, 1998; Horton *et al.*, 2001; Law *et al.*, 2001; Chen *et al.*, 2002; Huxman *et al.*, 2004). Although free air carbon enrichment (FACE), open top chamber, and eddy covariance technologies are being used more widely to thoroughly examine gas exchange at the canopy level (McLeod & Long, 1999; King *et al.*, 2004; DeLucia *et al.*, 2005; Norby *et al.*, 2006), a lack of control over climatic variables across the entire stand hinders experimental study of T and VPD in a factorial setting.

Leaf-level respiration responses may differ depending on whether temperature manipulations are done on a leaf or on the whole tree (Griffin *et al.*, 2002a). When traditional gas exchange equipment is used to manipulate conditions inside the leaf chamber, measures of leaf-level net CO<sub>2</sub> uptake or stomatal conductance can be flawed because of leakage

associated with leaf anatomy (Amthor *et al.*, 2001; Jahnke, 2001; Jahnke & Krewitt, 2002; Pieruschka *et al.*, 2005; Pieruschka *et al.*, 2006). Typically, the effect of factors such as VPD and T on canopy-level photosynthesis, conductance, and transpiration are not measured directly, but rather, are estimated using a variety of scaling methods or process modeling frameworks (Smith *et al.*, 1991; Cienciala *et al.*, 1992; Granier & Breda, 1996; Lindroth & Cienciala, 1996; Oren *et al.*, 1998; Clearwater *et al.*, 1999; Oren *et al.*, 1999a; Guevara-Escobar *et al.*, 2000; Law *et al.*, 2001; Hanson *et al.*, 2005; Yopez *et al.*, 2005). With increasing interest in larger-scale implications of global warming for ecosystem productivity and stability, leaf-level measurements are used to model canopy-level physiological responses. It is not clear how well leaf-level responses translate to or can be directly compared with whole-canopy level responses (Lin *et al.*, 1998). In order to model field responses with greater fidelity, we need to be able to measure the responses of both the leaf and the canopy to changes in T and VPD simultaneously.

Here we describe a large-scale experiment on eastern cottonwoods (*Populus deltoides*), that quantifies the effects of increasing T and VPD on leaf- and stand-level CO<sub>2</sub> exchange, water-use efficiency and productivity. Soil moisture was kept relatively constant, eliminating the confounding influence of soil water stress under any of the four treatment conditions. Using the uniquely large, semiclosed facility of the Biosphere 2 Laboratory, our objectives were to: simultaneously quantify leaf- and stand-level gas exchange in a stand of *P. deltoides* subjected to a factorial combination of low and high treatments of T and VPD; and compare leaf- and stand-level gas exchange responses to T and VPD by calculating canopy-level measures on a projected leaf area basis. We hypothesized that T and VPD would have different interactive effects at the leaf- and canopy-level on gas exchange because of leaf heterogeneity within the canopy and modification of the local environment by transpiration within the canopy. We discuss these data in the context of extrapolations from leaf- to stand-level processes.

## Materials and Methods

### Site characteristics

This experiment was conducted within the Intensively-managed Forest Mesocosm (IFM) at the Biosphere 2 Laboratory located in Oracle, Arizona, USA (32° 37.13'N; 110° 47.05'W, 1200 m asl). Treatments were applied to a stand of 35 eastern cottonwood trees (*Populus deltoides* Bartr.) enclosed in a mesocosm chamber with a soil volume of 550 m<sup>3</sup> and an estimated total air volume of 11873 m<sup>3</sup> that was maintained at *c.* 400 μmol mol<sup>-1</sup> [CO<sub>2</sub>]. The atmosphere surrounding the enclosed stand was mixed by fans, and outer canopy leaves in edge trees fluttered visibly. Measurements were made during the peak of the growing season after full-canopy development, and system-level performance before and after the experiments

**Table 1** Average air and mid-canopy *Populus deltoides* leaf temperature, vapor pressure deficit (VPD) and soil moisture for the four treatment combinations

Treatment	Canopy position	Air temperature		Leaf temperature		VPD		Soil moisture (v : v, %)
		Day (°C)	Night (°C)	Day (°C)	Night (°C)	Day (kPa)	Night (kPa)	
Low T + Low VPD	Mesocosm	24.39 (0.18)	21.59 (0.19)			0.76 (0.06)	0.74 (0.05)	28.7 (0.1)
	Lower	23.30 (0.20)	20.96 (0.19)			0.75 (0.05)	0.73 (0.06)	
	Middle	24.13 (0.16)	20.56 (0.14)	20.62 (0.18)	20.18 (0.18)	0.77 (0.06)	0.75 (0.05)	
	Upper	26.48 (0.23)	21.39 (0.20)			0.77 (0.07)	0.75 (0.07)	
Low T + High VPD	Mesocosm	24.42 (0.20)	22.21 (0.18)			1.73 (0.05)	1.50 (0.04)	28.3 (0.2)
	Lower	24.69 (0.19)	21.83 (0.17)			1.72 (0.04)	1.49 (0.04)	
	Middle	24.55 (0.16)	21.96 (0.18)	20.19 (0.16)	19.99 (0.17)	1.75 (0.06)	1.51 (0.05)	
	Upper	26.50 (0.22)	21.86 (0.23)			1.76 (0.06)	1.51 (0.06)	
High T + Low VPD	Mesocosm	30.42 (0.14)	26.09 (0.12)			0.74 (0.05)	0.79 (0.04)	28.9 (0.1)
	Lower	30.10 (0.16)	25.11 (0.15)			0.74 (0.04)	0.77 (0.05)	
	Middle	31.50 (0.16)	25.25 (0.13)	24.94 (0.18)	24.91 (0.15)	0.75 (0.06)	0.79 (0.06)	
	Upper	33.72 (0.22)	25.22 (0.18)			0.76 (0.07)	0.80 (0.07)	
High T + High VPD	Mesocosm	30.26 (0.13)	26.34 (0.11)			1.78 (0.06)	1.81 (0.05)	28.4 (0.1)
	Lower	29.95 (0.16)	25.36 (0.18)			1.76 (0.05)	1.78 (0.05)	
	Middle	30.21 (0.17)	25.83 (0.14)	24.2 (0.15)	23.7 (0.17)	1.77 (0.05)	1.80 (0.04)	
	Upper	32.92 (0.21)	24.98 (0.19)			1.78 (0.07)	1.81 (0.07)	

Hourly means were calculated from observations recorded every 15 min for each day and then treatment means  $\pm$  SE were calculated from the three subsequent days ( $n = 3$ ) used for each treatment. 'Mesocosm' denotes open-air temperature within the stand. Leaf temperatures obtained from infrared thermocouples positioned within the mid-canopy ( $n = 3$ ) were calculated from observations recorded every 15 min as above. Values in parentheses indicate  $\pm 1$  SE.

has been reported previously (Barron-Gafford *et al.*, 2005). At the beginning of this experiment, the trees averaged  $11.3 \pm 1.6$  m in height and  $93.8 \pm 11.9$  mm in basal diameter. The size of the trees had not changed significantly by the end of the experiment, averaging  $11.4 \pm 1.3$  m in height and  $95.2 \pm 11.2$  mm in basal diameter. Further details on climate control and the structure of the IFM can be found elsewhere (Lin *et al.*, 1998; Dempster, 1999; Zabel *et al.*, 1999; Griffin *et al.*, 2002a,b; Murthy *et al.*, 2003; Engel *et al.*, 2004; Barron-Gafford *et al.*, 2005; Murthy *et al.*, 2005). Soil depth averaged 1 m and approximated a rich agricultural loam (Torbert & Johnson, 2001) with microflora characteristic of agricultural and natural soils (Lipson *et al.*, 2006). The soil profile showed a normal bulk density distribution with depth, nitrogen (N) concentrations of  $2\text{--}3$  mg g<sup>-1</sup>, and a carbon (C) : N ratio of  $c. 10$  (Martens & McLain, 2003; Barron-Gafford *et al.*, 2005).

### Experimental protocol

Treatments applied to the trees were variations in ambient air temperature (T) and atmospheric vapor pressure deficit (VPD). Day : night T treatments were either 24 : 22°C (low) or 30 : 26°C (high), and day : night VPD was maintained at either 0.75 : 0.77 kPa (low) or 1.75 : 1.77 kPa (high). During the 33-d experiment, particular combinations of T and VPD were maintained for 3 d each. Each of the four treatments was separated by a 7-d transition period to wash-out any 'carry-over' effects of previous treatments, and treatments

were arranged in the sequence low VPD/low T to high VPD/high T to minimize any potential 'carry-over' effects between subsequent treatments in the sequence (Table 1). Although total daily photosynthetically active radiation (PAR) declined due to decreasing day length, average mid-day (10 : 30–14 : 00 h) PAR was not significantly different between the four treatments (see Fig. 2). Leaf gas-exchange measurements were repeated four times per day on day 3 of each treatment. Canopy-level gas exchange measurements were taken continuously throughout the day.

Relative humidity, T, atmospheric pressure, VPD, and PPFD were measured every 15 s using sensors placed at three canopy heights at four locations within the stand, averaged and stored every 15 min using data-loggers (Campbell-CR10X; Campbell Scientific Inc., Logan, UT, USA). Average VPD and T were tightly controlled and did not fluctuate significantly during measurement days (Table 1). Temperatures of three mid-canopy leaves were measured throughout the experiment using infrared thermocouple sensors (IRTS-P; Apogee Instruments Inc., Logan, UT, USA) and 15-min averages were also stored using data-loggers. Volumetric soil moisture was monitored continuously using 12 probes (CS615; Campbell Scientific) installed at three depths in four locations throughout the stand. Soil moisture was maintained at a constant volumetric water content of approx. 28% (v : v) using drip irrigation and did not differ substantially between treatments (Table 1). The day : night CO<sub>2</sub> concentration set points were maintained at 400 : 500  $\mu\text{mol mol}^{-1}$ , respectively.

## Measurements

**Leaf gas exchange** Leaf gas-exchange measurements of CO<sub>2</sub> uptake, conductance, and transpiration were taken at 08 : 00, 10 : 30, 14 : 00 and 16 : 30 h using a portable photosynthesis system (LI-6400; LI-COR Inc., Lincoln NE, USA). Two south-facing fully expanded leaves of like age from each of three canopy positions (lower, middle and upper) were measured on four trees on day 3 of each treatment. Because the two subsampled leaves within each canopy position per tree did not differ greatly, they were averaged to give a single value per tree. Photosynthetically active radiation, VPD, and T within the leaf cuvette were set to reflect the controlled ambient conditions of the mesocosm at the time of each sampling. Leaf photosynthetic rates were calculated within the LI-COR system according to von Caemmerer & Farquhar (1981), using the physiological convention with positive values corresponding to net photosynthetic CO<sub>2</sub> uptake. Because leaf level gas exchange rates were greatest during the midday (10 : 30 and 14 : 00 h), and light intensities were not significantly different between these times, treatment comparisons were based on averages obtained over this midday period ( $n = 8$ , two time periods with four sample trees each) per canopy position. Pre-dawn and mid-day water potentials were also measured on adjacent leaves using the pressure chamber method (Koide *et al.*, 1991) (model 610 Pressure Chamber; PMS Instrument Co., Corvallis, OR, USA).

**Continuous measures of stand gas exchange** During the experimental period, the stand was operated in an 'open' state during the night and a 'closed' state during the day, referring to whether the fans that exchange air with the outside were open or closed, respectively. System Net CO<sub>2</sub> Exchange Rate (*SNCER*,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , soil surface area basis) was calculated from changes in the mesocosm [CO<sub>2</sub>] measured at 15-min intervals in the light (*SNCER*<sub>Light</sub>) and dark (*SNCER*<sub>Dark</sub>), and is shown using the atmospheric convention as negative and positive, respectively.

Leaks between the IFM chamber and the outside environment were determined before and during each experimental setting by tracking the dilution of a trace gas sulphahexafluoride (SF<sub>6</sub>). Rates of *SNCER* were then corrected based on leak rates obtained from SF<sub>6</sub> calculations. Further details on the system-level measurements can be found in Barron-Gafford *et al.* (2005). *SNCER* was also adjusted for soil CO<sub>2</sub> efflux and expressed per unit projected leaf area to make comparisons with leaf-level net photosynthetic rates as detailed in Murthy *et al.* (2005). Projected leaf area is being defined as the leaf area projected horizontally onto the ground surface. Soil CO<sub>2</sub> efflux was estimated from *SNCER*<sub>Dark</sub> values corresponding to total (soil and root) respiration for this stand (Murthy *et al.*, 2003; Barron-Gafford *et al.*, 2005; Murthy *et al.*, 2005).

$$\text{Net above-ground CO}_2 \text{ exchange rate (NACER)} = \text{SNCER}_{\text{Light}} - \text{SNCER}_{\text{Dark}} \quad \text{Eqn 1}$$

When *NACER* was divided by projected leaf area, we obtained a net photosynthetic rate per unit leaf area ( $A_C$ ). This value represents the average net photosynthetic rate per unit leaf area within the canopy. Leaf area was estimated from allometric regressions based on extensive physical measurements on a subset of trees, as described in Murthy *et al.* (2005). Comparisons of leaf- and stand-level gas exchange rates were always based on the average midday rate between 10 : 30 and 14 : 00 h.

**Stand water dynamics** Average sap flux on a sapwood area basis ( $\text{mm h}^{-1}$ ; Edwards *et al.*, 1996) was obtained every 15 min using Granier-type heat dissipation sensors installed on nine sample trees (Engel *et al.*, 2004). Whole-canopy transpiration ( $\text{kg H}_2\text{O m}^{-2} \text{ ground area s}^{-1}$ ) was calculated by multiplying sapwood area index (total stem basal area/planting area) by average sap flux. Transpiration rate per unit leaf area ( $E_C$ ) was calculated by dividing whole canopy transpiration by projected leaf area index (Granier, 1987; Cienciala *et al.*, 1992; Martin *et al.*, 1997; Clearwater *et al.*, 1999; Oren *et al.*, 1999b). Sap flux probes were used because sensors to derive whole stand transpiration from greenhouse gas exchange similar to *SNCER* were inadequate at the time. Canopy conductance per unit leaf area ( $g_{sC}$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) was calculated using the following equation:

$$g_{sC} = 55.6 \times E_C \times P / \text{VPD} \quad \text{Eqn 2}$$

( $P$  represents atmospheric pressure (kPa) and the coefficient 55.6 converts  $E_C$  from  $\text{kg H}_2\text{O m}^{-2} \text{s}^{-1}$  to  $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ; Engel *et al.*, 2004).

Instantaneous water-use efficiencies at the leaf-level ( $WUE_L$ ) were calculated as the ratio of rates of leaf-level net assimilation over leaf-level transpiration ( $A_L/E_L$  measured as  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ). Whole-canopy water use efficiency was estimated in two manners: (1) instantaneous ( $WUE_C$ ; canopy CO<sub>2</sub> gain/canopy H<sub>2</sub>O loss in  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) and (2) cumulative daily ( $WUE_D$ ; total daily canopy CO<sub>2</sub> gain/total daily canopy H<sub>2</sub>O loss in  $\text{g C l}^{-1} \text{ H}_2\text{O}$ ). Values for  $WUE_D$  were calculated by summing the total daily carbon uptake data (from  $A_C$ ) and dividing it by the cumulative amount of water transpired by the stand (from  $E_C$ ) over a 24-h period.

## Statistical analysis

Data were analysed using the general linear models and mixed procedures for repeated measures within the SAS statistical software package (1996, SAS Institute Inc., Cary, NC, USA). Analysis of rates of midday (average of 10 : 30 h and 14 : 00 h) leaf-scale gas exchange, sap flux and stand level net ecosystem CO<sub>2</sub> exchange was based on a split plot design. Within this design the whole-plot fixed factors were the treatments T and VPD and the subplot factors were canopy positions.



**Table 2** Degrees of freedom and *F*-statistics from the statistical analysis on *Populus deltoides* trees in response to factorial combinations of treatments of low and high temperature and vapor pressure deficit (VPD) through time

Factors	df	Physiological variable								
		$A_L$	$g_{SL}$	$E_L$	$WUE_L$	$A_C$	$g_{SC}$	$E_C$	$WUE_C$	$WUE_D$
Time	1	0.36	0.53	2.33	0.04	0.14	0.62	0.68	0.09	0.01
Temperature	1	3.43*	10.76**	83.05***	6.31**	153.4***	42.18**	1780***	25.47*	0.00
VPD	1	3.06*	7.34**	1.07	0.04	835.8***	937.14***	1750***	3505***	15.29**
Canopy height	2	21.91***	7.80**	21.15**	1.88	0.	0.	0.	0.	0.
Temperature × VPD	1	0.58	2.10	0.75	0.21	6.74*	0.25	2932***	631.8***	1.30
Temperature × Canopy height	2	1.18	1.24	5.74**	0.24	0.	0.	0.	0.	0.
VPD × Canopy Ht	2	3.57*	1.81	0.85	0.39	0.	0.	0.	0.	0.
Temperature × VPD × Canopy height	2	0.09	0.39	0.17	0.14	0.	0.	0.	0.	0.

\*, \*\*, \*\*\*, Significance at  $P < 0.10$ ,  $P < 0.05$  and  $P < 0.001$ , respectively.

$A_C$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), canopy-level net  $\text{CO}_2$  uptake per unit leaf area;  $A_L$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), leaf-level net  $\text{CO}_2$  uptake;  $g_{SC}$  ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), canopy-level stomatal conductance per unit leaf area

$g_{SL}$  ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), leaf-level stomatal conductance;  $E_C$  ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), canopy-level transpiration per unit leaf area;  $E_L$  ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), leaf-level transpiration;

$WUE_C$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} : \text{mol H}_2\text{O}$ ), canopy-level instantaneous water use efficiency;  $WUE_D$  ( $\text{g C} : \text{l H}_2\text{O}$ ), canopy-level cumulative daily carbon uptake per litre of water transpired;  $WUE_L$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} : \text{mol H}_2\text{O}$ ), leaf-level instantaneous water-use efficiency.

## Results

### Leaf gas exchange

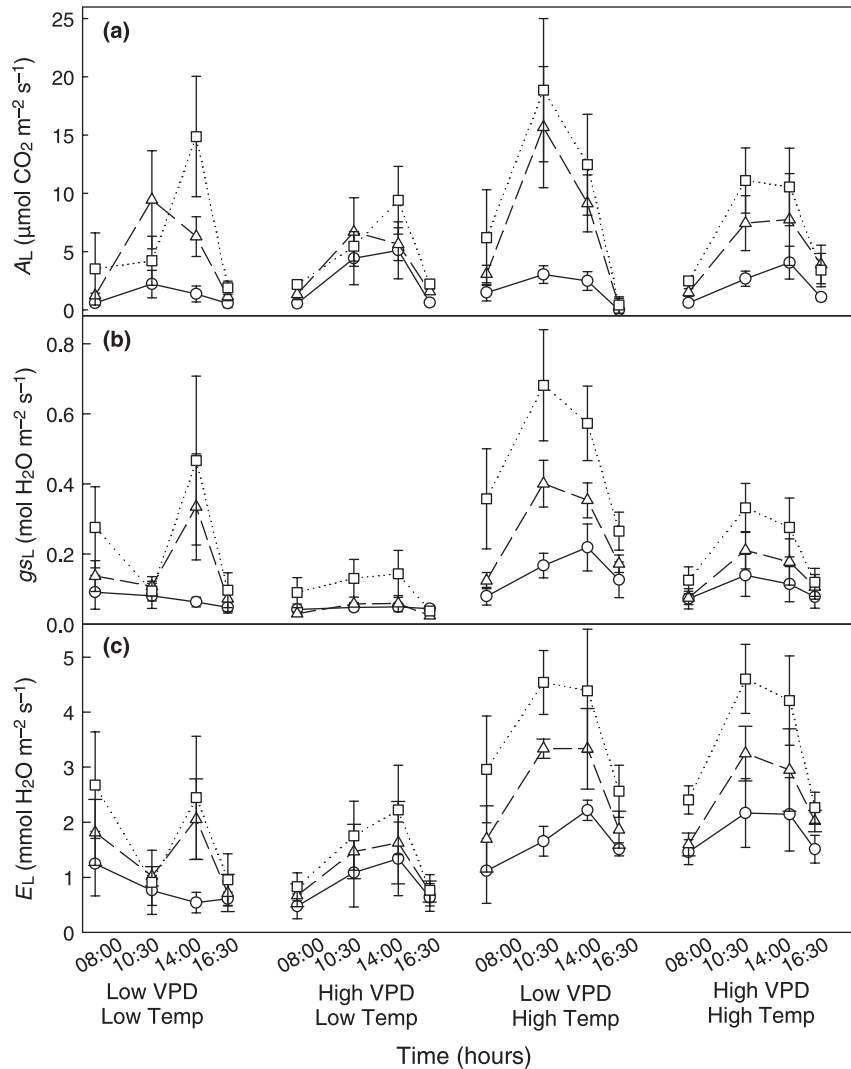
Measures of midday (10 : 30 k and 14 : 00 h) leaf-level net  $\text{CO}_2$  uptake ( $A_L$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) were significantly affected by temperature (T), vapor pressure deficit (VPD) and canopy height, but there was not a significant T × VPD interaction (Table 2). A significant VPD × canopy height interaction for  $A_L$  was detected (Table 2). Increasing T from the low to high treatments increased midday, mid-canopy  $A_L$  by 58% and 23% at low and high VPD, respectively (Fig. 1a). Increasing VPD reduced midday  $A_L$  by 22% and 39% at low and high temperatures, respectively. The mid- and upper-canopies were the most influenced by changes in T and VPD.

Maximum midday conductance ( $g_{SL}$ ) was significantly affected by T, VPD, and canopy height, but there was no significant T × VPD interaction (Table 2). Increasing temperature  $6^\circ\text{C}$  increased midday  $g_{SL}$  by 71% and 234% within the low and high VPD treatments, respectively (Fig. 1b). As with  $A_L$ , this trend was especially apparent within the mid- and upper-canopy heights. Increasing VPD decreased midday  $g_{SL}$  by 74% and 49% in the low- and high-temperature treatments, respectively. Midday leaf-level transpiration rates ( $E_L$ ) were significantly influenced by T, canopy height and their interaction, but were unaffected by VPD (Table 2). No T × VPD interaction was detected. Transpiration rates were greater under elevated T than low T in both VPD treatments (Fig. 1c). On average,  $E_L$  increased by 109% with increasing T under both VPD treatments. Transpiration rates were relatively unresponsive to changes in VPD regardless of T treatments (Table 2).

### Stand-level gas exchange

Values of net above-ground  $\text{CO}_2$  exchange were calculated on a projected leaf area basis to yield  $A_C$  ( $\mu\text{mol CO}_2 \text{ m}^{-2}$  projected leaf area  $\text{s}^{-1}$ ). Rates of midday (between 10 : 30 h and 14 : 00 h)  $A_C$  were significantly influenced by increasing T and VPD, and there was a significant T × VPD interaction (Table 2). Elevated T increased (more negative, greater  $\text{CO}_2$  sink) midday  $A_C$  13% from  $-17.2$  to  $-19.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and 15% from  $-12.5$  to  $-14.4 \mu\text{mol m}^{-2} \text{ s}^{-1}$  within the low and high VPD treatments, respectively (Fig. 2). The  $A_C$  rates were greater under low VPD conditions than under high VPD. Increased VPD reduced midday  $A_C$  by 28% and 26% at low and high temperatures treatments, respectively.

Predawn leaf water potential ( $\Psi_L$ ) was differentially influenced by T and VPD because of a strong T × VPD interaction ( $P < 0.0001$ ). An increase in T under low VPD resulted in a statistically nonsignificant change ( $P = 0.5175$ ) in  $\Psi_L$ , but under high VPD conditions, increased T resulted in a statistically significant 24% decrease (more negative) in  $\Psi_L$  ( $P = 0.0195$ ; Fig. 3a). The value for  $\Psi_L$  did not change with increasing VPD at lower T ( $P = 0.9987$ ), but increasing VPD under high T treatments resulted in a significant 40.2% decrease ( $P = 0.0010$ ; Fig. 3a). These trends indicate that the trees were experiencing greatest above-ground water stress under high T/high VPD conditions (Fig. 3a). Increasing VPD decreased average midday canopy stomatal conductance ( $g_{SC}$ ) throughout the canopy, regardless of atmospheric T conditions (Fig. 3b, Table 2). As with  $A_C$ , increasing VPD resulted in a greater change in  $g_{SC}$  than did increasing T. Increasing VPD within low and high T treatments resulted in a 50% and 42% decrease, respectively, in  $g_{SC}$  (Fig. 3b).



**Fig. 1** Average diurnal leaf-level (a) net photosynthesis ( $A_L$ ), (b) conductance ( $g_{sL}$ ) and (c) transpiration ( $E_L$ ) rates for south-facing leaves of eastern cottonwoods (*Populus deltoides*) at three canopy positions (lower, circles; middle, triangles; upper, squares) for the temperature and vapor pressure deficit (VPD) treatment combinations. Vertical bars represent  $\pm 1$  SE of the mean.

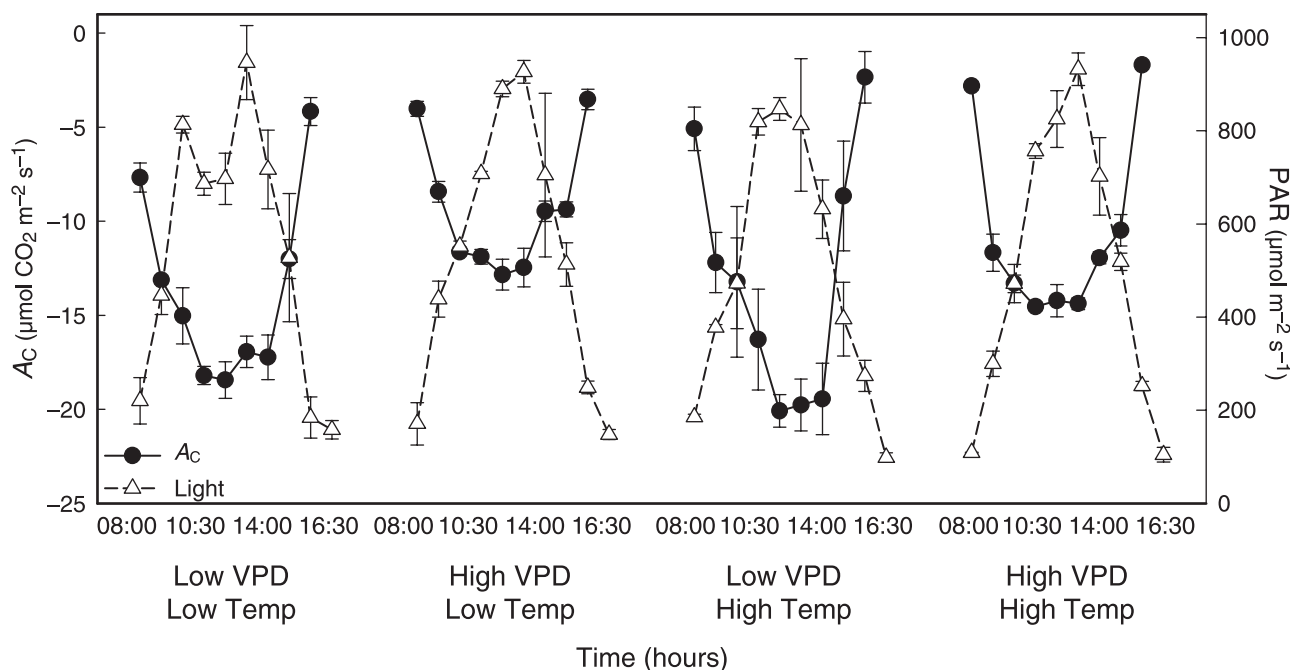
Absolute values of  $g_{sC}$  estimated from the sap flow data were nearly five times smaller than  $g_{sL}$ . Midday transpiration per unit leaf area ( $E_C$ ) was significantly affected by T and VPD, but unlike  $g_{sC}$ , a very significant T  $\times$  VPD interaction was detected ( $P < 0.0001$ ; Table 2). Transpiration rates were greatest under high T/high VPD conditions (Fig. 3c). The combined effect of increased T and VPD resulted in a 30% increase in  $E_C$  over low T/low VPD conditions from 0.753 to 0.980 mmol m<sup>-2</sup> s<sup>-1</sup>.

#### Instantaneous water-use efficiencies

Leaf-level instantaneous water use efficiency ( $WUE_L$ ) was greatest under low T/low VPD conditions and lowest under high T/high VPD conditions (Fig. 4a). Increasing T significantly influenced  $WUE_L$  (Table 2), and resulted in an average 48% decrease in midday  $WUE_L$  across VPD treatments. Increasing VPD did not result in a statistically significant change in average midday  $WUE_L$  (Table 2). Ratios of midday canopy

CO<sub>2</sub> gain/canopy H<sub>2</sub>O loss ( $\mu\text{mol CO}_2$  per mmol H<sub>2</sub>O) were examined to give an indication of whole-canopy instantaneous water-use efficiency ( $WUE_C$ ; Fig. 4b). Overall  $WUE_C$  was significantly influenced by T, VPD and the interaction between the two variables (Table 2). It was not greatly influenced by the 6°C increase in T at low VPD, but at high VPD this increase in T decreased  $WUE_C$  from 17.3 to 14.7  $\mu\text{mol CO}_2$  per mmol H<sub>2</sub>O. Increasing VPD resulted in a 26% decrease in average mid-day  $WUE_C$  under low T from 23.2 to 17.3  $\mu\text{mol CO}_2$  per mmol H<sub>2</sub>O and a 40% decrease under high T from 24.4 to 14.7  $\mu\text{mol CO}_2$  per mmol H<sub>2</sub>O (Fig. 4b). These trends parallel many of the observations of  $WUE_L$ .

A 6°C increase in T resulted in an 11% decrease ( $P = 0.0056$ ) in cumulative daily carbon uptake of the entire stand under low VPD conditions, but a nonsignificant change under higher VPD conditions ( $P = 0.3355$ ; data not shown). An increase in VPD decreased cumulative daily carbon uptake by 27% ( $P < 0.0001$ ) when T was maintained at 24°C and by 11% ( $P = 0.0409$ ) when maintained at 30°C (data not



**Fig. 2** Average diurnal stand canopy-level net ecosystem carbon dioxide exchange rates per unit projected leaf area ( $A_c$ , circles) and average photosynthetically active radiation (PAR, triangles; shown on the secondary y axis) for the stand of eastern cottonwoods (*Populus deltoides*) for the temperature and vapor pressure deficit (VPD) treatment combinations. Data are means of daily averages ( $n = 3$ ), and vertical bars represent  $\pm 1$  SE of the mean.

shown). Cumulative daily carbon uptake per liter of daily total water lost via transpiration was examined to illustrate the effects of changes in T and VPD on total daily  $WUE$  of the stand ( $WUE_D$ ; cumulative  $\text{g C l}^{-1} \text{ H}_2\text{O}$ , Fig. 4c). Overall  $WUE_D$  was not affected by T, but was significantly influenced by VPD (Table 2). Trends in total daily  $WUE_D$  were very similar to  $WUE_C$ , with changes in VPD having the greatest influence regardless of T conditions (Fig. 4b,c).

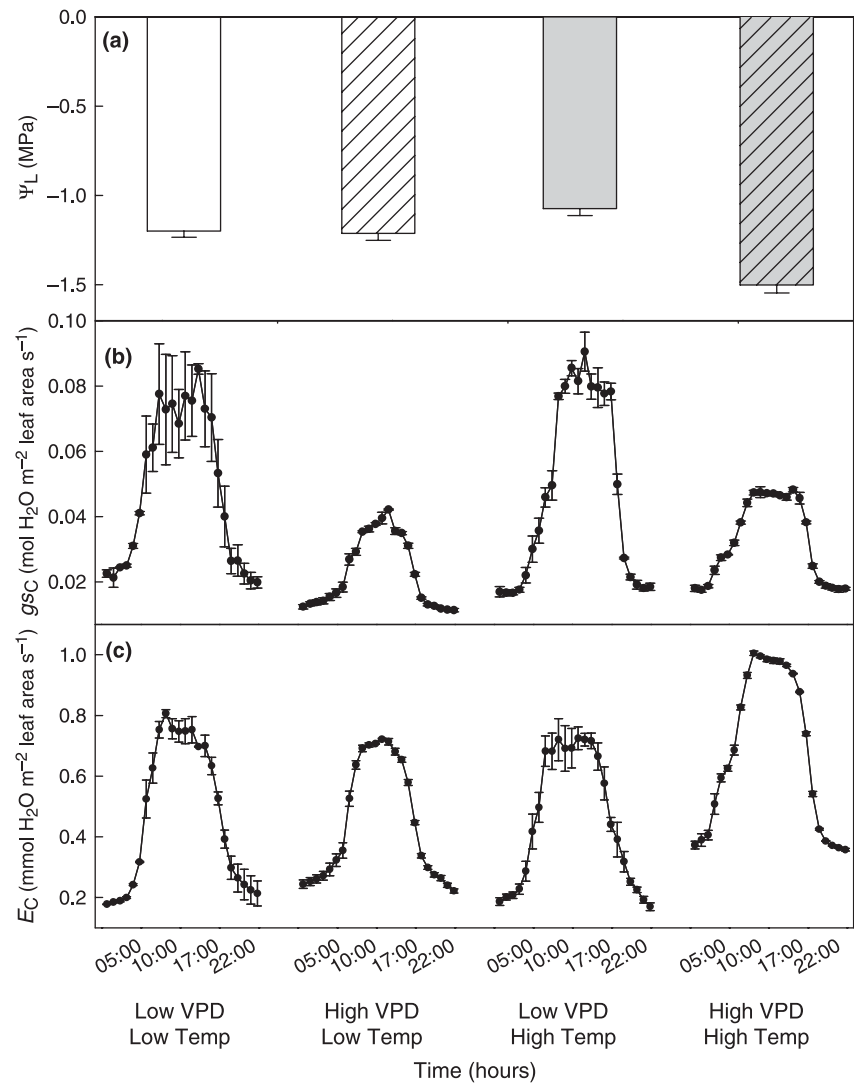
#### Mid-canopy leaf vs air temperature

Leaf temperatures within the mid-canopy were significantly lower than both the average mesocosm and the mid-canopy atmospheric air temperatures (Table 1). Midday leaf temperatures averaged  $3.5^\circ\text{C}$  lower than mid-canopy air temperature under the low T/low VPD treatment ( $P < 0.0001$ ), and were  $4.4^\circ\text{C}$  lower under the low T/high VPD treatment ( $P < 0.0001$ ). The differences between mid-canopy air and leaf temperatures were even greater under high T treatments (Table 1). Leaf temperatures averaged  $6.6$  and  $6.0^\circ\text{C}$  lower than mid-canopy air temperatures under the low and high VPD treatments when the mesocosm was maintained at  $30^\circ\text{C}$  ( $P < 0.0001$  for both treatments).

#### Discussion

Chen *et al.* (2002) concluded that although many environmental variables jointly control net  $\text{CO}_2$  uptake in trees, temperature

(T), vapor pressure deficit (VPD), and available energy are the key factors in determining differences in net  $\text{CO}_2$  uptake of an ecosystem. The relative influences of T and VPD are often merged together as a combined effect because of the strong correlation between the two parameters (Gonzalez-Rodriguez *et al.*, 2001; Medlyn *et al.*, 2002). In the experiment described here we were able to independently manipulate settings of both T and VPD so we could study each of these variables separately, while maintaining the ability to examine interactions. Soil moisture was kept constant throughout this study so that any water stress was imposed by atmospheric T and VPD alone. In fact, we found no significant interaction between T and VPD for leaf-level measures of net  $\text{CO}_2$  uptake ( $A_L$ ), stomatal conductance ( $g_{sL}$ ), transpiration ( $E_L$ ), instantaneous water use efficiency ( $WUE_L$ ), or for canopy-level stomatal conductance ( $g_{sC}$ ) or total daily  $WUE$  of the stand ( $WUE_D$ ). However, a significant interaction between the T and VPD was found, for canopy-level net  $\text{CO}_2$  uptake ( $A_C$ ), canopy-level transpiration per unit leaf area ( $E_C$ ), and canopy-level instantaneous water use efficiency ( $WUE_C$ ; Table 2). Increasing T alone did not increase  $E_C$  as it did  $E_L$ , but a combination of increasing T and increasing VPD resulted in more than a 33% increase in maximum sap flux and  $E_C$  (Fig. 3c). The T  $\times$  VPD interaction we observed could have been the result of increased evaporative cooling of the leaves, in combination with higher VPD, resulting in a large rise in  $E_C$ . We did find that leaves within the mid-canopy were approximately  $6^\circ\text{C}$  cooler than atmospheric temperatures under both high



**Fig. 3** Average (a) predawn leaf water potential ( $\Psi_L$ ) (b) canopy conductance ( $g_{sC}$ ), and (c) transpiration ( $E_C$ ) per unit projected leaf area for the stand of eastern cottonwoods (*Populus deltoides*) for the temperature and vapor pressure deficit (VPD) treatment combinations. Data are means of daily averages ( $n = 3$ ), and vertical bars represent  $\pm 1$  SE of the mean.

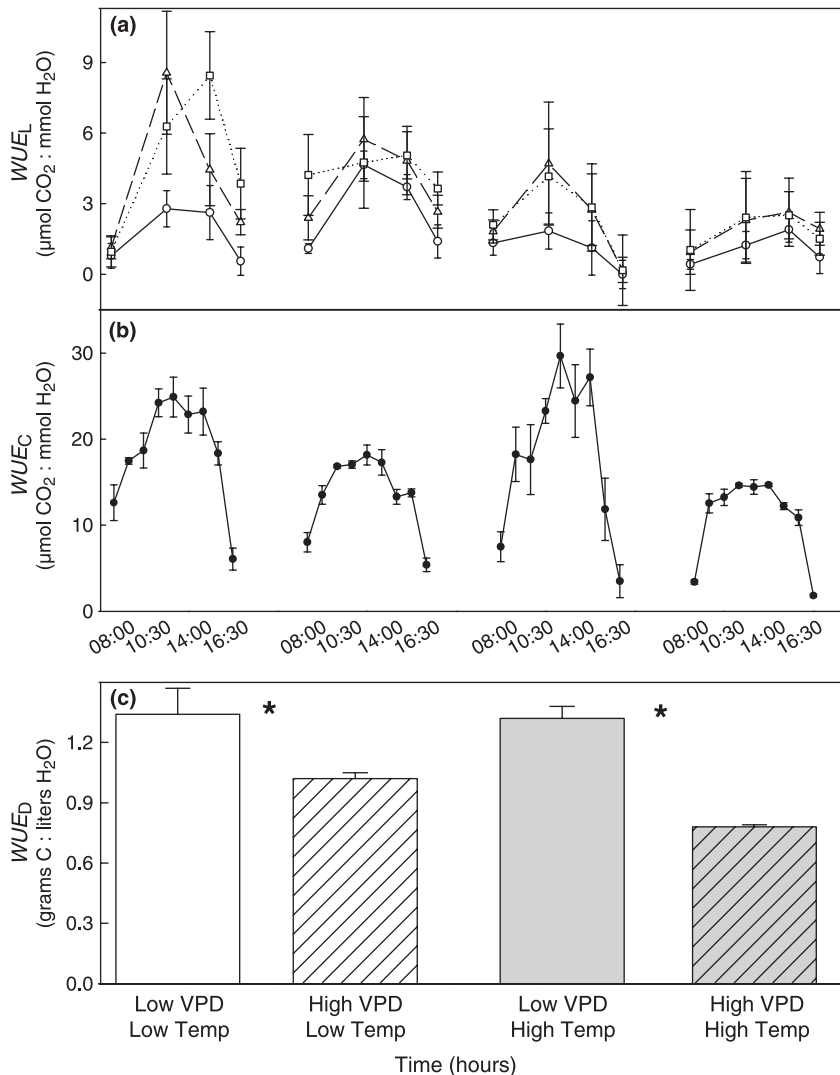
T treatments, which is potential evidence for increased evaporative cooling (Table 1). Any evaporative cooling that may have occurred locally by individual leaves was not detected by T or VPD sensors within the different canopy positions (Table 1), though all of these sensors were at least 0.5 m from the nearest leaf. Unfortunately, we had no similar means of making mid-canopy measures of leaf-level VPD at the time of this study. The lack of interaction between T and VPD for most measures of gas exchange suggests intracanopy transpiration considerably modified local T, and potentially local VPD for individual leaves, making it difficult to extrapolate from leaf-level data to the canopy-level.

As expected, an increase in T resulted in increased rates of both leaf- and canopy-level  $A$ ,  $g_s$  and  $E$ . This increased  $A$  in response to increasing T has been broadly shown in other experimental and observational studies (Bassman & Zwier, 1991; Cowling & Sage, 1998; Horton *et al.*, 2001; Medlyn *et al.*, 2002; Turnbull *et al.*, 2002). Mid-canopy leaf-level

values of net  $\text{CO}_2$  uptake ( $A_L$ ) and stomatal conductance ( $g_s$ ) averaged over the four treatments were  $11.9 \mu\text{mol m}^{-2}\text{s}^{-1}$  and  $0.260 \text{ mol m}^{-2}\text{s}^{-1}$ , respectively. These values are consistent with field-based leaf gas exchange rates of Fremont cottonwood (*Populus fremontii*; Horton *et al.*, 2001) and other tree species (Bassman & Zwier, 1991; Will & Teskey, 1999; Gunderson *et al.*, 2002). Midday measures of  $WUE_L$  were lower under higher temperature conditions, regardless of VPD treatments, principally due to the greater  $E_L$ .

An increase in VPD resulted in decreased leaf- and canopy-level  $A$  and  $g_s$ . Decreases in  $g_s$  with increasing VPD have been shown for several species at the leaf- (McCaughy & Iacobelli, 1994; Monteith, 1995; Medlyn *et al.*, 2002) and canopy-levels (Cienciala *et al.*, 1992; Granier & Breda, 1996; Martin *et al.*, 1997; Oren *et al.*, 1999a; Chen *et al.*, 2002). Interestingly, while both  $A$  and  $g_s$  decreased with elevated VPD, the relative decrease in  $A$  was less than the decrease in  $g_s$  at both the leaf and stand levels. These results suggest that, regardless of scale,





**Fig. 4** (a) Instantaneous  $WUE_L$  (leaf  $\text{CO}_2$  gain/leaf  $\text{H}_2\text{O}$  loss,  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) at the three canopy heights (lower, circles; middle, triangles; upper, squares), (b) instantaneous  $WUE_C$  (canopy  $\text{CO}_2$  gain/canopy  $\text{H}_2\text{O}$  loss,  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) and (c) cumulative daily total carbon uptake by the canopy per daily total  $\text{H}_2\text{O}$  loss ( $WUE_D$ ) for a stand of eastern cottonwoods (*Populus deltoides*). \*, Increased vapor pressure deficit (VPD) resulted in a significant decrease in  $WUE_D$ , regardless of temperature treatment, but increasing temperature did not result in any significant changes in  $WUE_D$ . Vertical bars represent  $\pm 1$  SE of the mean.

$g_s$  may be more sensitive than  $A$  to increasing VPD under both moderate and high T, indicating a stomatal regulation rather than metabolic limitation of photosynthesis. Comparable trends have been reported for *Picea rubens* (Day, 2000) and *Picea sitchensis* (Sandford & Jarvis, 1986). Both  $WUE_C$  and  $WUE_D$  were decreased by increasing VPD, likely owing to a decrease in  $A_C$  but only a slight increase in  $E_C$ . Lindroth & Cienciala (1996) found a similar decrease in  $WUE$  under increased VPD in short-rotation *Salix viminalis*.

Within this study, responses of  $A$  and  $g_s$  to T and VPD were often similar at the leaf- and canopy-levels, but the scale of response to treatment was often greater at the leaf-level than at the canopy-level. This suggests that whole-canopy responses are more dampened than those of individual leaves, and this was particularly true in terms of T-induced changes. Rates of midday canopy  $\text{CO}_2$  exchange ( $A_C$ ) were consistently slightly greater than  $A_L$ . This discrepancy could result from heterogeneity in the canopy or possibly could be attributed to the manner in which  $A_C$  was calculated (i.e. by dividing

above-ground net  $\text{CO}_2$  uptake by projected leaf area). The use of a projected leaf area, which gives weight to the surface of a canopy, may explain why  $A_C$  values best match  $A_L$  measured in the mid- and upper canopy. Clearly, lower canopy leaves contribute only a small part of the whole canopy exchange. This comparison also highlights the need for further investigation into the use of projected vs total leaf area and reiterates the importance of different scaling values for each canopy position within a stand.

Leaf-level water vapor exchange parameters ( $g_{sL}$  and  $E_L$ ) were consistently higher than those obtained at the whole-canopy level calculated on a projected leaf area basis. The discrepancy between the rates presumably reflects the possibility that although bulk air T and VPD were well controlled in the mesocosms, intracanopy conditions (especially VPD) may have been rather different because of the low wind speeds achieved in the canopy. A greater canopy boundary layer relative to the mixing rates achieved in the leaf gas exchange cuvette may have developed. The indirect means of estimating

$E_C$  could have also contributed to this discrepancy between leaf- and canopy-levels, however, a better means of determining  $E_C$  was not available. Theoretically,  $g_{sC}$  should be greater than  $g_{sL}$  because the canopy has a much greater boundary layer than a single leaf because of its relatively greater size and spatial architecture. Discrepancies between leaf- and canopy-level measures of  $g_s$  and  $E$  could also be attributed to the use of a single scaling value, rather than determining leaf area within different canopy heights and multiplying those canopy-specific leaf areas by canopy specific rates of measured  $g_{sL}$ . Recent studies have shown upper canopy leaves to have greater rates of  $g_{sL}$  than middle or lower canopy leaves in other tree species (Gunderson *et al.*, 2002; Sellin & Kupper, 2005).

Eastern cottonwood stands occur as a codominant or dominant component of bottomland hardwood or floodplain forests subjected to flooding. This mesic species, which is naturally acclimatized to high levels of both soil and air moisture, may principally regulate  $E_L$  by decreasing stomatal conductance in response to small changes in VPD. This interpretation is supported by the absence of change in leaf water potential with increased VPD at lower temperatures (Fig. 3a). Many studies have examined the relationship between leaf water potential and stomatal responses for a variety of species (Teskey *et al.*, 1983; Tyree & Sperry, 1988; Saliendra *et al.*, 1995; Marron *et al.*, 2002; Brodribb & Holbrook, 2003), but the level of control over stomatal behavior in response to VPD evidently differs between species (Flexas & Medrano, 2002). Regardless of T treatment, increasing VPD caused a reduction in  $g_{sL}$ , which in turn led to decreased  $A_L$ , but did not yield a reduction in  $E_L$ . These trees appeared to be regulating their stomata to remain below a threshold level of water stress. Tyree & Sperry (1988) have discussed this concept of woody plants operating near the point of catastrophic xylem dysfunction.

## Conclusions

A 6°C increase (from 24 to 30°C) in daytime air temperature resulted in a significant increase in total daily carbon uptake of a stand of eastern cottonwoods, and a 1 kPa increase in VPD decreased total daily carbon uptake of the stand significantly, regardless of temperature. Surprisingly, only a minimal interaction between T and VPD was found to influence the responses of CO<sub>2</sub> uptake and stomatal conductance at either level. The disparities found between leaf- and canopy-level responses underline the need for further large-scale mesocosm studies to examine some of the assumptions made when scaling leaf-level rates obtained using traditional gas exchange equipment to the stand scale. Better engineering of large-scale controlled environment facilities and close attention to measurements of intracanopy environments are needed to extrapolate results from leaf-level measurements to canopy performance, and to longer time-scales relevant to global climate change.

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