

# Acclimation of leaf hydraulic conductance and stomatal conductance of *Pinus taeda* (loblolly pine) to long-term growth in elevated CO<sub>2</sub> (free-air CO<sub>2</sub> enrichment) and N-fertilization

JEAN-CHRISTOPHE DOMECH<sup>1,3</sup>, SARI PALMROTH<sup>1</sup>, ERIC WARD<sup>1</sup>, CHRIS A. MAIER<sup>4</sup>, M. THÉRÉZIEN<sup>2</sup> & RAM OREN<sup>1</sup>

<sup>1</sup>Nicholas School of the Environment and Earth Sciences, Box 90328, <sup>2</sup>Department of Civil and Environmental Engineering, Box 90287, Duke University, Durham, NC 27708, USA, <sup>3</sup>Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA and <sup>4</sup>Southern Research Station, USDA Forest Service, 3041 Cornwallis Road, Research Triangle Park, NC 27709, USA

## ABSTRACT

We investigated how leaf hydraulic conductance ( $K_{\text{leaf}}$ ) of loblolly pine trees is influenced by soil nitrogen amendment (N) in stands subjected to ambient or elevated CO<sub>2</sub> concentrations (CO<sub>2</sub><sup>a</sup> and CO<sub>2</sub><sup>e</sup>, respectively). We also examined how  $K_{\text{leaf}}$  varies with changes in reference leaf water potential ( $\Psi_{\text{leaf-ref}}$ ) and stomatal conductance ( $g_{\text{s-ref}}$ ) calculated at vapour pressure deficit,  $D$  of 1 kPa. We detected significant reductions in  $K_{\text{leaf}}$  caused by N and CO<sub>2</sub><sup>e</sup>, but neither treatment affected pre-dawn or midday  $\Psi_{\text{leaf}}$ . We also detected a significant CO<sub>2</sub><sup>e</sup>-induced reduction in  $g_{\text{s-ref}}$  and  $\Psi_{\text{leaf-ref}}$ . Among treatments, the sensitivity of  $K_{\text{leaf}}$  to  $\Psi_{\text{leaf}}$  was directly related to a reference  $K_{\text{leaf}}$  ( $K_{\text{leaf-ref}}$  computed at  $\Psi_{\text{leaf-ref}}$ ). This liquid-phase response was reflected in a similar gas-phase response, with  $g_{\text{s}}$  sensitivity to  $D$  proportional to  $g_{\text{s-ref}}$ . Because leaves represented a substantial component of the whole-tree conductance, reduction in  $K_{\text{leaf}}$  under CO<sub>2</sub><sup>e</sup> affected whole-tree water use by inducing a decline in  $g_{\text{s-ref}}$ . The consequences of the acclimation of leaves to the treatments were: (1) trees growing under CO<sub>2</sub><sup>e</sup> controlled morning leaf water status less than CO<sub>2</sub><sup>a</sup> trees resulting in a higher diurnal loss of  $K_{\text{leaf}}$ ; (2) the effect of CO<sub>2</sub><sup>e</sup> on  $g_{\text{s-ref}}$  was manifested only during times of high soil moisture.

**Key-words:** capacitance; embolism; reference conductances; reference leaf water potential; time constant; turgor loss point.

## INTRODUCTION

The magnitude of plant response to elevated atmospheric CO<sub>2</sub> concentration (CO<sub>2</sub><sup>e</sup>) often depends on the availability of other resources, such as nutrients and water (Monje & Bugbee 1998; Oren *et al.* 2001; Hyvönen *et al.* 2007). For example, although leaf level photosynthesis is generally

stimulated under CO<sub>2</sub><sup>e</sup> (Medlyn *et al.* 1999; Bernacchi *et al.* 2003), gas exchange over diurnal and repeated drying cycles may be often dominated by the nitrogen availability and the hydraulic regulation of the stomata (Katul, Leuning & Oren 2003). Understanding the effects of CO<sub>2</sub><sup>e</sup> on gas exchange and on tree water relations over a range of nutrient and water availabilities is therefore critical to our ability to predict forest productivity as the climate changes (Wullschlegel, Tschaplinski & Norby 2002). However, at present, the interaction effects between CO<sub>2</sub>, nitrogen (N), and water availabilities on plant hydraulics are poorly understood.

In isohydric species, stomata conservatively regulate plant water status by controlling the rate of water loss to the atmosphere such that it matches the capacity of the soil-plant hydraulic system to supply water to leaves. Should stomata fail to sense and respond to a lower capacity of the soil-plant system to supply water, xylem would embolize rapidly, increasing the risk of hydraulic dysfunction and dehydration of leaves (Maseda & Fernández 2006). However, stomatal regulation of transpiration rate imposes limits on photosynthesis. Thus, water availability is one of the most important factors limiting productivity and has likely been an important selective regime influencing the evolution of plant physiology (Schulze *et al.* 1987). Despite decades of research on the physiology of stomata, the specific mechanisms that permit coordination of stomatal conductance ( $g_{\text{s}}$ ) with plant water balance and hydraulic properties remain elusive (Meinzer 2002; Buckley 2005). Nevertheless, there does seem to be a general agreement that stomata sense leaf water potential ( $\Psi_{\text{leaf}}$ ) somewhere within the leaf so that similarity exists in the response of both  $g_{\text{s}}$  and leaf xylem cavitation to decreasing  $\Psi_{\text{leaf}}$  (Brodribb & Cochard 2009; Domec *et al.* 2009).

The diversity in form and longevity of leaves translates to large differences in water transport capacity and, thus, in gas exchange (Sack & Holbrook 2006). Water transport capacity can be quantified in terms of leaf hydraulic conductance

Correspondence: J.-C. Domec. Fax: +1 919 513 2978; e-mail: jc.domec@duke.edu

( $K_{\text{leaf}}$ , Aasamaa, Sober & Rahi 2001; Domec *et al.* 2009). Consistent with earlier works documenting coordination of  $g_s$  with overall plant hydraulic conductance (Meinzer *et al.* 1995; Cochard *et al.* 2002),  $K_{\text{leaf}}$  and its relationship to stomatal control has recently been investigated, revealing that maximum  $g_s$  is very sensitive to  $K_{\text{leaf}}$  within and among species (Brodribb *et al.* 2003; Woodruff *et al.* 2007). Leaves comprise the terminal portion of the liquid water transport pathway and their xylem is under greater tension than in stems, yet leaf xylem appears to be generally more vulnerable to embolism than that of the stems. This low resistance to embolism results in large decreases of  $K_{\text{leaf}}$  on a regular basis, even under non-extreme environmental conditions (Brodribb & Holbrook 2003; Bucci *et al.* 2003). Thus, although the hydraulic system of leaves represent less than 5% of the hydraulic pathway it constitutes a substantial (30–80%) and variable part of the resistance to water flow through plants (Sack & Holbrook 2006; Domec *et al.* 2009). These observations imply that the large diurnal declines of  $K_{\text{leaf}}$  may be an inherent component of the stomatal regulatory system acting as a signal rather than a catastrophe to be avoided.

Environmental changes that increase the availability of resources (either above- or below-ground) result in the long-term acclimation of a less conductive (per unit leaf area) hydraulic system because of anatomical acclimation (Mencuccini 2003). For example, loblolly pine trees growing under  $\text{CO}_2^e$  and N-fertilization exhibit some differences in wood properties such as shorter tracheids and lower wood density (Oren *et al.* 2001; McCarthy *et al.* 2006a). In addition, when soil nutrient availability is low, plants allocate more carbon to roots compared with leaves to enhance nutrient uptake (Gerbauer, Reynolds & Strain 1996; Palmroth *et al.* 2006). On the other hand, when nutrient limitation is relieved by fertilization, the pattern of biomass allocation may change, favouring the expansion of the leaf surface area to enhance  $\text{CO}_2$  capture at the expense of below-ground plant parts (King, Thomas & Strain 1997; McCarthy *et al.* 2006b). The effect of nutrient limitation on carbon partitioning might impact leaf hydraulic architecture and, consequently, leaf water relations. For example, increase in canopy leaf area with increasing nutrient availability and  $\text{CO}_2^e$  (McCarthy *et al.* 2007) should cause  $K_{\text{leaf}}$  to decrease if not accompanied by a proportional increase in sapwood area and root area (Ewers, Oren & Sperry 2000; Addington *et al.* 2006). This reduction in  $K_{\text{leaf}}$  may then impose a series of constraints on the water economy of the plant, with ultimate impacts on the carbon economy (Sperry *et al.* 2002; Bucci *et al.* 2006).

Under  $\text{CO}_2^e$ ,  $g_s$  in most species decreases (e.g. Medlyn *et al.* 2001), reducing transpiration per unit leaf area. Coniferous species generally have stomata that are less responsive to  $\text{CO}_2^e$  than broad-leaved species (Pataki, Oren & Tissue 1998; Ellsworth 1999; Maier, Palmroth & Ward 2008). However, it has also been proposed that  $g_s$  might decrease under  $\text{CO}_2^e$  only in longer-term (>1 year) experiments, and that the seasonal timing of data collection was important (Medlyn *et al.* 2001; Wang *et al.* 2005). The

magnitude of the response of stomata to  $\text{CO}_2^e$  also depends on the sensitivity of  $g_s$  to soil moisture and vapour pressure deficit (Ainsworth & Rogers 2007). In loblolly pine trees, it has been shown that  $g_s$  under  $\text{CO}_2^e$  is similar to that under ambient  $\text{CO}_2$  concentration ( $\text{CO}_2^a$ ) when subjected to water stress, but lower without moisture limitation (Murthy *et al.* 1996). Therefore seasonal monitoring of stomatal response to  $\text{CO}_2^e$  may be necessary to understand stomatal response under varying soil moisture and evaporative demand.

Here we focused on the possible effects of  $\text{CO}_2^e$  and N-fertilization on leaf water transport traits and their consequence for long distance water transport and drought tolerance of *Pinus taeda* L. (loblolly pine) trees, species very sensitive to low soil moisture (Noormets *et al.* 2009). We specifically investigated the effects on  $g_s$  regulation and possible compensatory adjustments. The 2007 severe summer drought in the Atlantic Southeast of the United States provided an opportunity to study whether: (1) N-fertilization and  $\text{CO}_2^e$  lower leaf and whole tree hydraulic conductance; (2)  $K_{\text{leaf}}$  and  $g_s$  show coordinated acclimation to long term N-fertilization and  $\text{CO}_2^e$ ; and (3) N-fertilization and  $\text{CO}_2^e$  result in a reduced sensitivity of  $g_s$  to declining vapour pressure deficit and soil moisture. It was hypothesized that when compared with unfertilized trees under  $\text{CO}_2^a$ , water transport capacity would adjust to nitrogen availability and  $\text{CO}_2^e$ , with  $K_{\text{leaf}}$  and  $g_s$  being lower in fertilized trees and under  $\text{CO}_2^e$ .

## MATERIALS AND METHODS

### Setting

The treatments have been administered in a loblolly pine plantation established in 1983 on low fertility, acidic clay loam of the Enon series, in the Blackwood Division of Duke University Forest, in Orange County, North Carolina (35°58'N, 79°08'W). The average height of pines was ~19 m in 2007. Loblolly pine accounts for up to 90% of the basal area (McCarthy *et al.* 2007). The most prevailing co-dominant species is *Liquidambar styraciflua*, and the most common sub-canopy species are *Acer rubrum*, *Ulmus alata* and *Cornus florida*. Mean annual temperature is 15.8 °C and mean annual precipitation is 1150 mm, with usually an even distribution throughout the year. Summers are warm and humid with a growing season mean temperature of 22.1 °C. Further details about the site can be found in Oren *et al.* (1998a).

The experimental site consisted of four plots exposed to ambient  $\text{CO}_2$  ( $\text{CO}_2^a$ ) and four plots targeted at +200  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  ( $\text{CO}_2^e$ ) above current, with half of each plot fertilized with N (Oren *et al.* 2001; Schäfer *et al.* 2002).  $\text{CO}_2$  enrichment is implemented according to the free-air  $\text{CO}_2$  enrichment (FACE) protocol throughout the year whenever ambient temperature is above 5 °C and wind speed is below 5  $\text{m s}^{-1}$  (Hendrey *et al.* 1999). In 1994, two 30-m-diameter plots were established: the FACE prototype plot (Plot 7) and its adjacent untreated reference plot (Plot 8). In 1996, six additional plots (replicated FACE; Plots 1 to 6)

were established, three of which received CO<sub>2</sub><sup>c</sup>. In 1998, the prototype plot and its reference plot were halved using a ditch and a barrier, and one-half of each has received annual nitrogen fertilization (N) of 11.2 g N m<sup>-2</sup> (Oren *et al.* 2001); the same design was implemented in the rest of the plots in 2005.

### Measurement of leaf hydraulic conductance ( $K_{\text{leaf}}$ )

Aiming to ensure long-term integrity of the Duke FACE experiment, only limited sampling is permitted. Thus, one terminal branch per tree was collected from four trees per plot (two trees from each half). Branches were collected at pre-dawn and enclosed in sealed plastic bags to prevent water loss.  $K_{\text{leaf}}$  was measured on single fascicles by assessing the rehydration kinetics of needles after detachment and determined as (Brodrribb & Holbrook 2003):

$$K_{\text{leaf}} = C_{\text{leaf}} \ln[\Psi_{\text{leaf}(o)}/\Psi_{\text{leaf}(t)}]/t \quad (1)$$

where  $K_{\text{leaf}}$  (mmol m<sup>-2</sup> MPa<sup>-1</sup> s<sup>-1</sup>) is the leaf hydraulic conductance,  $C_{\text{leaf}}$  (mmol m<sup>-2</sup> MPa<sup>-1</sup>) is the leaf capacitance,  $\Psi_{\text{leaf}(o)}$  (MPa) is the leaf water potential prior to rehydration,  $\Psi_{\text{leaf}(t)}$  is the leaf water potential after rehydration, and  $t$  (s) is the duration of rehydration of needles detached under water from the stem.  $C_{\text{leaf}}$  was determined from the slope of relative water content to  $\Psi_{\text{leaf}}$  obtained from pressure–volume curves. All capacitance values reported and used to calculate  $K_{\text{leaf}}$  corresponded to  $C_{\text{leaf}}$  determined before the water potential at turgor loss point ( $\Psi_{\text{tp}}$ ) (Brodrribb & Holbrook 2003). Values of  $K_{\text{leaf}}$  were corrected for a viscosity of water at a temperature of 20 °C.

Pressure–volume analyses (Tyree & Hammel 1972) were conducted on single fascicles taken on the same trees used to determine  $K_{\text{leaf}}$ , between the months of January and February 2007, when soil moisture and pre-dawn water potentials were high. These samples were excised early in the morning prior to significant water loss, sealed in plastic bags with moist paper to prevent desiccation, and then stored in a refrigerator within 1 h of excision. Pressure–volume curves were initiated by first determining the fresh weight of the fascicle, and then measuring  $\Psi_{\text{leaf}}$  with a pressure chamber (PMS Instrument Company, Albany, OR). Alternate determinations of fresh weight and  $\Psi_{\text{leaf}}$  were repeated during slow dehydration on the laboratory bench until values of  $\Psi_{\text{leaf}}$  ranging from –4.0 MPa to –5.0 MPa were attained. The inverse of water potential was plotted against relative water content to establish a pressure–volume curve and determine  $\Psi_{\text{tp}}$ . For normalizing  $C_{\text{leaf}}$  on a leaf area basis, needle areas were obtained geometrically from dimensions measured using a digital caliper (series 500 Mitutoyo, Aurora, IL, USA) (Rundel & Yoder 1998).

Because differences may exist between  $K_{\text{leaf}}$  measured under field condition and  $K_{\text{leaf}}$  measured in the laboratory (Tyree *et al.* 2005; Sack & Holbrook 2006), we also estimated  $K_{\text{leaf}}$  based on field measurements on some of the same trees on which stomatal conductance ( $g_s$ ) and

transpiration ( $E$ ; see further discussion) were measured (Supporting Information Fig. S1). Field  $K_{\text{leaf}}$  was calculated as  $K_{\text{leaf}} = v/v_o E/(\Psi_{\text{stem}} - \Psi_{\text{leaf}})$ , where  $v$  and  $v_o$  are the kinematic viscosities of water at the measured leaf temperature and at 20 °C, respectively;  $E$  is the transpiration rate (mmol m<sup>-2</sup> s<sup>-1</sup>) measured with a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA), and  $\Psi_{\text{stem}}$  is the stem water potential estimated from non-transpiring covered shoots (Meinzer 2002). Although  $K_{\text{leaf}}$  estimated from the dehydration curves and  $\Psi_{\text{leaf}}$  was 12% lower than field  $K_{\text{leaf}}$ , the difference was not statistically significant ( $P = 0.11$ , paired  $T$ -test; Supporting Information Fig. S1).

### Field leaf water potential ( $\Psi_{\text{leaf}}$ ) and stomatal conductance ( $g_s$ )

Measurements of  $\Psi_{\text{leaf}}$  were conducted with a pressure chamber, and  $g_s$  with a LI-6400 portable photosynthesis system (Li-Cor Inc.). Measurements of  $\Psi_{\text{leaf}}$  and  $g_s$  were conducted on detached fascicles taken from the same shoot simultaneously every 90 min on one non-fertilized and one fertilized tree per ring for a total of 16 trees during each sampling period. Previous studies at the site has shown that there were no differences between excised and attached needle gas exchange when measurements were restricted to less than 15 min after excision (Maier *et al.* 2008). Vapour pressure deficit ( $D$ ) and average CO<sub>2</sub> concentration, inside the chamber followed ambient conditions, and photosynthetically active radiation were matched with the average conditions over a 15 min period prior the measurement. Diurnal measurements commenced prior to dawn and continued until 1500 h on 22 and 23 May, 24 and 25 July, and between 19 and 23 September 2007. These dates encompassed large differences in climatic and soil conditions allowing us to study a wide range of liquid- and gas-phase values.

In two of the plots where leaf area data were available for each treatment (rings 7 and 8), mean crown canopy conductances ( $G_s$ ) derived from basal sap flow were used to provide an independent assessment of stomatal control of transpiration. We used leaf area data measured in 2005 (McCarthy *et al.* 2007), which is thought to accurately represent leaf area of 2007 (McCarthy, personal communication). Sap flow was measured at breast height in at least five trees per treatment with 20 mm heat dissipation sensors installed at three depths in the sapwood. Sensor operation at the FACE site is detailed in Schäfer *et al.* (2002). The sensor signal was converted to sap flux density ( $J_s$ , in g m<sup>-2</sup> s<sup>-1</sup>) according to Granier (1987) and accounted for the effects of non-zero night-time fluxes (Kim, Oren & Hinckley 2008; Oishi, Oren & Stoy 2008). Sap flux density was scaled and converted to a tree-scale average transpiration per unit leaf area ( $E$ , in mmol m<sup>-2</sup> s<sup>-1</sup>; Oren *et al.* 1998b; Schäfer *et al.* 2002). Basal  $J_s$  values were lagged by half an hour in order to take into account water use from capacitance and thus to more closely approximate the relationship between  $D$  and transpiration in the crown (Phillips *et al.* 1997). The half-hour lag time was derived from the observed time lag of  $J_s$  with respect to  $D$  (Ewers & Oren

2000; Chuang *et al.* 2006). The sap-flux based  $G_s$  was calculated from  $E$  and  $D$ , using the simplification of the inversion of Penman–Monteith model (Ewers & Oren 2000). The simplified calculation was permitted because in all treatments  $D$  was close to the leaf-to-air vapour pressure deficit because of the high boundary-layer conductance. Indeed, given that >90% of the daytime mean wind velocity was  $>0.7 \text{ m s}^{-1}$ , and that leaf dimension never exceeded  $0.0017 \text{ m}$ , we estimated using Jones (1992) that the mean daytime boundary-layer conductance averaged 65 times  $G_s$ .

### Analysis of the response of liquid and gas conductance to changes in driving force

Stomata of isohydric plants respond to  $D$  in a manner consistent with protection of the xylem integrity for water transport. The emergent behaviour is a decreasing  $g_s$  with increasing  $D$  at a rate that is predictable and proportional to  $g_s$  at low  $D$  (Oren *et al.* 1999). Thus,  $g_s$  data from each treatment were analysed based on:

$$g_s = b - m \times \ln(D) \quad (2)$$

where  $b$  is  $g_s$  at  $D = 1 \text{ kPa}$  (hereafter designated as reference stomatal conductance,  $g_{s\text{-ref}}$ ) and  $m$  is the sensitivity of  $g_s$  to  $D$  [ $-\text{d}g_s/\text{d}\ln D$ , in  $\text{mmol m}^{-2} \text{ s}^{-1} (\ln \text{kPa})^{-1}$ ]. Based on the stated hydraulic consideration,  $-\text{d}g_s/\text{d}\ln D$  is proportional to  $g_{s\text{-ref}}$  with the proportionality averaging  $\sim 0.60$ , and varying predictably depending on the range of  $D$  used in the analysis (Oren *et al.* 1999; Ewers *et al.* 2007; Kim *et al.* 2008; Ward *et al.* 2008).

Similarly, we evaluated whether the sensitivity of xylem conductivity to  $\Psi_{\text{leaf}}$  is related to  $K_{\text{leaf}}$  by generating a slope of the reduction in  $K_{\text{leaf}}$  versus the natural logarithm of  $\Psi_{\text{leaf}}$  [ $\text{d}K_{\text{leaf}}/\text{d}\ln \Psi_{\text{leaf}}$  in  $\text{mmol m}^{-2} \text{ s}^{-1} (\ln \text{MPa})^{-1}$ ] (Ewers *et al.* 2000). To analyse the effect of  $K_{\text{leaf}}$  on whole tree hydraulic

conductance ( $K_t$ ),  $K_t$  was calculated from the slope of the relationship between  $E$  and  $\Psi_{\text{leaf}}$  (Loustau, Domec & Bosc 1998).

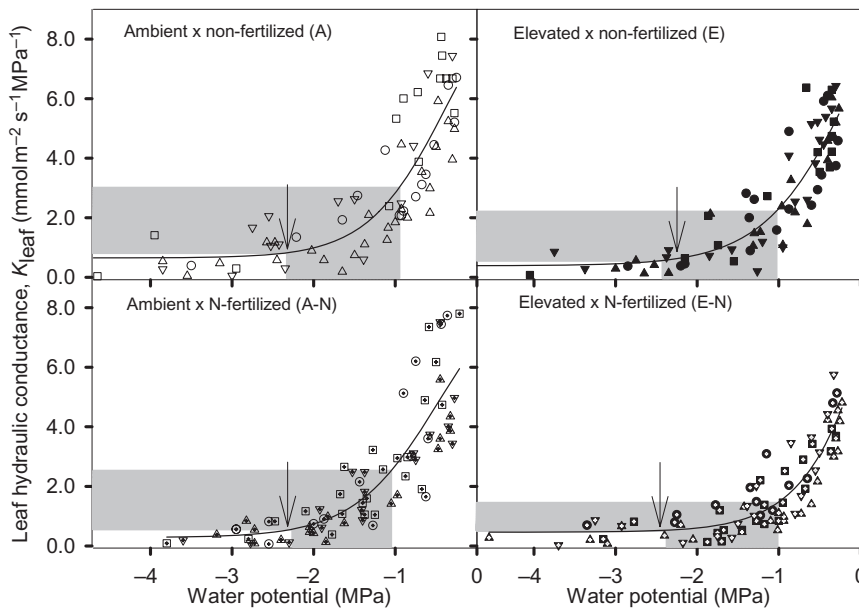
### Statistical analysis

The effects of  $\text{CO}_2^e$  and N on  $K_{\text{leaf}}$ ,  $C_{\text{leaf}}$ ,  $\Psi_{\text{leaf}}$ ,  $\Psi_{\text{tp}}$  and  $g_{s\text{-ref}}$  were tested through analysis of variance (ANOVA) based on a split-plot design.  $\text{CO}_2$  concentration and N were the main and split-plot effects, respectively, and individual plots were used as replicates. Measurements made on multiple dates were analysed by repeated measure ANOVA. Statistical analyses were performed using SAS (version 9.1, Cary, NC, USA) and curve fits were performed using Sigmaplot (version 9.0, SPSS Inc., San Rafael, CA, USA).

### RESULTS

Leaf hydraulic conductance ( $K_{\text{leaf}}$ ) determined in the laboratory declined exponentially with declining  $\Psi_{\text{leaf}}$  (Fig. 1;  $R^2$  ranging from 0.74 to 0.89;  $P < 0.01$ ). We obtained the treatment means of maximum  $K_{\text{leaf}}$  and the water potentials at which 50% of maximum  $K_{\text{leaf}}$  was lost ( $\Psi_{50}$ ) from curves fitted to data from each tree.  $\text{CO}_2^e$  and N significantly decreased maximum  $K_{\text{leaf}}$  by 21% and 13%, respectively (Table 1). No treatment effects were observed in  $\Psi_{50}$ .

Although  $\text{CO}_2^e$  decreased the water potential at the turgor loss point ( $\Psi_{\text{tp}}$ ) by 0.12 MPa and  $C_{\text{leaf}}$  by 15%, no treatment effects were observed in the osmotic potential at full hydration, although a trend was discernable (Table 1). There was no  $\text{CO}_2^e \times \text{N}$  interaction effects on any parameter measured in the laboratory (Table 1). Among the treatments, the maximum  $K_{\text{leaf}}$  and  $C_{\text{leaf}}$  decreased linearly with decreasing  $\Psi_{\text{tp}}$  (Fig. 2). Extrapolating the regression lines to zero  $K_{\text{leaf}}$  and zero  $C_{\text{leaf}}$  generated similar values of  $\Psi_{\text{tp}}$  ( $-2.99 \text{ MPa}$  in Fig. 2a, and  $-3.08 \text{ MPa}$  in Fig. 2b, respectively).



**Figure 1.** Leaf hydraulic conductance ( $K_{\text{leaf}}$ ) in relation to leaf water potential ( $\Psi_{\text{leaf}}$ ) for foliage samples obtained from the upper crowns of loblolly pine trees growing under ambient ( $\text{CO}_2^a$ ), elevated ( $\text{CO}_2^e$ ) and/or N-fertilized conditions (Duke free-air  $\text{CO}_2$  enrichment site). Different symbols indicate individual trees within each treatment. Symbols with cross inside are from N plots. Closed symbols are for  $\text{CO}_2^e$ , and open symbols are for  $\text{CO}_2^a$ . Within ambient or elevated plots, same shaped symbols are from the same plots. The grey-shaded areas represent the range of  $K_{\text{leaf}}$  experienced over the seasonal range of measured  $\Psi_{\text{leaf}}$ . Arrows indicate the  $\Psi_{\text{leaf}}$  at the turgor loss point ( $\Psi_{\text{tp}}$ ).

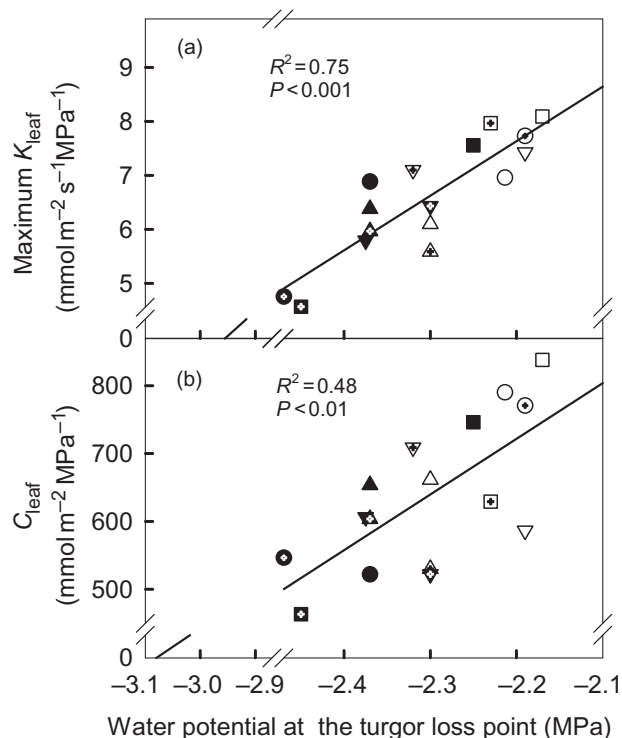
**Table 1.** Maximum leaf hydraulic conductance (max.  $K_{\text{leaf}}$ ,  $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ), water potential that induces 50% loss of  $K_{\text{leaf}}$  ( $\Psi_{50}$ , MPa); water potential at the turgor loss point ( $\Psi_{\text{tp}}$ , MPa), osmotic potential at full hydration ( $\Pi$ , MPa), leaf capacitance on a volume basis ( $C_{\text{leaf-RWC}}$ ,  $\% \text{RWC MPa}^{-1}$ ) and leaf capacitance on a leaf area basis ( $C_{\text{leaf}}$ ,  $\text{mmol m}^{-2} \text{MPa}^{-1}$ ) for foliage samples obtained in the upper crowns of loblolly pine trees, growing under ambient carbon dioxide concentration, elevated and/or N-fertilized conditions

	Ambient–non-fertilized	Ambient–fertilized	Elevated–non-fertilized	Elevated–fertilized	ANOVA		
					CO <sub>2</sub>	N	CO <sub>2</sub> × N
Max. $K_{\text{leaf}}$	6.38 ± 0.48	5.95 ± 0.51	5.48 ± 0.36	4.63 ± 0.27	0.035	0.014	0.104
$\Psi_{50}$	−0.91 ± 0.014	−0.92 ± 0.17	−1.01 ± 0.19	−0.73 ± 0.11	ns	ns	ns
$\Psi_{\text{tp}}$	−2.22 ± 0.02	−2.27 ± 0.03	−2.34 ± 0.03	−2.40 ± 0.03	0.016	ns	ns
$\Pi$	0.96 ± 0.06	1.13 ± 0.07	1.13 ± 0.05	1.21 ± 0.08	0.102	0.114	ns
$C_{\text{leaf-RWC}}$	10.1 ± 0.4	9.4 ± 0.7	8.5 ± 0.4	7.7 ± 0.6	0.041	ns	ns
$C_{\text{leaf}}$	771 ± 45	644 ± 59	641 ± 53	544 ± 35	0.030	ns	ns

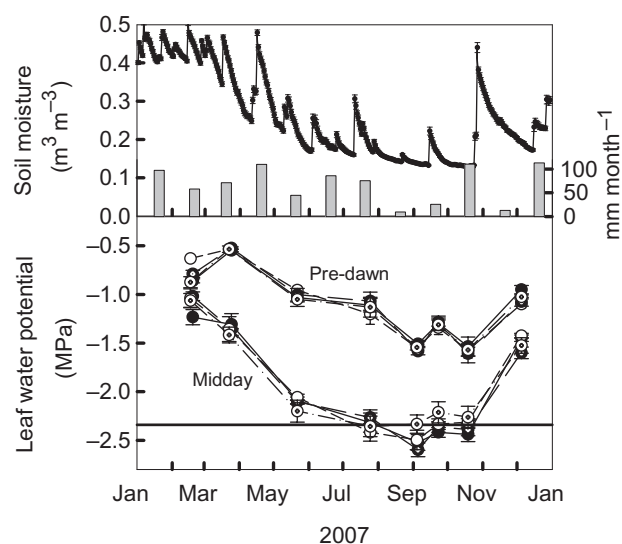
Analysis of variance (ANOVA) probability values for carbon dioxide concentration (CO<sub>2</sub>) and N-fertilization treatment (N) are also shown (the probability level  $P < 0.15$  was considered to indicate a trend). ns, not significant

In 2007, climate along the eastern seaboard of the United States was characterized by a long summer drought with only traces of precipitation from July through September (Fig. 3). Because the rooting zone is very shallow (~35 cm; Oren *et al.* 1998a), tree water uptake during a summer without significant rain events causes soil moisture to decline quickly. In 2007, extractable moisture from the

upper 30 cm was nearly exhausted by early August, and remained so until sizable rain events resumed in late October (Fig. 3). The seasonal pre-dawn  $\Psi_{\text{leaf}}$  decreased by 1.0 MPa from its highest in winter to its lowest following two dry months (<35 mm), averaging −1.31 MPa between May and October (Table 2). The minimum midday  $\Psi_{\text{leaf}}$ , which averaged −2.34 MPa throughout the season (Table 2, Fig. 3), never fell below −2.6 MPa. At a given sampling date neither leaf water potential variable differed between treatments ( $P > 0.21$ ). However, there was a date effect on  $\Psi_{\text{leaf}}$  (Table 3) because from May to September, pre-dawn and midday  $\Psi_{\text{leaf}}$  decreased by −0.33 MPa and −0.18 MPa, respectively.



**Figure 2.** (a) Maximum leaf hydraulic conductance (max.  $K_{\text{leaf}}$ ) and (b) leaf capacitance in relation to the water potential at the turgor loss point ( $\Psi_{\text{tp}}$ ) of loblolly pine trees growing under CO<sub>2</sub><sup>a</sup>, CO<sub>2</sub><sup>c</sup> and/or N-fertilized conditions. Each point represents samples taken from either the non-fertilized or N-fertilized half of a plot. Symbols are as in Fig. 1. Extrapolations of the regression lines to zero  $K_{\text{leaf}}$  and zero  $C_{\text{leaf}}$  are also shown.



**Figure 3.** Seasonal course of soil moisture and monthly precipitation and seasonal variation of leaf water potentials ( $\Psi_{\text{leaf}}$ ) for CO<sub>2</sub><sup>a</sup> (open symbols), CO<sub>2</sub><sup>a</sup> × N fertilization (open-crossed symbols), CO<sub>2</sub><sup>c</sup> (closed symbols) and CO<sub>2</sub><sup>c</sup> × N fertilization (closed-crossed symbols) loblolly pine trees. Soil moisture content data are shown for all treatments combined. The horizontal line in the lower panel represents the seasonal minimum  $\Psi_{\text{leaf}}$  across treatments (−2.34 MPa).

**Table 2.** Mean seasonal (between May and October) leaf water potential ( $\Psi_{\text{leaf}}$ , MPa) at midday (minimum) and at pre-dawn, mean seasonal leaf water potential at reference stomatal conductance ( $\Psi_{\text{leaf-ref}} = \Psi_{\text{leaf}}$  at  $g_{\text{s-ref}}$ ), daily percent loss of leaf hydraulic conductance at minimum  $\Psi_{\text{leaf}}$  ( $\text{PLK}_{\text{leaf}}$ ), daily percent loss of leaf hydraulic conductance at  $\Psi_{\text{leaf-ref}}$  ( $\text{PLK}_{\text{leaf-ref}}$ ) and mean seasonal leaf time constant ( $\tau$ , min) for foliage samples obtained in the upper crowns of loblolly pine trees, growing under  $\text{CO}_2^{\text{a}}$  (ambient),  $\text{CO}_2^{\text{e}}$  (elevated) and/or N-fertilized conditions

	Ambient–non-fertilized	Ambient–fertilized	Elevated–non fertilized	Elevated–fertilized
Pre-dawn $\Psi_{\text{leaf}}$	$-1.32 \pm 0.04$	$-1.32 \pm 0.05$	$-1.33 \pm 0.02$	$-1.27 \pm 0.03$
Minimum $\Psi_{\text{leaf}}$	$-2.33 \pm 0.05$	$-2.26 \pm 0.08$	$-2.39 \pm 0.05$	$-2.36 \pm 0.06$
$\Psi_{\text{leaf-ref}}$	$-1.41 \pm 0.09$	$-1.50 \pm 0.07$	$-1.53 \pm 0.07$	$-1.59 \pm 0.04$
$\text{PLK}_{\text{leaf}}$	$70 \pm 4$	$71 \pm 3$	$72 \pm 3$	$77 \pm 5$
$\text{PLK}_{\text{leaf-ref}}$	$24 \pm 2$	$33 \pm 3$	$34 \pm 1$	$42 \pm 4$
$\tau = C_{\text{leaf}}/K_{\text{leaf}}$	$7.0 \pm 0.7$	$8.2 \pm 1.6$	$9.9 \pm 1.15$	$16.5 \pm 1.3$

Diurnal measurements showed a typical pattern of  $g_{\text{s}}$  increasing in the early morning once sunlight reached the foliage and then declining from late morning (Fig. 4). Similar trends were observed in both sap flow-based crown canopy conductance ( $G_{\text{s}}$ ) and porometry-based  $g_{\text{s}}$ . At the end of May,  $\text{CO}_2^{\text{e}}$  decreased the daily maximum  $g_{\text{s}}$  by 31% ( $P = 0.018$ , Fig. 4) but, because in July and September  $\text{CO}_2^{\text{e}}$  had no effect on  $g_{\text{s}}$  ( $P > 0.55$ ),  $\text{CO}_2^{\text{e}}$  had no significant effect on  $g_{\text{s}}$  ( $P = 0.19$ ) when assessed over all measurement dates. There was no  $\text{CO}_2^{\text{e}} \times \text{N}$  interaction ( $P = 0.85$ ) on  $g_{\text{s}}$ . In this study N did not significantly affect  $g_{\text{s}}$  ( $P = 0.38$ ). There was no consistent relationship between  $g_{\text{s}}$  and  $\Psi_{\text{leaf}}$ . Unlike the peaking pattern described for  $g_{\text{s}}$ ,  $\Psi_{\text{leaf}}$  declined continuously during the day (Fig. 4).

Compared with trees growing under  $\text{CO}_2^{\text{a}}$ , trees growing under  $\text{CO}_2^{\text{e}}$  tended to have lower  $K_{\text{leaf}}$  at high  $\Psi_{\text{leaf}}$  and lower  $g_{\text{s}}$  at low  $D$  and showed reduced hydraulic sensitivity to  $\Psi_{\text{leaf}}$  and reduced  $g_{\text{s}}$  sensitivity to  $D$  (Fig. 5a,b). Using the diurnal relationships between  $\Psi_{\text{leaf}}$  and  $D$ , we were able to calculate  $\Psi_{\text{leaf}}$  at  $g_{\text{s-ref}}$  taken as the  $\Psi_{\text{leaf}}$  at  $D = 1$  kPa (hereafter,  $\Psi_{\text{leaf-ref}}$ ). Similarly,  $K_{\text{leaf-ref}}$  ( $K_{\text{leaf}}$  at  $D = 1$  kPa) was calculated from  $\Psi_{\text{leaf-ref}}$  and the relationships between  $K_{\text{leaf}}$  and  $\Psi_{\text{leaf}}$  (Fig. 1). There was a date effect on  $\Psi_{\text{leaf-ref}}$  (Table 3), generated by a  $-0.31$  MPa drop from May to September. There was a marginal decrease in the  $\Psi_{\text{leaf-ref}}$  under  $\text{CO}_2^{\text{e}}$  (Table 3). Between treatments, the sensitivity of  $K_{\text{leaf}}$  to  $\Psi_{\text{leaf}}$

increased linearly with  $K_{\text{leaf-ref}}$  (Fig. 5c). We also tested whether the coefficients from Equation 2, used on  $g_{\text{s}}$  in all measurement days, conformed to the theoretical expectations of the response of  $g_{\text{s}}$  to  $D$  (Oren *et al.* 1999). In all treatments  $g_{\text{s}}$  showed an expected sensitivity to  $D$  that was proportional to  $g_{\text{s-ref}}$ . The slope was equal to 0.64, similar to the general slope of 0.60 ( $P = 0.13$ ; Fig. 5d).

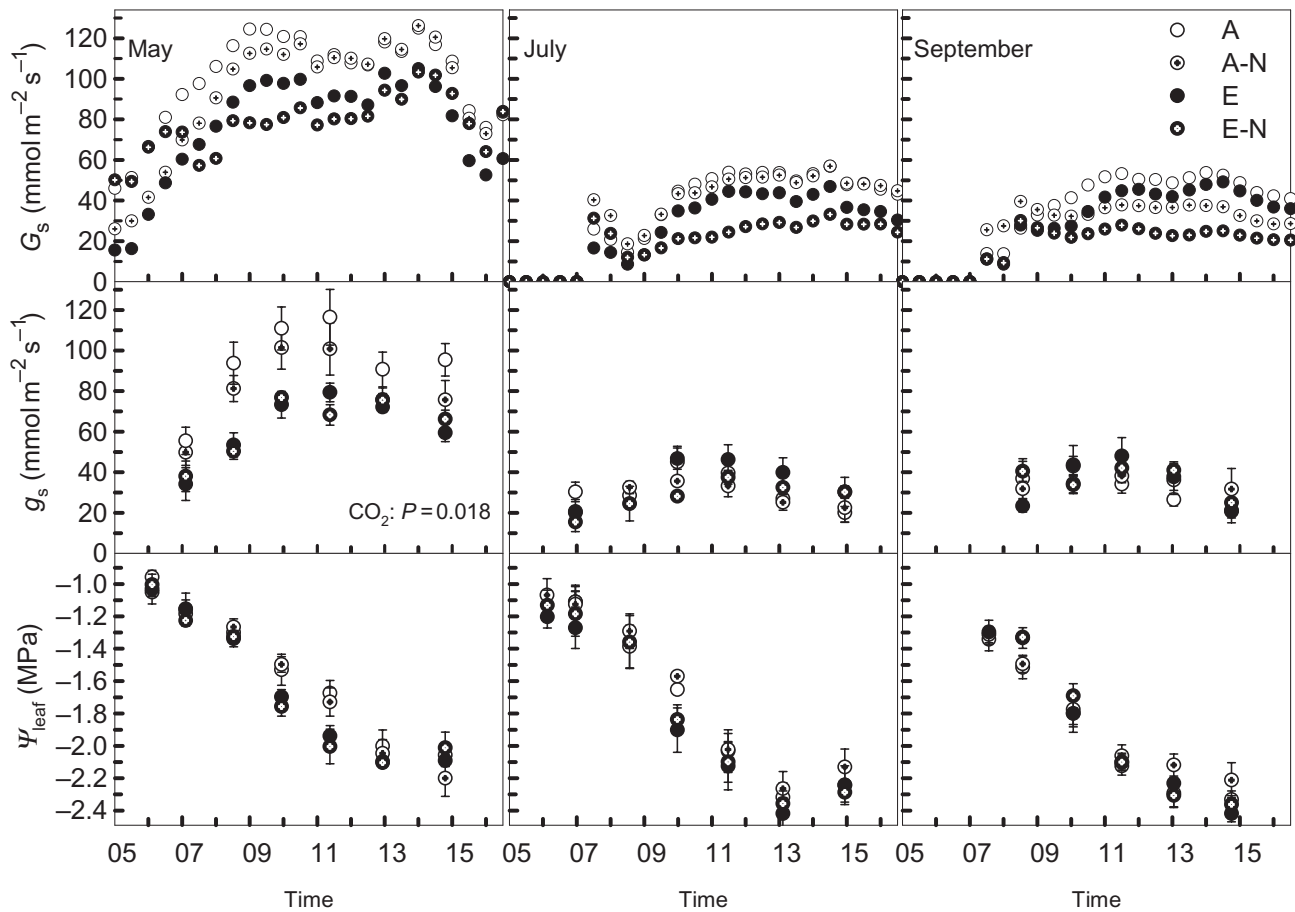
With rapidly decreasing pre-dawn  $\Psi_{\text{leaf}}$  and soil moisture over the growing season, a date effect was apparent with a sharp decrease in  $g_{\text{s-ref}}$  (Table 3; Fig. 6). There was no N effect on  $g_{\text{s-ref}}$  (Table 3). The decline in  $g_{\text{s-ref}}$  associated with a decline in soil moisture was more pronounced ( $P = 0.02$ , slope analysis) in the  $\text{CO}_2^{\text{a}}$  plots (50%) than in the  $\text{CO}_2^{\text{e}}$  plots (33%).

Values of field  $K_{\text{leaf}}$  were estimated using the relationships between  $\Psi_{\text{leaf}}$  and  $K_{\text{leaf}}$  established from laboratory measurements (Fig. 1) and  $\Psi_{\text{leaf}}$  measured in the field (Fig. 4). The percent loss of needle hydraulic conductance at the lowest measured  $\Psi_{\text{leaf}}$  ( $\text{PLK}_{\text{leaf}}$ ) and at  $\Psi_{\text{leaf-ref}}$  ( $\text{PLK}_{\text{leaf-ref}}$ ) were calculated by comparing  $K_{\text{leaf}}$  at pre-dawn to either  $K_{\text{leaf}}$  at midday or to  $K_{\text{leaf-ref}}$ , respectively. Averaged across all days,  $\text{PLK}_{\text{leaf-ref}}$  increased by  $\sim 10\%$  under  $\text{CO}_2^{\text{e}}$ , whereas  $\text{PLK}_{\text{leaf}}$  at minimum  $\Psi_{\text{leaf}}$  increased marginally by  $\sim 5\%$  (Tables 2 & 3). N marginally increased  $\text{PLK}_{\text{leaf-ref}}$  ( $\sim 8\%$ ), and there was no  $\text{CO}_2^{\text{e}} \times \text{N}$  interaction on  $\text{PLK}_{\text{leaf}}$  or on  $\text{PLK}_{\text{leaf-ref}}$ . Based on these field  $K_{\text{leaf}}$  and the calculated capacitance (Table 1), we

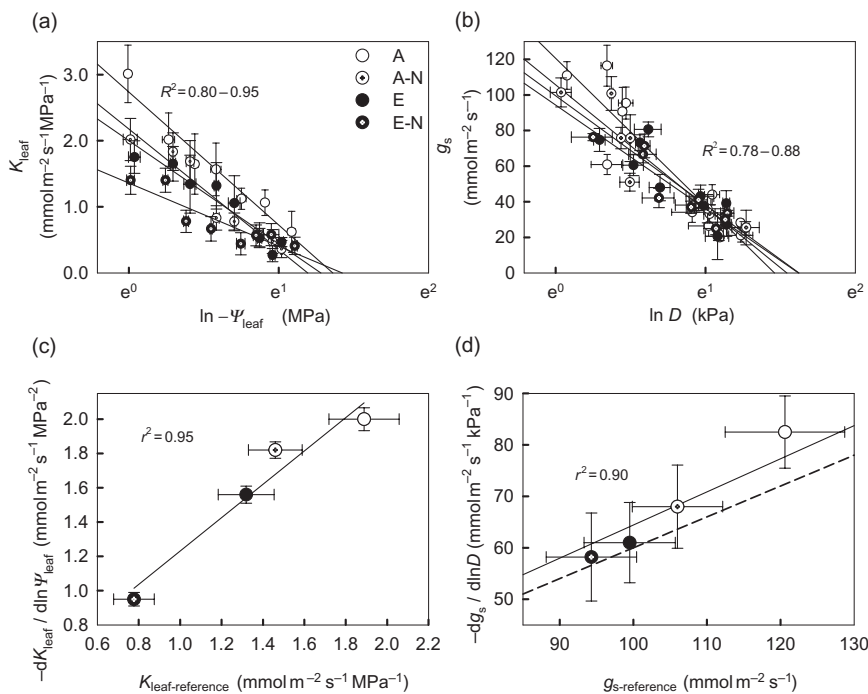
**Table 3.** Analysis of variance probability values for carbon dioxide concentration ( $\text{CO}_2$ ), N-fertilization treatment (N) and date of measurements (date) on pre-dawn and minimum (midday) leaf water potential ( $\Psi_{\text{leaf}}$ ), reference stomatal conductance ( $g_{\text{s-ref}} = g_{\text{s}}$  at  $D = 1$  kPa), leaf water potential at reference stomatal conductance ( $\Psi_{\text{leaf-ref}} = \Psi_{\text{leaf}}$  at  $g_{\text{s-ref}}$ ), daily percent loss of leaf hydraulic conductance at minimum leaf water potential ( $\text{PLK}_{\text{leaf}}$ ), daily percent loss of leaf hydraulic conductance at  $\Psi_{\text{leaf-ref}}$  ( $\text{PLK}_{\text{leaf-ref}}$ ), and the leaf time constant ( $\tau$ )

Effect	d.f.	Pre-dawn $\Psi_{\text{leaf}}$	Minimum $\Psi_{\text{leaf}}$	$g_{\text{s-ref}}$	$\Psi_{\text{leaf-ref}}$	$\text{PLK}_{\text{leaf}}$	$\text{PLK}_{\text{leaf-ref}}$	$\tau$
$\text{CO}_2$	1	ns	ns	ns	0.063	0.051	0.012	0.036
N	1	ns	ns	ns	ns	ns	0.075	<0.001
$\text{CO}_2 \times \text{N}$	1	ns	ns	ns	ns	ns	ns	0.013
Date	2	0.001	0.004	<0.001	0.03	0.022	0.017	0.059
$\text{CO}_2 \times \text{date}$	2	ns	ns	0.002	ns	ns	ns	ns
$\text{N} \times \text{date}$	2	ns	0.134	ns	ns	ns	ns	ns
$\text{CO}_2 \times \text{N} \times \text{date}$	2	ns	ns	ns	ns	ns	ns	ns

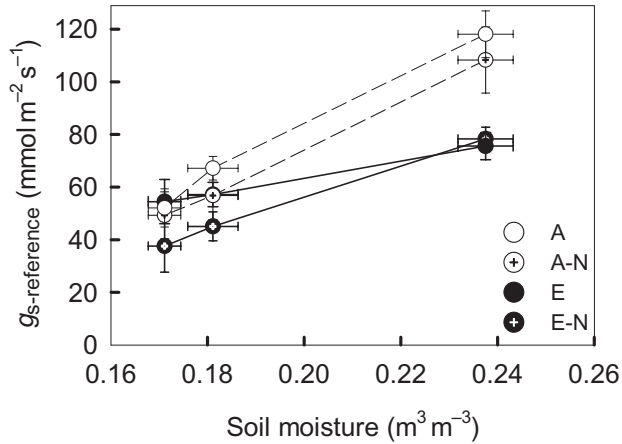
The probability level  $P < 0.15$  was considered to indicate a trend. ns, not significant; d.f., degrees of freedom.



**Figure 4.** Diurnal canopy stomatal conductance ( $G_s$ ), stomatal conductance ( $g_s$ ) and needle water potentials ( $\Psi_{\text{leaf}}$ ) at the beginning (May), in the middle (July) and at the end of the growing season (September) in  $\text{CO}_2^{\text{a}}$  (A),  $\text{CO}_2^{\text{a}} \times \text{N}$ -fertilization (A-N),  $\text{CO}_2^{\text{e}}$  (E) and  $\text{CO}_2^{\text{e}} \times \text{N}$ -fertilization (E-N) trees.



**Figure 5.** (a) Leaf hydraulic conductance ( $K_{\text{leaf}}$ ) versus the natural logarithm of leaf water potential ( $\Psi_{\text{leaf}}$ ), and (b) stomatal conductance ( $g_s$ ) versus the natural logarithm of air vapour pressure deficit ( $D$ ). (c) Slope of the response of  $K_{\text{leaf}}$  to the natural logarithm of  $\Psi_{\text{leaf}}$  versus  $K_{\text{leaf-reference}}$  ( $K_{\text{leaf}}$  at  $D = 1 \text{ kPa}$ ) and (d) slope of the response of  $g_s$  to the natural logarithm of  $D$  versus  $g_{s\text{-reference}}$  ( $g_s$  at  $D = 1 \text{ kPa}$ ). Dotted line in (d) represents the theoretical slope of 0.6 (Oren *et al.* 1999).



**Figure 6.** Effect of soil moisture on reference stomatal conductance ( $g_{s\text{-reference}}$ ) in  $\text{CO}_2^{\text{a}}$  (A),  $\text{CO}_2^{\text{a}} \times \text{N}$ -fertilization (A-N),  $\text{CO}_2^{\text{e}}$  (E) and  $\text{CO}_2^{\text{e}} \times \text{N}$ -fertilization (E-N) trees.

estimated the time constant ( $\tau = C_{\text{leaf}}/K_{\text{leaf}}$ ) of water flow in the leaf. The time constant represents the time required for  $\Psi_{\text{leaf}}$  to reach 63% of its steady state value after a step change in transpiration. Mean  $\tau$  varied from 7 min in non-fertilized trees growing under  $\text{CO}_2^{\text{a}}$  to 16 min in fertilized trees growing under  $\text{CO}_2^{\text{e}}$  (Tables 2 & 3) and there was a significant  $\text{CO}_2^{\text{e}} \times \text{N}$  interaction on  $\tau$  (Table 3).

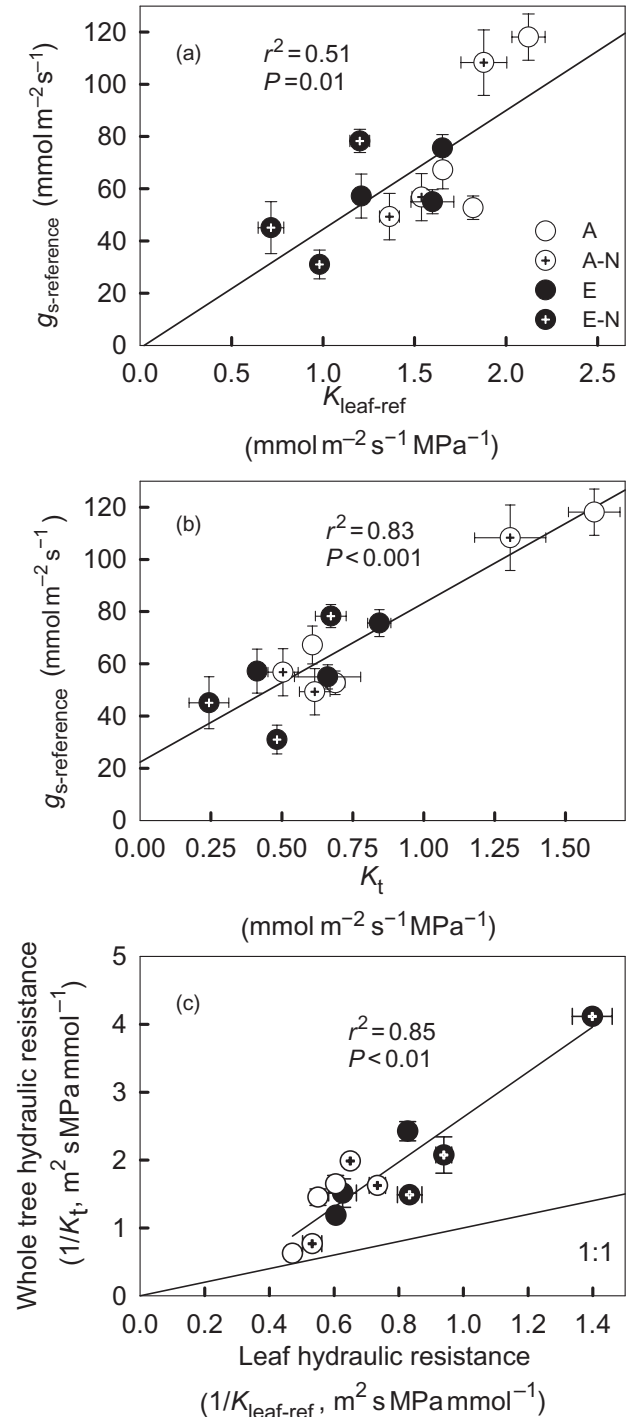
The  $g_{s\text{-ref}}$  obtained from the four treatments in the three measurement periods increased linearly and proportionally with  $K_{\text{leaf-ref}}$  (Fig. 7a). Similarly,  $g_{s\text{-ref}}$  increased with treatment and seasonally based increases in whole tree hydraulic conductance ( $K_t$ , calculated from the relationship between leaf transpiration and  $\Psi_{\text{leaf}}$ ; Fig. 7b), but the reduction in  $g_{s\text{-ref}}$  was less than proportional to the reduction in  $K_t$  (i.e. changes in  $g_{s\text{-ref}}$  were more sensitive to changes in  $K_{\text{leaf-ref}}$ ). Between May and September,  $K_t$  declined by 56% in trees growing under  $\text{CO}_2^{\text{a}}$ , and by 41% in trees growing under  $\text{CO}_2^{\text{e}}$ . There was a linear relationship between leaf hydraulic resistance ( $1/K_{\text{leaf-ref}}$ ) and whole tree hydraulic resistance ( $1/K_t$ ) (Fig. 7c). The contribution of  $1/K_{\text{leaf-ref}}$  to  $1/K_t$  decreased with  $1/K_{\text{leaf-ref}}$ , which was affected by treatments and changed seasonally (Fig. 7c). Therefore,  $K_{\text{leaf-ref}}$  exerted a greater constraint on whole-plant water transport under  $\text{CO}_2^{\text{a}}$  than under  $\text{CO}_2^{\text{e}}$ , and mostly in May, when  $K_{\text{leaf-ref}}$  was high (or  $1/K_{\text{leaf-ref}}$  was low), than in July or September. As a consequence, the contribution of the woody parts (root-to-branch hydraulic resistance) to the whole tree hydraulic resistance increased from ~50% in May to ~70% in July–September ( $P = 0.007$ ).

Across treatments,  $\Psi_{\text{leaf-ref}}$  was higher (less negative) but strongly correlated with  $\Psi_{\text{tp}}$  ( $P < 0.001$ ; Fig. 8), indicating that stomata tended to begin closing before the leaf reached its turgor loss point. The minimum measured field  $\Psi_{\text{leaf}}$  on the other hand were similar to  $\Psi_{\text{tp}}$  ( $P = 0.21$ ).

## DISCUSSION

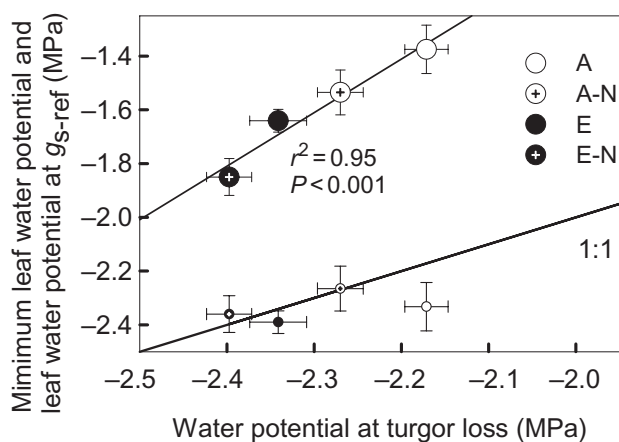
There have been no studies on the effects of  $\text{CO}_2^{\text{e}}$  and N availability on leaf hydraulics and their downstream effect

on gas exchange. Our study revealed that  $\text{CO}_2^{\text{e}}$  and N had a significant effect on maximum  $K_{\text{leaf}}$ , and that both treatment-induced seasonal variation in  $K_{\text{leaf-ref}}$  translated to similar variation in  $g_{s\text{-ref}}$  and, thus, gas exchange. The similarity of trends in liquid- and gas-phase conductances



**Figure 7.** Reference stomatal conductance ( $g_{s\text{-ref}}$ ) as a function of (a) reference leaf hydraulic conductance ( $K_{\text{leaf-ref}}$ ) and (b) whole tree hydraulic conductance ( $K_t$ ). (c) Whole tree hydraulic resistance ( $1/K_t$ ) as a function of reference leaf hydraulic resistance ( $1/K_{\text{leaf-ref}}$ ).





**Figure 8.** Mean seasonal leaf water potential at maximum stomatal conductance ( $\Psi_{\text{leaf}}$ ) (small symbols) and mean seasonal leaf water potential at reference stomatal conductance ( $\Psi_{\text{leaf-ref}} = \Psi_{\text{leaf}}$  at  $g_{s\text{-ref}}$ ) (big symbols) as a function of water potential at the turgor loss point ( $\Psi_{\text{tp}}$ ) in CO<sub>2</sub><sup>a</sup> (A), CO<sub>2</sub><sup>a</sup> × N-fertilization (A-N), CO<sub>2</sub><sup>e</sup> (E) and CO<sub>2</sub><sup>e</sup> × N-fertilization (E-N) trees.

implies that functional adjustments under CO<sub>2</sub><sup>e</sup> and N contributed to homeostasis in the operation of the hydraulic regulatory systems that were driven by  $K_{\text{leaf-ref}}$  and the point of turgor loss. The results of this study show that, in loblolly pine, it required a structural change of the hydraulic pathway to produce stomatal closure under CO<sub>2</sub><sup>e</sup>. This contrasts with the current view that CO<sub>2</sub><sup>e</sup> directly causes stomatal closure (Ainsworth & Rogers 2007).

### Effect of elevated CO<sub>2</sub> and N-fertilization on $K_{\text{leaf}}$ , $C_{\text{leaf}}$ and $\Psi_{\text{tp}}$

As shown in a study on needle ‘vulnerability curves’ of *Pseudotsuga menziesii* (Woodruff *et al.* 2007),  $K_{\text{leaf}}$  was highly sensitive to  $\Psi_{\text{leaf}}$  (Fig. 1). However, compared with trees growing under CO<sub>2</sub><sup>e</sup>, trees growing under CO<sub>2</sub><sup>a</sup> showed higher maximum  $K_{\text{leaf}}$  and higher sensitivity of  $K_{\text{leaf}}$  to  $\Psi_{\text{leaf}}$ . The differences observed in maximum  $K_{\text{leaf}}$  among treatments was not associated with differences in  $\Psi_{50}$ , in contrast to the pattern observed in other tree organs (Tyree & Zimmermann 2002; Domec *et al.* 2008). This lack of trade-off between hydraulic efficiency and hydraulic safety points to the more complex role  $K_{\text{leaf}}$  plays in water transport, and to the refilling dynamics of leaves. The mechanism explaining the reduction in  $K_{\text{leaf}}$  with  $\Psi_{\text{leaf}}$  likely involves cavitation-induced embolism (Johnson *et al.* 2009), although we can not rule out that needle xylem might have collapsed at lower  $\Psi_{\text{leaf}}$  (Cochard *et al.* 2004; Brodrribb & Holbrook 2005). The decrease in maximum  $K_{\text{leaf}}$ ,  $C_{\text{leaf}}$ , and their lower sensitivities to  $\Psi_{\text{leaf}}$  in trees growing under CO<sub>2</sub><sup>e</sup> and higher soil fertility, as hypothesized (first hypothesis), may be partly related to the development of conducting tissue with different hydraulic characteristics (Centritto *et al.* 1999). Treatment-induced structural and anatomical adjustments, such as a decrease in the length and diameter

of leaf tracheids (Prior *et al.* 1997; Woodruff, Meinzer & Lachenbruch 2008), and probably in the size of connecting pit membranes between adjacent conduits, would reduce  $K_{\text{leaf}}$ . Such structural modifications may also explain the lower  $\Psi_{\text{tp}}$  because thick and stiff cell walls have been shown to reduce turgor loss (Marshall & Dumbroff 1999).

### Coordination between liquid- and gas-phase leaf conductances

Following our second hypothesis, treatment-induced reductions in  $g_{s\text{-ref}}$  were consistent with those in  $K_{\text{leaf-ref}}$  (Table 3). However, and in contrast to another study on loblolly pine (Ewers *et al.* 2000), N did not affect  $g_{s\text{-ref}}$ , although a tendency for reduced  $g_s$  was observed in May (Fig. 4). The reason for lack of statistical difference may simply be the power of the experiment, owing to the low number of replicates ( $n = 4$ ) and the split-plot N factor. Furthermore, stomata of trees growing under CO<sub>2</sub><sup>e</sup> and high soil fertility were less sensitive to  $D$  than those of trees growing under CO<sub>2</sub><sup>a</sup>, unfertilized conditions (Fig. 5). These results also showed that the sensitivity of  $g_s$  to  $D$  was related to the variation in  $g_{s\text{-ref}}$ , meaning that CO<sub>2</sub><sup>e</sup> and N-fertilization did not affect the relative sensitivity of  $g_s$  to  $D$ , a behaviour consistent with an isohydric regulation of water potential (McNaughton & Jarvis 1991; Oren *et al.* 1999). These results are consistent with the third hypothesis.

Mirroring the stomatal behaviour, the sensitivity of  $K_{\text{leaf}}$  to  $\Psi_{\text{leaf}}$  was related to  $K_{\text{leaf-ref}}$ , as has been found in roots of the same species (Ewers *et al.* 2000). Furthermore, the common patterns in the sensitivity of  $K_{\text{leaf}}$  to  $\Psi_{\text{leaf}}$  and  $g_s$  to  $D$  were additional evidence of the strong coordination between liquid- and gas-phase conductances (Fig. 5). Because of this coordinated sensitivity between the liquid- and gas-phase fluxes, treatment-induced differences in  $K_{\text{leaf-ref}}$  and  $g_{s\text{-ref}}$  have implications to gas exchange on both the short (diurnal) and long (drying cycle) time scales (Domec *et al.* 2009).

Diurnally, the reduction in  $g_s$  with  $D$  is proportional to  $g_{s\text{-ref}}$ , meaning that on an absolute basis, treatments with higher  $g_{s\text{-ref}}$  experienced a greater loss of CO<sub>2</sub> uptake than those with lower  $g_{s\text{-ref}}$ . The diurnal patterns in  $K_{\text{leaf}}$ ,  $\Psi_{\text{leaf}}$  and  $g_s$  provide insight to a possible mechanism involved in the coordination between the liquid- and gas-phase water flows. The patterns indicate that  $K_{\text{leaf}}$  does not directly control  $g_s$ . For example,  $K_{\text{leaf}}$  begins to decline immediately after dawn whereas  $g_s$  is increasing with light, generating a fast decrease in  $\Psi_{\text{leaf}}$  even though  $D$  is not very high at this time. This sets up the leaf hydraulic system to a state in which  $g_s$  will respond quickly to increasing  $D$  later in the morning, thus regulating minimum  $\Psi_{\text{leaf}}$ . Indeed, later,  $g_s$  decreases sharply in response to  $D$  whereas  $K_{\text{leaf}}$  decreases slowly, mirroring the slow decrease in  $\Psi_{\text{leaf}}$ . The balance between xylem tension and the loss of conductivity has caused several authors to suggest a functional role for cavitation as part of a feedback mechanism linking stomatal regulation to hydraulic conductance and plant water status (Sperry 2000; Meinzer 2002). The diurnal patterns described earlier

suggest that hypersensitive leaf xylem serves to protect the integrity of the upstream woody portion of the hydraulic pathway by causing a rapid stomatal closure before water potential drops in the woody xylem, consistent with the hydraulic segmentation hypothesis (Sperry 1986).

During drying cycles, or seasons in areas where soil moisture becomes progressively limiting, the coordinated sensitivity between the liquid- and gas-phase fluxes coupled with treatment-induced differences in  $K_{\text{leaf-ref}}$  and  $g_{\text{s-ref}}$  have different implications to gas exchange. Lower values of  $g_{\text{s-ref}}$  at the beginning of the growing season, and the lower seasonal reduction in  $g_{\text{s-ref}}$  in trees growing under  $\text{CO}_2^{\text{e}}$  and N were related to lower reductions in  $K_{\text{leaf-ref}}$  and  $K_{\text{t}}$  (Fig. 7). Reduced  $K_{\text{t}}$  has seldom been explicitly reported in studies comparing trees growing under  $\text{CO}_2^{\text{a}}$  and  $\text{CO}_2^{\text{e}}$  (Bunce & Ziska 1998; Kupper *et al.* 2006). In our study, treatment-induced differences in  $K_{\text{t}}$  were to a great extent explained by differences in  $K_{\text{leaf-ref}}$  because the short portion of the pathway through the needles constituted a major part (30–50%) of the whole tree hydraulic resistance to water flow ( $1/K_{\text{t}}$ ). Seasonally, the effect of  $K_{\text{leaf-ref}}$  on  $K_{\text{t}}$  decreased in all treatments (Fig. 7c). In May,  $K_{\text{leaf-ref}}$  dominated  $K_{\text{t}}$ , whereas in July and September,  $K_{\text{t}}$  became less limited by  $K_{\text{leaf-ref}}$ , probably because of a large decrease in root and stem hydraulic conductances as soil moisture declined (Hacke *et al.* 2000; Domec *et al.* 2009).

The treatment-induced differences in the sensitivity of  $g_{\text{s}}$  to  $D$  remained proportional to  $g_{\text{s-ref}}$  on both diurnal and seasonal time scales. However, treatment effects on  $g_{\text{s-ref}}$  changed over the season as the soil dried (Fig. 6). As a result of a greater sensitivity of  $g_{\text{s-ref}}$  to soil moisture under  $\text{CO}_2^{\text{a}}$ , the differences in  $g_{\text{s}}$  observed early in the season among treatments disappeared by the end of the season even though soil moisture was similar in all treatments. Indicative of a severe soil water depletion, values pre-dawn  $\Psi_{\text{leaf}}$  fell below  $-1.5$  MPa, which has already been reported for this site during a previous dry year (Ellsworth 1999). Although pre-dawn  $\Psi_{\text{leaf}}$  was similar in all treatments during the growing season (Fig. 3), this might not reflect access to the same amount of water as the soil dries (Donovan, Richards & Linton 2003). The lower sensitivity to soil drying of  $g_{\text{s-ref}}$  under  $\text{CO}_2^{\text{e}}$  might reflect deeper penetration of fine roots (King *et al.* 2001; Pritchard *et al.* 2008), accessing soil moisture beyond the measurement depth, as reflected in a lesser seasonal decrease in  $K_{\text{t}}$  of  $\text{CO}_2^{\text{e}}$  trees (Fig. 7b). Regardless of whether  $\text{CO}_2^{\text{e}}$  trees had access to more moisture at the end of the year, the differences in  $g_{\text{s}}$  observed early in the season but not late, might also explain differences among studies on the effect of  $\text{CO}_2^{\text{e}}$  on  $g_{\text{s}}$ .

### Consequences of adjustments in turgor loss, $K_{\text{leaf}}$ and time constant on tree water economy

The minimum  $\Psi_{\text{leaf}}$  observed in field measurements corresponded across treatments to the  $\Psi_{\text{leaf}}$  at turgor loss (Fig. 8). Leaves of trees in treatments (ambient and unfertilized) with higher (less negative)  $\Psi_{\text{tp}}$  began regulating stomata at

a higher  $\Psi_{\text{leaf}}$ . It is possible that the strong linear correlation across treatment between  $\Psi_{\text{tp}}$  and  $\Psi_{\text{leaf-ref}}$  means that declining  $K_{\text{leaf}}$  was related to declining cell turgor in the leaf prior to the onset of cavitation in the needle xylem (Brodribb & Holbrook 2005; Woodruff *et al.* 2007). As result, the rate of decrease in  $\Psi_{\text{leaf}}$  is greater under  $\text{CO}_2^{\text{a}}$  and without fertilization between the time in which stomata begin to respond to  $D$  and the time in which  $\Psi_{\text{leaf}}$  reaches its minimum value. Moreover, in trees growing under  $\text{CO}_2^{\text{e}}$ , more negative  $\Psi_{\text{tp}}$  allowed stomata to remain open at lower  $\Psi_{\text{leaf}}$ , which decreased the effect of drought on  $g_{\text{s-ref}}$ . However, lower  $K_{\text{leaf}}$  and lower sensitivity to  $\Psi_{\text{leaf}}$  under  $\text{CO}_2^{\text{e}}$  induced a steeper drop in  $\Psi_{\text{leaf}}$  at  $g_{\text{s-ref}}$  relative to the decrease in turgor loss points, and therefore increased the percent loss of needle hydraulic conductance (Table 2).

Another consequence of the differences in  $C_{\text{leaf}}$  and maximum  $K_{\text{leaf}}$  among treatments was reflected in the time constants (Table 2). The time constant of the needles growing under ambient conditions represented around half the time constant of the soil–trunk–leaf compartment calculated at the same site (Phillips *et al.* 1997), implying that woody tissue and leafy tissue both exhibit a similar time constant. An important consequence of the increase in time constant in trees growing under  $\text{CO}_2^{\text{e}}$  and N is that, under dry conditions, these trees would have less time to restore their equilibrium water content and could experience a greater degree of leaf dehydration.

The results from this study are useful in a number of ways. Firstly, we show that in species such as loblolly pine,  $\text{CO}_2^{\text{e}}$  and N-fertilization alter the hydraulic pathway, most likely structurally, affecting the liquid phase transport, thus reducing stomatal conductance. This contrasts with previous studies focusing on a direct stomatal response to  $\text{CO}_2^{\text{e}}$ . We also show evidence that the hydraulic changes allow plants growing under  $\text{CO}_2^{\text{e}}$  to reduce stomatal conductance less under drought than plants growing under current  $\text{CO}_2$ . These conclusions will inform models employed to predict ecosystem responses to climate change, and biosphere-atmosphere interactions under current and future climates. Secondly, the results show that clear effects of  $\text{CO}_2$  and N-fertilization treatments on  $g_{\text{s}}$  can be observed only under certain conditions. This may explain some of the contradictions in published responses. Furthermore, stomatal response to elevated  $\text{CO}_2^{\text{e}}$  has been investigated in two types of studies. One type exposes leaves grown under  $\text{CO}_2^{\text{a}}$  to  $\text{CO}_2^{\text{e}}$ , detecting clear stomatal closure in most species but conifers (Murthy *et al.* 1996; Saxe, Ellsworth & Heath 1998). The other type grows plants under  $\text{CO}_2^{\text{e}}$  and compares their  $g_{\text{s}}$  with that of plants grown under  $\text{CO}_2^{\text{a}}$ . In broadleaved species, studies of this type find similar responses to the first type, but the responses in conifers are more variable, with a tendency for the longer studies to find an effect (Medlyn *et al.* 2001; Wang *et al.* 2005). If the response we describe is particularly important in conifers, the length of exposure to  $\text{CO}_2^{\text{e}}$  necessary to produce a reduction in  $g_{\text{s}}$  would depend on the time it takes the species to replace a large proportion of pretreatment foliage with foliage produced under  $\text{CO}_2^{\text{e}}$ .

## ACKNOWLEDGMENTS

The Duke FACE research site was supported by the United States Department of Energy (DOE) through the Office of Biological and Environmental Research (BER) Terrestrial Carbon Processes (TCP) programme (DE-FG02-95ER62083). Support for S. Palmroth also came from the National Science Foundation (NSF-EAR 0628342).

## REFERENCES

- Aasamaa K., Sober A. & Rahi M. (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* **28**, 765–774.
- Addington R.N., Donovan L.A., Mitchell R.J., Vose J.M., Pecot S.D., Jack S.B., Hacke U.G., Sperry J.S. & Oren R. (2006) Adjustments in hydraulic architecture of *Pinus palustris* maintain similar stomata conductance in xeric and mesic habitats. *Plant, Cell & Environment* **29**, 535–545.
- Ainsworth E.A. & Rogers A. (2007) The response of photosynthesis and stomatal conductance to rising [CO<sub>2</sub>]: mechanisms and environmental interactions. *Plant, Cell & Environment* **30**, 258–270.
- Bernacchi C.J., Calafapietra C., Davey P.A., Wittig V.E., Scarascia-Mugnozza G.E., Raines C.A. & Long S.P. (2003) Photosynthesis and stomatal conductance responses of poplars to free-air CO<sub>2</sub> enrichment (PopFACE) during the first growth cycle and immediately following coppice. *New Phytologist* **159**, 609–621.
- Brodribb T.J. & Cochard H. (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology* **149**, 575–584.
- Brodribb T.J. & Holbrook N.M. (2003) Stomatal Closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* **132**, 2166–2173.
- Brodribb T.J. & Holbrook N.M. (2005) Water stress deforms tracheids peripheral to the leaf vein of a tropical conifer. *Plant Physiology* **137**, 1139–1146.
- Brodribb T.J., Holbrook N.M., Edwards E.J. & Gutiérrez M.V. (2003) Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell & Environment* **26**, 443–450.
- Bucci S.J., Scholtz F.G., Goldstein G., Meinzer F.C. & Sternberg L. (2003) Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell & Environment* **26**, 1633–1645.
- Bucci S.J., Scholtz F.G., Goldstein G. & Meinzer F.C. (2006) Nutrient availability constrains the hydraulic architecture and water relations of savannah trees. *Plant, Cell & Environment* **29**, 2153–2167.
- Buckley T.N. (2005) The control of stomata by water balance. *New Phytologist* **168**, 275–292.
- Bunce A. & Ziska L.H. (1998) Decreased hydraulic conductance in plants at elevated carbon dioxide. *Plant, Cell & Environment* **21**, 121–126.
- Centritto M., Magnani F., Helen S., Lee J. & Jarvis P.G. (1999) Interactive effects of elevated and drought on cherry (*Prunus avium*) seedlings. II. photosynthetic capacity and water relations. *New Phytologist* **141**, 141–153.
- Chuang Y.-L., Oren R., Bertozzi A.L., Phillips N. & Katul G.G. (2006) The porous media model for the hydraulic system of a tree: from sap flux data to transpiration rate. *Ecological Modelling* **191**, 447–468.
- Cochard H., Coll L., Le Roux X. & Améglio T. (2002) Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. *Plant Physiology* **128**, 282–290.
- Cochard H., Froux F., Mayr S. & Coutand C. (2004) Xylem wall collapse in water-stressed pine needles. *Plant Physiology* **134**, 401–408.
- Domec J.-C., Lachenbruch B., Meinzer F.C., Woodruff D.R., Warren J.M. & McCulloh K.A. (2008) Maximum height in a conifer is associated with conflicting requirements for xylem design. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 12069–12074.
- Domec J.-C., Noormets A., King J.S., Sun G., McNulty S.G., Gavazzi M.J., Boggs J.L. & Treasure E.A. (2009) Decoupling the influence of leaf and root hydraulic conductances on stomatal conductance and its sensitivity to vapor pressure deficit as soil dries in a drained loblolly pine plantation. *Plant, Cell & Environment* (in press). doi:10.1111/j.1365-3040.2009.01981.x.
- Donovan L.A., Richards J.H. & Linton M.J. (2003) Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials in desert shrubs. *Ecology* **84**, 463–470.
- Ellsworth D.S. (1999) CO<sub>2</sub> enrichment in a maturing pine forest: are CO<sub>2</sub> exchange and water status in the canopy affected? *Plant, Cell & Environment* **22**, 461–472.
- Ewers B.E. & Oren R. (2000) Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. *Tree Physiology* **20**, 579–589.
- Ewers B.E., Oren R. & Sperry J.S. (2000) Influence of nutrient versus water supply on hydraulic architecture and water balance in *Pinus taeda*. *Plant, Cell & Environment* **23**, 1055–1066.
- Ewers B.E., Oren R., Kim H.S., Bohrer B. & Lai C.T. (2007) Effects of hydraulic architecture and spatial variation in light on mean stomatal conductance of tree branches and crowns. *Plant, Cell & Environment* **30**, 483–496.
- Gerbauer R.L.E., Reynolds J.F. & Strain B.R. (1996) Allometric relations and growth in *Pinus taeda*: the effect of elevated CO<sub>2</sub> and changing N Availability. *New Phytologist* **134**, 85–93.
- Granier A. (1987) Evaluation of transpiration in a Douglas-Fir stand by means of sap flow measurements. *Tree Physiology* **3**, 309–320.
- Hacke U.G., Sperry J.S., Ewers B.E., Ellsworth D.S., Schäfer K.V.R. & Oren R. (2000) Influence of soil porosity on water use in *Pinus taeda*. *Oecologia* **124**, 495–505.
- Hendrey G.R., Ellsworth D.S., Lewin K.F. & Nagy J. (1999) A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO<sub>2</sub>. *Global Change Biology* **5**, 393–310.
- Hyvönen R., Agren G.I., Linder S., *et al.* (2007) The likely impact of elevated [CO<sub>2</sub>], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist* **173**, 463–480.
- Johnson D.M., Meinzer F.C., Woodruff D.R. & McCulloh K.A. (2009) Leaf xylem embolism, detected acoustically and by cryo-SEM, corresponds to decreases in leaf hydraulic conductance in four evergreen species. *Plant, Cell & Environment* **32**, 828–836.
- Jones H.G. (1992) *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*. Cambridge University Press, Cambridge, UK.
- Katul G.G., Leuning R. & Oren R. (2003) Relationship between plant hydraulic and biochemical properties derived from a steady-state coupled water and carbon transport model. *Plant, Cell & Environment* **26**, 339–350.

- Kim H.-S., Oren R. & Hinckley T.M. (2008) Actual and potential transpiration and carbon assimilation in an irrigated poplar plantation. *Tree Physiology* **28**, 559–577.
- King J.S., Thomas R.S. & Strain B.R. (1997) Morphology and tissue quality of seedling root systems of *Pinus taeda* and *Pinus ponderosa* as affected by varying CO<sub>2</sub>, temperature, and nitrogen. *Plant and Soil* **195**, 107–119.
- King J.S., Pregitzer K.S., Zak D.R., Sober J., Isebrands J.G., Dickson R.E., Hendrey G.R. & Karnosky D.F. (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO<sub>2</sub> and tropospheric O<sub>3</sub>. *Oecologia* **128**, 237–250.
- Kupper P., Sellin A., Klimánková Z., Pokorný R. & Puértolas J. (2006) Water relations in Norway spruce trees growing at ambient and elevated CO<sub>2</sub> concentrations. *Biologia Plantarum* **50**, 594–603.
- Loustau D., Domec J.C. & Bosc A. (1998) Interpreting the variations in xylem sap flux density within the trunk of maritime pine (*Pinus pinaster* Ait.): application of a model for calculating water flows at tree and stand levels. *Annales Des Sciences Forestieres* **55**, 29–46.
- McCarthy H.R., Oren R., Kim H.-S., Johnsen K.H., Maier C., Pritchard S.G. & Davis M.A. (2006a) Interaction of ice storms and management practices on current carbon sequestration in forests with potential mitigation under future CO<sub>2</sub> atmosphere. *Journal of Geophysical Research* **111**, 1–10.
- McCarthy H.R., Oren R., Finzi A.C. & Johnsen K.H. (2006b) Canopy leaf area constrains [CO<sub>2</sub>]-induced enhancement of productivity and partitioning among aboveground carbon pools. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 19356–19361.
- McCarthy H.R., Oren R., Finzi A.C., Ellsworth D.S., Kim H.-S., Johnson K.H. & Millar B. (2007) Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO<sub>2</sub>. *Global Change Biology* **13**, 2479–2497.
- McNaughton K. & Jarvis P.G. (1991) Effects of spatial scale on stomatal control of transpiration. *Agricultural and Forest Meteorology* **54**, 279–302.
- Maier C.A., Palmroth S. & Ward E. (2008) Branch growth and gas exchange in 13-year-old loblolly pine (*Pinus taeda*) trees in response to elevated carbon dioxide concentration and fertilization. *Tree Physiology* **28**, 1093–1106.
- Marshall J.G. & Dumbroff E.B. (1999) Turgor regulation via cell wall adjustment in white spruce. *Plant Physiology* **119**, 313–320.
- Maseda P.H. & Fernández R.J. (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *Journal of Experimental Botany* **57**, 3963–3977.
- Medlyn B.E., Badeck F.W., De Pury D.G.G., et al. (1999) Effects of elevated [CO<sub>2</sub>] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant, Cell & Environment* **22**, 1475–1495.
- Medlyn B.E., Barton C.V.M., Broadmeadow M.S.J., et al. (2001) Stomatal conductance of forest species after long-term exposure to elevated CO<sub>2</sub> concentration: a synthesis. *New Phytologist* **149**, 247–264.
- Meinzer F.C. (2002) Co-ordination of vapour and liquid phase water transport properties in plants. *Plant, Cell & Environment* **25**, 265–274.
- Meinzer F.C., Goldstein G., Jackson P., Holbrook N.M., Gutiérrez M.V. & Cavelier J. (1995) Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic properties. *Oecologia* **101**, 514–522.
- Mencuccini M. (2003) The ecological significance of long distance water transport: short term regulation, long term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell & Environment* **26**, 163–182.
- Munje O. & Bugbee B. (1998) Adaptation to high CO<sub>2</sub> concentration in an optimal environment: radiation capture, canopy quantum yield and carbon use efficiency. *Plant, Cell & Environment* **21**, 315–324.
- Murthy R., Dougherty P.M., Zarnoch S.J. & Allen H.L. (1996) Effects of carbon dioxide, fertilization, and irrigation on photosynthetic capacity of loblolly pine trees. *Tree Physiology* **16**, 537–546.
- Noormets A., Gavazzi M., McNulty S., Sun G., Domec J.-C., King J. & Chen J. (2009) Response of carbon fluxes to drought in a coastal plain loblolly pine forest. *Global Change Biology* (in press). doi:10.1111/j.1365-2486.2009.01928.x.
- Oishi A.C., Oren R. & Stoy P.C. (2008) Estimating components of forest evapotranspiration: a footprint approach for scaling sap flux measurements. *Agricultural and Forest Meteorology* **148**, 719–732.
- Oren R., Ewers B.E., Todd P., Phillips N. & Katul G. (1998a) Water balance delineates the soil layer in which soil moisture affects canopy conductance. *Ecological Applications* **8**, 990–1002.
- Oren R., Phillips N., Katul G., Ewers B.E. & Pataki D.E. (1998b) Scaling xylem sap flux and soil water balance and calculating variance: a method for partitioning water flux in forests. *Annales Des Sciences Forestieres* **55**, 191–216.
- Oren R., Sperry J.S., Katul G.G., Pataki D.E., Ewers B.E., Phillips N. & Schäfer K.V.R. (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell & Environment* **22**, 1515–1526.
- Oren R., Ellsworth D.S., Johnsen K.H., et al. (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO<sub>2</sub>-enriched atmosphere. *Nature* **411**, 469–472.
- Palmroth S., Oren R., McCarthy H.R., Johnsen K.H., Finzi A.C., Butnor J.R., Ryan M.G. & Schlesinger W.H. (2006) Above-ground sink strength in forests controls the allocation of carbon belowground and its [CO<sub>2</sub>]-induced enhancement. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 19362–19367.
- Pataki D.E., Oren R. & Tissue D.T. (1998) Elevated carbon dioxide does not affect average canopy stomatal conductance of *Pinus taeda* L. *Oecologia* **117**, 47–52.
- Phillips N., Nagchaudhuri A., Oren R. & Katul G. (1997) Time constant for water transport in loblolly pine trees estimated from time series of evaporative demand and stem sapflow. *Trees* **11**, 412–419.
- Prior S.A., Pritchard S.G., Runion G.B. & Rogers H.H. (1997) Influence of atmospheric CO<sub>2</sub> enrichment, soil N, and water stress on needle surface wax formation in *Pinus palustris* (Pinaceae). *American Journal of Botany* **84**, 1070–1077.
- Pritchard S.G., Strand A.E., McCormack M.L., Davis M.A., Finzi A.C., Jackson R.B., Matamala R., Rogers H.H. & Oren R. (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air-CO<sub>2</sub>-enrichment: a six-year-minirhizotron study. *Global Change Biology* **14**, 588–602.
- Rundel P.W. & Yoder B.J. (1998) Ecophysiology of pinus. In *Ecology and Biogeography of Pinus* (ed. D.M. Richardson) pp. 296–323. Cambridge University Press, Cambridge, UK.
- Sack L. & Holbrook N.M. (2006) Leaf hydraulics. *Annual Review of Plant Biology* **57**, 361–381.
- Saxe H., Ellsworth D.S. & Heath J. (1998) Tree and forest functioning in an enriched CO<sub>2</sub> atmosphere. *New Phytologist* **139**, 395–436.
- Schäfer K.V.R., Oren R., Lai C.-T. & Katul G.G. (2002) Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO<sub>2</sub> concentration. *Global Change Biology* **8**, 895–911.

- Schulze E.D., Robichaux R.H., Grace J., Rundel P.W. & Ehleringer J.R. (1987) Plant water balance. *Bioscience* **37**, 30–37.
- Sperry J.S. (1986) Relationship of xylem pressure potential, stomatal closure and shoot morphology in the palm *Rhapis excelsa*. *Plant Physiology* **80**, 110–116.
- Sperry J.S. (2000) Hydraulic constraints on plant gas exchange. *Agricultural and Forest Meteorology* **104**, 13–23.
- Sperry J.S., Hacke U.G., Oren R. & Comstock J.P. (2002) Water deficits and hydraulic limits to leaf water supply. *Plant, Cell & Environment* **25**, 251–263.
- Tyree M.T. & Hammel H.T. (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany* **23**, 267–282.
- Tyree M.T. & Zimmermann M.H. (2002) *Xylem Structure and the Ascent of Sap*, 2nd edn. Springer, New York, NY, USA.
- Tyree M.T., Nardini A., Salleo S., Sack L. & El Omari B. (2005) The dependence of leaf hydraulic conductance on irradiance during HPFM measurements: any role for stomatal response? *Journal of Experimental Botany* **56**, 737–744.
- Wang K.Y., Kellomäki S., Zha T. & Peltola H. (2005) Annual and seasonal variation of sap flow and conductance of pine trees grown in elevated carbon dioxide and temperature. *Journal of Experimental Botany* **56**, 155–165.
- Ward E.J., Oren R., Sigurdsson B.D., Jarvis P.G. & Linder S. (2008) Fertilization effects on mean stomatal conductance are mediated through changes in the hydraulic attributes of mature Norway spruce trees. *Tree Physiology* **28**, 579–596.
- Woodruff D.R., McCulloh K.A., Warren J.M., Meinzer F.C. & Lachenbruch B. (2007) Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. *Plant, Cell & Environment* **30**, 559–569.
- Woodruff D.R., Meinzer F.C. & Lachenbruch B. (2008) Height-related trends in leaf xylem anatomy and shoot hydraulic characteristics in a tall conifer: safety versus efficiency in water transport. *New Phytologist* **180**, 90–99.
- Wullschlegel S.D., Tschaplinski T.J. & Norby R.J. (2002) Plant water relations at elevated CO<sub>2</sub> – implications for water-limited environments. *Plant, Cell & Environment* **25**, 319–331.

Received 17 March 2009; received in revised form 25 May 2009; accepted for publication 26 May 2009

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Leaf hydraulic conductance ( $K_{\text{leaf}}$ ) in relation to leaf water potential ( $\Psi_{\text{leaf}}$ ) for foliage samples obtained from the upper crowns of loblolly pine trees growing under ambient conditions. Closed symbols are from dehydration curves obtained in the laboratory (see Eq. 1). Open symbols are from field measurements using the evaporative flux method and calculated as  $K_{\text{leaf}} = v/v_0 E/(\Psi_{\text{stem}} - \Psi_{\text{leaf}})$ , where  $v$  and  $v_0$  are respectively the kinematic viscosities of water at the measured leaf temperature and at 20 °C,  $E$  is the transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) measured with a LI-6400 portable photosynthesis system, and  $\Psi_{\text{stem}}$  is the stem water potential estimated from non-transpiring covered shoots.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.