Research paper

Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest

Jeffrey M. Warren[1], Richard J. Norby and Stan D. Wullschleger

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6301, USA; [1]Corresponding author (warrenjm@ornl.gov)

In 2007, an extreme drought and acute heat wave impacted ecosystems across the southeastern USA, including a 19-year-old *Liquidambar styraciflua* L. (sweetgum) tree plantation exposed to long-term elevated (E_{CO_2}) or ambient (A_{CO_2}) CO_2 treatments. Stem sap velocities were analyzed to assess plant response to potential interactions between CO_2 and these weather extremes. Canopy conductance and net carbon assimilation (A_{net}) were modeled based on patterns of sap velocity to estimate indirect impacts of observed reductions in transpiration under E_{CO_2} on premature leaf senescence. Elevated CO_2 reduced sap flow by 28% during early summer, and by up to 45% late in the drought during record-setting temperatures. Modeled canopy conductance declined more rapidly in E_{CO_2} plots during this period, thereby directly reducing carbon gain at a greater rate than in A_{CO_2} plots. Indeed, pre-drought canopy A_{net} was similar across treatment plots, but declined to ~40% less than A_{net} in A_{CO_2} as the drought progressed, likely leading to negative net carbon balance. Consequently, premature leaf senescence and abscission increased rapidly during this period, and was 30% greater for E_{CO_2}. While E_{CO_2} can reduce leaf-level water use under droughty conditions, acute drought may induce excessive stomatal closure that could offset benefits of E_{CO_2} to temperate forest species during extreme weather events.

Keywords: canopy conductance, FACE, leaf litter, root mortality, sap flow, sweetgum.

Introduction

Terrestrial ecosystems are expected to experience not only climate change, but also an increase in climate variability (Christensen et al. 2007, Meehl et al. 2007, Jentsch and Beierkuhnlein 2008). Future conditions will subject plants to increases in mean temperatures and to climatic variation that could manifest itself in the form of extreme events. Temporal morphological and biochemical adjustments allow plants to adapt to a broad range of environmental conditions, including increases in atmospheric CO_2 concentrations [elevated CO_2 (E_{CO_2})]. Plants exposed to moderate temperature increases or drought may benefit under future E_{CO_2} conditions through CO_2 enhancement of the thermal optimum for photosynthesis (Long 1991, Nowak et al. 2004), and consequential effects on net carbon balance. In addition, for many C_3 plants stomatal

conductance (g_s) is reduced under E_{CO_2} (Ainsworth and Rogers 2007), which reduces the rate of leaf-level water loss and soil water extraction, and thereby may reduce water stress during drought.

Alternatively, the benefit to plants from E_{CO_2} may be limited under more extreme atmospheric conditions or events. Chronic drought and acute heat events can cause significant damage to total plant productivity in natural ecosystems, as exhibited in the large regional European drought of 2003 (Ciais et al. 2005). Elevated CO_2 can buffer and maintain photosynthetic capacity of thermophilic species exposed to experimental drought or heat (~45 °C) events, as demonstrated for *Larrea tridenta* seedlings (Hamerlynck et al. 2000), evergreen *Quercus suber* seedlings (Faria et al. 1996) and *Eucalyptus* sp. seedlings (Roden and Ball 1996). In contrast, in more mesic species, E_{CO_2}

has been linked to greater heat-stress damage (including leaf loss and mortality) for temperate tree seedlings (*Betula populifolia*, *Betula alleghaniensis* and particularly for *Acer pensylvanicum*) exposed to a 45 °C heat event (Bassow et al. 1994). This damage may be attributable to E_{CO_2} reduction of g_s, leading to reduced carbon uptake and excessive leaf temperatures, or through some E_{CO_2}-mediated change in the sensitivity of xylem hydraulic conductivity to higher T; however, few observations exist of these phenomena for mesic tree species exposed to E_{CO_2} under natural field conditions.

Ecosystem-level studies have provided substantial evidence of E_{CO_2} reduction in g_s across terrestrial C_3 plant communities, including natural and managed herbaceous species and woody shrubs and trees (Medlyn et al. 2001, Ainsworth and Rogers 2007). The reduction in g_s, however, may be species specific (Keel et al. 2007) and has been shown to be transitory in some free-air CO_2 enrichment (FACE) studies (Uddling et al. 2009). In other FACE studies, E_{CO_2} reduced stomatal conductance in *Liquidambar styraciflua* trees (Gunderson et al. 2002, Herrick et al. 2004) and reduced whole-tree and site-level water use by up to 20% based on sap flow measurements (Wullschleger and Norby 2001, Norby et al. 2006). In addition to reducing site water use, E_{CO_2} stimulated an increase in fine-root production by *L. styraciflua* trees (Norby et al. 2004), especially at deeper depths up to 60 cm (Iversen et al. 2008), potentially increasing the capacity for soil water extraction, and whose turnover into the soil organic matter pool ultimately could affect soil water holding capacity (Warren et al. 2010). If E_{CO_2} does not increase leaf area index (LAI) through enhanced foliar production, then a reduction in stomatal conductance should lead to reduced stand-level water use, and thus reduce plant water stress during droughty periods.

In contrast to the benefits of reduced g_s for stand water use, reduced g_s directly limits diffusion of CO_2 into the leaf and thus reduces carbon uptake. Elevated CO_2 can offset the increased stomatal resistance to diffusion and thus maintain greater assimilation than in ambient CO_2 (A_{CO_2}) leaves. However, if g_s is further reduced for E_{CO_2} leaves as compared with A_{CO_2} leaves, the benefit of enhanced CO_2 substrate may be lost. Reduced foliar water loss also reduces latent heat loss through evaporation, which may result in higher leaf temperature and thereby greater respiratory carbon loss. Indeed, as a consequence of E_{CO_2}-reduced evapotranspiration, daytime leaf temperature (T_{leaf}) can increase by up to 3 °C (Kimball and Bernacchi 2006, Leuzinger and Körner 2007a, Barker et al. 2005). As g_s declines, T_{leaf} can exceed that of air (T_{air}) by 5–10 °C or more, depending on leaf characteristics and environmental conditions (Ball et al. 1988, Barker et al. 2005, Leuzinger and Körner 2007b), which will result in direct reductions in photosynthesis through reduced CO_2 substrate, potential biochemical limitations if thermal optima are exceeded, and through higher respiratory costs.

In addition to physiological effects on leaf carbon balance, an extreme heat/drought event will dramatically increase the vapor pressure deficit (D), which drives water flux from the plant to the atmosphere. Isohydric species such as *L. styraciflua* regulate stomatal aperture in response to increasing D in order to minimize excessive water loss and thereby xylem embolism and loss of hydraulic conductivity. Reduced conductance is balanced by larger D such that total site evapotranspiration can remain fairly uniform across droughts and mesic periods (Oishi et al. 2010). Maintenance of site water extraction during extended drought can lead to substantial damage to this bottomland species, as evidenced in 'sweetgum blight' decline in the 1950s, a condition attributed to abiotic stress that correlated low soil water availability with fine-root mortality and shoot dieback (Toole and Broadfoot 1959). A sudden and progressive increase in drought stress could lead to negative leaf carbon balance or to hydraulic failure, which reduces hydraulic capacity, and, by necessity, leaves would be shed to balance this loss (hydraulic adjustment). Small *L. styraciflua* trees growing under E_{CO_2} do display changes in xylem hydraulic characteristics, including greater specific conductivity and enhanced vulnerability to xylem embolism in branches (Domec et al. 2009). In other diffuse porous species, E_{CO_2} reduced petiole xylem vessel area in 4-year-old birch (Eguchi et al. 2008) and stem vessel area in 3-year-old beech (Overdieck et al. 2007), but increased xylem cell size in dogwood (Domec et al. 2010), thereby decreasing or increasing hydraulic conductivity, respectively. Elevated CO_2-mediated shifts in carbon availability during xylem development will have impacts on hydraulic characteristics, through regulation of allocation between above- and below-ground sinks, and resultant xylem structural properties (e.g., cell size, cell wall or perforation plate characteristics, chemical composition).

Under future climate change conditions, plants exposed to increased atmospheric E_{CO_2}, severe drought and high T_{air} could experience reductions in net carbon uptake or hydraulic conductance, although the thresholds for such damage are not well known and will be related to variability in underlying genetic/environmental phenotypes, site resources and species-specific plasticity of response (Bassow et al. 1994). Although manipulative studies have investigated plant response to various stressors, seldom does an opportunity arise to study tree response to the coupling of extreme climatic events and CO_2 enrichment in a natural environment—a rare glimpse into a future ecosystem scenario. At the Oak Ridge National Laboratory (ORNL)'s FACE site in southeastern Tennessee, USA, extreme drought in 2007 was exacerbated by 2 years of below-average rainfall, and resulted in some of the driest periods on record (since the year 1895), 30% drier than 50-year-average precipitation. In addition, there was an acute drought in August, when this *L. styraciflua* plantation received <5 mm of precipitation amid record-high temperatures (Figure 1a and b).

Therefore, the primary objective of this study was to assess whether E_{CO_2} buffered or exacerbated tree stress during an extreme drought and heat event. We hypothesized that drought would reduce transpiration in both E_{CO_2} and A_{CO_2} treatments, and that the reduction would be less in E_{CO_2} stands due to greater residual soil moisture linked to lower g_s. We hypothesized that thresholds for catastrophic drought-, temperature- and conductivity-dependent reductions in net carbon uptake would not be exceeded, and responses would not differ substantially between the treatments. As such, we hypothe-

sized that E_{CO_2} plots would maintain greater relative productivity in 2007 than exhibited in earlier years by mitigation of the drought stress through greater residual soil moisture.

Methods

Site description and CO_2 treatments

The research took place in a 19-year-old sweetgum (*L. styraciflua* L.) tree plantation established in Oak Ridge National Environmental Research Park in eastern Tennessee, USA (35°54′N; 84°20′W). One-year-old sweetgum seedlings were planted in 1988 at 2.3 × 1.2 m spacing on previously cultivated alluvial land along the Clinch River. A FACE system (Hendrey et al. 1999) was installed in four 25-m-diameter plots within the closed-canopy stand in 1997 (Norby et al. 2001). The FACE system regulates release of CO_2 from vertical PVC pipes located in a ring around each plot based on wind speed, wind direction and in situ measurements of current CO_2 within the canopy. Since 1998, E_{CO_2} has been released in two treatment rings during each growing season, while ambient air was circulated in two control rings. One additional 25-m plot was established to act as an additional ambient control, without any FACE infrastructure installed. Daytime atmospheric CO_2 in elevated plots ranged from 525 to 555 ppm in 2007, ~40% greater than CO_2 in ambient plots (380–400 ppm). In 2007, mean tree height was 17.9 m (7.8–23.6 m) and mean tree basal area (BA) was 178 cm^2 (19–491 cm^2).

Mean annual temperature at the site in 2007 was 15.5 °C, and annual precipitation was 905 mm, warmer and much drier than the 50-year average for nearby Oak Ridge, TN (1957–2007; 14.3 °C, 1390 mm; National Climatic Data Center: http://cdo.ncdc.noaa.gov). Climate data were collected onsite with micrometeorological equipment including measurement of precipitation, wind, upper soil water content (0–20 cm; TDR probes), and photosynthetically active radiation (PAR), air temperature and relative humidity above (22 m) and beneath (2 m) the canopy. The site, experimental design and FACE apparatus have been previously described (Norby et al. 2001), and research results including micrometeorological data are documented and archived for public use at http://public.ornl.gov/face/ORNL/ornl_data.shtml.

Stand characteristics

Seasonal patterns of foliar emergence, expansion, senescence and abscission were assessed by measurement of canopy attenuation of PAR using quantum sensors placed above and below the canopy, by frequent collection of leaf litter from seven 0.2 m^2 litter traps per plot, and by establishment of leaf area to leaf mass allometry as previously described (Norby et al. 2003). Nitrogen (N) concentration was assessed for oven-dry (70 °C) foliar litter using an elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA).
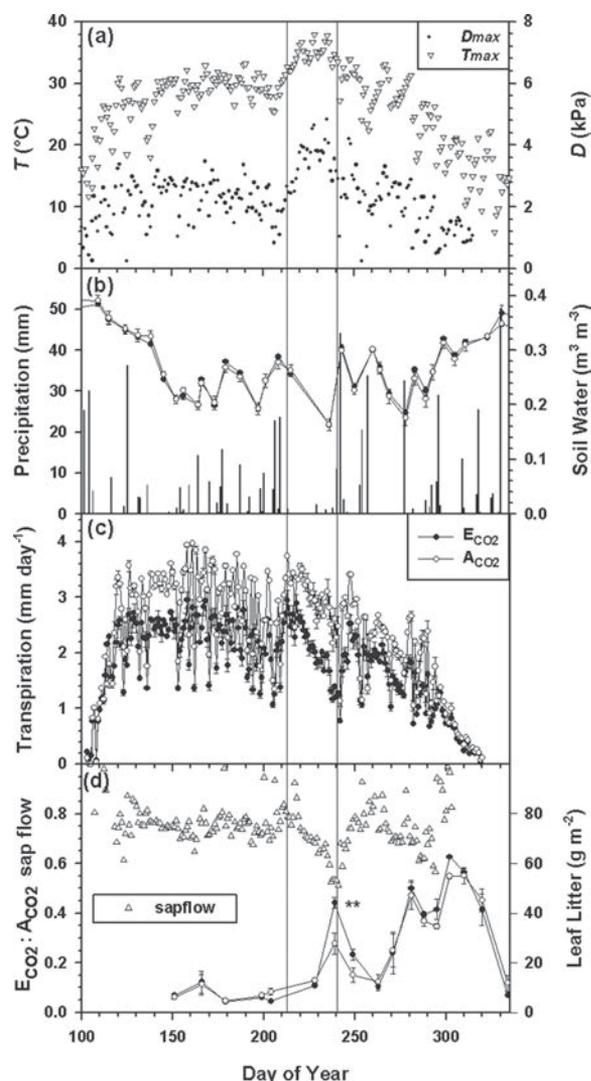

Figure 1. (a) Maximum vapor pressure deficit (*D*) and maximum air temperature (*T*) measured above the canopy (22 m). (b) Daily precipitation and soil water content (0–20 cm) during 2007 at a 19-year-old sweetgum plantation exposed to long-term CO_2 treatments. (c) Seasonal patterns of stand-level transpiration for sweetgum trees exposed to E_{CO_2} or A_{CO_2} treatments as scaled from stem sap flow measurements in eight trees per treatment. (d) Relative sap flow ratio between treatments and mass of leaf litter collected by treatment (ground area basis). Double asterisks denote significant treatment difference in litter at *P* < 0.01. The vertical lines delineate an extreme drought period.

Seasonal dynamics of LAI from non-drought, drought and post-drought years were used to assess the impact of the 2007 drought, and were supported by data collected in previous years (Norby et al. 2003, Norby and Tharp 2007), including from a prior drought period in 2002.

Current and previous year branch growth (as length increment) were measured in situ and from excised branches accessed via the FACE infrastructure towers surrounding each ring in fall 2007, and in summer 2008 after full canopy expansion. Root length production and mortality were assessed every 2 weeks using minirhizotron imaging to a depth of 60 cm ($n = 5$ per ring) (Norby et al. 2004, Iversen et al. 2008), and data are available through the FACE website (see above). Results were scaled by root standing crop for each tube to estimate relative treatment differences during the drought period.

The BA growth increment of all trees in each ring was measured monthly during each growing season using previously installed metal dendrometer bands (Norby et al. 2001). Sapwood areas (SAs) of all trees in each ring were determined after expanding the relationship between BA and bark depth previously established onsite for 58 trees (3.1–16.3 cm diameter at breast height; Wullschleger and Norby 2001) to include larger trees. Bark depth was measured at breast height at four locations around the bole of 12 additional trees with diameters ranging from 15.0 to 21.8 cm. Sapwood area (cm^2) was linearly related to BA (including bark) for all 70 sample trees:

$$SA = 0.792 \times BA - 5.18 \ (R^2 = 0.99) \qquad (1)$$

Sapwood depth was assumed to extend from the phloem–xylem interface to the center of the tree.

Sap flow analysis

Sap velocity (or sap flux density) was quantified using Granier-style thermal dissipation probe sets installed at multiple depths into the xylem of four trees in each of the two E_{CO_2} and two A_{CO_2} treatment rings with FACE apparatus. Trees were selected based on canopy class: one suppressed, two co-dominant and one dominant, which reflected the current distribution of trees at the site. Probe sets consisting of an unheated and a heated probe spaced 5 cm apart (Dynamax Inc., Houston, TX, USA) were installed radially at depths of 1.5 and 2.5 cm in all trees, with an additional probe set installed at 7 cm in only the dominant tree in each ring. Probe sets were installed at 1.3 m on the north aspect of the trees, vertically and horizontally offset from other probe sets by 5–10 cm. Probe sets were insulated with polystyrene foam and reflective bubble insulation to reduce confounding thermal variation. Assessment of potential inter-tree variability can require a greater number of deployed sensors than used in this study, as demonstrated for multi-aged *L. styraciflua* growing in pine-dominated forest (Oren et al.

1998). In our even-aged, planted stand, we assumed that radial and diameter-based sensor deployment represented the majority of sap flow variation. We further assumed uniform sap flow circumferentially around the bole for this comparative study.

The temperature (T) difference between the heated and unheated probe is proportional to the electrical potential difference (ΔV) within the probe set and can be related to sap velocity (v_s; m^3 m^{-2} s^{-1}) based on the empirical relationship between ΔV and the heat dissipation attributable to sap flow near the heated probe (Granier 1985, Lu et al. 2004):

$$v_s = 118.99 \times 10^{-06} \times \left(\frac{\Delta V_{max} - \Delta V}{\Delta V} \right)^{1.231} \qquad (2)$$

where ΔV_{max} is the voltage difference between probes when sap flow is zero. The Dynamax sensor design varies from Granier's original design; thus extension of his empirical relationship to this sensor, in different species, under different conditions may result in scaling errors. Indeed, this thermal dissipation sensor can substantially underestimate total sap flow when using the standard Granier relationship, which suggests that species-specific calibrations are needed to assess absolute flow (Steppe et al. 2010). Even so, the sensors do provide rigorous relative values of sap flow that allow one to test for treatment effects, as in this comparative study.

Nocturnal recharge of capacitance and nocturnal transpiration can prevent daily attainment of zero-flow depending on physiological and environmental conditions, which can result in measured ΔV_{max} values that are lower than actual $\Delta V_{max.}$, which would lead to underestimates of flux if not corrected. In addition, xylem response to wounding from probe installation or fluctuations in xylem water content may lead to seasonal shifts in ΔV_{max} between the heated and unheated probes (Lu et al. 2004). Therefore, we modeled ΔV_{max} for each sensor based on linear regression of the multi-day maximum value of ΔV_{max} within successive 5-day intervals through the season. Interval size was selected based on the maximum interval that fitted the seasonal fluctuations of both declining trends in ΔV_{max} associated with water deficit and increasing trends in ΔV_{max} following significant precipitation events. These modeled, near-zero-flow values were used to reduce error in the calculation of sap flux density across periods when zero-flow conditions were likely not achieved.

In order to minimize background error associated with probe-, tree- and depth-specific patterns of ambient T fluctuations, heaters on all probes were periodically (every 3–4 weeks) unplugged for 2–3 days. The resulting diurnal patterns of probe ΔV during unheated periods were then subtracted from calculated fluxes during periods when power was supplied to the probe heaters. Voltage differences between probes were sampled every 15 min during the growing season and

stored on a data logger (model CR10X; Campbell Scientific, Logon, UT, USA).

Radial patterns of sap velocity were established using measurements of sap flux density at 1.5, 2.5 and 7.0 cm within the dominant trees. Whole-tree sap flow (Q; m^3 m^{-2} s^{-1}) was calculated by linear interpolation of radial patterns of sap velocity at 15 min intervals through each consecutive annulus of SA bound by sensors installed at different depths:

$$Q = \Sigma(v_s \times SA)_i, \quad (3)$$

where i represents the different radial depth annuli sampled.

Diurnal patterns of whole-tree sap flow for sampled trees in each ring were estimated using treatment-specific linear regressions of Q against SA ($Q = aSA + b$; $n = 8$) at 15 min intervals from the eight measurement trees in each treatment. These regression equations were applied individually to all trees ($n = 74$–90 trees per ring) at 15 min intervals within each treatment ring, and then summed to yield comparative diurnal patterns of stand-level sap flow across the season.

Modeled canopy conductance and A_{net}

Canopy conductance was estimated during the daytime using an inversion of the Penman–Monteith equation as previously performed at this site (Wullschleger et al. 2002):

$$g_c = \frac{\gamma \lambda E_c g_a}{s(R_{net} - G) + \rho c_p D g_a - \lambda(s + \gamma)E_c} \quad (4)$$

where γ is the psychrometer constant (kPa °C^{-1}), λ is latent heat of vaporization (J kg^{-1}), E_c is canopy transpiration (kg m^{-2} s^{-1}), g_a is aerodynamic conductance (m s^{-1}) (Granier et al. 2000) and s (kPa °C^{-1}) is the slope of the saturation vapor pressure function at T_{air} adjusted by elevation (230 m). R_{net} (J m^{-2} s^{-1}) is the leaf-level balance of absorbed and emitted short- and long-wave radiation, which assumed 50% absorbance of direct incident short-wave radiation (measured) at the abaxial leaf surface, 10% reflected shortwave radiation at the adaxial leaf surface, where $T_{soil\ surface}$ (modeled from measured $T_{soil\ 10cm}$; $T_{air\ 2m}$), and horizontal leaf display in full sun in the upper canopy. G is soil heat flux (assumed to be 10% of R_{net} based on maximal summer values at a nearby site; Wilson and Baldocchi 2000), ρ is the density of dry air (kg m^{-3}), c_p is the specific heat of dry air at constant pressure (29.3 J kg^{-1} °C^{-1}) and D is the leaf-air vapor pressure deficit (kPa). g_c was converted from ground-area-based units (m s^{-1}) to leaf-area-based units (mmol m^{-2} s^{-1}) by multiplying by a unit conversion factor (Pearcy et al. 1989) and dividing by LAI, thereby reflecting average canopy stomatal conductance.

The sensitivity of canopy conductance to changes in vapor pressure deficit was assessed using an empirical equation consistent with theoretical stomatal behavior to regulate leaf water potential. The model scales the response of g_c to D based on a reference conductance, g_{cref} (g_c at $D = 1.0$ kPa), where $g_c = -m \ln(D) - g_{cref}$ (Oren et al. 1999). The slope of the model, m, represents the absolute stomatal sensitivity to D. Boundary line analysis of the relationship between g_c and D was assessed at multiple PAR levels (Oren et al. 1999, Schäfer et al. 2000).

Gross primary production, or net canopy photosynthesis (Kim et al. 2008), was estimated from canopy conductance based on Fick's law:

$$A_{net} = g_c C_a \left(1 - \frac{C_i}{C_a}\right) \quad (5)$$

where diffusive carbon uptake through stomata is approximately equivalent to diffusive water loss from stomata divided by their relative diffusivity (1.6), C_a is atmospheric CO$_2$ concentration (measured above the canopy) and C_i/C_a is the ratio of intercellular to atmospheric CO$_2$ concentrations based on A–C_i curves collected from the mid- to upper canopy from late July through September 1998–2000 (Gunderson et al. 2002), 2008 and 2009. There was no treatment difference in the declining linear relationship between canopy conductance and C_i/C_a in late summer, so a single relationship was used: $C_i/C_a = 0.631 + 0.267 g_c$ ($n = 19$; $R^2 = 0.47$). We computed g_c on an hourly time step when PAR >50 μmol m^{-2} s^{-1}, $D > 0.1$ kPa and excluding precipitation events. All model inputs were assumed equivalent between treatments except for the seasonal pattern of canopy stomatal conductance and its dependence on D, LAI, C_a and C_i/C_a.

This approach is similar to the canopy conductance constrained carbon assimilation (4C-A) method first introduced by Schäfer et al. (2003) and further improved by Kim et al. (2008); however, our analysis did not include the detailed assessment of differential functional responses across multiple canopy layers. While other CO$_2$-driven changes in leaf anatomical or physiological characteristics, as well as structure or environmental conditions through the canopy, can impact thermal heat exchange, leaf conductance and estimates of A_{net} (e.g., Schäfer et al. 2003, Kim et al. 2008), they were not measured or modeled for this exercise. Missing data (~19%; primarily during periods when sap flow heaters were off and early/late in the season) were gap-filled using linear regression techniques at various time bin intervals; D, PAR, precipitation and day of year (DOY) were independent variables.

Physiological response to stress

To assess short-term temperature impacts on carbon uptake in healthy leaves, foliar photosynthetic capacity was sampled across an imposed thermal gradient in September 2008 (1 year post-drought). Excised, dominant upper canopy branches were re-cut under water and fully hydrated tissue

was placed in a growth chamber. Light-saturated photosynthesis (A_{max}) at treatment growth CO_2 (400 or 550 ppm) was assessed by infrared gas analysis (model 6400XT; LI-COR Biosciences) as branches were exposed to progressively increasing T (25–45 °C over ~4 h; n = 14–19 per treatment).

To assess potential loss of conductivity under drying conditions, upper canopy branches were collected following the extreme drought event in September 2007. Specific hydraulic conductivity was measured by perfusion of a filtered 0.01 M HCl solution at low pressure (0.006 MPa) through current, 1- and 2-year-old branch segments (diameter 5–10 mm; length 20–35 cm; n = 27–37 per treatment). After assessment of native conductivity (native k_s), the branches were submerged in solution and placed under vacuum to remove emboli. Maximum conductivity (k_{s-max}) was measured in the rehydrated branches, and then a subset of 1-year-old branch segments (n = 8 per treatment) was used to measure xylem vulnerability to embolism by the air-injection method (Sperry and Saliendra 1994, Domec et al. 2004). In this technique, a pressure chamber is sealed around the sample such that the ends of the branch protrude from the chamber. The branch is subjected to stepwise increases in air pressure that induce xylem cavitation and the progressive loss of k_s. Relative conductance is plotted as a function of pressure to produce a xylem vulnerability to embolism curve that describes the potential loss of hydraulic function in relation to drought stress. The pressure at which 50% of conductivity is lost (P50) was used to test treatment differences in potential vulnerability to embolism.

Statistics

Treatment differences in growth parameters and LAI were analyzed using analysis of variance techniques (n = 2 replicate rings for E_{CO_2} and n = 3 for A_{CO_2}). A repeated measures mixed model was used to test for significant treatment differences in sap flow, soil moisture content, LAI, leaf litter biomass, or relative root production and mortality through time (contrasting treatment × date interactions). Treatment effects based on sap flow were assessed in only two rings per treatment. Data manipulation and statistical procedures were completed using SAS statistical software (ver. 9.1.3, SAS Institute, Cary, NC, USA). Differences were considered statistically significant at P < 0.1.

Results

Site water dynamics

Stand-level transpiration in E_{CO_2} plots was 72% of transpiration in A_{CO_2} plots during early and mid-summer (t = 5.4; P < 0.05; Figure 1c). Scaling transpiration from whole-tree sap velocity (Q) to the stand level through linear regression ($Q = aSA + b$) was largely controlled by treatment differences in mean values of SA distribution. The regression parameter estimate (slope)

varied during the day, and the maximum slope was twice as steep for E_{CO_2} compared with A_{CO_2}. The intercept (b) was generally higher for A_{CO_2}, which resulted in greater sap flow in A_{CO_2} small trees but less flow in A_{CO_2} large trees, compared with E_{CO_2} trees.

Transpiration increased in both treatments in response to precipitation events, but subsequently declined as water became more limiting. There was no evidence of a treatment × timing interaction on transpiration under drying conditions until late summer as the drought developed (Figure 1c). Transpiration declined linearly during the drought for both treatments; however, the CO_2 reduction of transpiration increased such that by late August E_{CO_2} trees used only 55% that of A_{CO_2} trees. Transpirational response to the drought increased with tree size, but there was no significant treatment difference between the relationships (Figure 2). Sap flow was reduced by ~60% during the drought in the largest trees with greater stand dominance and canopy area than smaller trees, where sap flow was only reduced by ~20%. Differences in BA distribution (see Plant productivity below) led to much greater stand-level reductions in transpiration in E_{CO_2} plots. Elevated CO_2 sap flow was lower across tree size, and flux was more depressed by drought than A_{CO_2} trees. In both treatments the flux in large trees decreased more during drought than in small trees.

Soil water content in the upper 20 cm declined from field capacity in early April to ~0.15 m^3 m^{-3} by the end of the acute drought period (Figure 1b), approaching a soil water potential of −1 MPa based on the soil water release curve (data not shown). This is a steep region of the soil water release curve
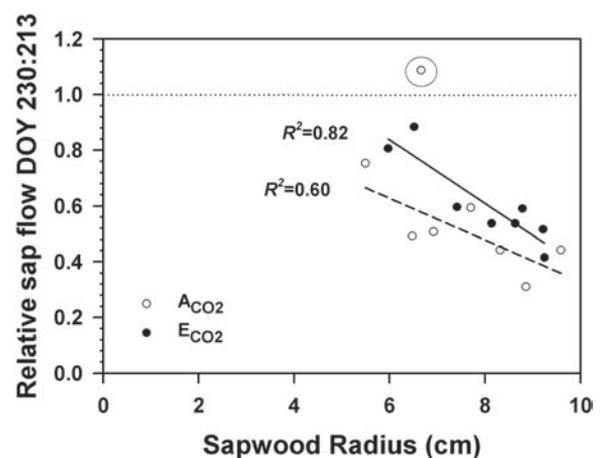

Figure 2. Relative sap flow decline in relation to tree size (as sapwood radius) in individual A_{CO_2} or E_{CO_2} treatment trees during the acute drought. Sap flow declined linearly in 15 of the 16 trees, and was expressed as the ratio of total daily sap flow toward the end of the drought (DOY 230) relative to sap flow at the beginning of the drought (DOY 213). Sap flow in one A_{CO_2} tree (circled) peaked at DOY 216 and then remained constant throughout the drought, and was not used in regressions.

(e.g., 0.13 m^3 m^{-3} ~ −2.5 MPa), resulting in very little upper soil water availability during the drought. There was no difference in upper soil water content between treatments throughout the summer.

Canopy conductance and A$_{net}$

Canopy conductance (g_c) was primarily dependent on PAR and vapor pressure deficit (D), and declined exponentially with increasing D for both treatments (Figure 3a). The response of g_c to D (stomatal sensitivity) was proportional to a reference conductance at low D (g_c at $D = 1$ kPa). The relative stomatal sensitivity (the slope of the response in relation to the reference conductance) was conserved across CO$_2$ treatments at 0.62 (Figure 3b), similar to values reported for various tree species earlier (Oren et al. 1999, Kim et al. 2008). Absolute stomatal sensitivity to D was 27–42% lower for E$_{CO_2}$ plots relative to A$_{CO_2}$ plots for PAR levels >150 µmol m^{-2} s^{-1} (Figure 3c), in agreement with results from small *L. styraciflua* trees at a FACE site in North Carolina, USA (Duke FACE) in 2000 (Domec et al. 2010). At lower PAR levels, there was no relationship between g_c and ln D ($R^2 < 0.2$) as stomata are more strongly controlled by PAR, and because under low D and low sap flow measurement uncertainties are relatively high (Ewers and Oren 2000, Schäfer et al. 2000). Mean daily g_c declined substantially during the extreme drought period (Figure 4) as D consistently exceeded 3 kPa and approached 5 kPa late in the drought. The decline in g_c correlated with declining soil water content, increasing T and increasing D (Figure 1a and b). Elevated CO$_2$ treatments resulted in significantly lower g_c than A$_{CO_2}$ treatments, and the relative difference increased twofold during the drought (Figure 4, inset).

Elevated CO$_2$ did not increase modeled mean daily A_{net} during the moderate drought period (DOY 119–210) as compared with A$_{CO_2}$ plots (E$_{CO_2}$: A$_{CO_2}$ A_{net} ~1.00). A_{net} increased as leaves expanded in spring, reaching up to ~15 g C m^{-2} day^{-1} for some days during mid-summer (Figure 5). A_{net} declined across all plots during the acute drought period beginning DOY 210; however, the decline was much steeper in E$_{CO_2}$ plots. This led to progressively less A_{net} in E$_{CO_2}$ plots relative to A$_{CO_2}$ plots during the drought, i.e., −39% by the end of the drought (Figure 5, inset). Across the drought period, there was a 15% cumulative reduction in A_{net} for E$_{CO_2}$ plots relative to A$_{CO_2}$ plots (August). The relative benefit of E$_{CO_2}$ to A_{net} returned a week after a significant rainfall event ended the drought, exhibited by E$_{CO_2}$: A$_{CO_2}$ A_{net} ~0.96 (September), similar to the pre-drought ratio (0.99; July), although absolute values of A_{net} did not fully return to pre-drought levels (Figure 5).

Foliar dynamics

The acute drought during the growing season significantly reduced stand transpiration, especially in E$_{CO_2}$ plots. As transpiration declined and the ratio of E$_{CO_2}$: A$_{CO_2}$ sap flow declined,

there was a concurrent increase in premature foliar abscission, especially under E$_{CO_2}$ (Figure 1d). Foliar loss was 30% greater for E$_{CO_2}$ trees in comparison with A$_{CO_2}$ trees during the late drought (DOY 228–239; $t = 2.8$, $P = 0.008$), with a loss of
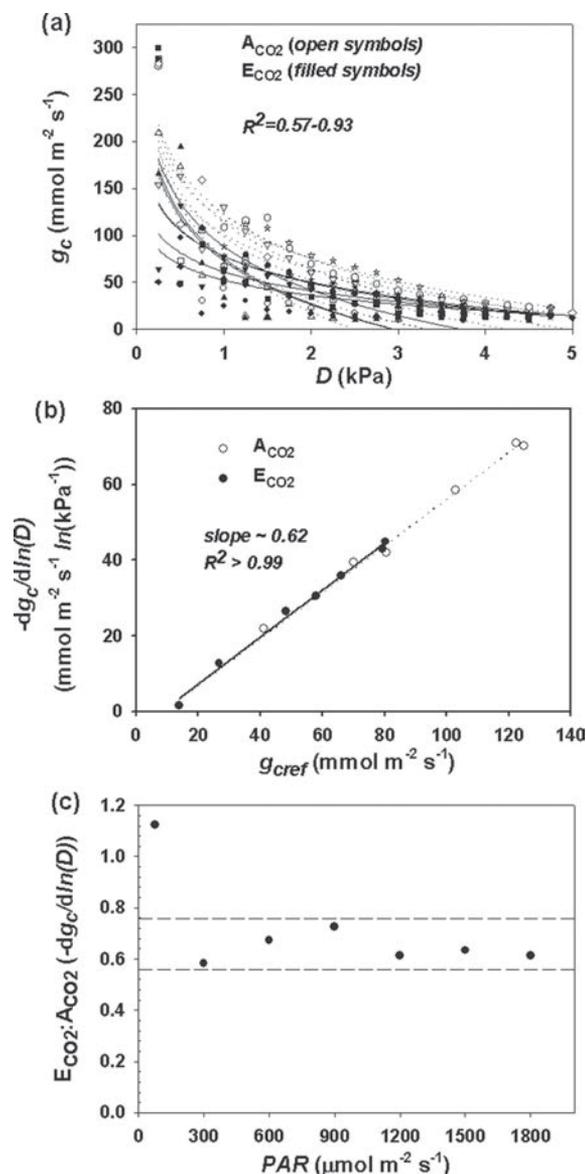

Figure 3. Canopy conductance (g_c) in relation to vapor pressure deficit (D) for sweetgum exposed to E$_{CO_2}$ or A$_{CO_2}$ treatments derived from sap flow measurements mid-May through the end of an extreme droughty period in August 2007. (a) Boundary line analysis of (maximum) canopy conductance at seven levels of PAR (<150, 150–450, 450–750, 750–1050, 1050–1350, 1350–1650 and >1650 µmol m^{-2} s^{-1}), depicted by different symbols. Magnitude of g_c was positively related to PAR level. Two-parameter logarithmic regressions were fitted for A$_{CO_2}$ (dotted lines) or E$_{CO_2}$ (solid lines) treatments (R^2 increasing with PAR level). (b) Sensitivity (slope) of canopy conductance to vapor pressure deficit ($m = −dg_c/d$ ln (D)) in relation to a reference conductance (g_{cref}) that was dependent upon magnitude of PAR (derived from panel a). (c) Relative CO$_2$-treatment sensitivity of canopy conductance to vapor pressure deficit (ratio of E$_{CO_2}$: A$_{CO_2}$) in relation to increasing magnitude of PAR.
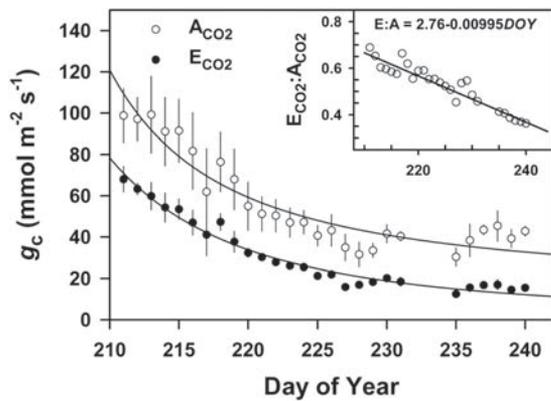
Figure 4. Daily mean canopy conductance (g_c) during the extreme droughty period (DOY 210–240) for sweetgum exposed to E_{CO_2} or A_{CO_2} treatments; (inset) relative g_c ratio of E_{CO_2} : A_{CO_2}.
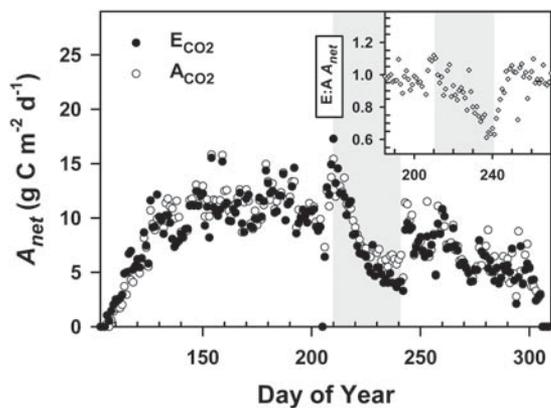

Figure 5. Modeled daily net canopy photosynthesis (A_{net}) during 2007 for sweetgum exposed to E_{CO_2} or A_{CO_2} treatments; (inset) relative A_{net} ratio of E_{CO_2}: A_{CO_2}.

15 or 10% of total leaf area, respectively. All other litter collection periods yielded no difference between treatments.

Nitrogen concentration of litter collected during the acute drought (6.6 mg g^{-1}) was only 55% of the N concentration in green leaf litter collected during the summer prior to the drought (11.9 mg g^{-1}), indicating that much of the N was translocated from the leaf prior to premature senescence in August. Nonetheless, these values were ~15% greater ($F = 3.5$; $P < 0.1$) than N concentration of the bulk senescent litter collected during the seasonal autumn leaf fall (5.8 mg g^{-1}), suggesting that N translocation from leaves shed during August was incomplete. There was no treatment effect on litter N content.

Premature foliar abscission occurred during the hottest time of year as T_{air} reached 38 °C. Both plots experienced a strong decline in g_c and thus latent heat loss through transpiration, which likely resulted in leaf temperatures (T_{leaf}) at the canopy surface exceeding 40 °C in both plots. Due to greater

relative reductions in g_c for E_{CO_2}, T_{leaf} in E_{CO_2} plots was expected to be slightly greater than T_{leaf} in A_{CO_2} plots under low wind conditions. To estimate potential treatment effects on T_{leaf}, we modeled fully exposed, average-sized leaves horizontally displayed at the upper canopy surface using canopy-averaged stomatal conductance and based on the energy transport equations described by Campbell and Norman (1998). The rough calculations from this hypothetical model suggest that E_{CO_2} reductions in g_c during drought could lead to midday T_{leaf} 1–2 °C higher in E_{CO_2} trees than in A_{CO_2} trees (data not shown).

Plant productivity

Leaf area index peaked at 5.4 for both treatments, but total annual leaf litter mass was ~7% greater in E_{CO_2} treatments ($t = 5.8$; $P < 0.01$), a consequence of ~8% greater ($t = 4.2$; $P < 0.1$) leaf mass per area for E_{CO_2} treatments as compared with A_{CO_2} treatments, consistent with previous measurements in 1999 and 2000 (Norby et al. 2003). Leaf area index was on average 8–9% greater in the E_{CO_2} rings than in A_{CO_2} rings during mid-summer in non-drought years ($F = 3.8$; $P = 0.10$; Figure 6a and b). In 2006, maximum LAI in E_{CO_2} rings was ~10% greater than in A_{CO_2} rings (Figure 6b), and this difference was maintained across the entire growing season ($F = 5.9$; $P < 0.1$). In 2007, there was no difference in LAI between plots until late summer when relative LAI declined in E_{CO_2} plots as compared with A_{CO_2} plots in response to enhanced early foliar senescence (Figure 6a). Similar patterns of drought-induced acceleration of leaf loss in E_{CO_2} plots were observed during a previous drought at the site. In 2001 (non-drought year), LAI was greater in E_{CO_2} plots in mid–late summer ($t > 2.6$; $P < 0.01$). In 2002 (drought year), LAI was the same across treatments in mid-summer, but dramatically lower in E_{CO_2} plots following extended drought in late summer (Figure 6a and b).

Mean BA increment (BAI) growth was not significantly affected by the 2007 drought and was within 95% of 2006 (non-drought) values. Basal area increment in suppressed and intermediate E_{CO_2} trees (BA < median) was 30% lower than in A_{CO_2} trees in both drought and non-drought years ($t = 1.7$–2.0; $P = 0.15$–0.23), while dominant tree (largest ~15–20%) BAI was not statistically different between E_{CO_2} (10.0–10.6 cm^2 tree^{-1}) and A_{CO_2} (9.4–10.0 cm^2 tree^{-1}) treatments. There was no treatment effect on stand-level BAI in either pre- or post-drought years. Mean annual height growth increment declined to <10 cm for both treatments in 2007, which continued a slow multi-year trend and thereby cannot be directly linked to the drought, but rather to reductions in N availability linked to stand development (Norby et al. 2010).

Dominant, upper canopy branch growth in 2007 (drought) and 2008 (post-drought) was 40% lower than growth in 2005 or 2006 ($P < 0.01$, $n = 12$–31 per treatment per year).
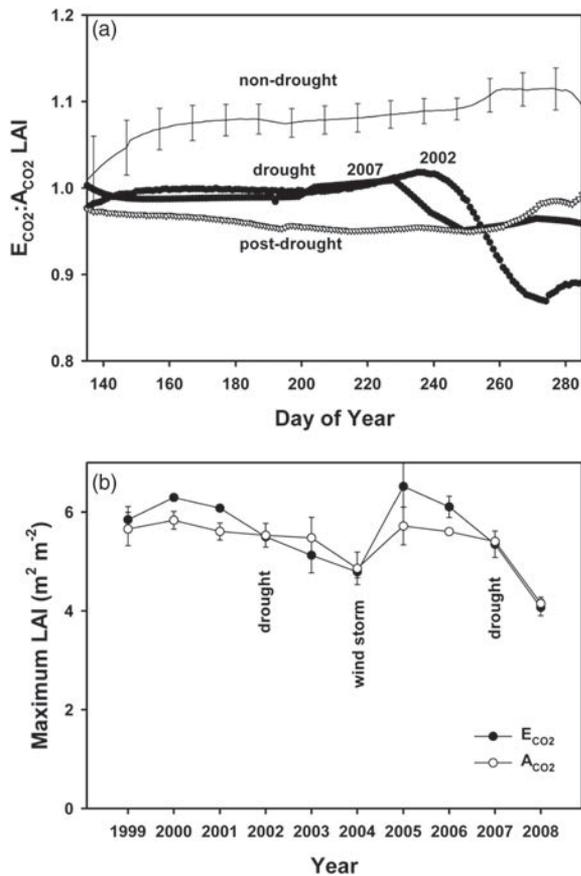
Figure 7. Light-saturated photosynthesis at growth CO$_2$ concentrations (400 or 550 ppm) in response to increasing T for fully rehydrated branches excised from A$_{CO_2}$ or E$_{CO_2}$ treatments ($n = 14$–19 per treatment).

Figure 6. (a) Ratio of LAI in E$_{CO_2}$ to that in A$_{CO_2}$ treatment plots from June to October by year (1999–2007), displaying non-drought years ($n = 6$; ±SE), drought years (2002, 2007) and a post-drought year (2003). Significant E$_{CO_2}$: A$_{CO_2}$ LAI reductions during drought years occurred by DOY 260 in 2002 ($t = 2.3$; $P < 0.1$) and DOY 250 in 2007 ($t = 1.6$; $P = 0.11$) based on a repeated measures mixed model. (b) Peak seasonal LAI across years for E$_{CO_2}$ ($n = 2$) and A$_{CO_2}$ ($n = 3$) treatment plots; ±SE. Treatment differences were significant at $P < 0.15$ for 2000–01 and at $P < 0.07$ for 2006 based on analysis of variance.

Mid-lower canopy growth rates in 2007 (~8.0 ± 1.8 cm) were less than upper canopy growth (~17.0 ± 1.5 cm), and not noticeably affected by the drought. There was no treatment effect on branch elongation before, during or after the extreme weather of 2007.

Average fine-root production was 75% lower in the E$_{CO_2}$ rings compared with the A$_{CO_2}$ rings during the extreme drought period, but results were not significant ($P = 0.18$, data not shown). Root production was evident in 40–60% of sampled minirhizotron tubes for both treatments during the summer drought. In addition, across all minirhizotron tubes, there was a fivefold increase in relative root mortality for E$_{CO_2}$ rings compared with A$_{CO_2}$ rings as the drought progressed ($P = 0.14$). Sixty percent of E$_{CO_2}$ minirhizotron tubes displayed evidence of root mortality during this extreme drought period as compared with just 15% of A$_{CO_2}$ tubes. Wide spatial and temporal variability
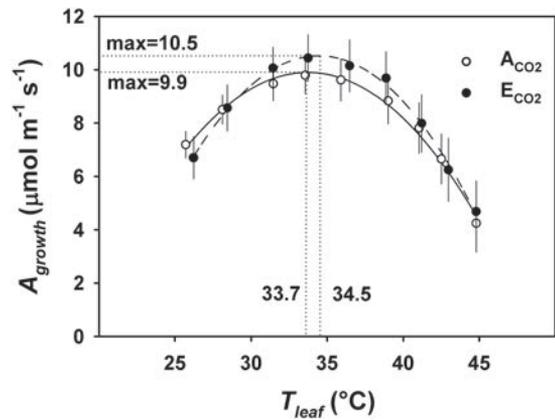
in root distribution and interception with minirhizotron tubes limits the statistical significance of results.

Physiological response to stress

The optimal T for light-saturated photosynthesis was ~34 °C, and not significantly different between treatments, although mean values of A were ~6% greater for E$_{CO_2}$ trees (Figure 7). Photosynthesis declined by 50% from peak values as T_{leaf} increased from 34 to 45 °C.

Branch specific hydraulic conductivity was not affected by treatments (Figure 8a). The mean values of native k_s were 10% ($P = 0.17$; A$_{CO_2}$) or 15% ($P = 0.10$; E$_{CO_2}$) lower than $k_{s\text{-}max}$. One-year-old E$_{CO_2}$ branches displayed greater potential loss of conductivity under drying conditions as measured by P50 based on sigmoid regressions ($P = 0.016$; E$_{CO_2}$ P50 = 5.15–6.43; A$_{CO_2}$ P50 = 5.67–6.91 MPa). At the beginning of the drought leaf water potential was −0.3 MPa at dawn and increased to −2 MPa by midday, with no difference between treatments (data not shown), a range implying that branches of either treatment did not suffer excessive hydraulic failure.

Discussion

Elevated CO$_2$ substantially reduced stand-level transpiration of *L. styraciflua* trees at ORNL FACE during an acute drought and heat event, which coincided with significant premature leaf abscission. Elevated CO$_2$ reduced stomatal conductance (g_s) and thereby directly reduced photosynthetic carbon gain. In addition, a decline in latent heat loss through evaporation may increase leaf temperature and leaf respiration, further affecting net carbon balance. Foliar stress under E$_{CO_2}$ may be manifest directly in net carbon balance or indirectly through impacts on hydraulic conductance, and in this stand resulted in enhanced premature leaf senescence and abscission for trees exposed to E$_{CO_2}$.
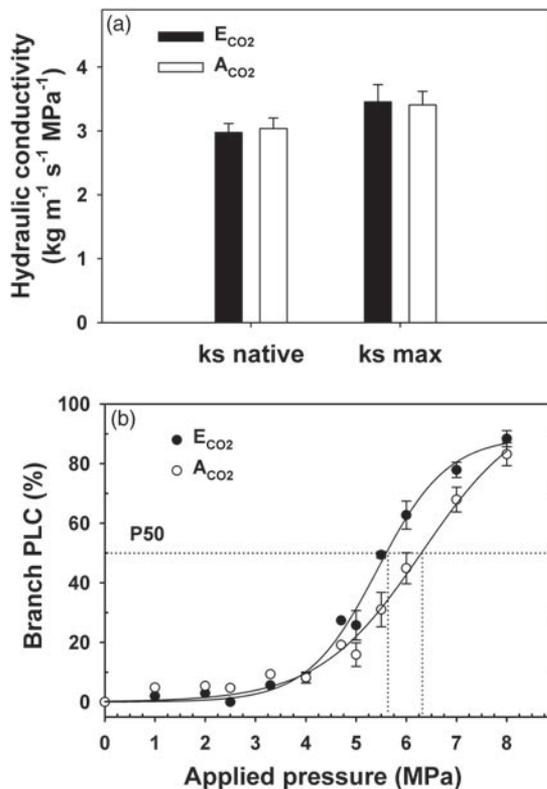
Figure 8. (a) Native and maximum xylem hydraulic conductivity (k_s) of upper canopy branches excised from A_{CO_2} or E_{CO_2} treatments in September 2007 and (b) vulnerability curves for 1-year-old branch segments that relate percent loss of hydraulic conductance (PLC) to applied pressure as an estimate of (–) xylem water potential ($n = 8$ per treatment).

Sap flow, g_c and A_{net}

The reduction in transpiration under E_{CO_2} in the current investigation was primarily dependent upon reduced g_s. Indeed, previous results for sweetgum trees in a mixed *Pinus taeda* stand and at ORNL FACE described a 28–44% reduction in leaf level g_s under E_{CO_2} treatments (Wullschleger et al. 2002, Herrick et al. 2004, Norby et al. 2006). Elevated CO_2 reduced sap flow by up to 25% for mid-canopy *L. styraciflua* trees in a *P. taeda* stand exposed to FACE (Schäfer et al. 2002), similar to the current and past response at ORNL FACE (Wullschleger et al. 2002, Norby et al. 2006, Warren et al. 2010). An expanded examination of sap flow in additional trees at the site in 2008 describes a similar reduction in sap flow in E_{CO_2} plots (Warren et al. 2010).

Transpiration declined at a greater rate for E_{CO_2} trees than for A_{CO_2} trees during the severe drought period, such that by the end of the drought E_{CO_2} trees used ~45% less water than ambient trees. This was in contrast to results from *L. styraciflua* trees exposed to CO_2 treatments at the Duke FACE site, where sap flux was ~10% lower in E_{CO_2} treatments during moderate drought in July 1997 and 1998, but substantially lower (~25%) in E_{CO_2} trees during wetter periods in July 1999 and 2000

(Schäfer et al. 2002). In that study the CO_2 was initiated in 1996, and as the authors suggest there may have been progressively increasing shifts in physiological response that culminated in the ~25% reduction in E_{CO_2} sap flux density, regardless of exposure to moderate drought.

In our study under severe drought we hypothesized that E_{CO_2}-reduced g_s led to increased soil moisture that would maintain stomatal aperture and allow E_{CO_2} leaves to maintain greater C uptake than A_{CO_2} leaves. This hypothesis was rejected as E_{CO_2} trees experienced reduced canopy stomatal sensitivity and enhanced reduction in canopy conductance. The primary effect of this enhanced reduction in g_c during drought was a twofold decrease in assimilation for E_{CO_2} as compared with A_{CO_2} plots, which was directly correlated to the enhanced E_{CO_2} leaf loss. Drought-induced ABA may provide a mechanistic link to reduced g_s, as it has the potential to enhance stomatal response to E_{CO_2} (Leymarie et al. 1999), consistent with the greater reductions in E_{CO_2} g_s, although this response may be confounded by other environmental and physiological signals (Hetherington and Woodward 2003).

Soil water and root dynamics

Despite the significant reduction in E_{CO_2} tree water use, there was no detectable difference in upper soil water content (θ) between treatments. As upper soil water potential dropped toward −1 MPa, very little water remained available in either treatment by the end of the droughty period. It is likely that fine roots (mean diameter ~0.35 mm; Iversen et al. 2008) in that upper 20 cm largely cavitated across treatments during the drought, based on an estimated P50 from −0.1 to −0.3 MPa for small (1.6–4.1 mm) *L. styraciflua* roots at Duke FACE (Domec et al. 2009), although hydraulic redistribution could temper this response. A lack of treatment effects on upper soil water content was not unexpected, with recognition of the potential for temporal shifts in the depth of root water extraction during a prolonged dry season as upper roots cavitate (e.g., Domec et al. 2004, Warren et al. 2005). *Liquidambar styraciflua* can develop a significant taproot, and previously extracted root systems at this site revealed multiple large and small roots emanating downward from beneath the root crown to at least 1 m; roots have also been detected at depths up to 2 m during equipment installation. Elevated CO_2 has increased root production at depth in this stand based on minirhizotron analysis (Norby et al. 2004, Iversen et al. 2008). The greater root surface area available for water uptake at depth should enhance hydraulic capacity under droughty conditions and enhance hydraulic redistribution and recharge of the tree at night, thereby tempering the daily effect of VPD on conductance; however, higher resistance in the smallest-diameter roots may limit benefits to these ephemeral structures (Valenzuela-Estrada et al. 2009). Reduced water use and greater potential capacity for water uptake at depth did not

buffer trees against premature leaf loss in E$_{CO_2}$ plots. In fact, during the extreme drought, there was some evidence of enhanced fine-root mortality in E$_{CO_2}$ plots, an outcome that would be expected if carbon transport to roots declined or if E$_{CO_2}$ increased vulnerability to embolism.

Hydraulic conductivity

During drought, leaf hydraulic conductivity generally declines as embolism of stem, petiole and especially leaf xylem tissue progressively increases (Salleo et al. 2002). Larger conduit diameter is often associated with increased cavitation potential (Tyree et al. 1994), and tracheid or vessel size is increased by E$_{CO_2}$ in some studies (e.g., Ceulemans et al. 2002). Elevated CO$_2$ does increase year-old *L. styraciflua* branch vulnerability to embolism in this FACE study, which may be caused by reduced growth limitations for E$_{CO_2}$ tissues prior to severe water stress (e.g., increased vessel cell expansion, larger cells), or by other changes in xylem anatomical, structural or chemical properties that may impact hydraulic response to stress. Elevated CO$_2$ was also found to increase vulnerability to embolism in branches of *L. styraciflua* at Duke FACE (Domec et al. 2010). In that study, branch P50 was 1.1 (E$_{CO_2}$) and 2.0 MPa (E$_{CO_2}$), 3–5 times lower than our results, and perhaps attributable to use of branch segments <15 cm in length (we found that the longest vessel in *L. styraciflua* branches often exceeded 15 cm). Leaves are much more vulnerable to embolism than branches, and in our study leaf water potential reached at least −2 MPa, although differential treatment impacts of leaf sensitivity to embolism were not quantified. Some studies report increased leaf sensitivity to cavitation under E$_{CO_2}$, as demonstrated for *P. taeda* with greater daily relative reductions in leaf hydraulic conductance under E$_{CO_2}$ (Domec et al. 2009) resulting in greater water stress. Elevated CO$_2$ reduced leaf specific hydraulic conductivity and petiole vessel area:leaf area for birch and oak saplings exposed to E$_{CO_2}$ (Eguchi et al. 2008), demonstrating an E$_{CO_2}$ impact on hydraulic architecture and maximum conductance. In our study, it is possible that the extreme conditions in August induced catastrophic loss of leaf or root hydraulic conductance to a greater extent in E$_{CO_2}$ trees than in A$_{CO_2}$ trees. Loss of root conductive capacity would necessitate hydraulic adjustment such that leaf area shed during the drought may allow the trees to increase leaf specific hydraulic conductivity and thereby maintain positive carbon balance in the residual leaves. Along with direct E$_{CO_2}$ stomatal-dependent reductions in net carbon balance, potential loss of root hydraulic conductivity is consistent with enhanced root mortality and increased premature leaf abscission in E$_{CO_2}$ plots.

Leaf T

High air temperature is directly linked to greater *D*, and may exacerbate E$_{CO_2}$-dependent reductions in g_s through impacts on both assimilation and respiration. Increasing midday T_{air} from 45

to 53 °C reduced or had no effect on g_s in glasshouse-grown E$_{CO_2}$ seedlings of *Yucca* sp., while g_s increased for A$_{CO_2}$ control plants (Huxman et al. 1998). Similarly, E$_{CO_2}$ reduced g_s by 40% for cotton exposed to water deficit and 45.5 °C T_{air} in open top chambers, while A$_{CO_2}$ only reduced g_s by 7% (Radin et al. 1987), illustrating the potential for E$_{CO_2}$ to increase stomatal sensitivity to drought. Elevated CO$_2$ also reduced g_s under extreme heat in more mesic tree seedlings such as *A. pensylvanicum* (Bassow et al. 1994), and the consequential reduction in net carbon balance and potential heat stress was substantial enough to induce mortality. In our study, it is likely that maximum T_{leaf} during the drought peaked between 40 and 44 °C for both treatments, a range that remains conducive to photosynthesis under well-hydrated conditions, albeit at declining rates beyond the optimal *T*. Our rough calculations suggest that T_{leaf} in E$_{CO_2}$ could be greater than in A$_{CO_2}$ due to reduced g_s. Based on the *T* response curve (Figure 7), the benefit of E$_{CO_2}$ to assimilation (A$_{net}$) declines rapidly with increased T_{leaf} above the optimum. For example at 42 °C, A_{net} in E$_{CO_2}$ foliage is 6.5% greater than A_{net} in A$_{CO_2}$ foliage; however, if E$_{CO_2}$ increased T_{leaf} by 1 °C, to 43 °C, then A_{net} in E$_{CO_2}$ foliage is 7% less than A_{net} in A$_{CO_2}$ foliage. Thus, depending on diurnal cycles of *T*, E$_{CO_2}$ C uptake may be greater than, equal to or less than A$_{CO_2}$, while E$_{CO_2}$ C release through respiration will likely be equal to or greater than A$_{CO_2}$ C release. Greater loss of E$_{CO_2}$ foliage during extreme drought and record-high T_{air} was likely attributable to a combination of effects initiated by E$_{CO_2}$, including the primary effect of reduced g_s that directly reduced assimilation, and a potential secondary effect of increased T_{leaf} that could increase respiration, reduce assimilation and further affect net carbon balance.

Comparison with the 2002 drought

Drought-initiated premature foliar abscission and loss of LAI began a week earlier in 2007 than in 2002, despite earlier drought initiation in 2002. The two acute drought periods had similar amounts of precipitation: 9.8 mm in 2002 (DOY 196–225) or 6.2 mm in 2007 (DOY 210–239) as compared with 80–90 mm for average 30-day late-summer precipitation. The difference in drought response between years may be linked to temperature patterns. Mean maximum T_{air} was 2.8 °C higher in 2007 than in 2002 across the drought period. There was also a sustained, linear reduction in g_s and an increase in T_{air} as the 2007 drought progressed. In contrast, mean maximum T_{air} in 2002 remained fairly constant and well below the record highs experienced in 2007. The higher and progressively increasing *D* associated with the 2007 drought enhanced the reduction in g_s and thus net carbon balance likely declined at a greater rate than in 2002, resulting in earlier foliar loss.

Drought effects on productivity

Enhanced productivity in E$_{CO_2}$ plots exhibited in earlier years (Norby et al. 2005) has been largely reduced in recent years,

as N has become limited and photosynthetic capacity has reached minimal levels (Norby et al. 2010). Elevated CO_2 did not enhance any measure of tree biomass production during the drought relative to trees in A_{CO_2} plots based on BA, height or branch length increment. Rather, our analysis of canopy leaf area dynamics suggests that the extreme events of 2007 reduced productivity for E_{CO_2} trees relative to A_{CO_2} trees. Elevated CO_2 enhanced loss of LAI and C uptake during each of the drought years, and thereby also likely reduced over-winter carbon storage and thus carbon available for spring growth. As a result in 2003, following the 2002 drought, E_{CO_2} net primary productivity (NPP) enhancement fell to its lowest level since the beginning of the study. Similarly, in 2008, LAI for both treatments dropped to <80% of average maximum LAI over the course of the study, with evidence of only mini-mal NPP enhancement (Norby et al. 2010). The reduction in E_{CO_2} : A_{CO_2} LAI and productivity during drought years, and particularly during acute drought periods, highlights the potential negative consequences of E_{CO_2} trees in response to prolonged drought or heat events. Similar temporal results have been described for *L. styraciflua* and pine at the Duke FACE site where foliar loss occurred earlier during moderate drought years resulting in reductions in total canopy leaf area the following year, although in contrast to our study, they found no effect of E_{CO_2} on foliar loss (McCarthy et al. 2007).

Conclusions

Elevated CO_2 reduced transpiration at this site throughout the growing season, resulting in reduced soil water extraction, and concurrently increased production of fine roots deeper in the soil. These physiological responses to E_{CO_2} should buffer trees against droughty conditions and thereby reduce potential growth limitations. Despite these apparent benefits, large reductions in g_s lead directly to large reductions in assimilation that can result in a negative leaf carbon balance and ultimately leaf loss. The reductions in g_s may also reduce latent heat loss and thereby elevate leaf temperatures, both reducing photo-synthesis and increasing respiration, which could further exac-erbate impacts of the direct effects of g_s on assimilation. In addition, through anatomical changes, E_{CO_2} may reduce xylem hydraulic resistance to cavitation in ephemeral tissues that could compromise hydraulic capacity under drought. In this manner, E_{CO_2} may exacerbate, rather than reduce, the apparent stress response of foliage to extreme conditions, which is con-sistent with exceeding a critical threshold for maintenance of a positive leaf carbon balance that regulates foliar senescence and abscission. This research suggests that future plant response to extreme environmental conditions may be con-founded by the balance between positive and negative effects of increased atmospheric CO_2 concentrations. Characterization of the physiological and hydraulic mechanisms and thresholds responsible for loss of leaf function and premature leaf loss under future climate scenarios should be considered in the context of their temporal implications for plant vigor and net carbon exchange, and their underlying genetic and environ-mental drivers.

References

Ainsworth, E.A. and A. Rogers. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environ-mental interactions. Plant Cell Environ. 30:258–270.

Ball, M.C., I.R. Cowan and G.D. Farquhar. 1988. Maintenance of leaf temperature and the optimisation of carbon gain in relation to water loss in a mangrove forest. Aust. J. Plant Physiol. 15:263–276.

Barker, D.H., B.R. Loveys, J.J.G. Egerton, H. Gorton, W.E. Williams and M.C. Ball. 2005. CO_2 enrichment predisposes foliage of a eucalypt to freezing injury and reduces spring growth. Plant Cell Environ. 28:1506–1515.

Bassow, S.L., K.D.M. McConnaughay and F.A. Bazzaz. 1994. The response of temperate tree seedlings grown in elevated CO_2 to extreme temperature events. Ecol. Appl. 4:593–603.

Campbell, G.S. and J.M. Norman. 1998. An introduction to environmen-tal biophysics. 2nd edition. Springer-Verlag, New York, 286 p.

Ceulemans, R., M.E. Jach, R. Van De Velde, J.X. Lin and M. Stevens. 2002. Elevated atmospheric CO_2 alters wood production, wood quality and wood strength of Scots pine *Pinus sylvestris* L. after three years of enrichment. Global Change Biol. 8:153–162.

Christensen, J.H., B. Hewitson, A. Busuioc, et al. 2007. Regional cli-mate projections. *In* Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Ciais, Ph., M. Reichstein, N. Viovy. et al. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533.

Domec, J.-C., J.M. Warren, F.C. Meinzer, J.R. Brooks and R. Coulombe. 2004. Native root xylem embolism and stomatal closure in stands of

Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. Oecologia 141:7–16.

Domec, J.-C., S. Palmroth, E. Ward, C.A. Maier, M. Thérézien and R. Oren. 2009. Acclimation of leaf hydraulic conductance and stomatal conductance of *Pinus taeda* (loblolly pine) to long-term growth in elevated CO$_2$ (free-air CO$_2$ enrichment) and N-fertilization. Plant Cell Environ. 32:1500–1512.

Domec, J.-C., K. Schafer, R. Oren, H.S. Kim, H.R. McCarthy. 2010. Variable conductivity and embolism in roots and branches of four contrasting tree species and their impacts on whole-plant hydraulic performance under future atmospheric CO$_2$ concentration. Tree Physiol. 30:1001–1015.

Eguchi, N., N. Morii, T. Ueda, R. Funada, K. Takagi, T. Hiura, K. Sasa and T. Koike. 2008. Changes in petiole hydraulic properties and leaf water flow in birch and oak saplings in a CO$_2$-enriched atmosphere. Tree Physiol. 28:287–295.

Ewers, B.E. and R. Oren. 2000. Analysis of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. Tree Physiol. 20:579–589.

Faria, T., D. Wilkins, R.T. Besford, M. Vaz, J.S. Pereira and M.M. Chaves. 1996. Growth at elevated CO$_2$ leads to down-regulation of photosynthesis and altered response to high temperature in *Quercus suber* L. seedlings. J. Exp. Bot. 47:1755–1761.

Granier, A. 1985. Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. Ann. Sci. For. 42:193–200.

Granier, A., P. Biron and D. Lemoine. 2000. Water balance, transpiration and canopy conductance in two beech stands. Agric. For. Meteorol. 100:291–308.

Gunderson, C.A., J.D. Sholtis, S.D. Wullschleger, D.T. Tissue, P.J. Hanson and R.J. Norby. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum *Liquidambar styraciflua* L plantation during three years of CO$_2$ enrichment. Plant Cell Environ. 25:379–393.

Hamerlynck, E.P., T.E. Huxman, M.E. Loik and S.D. Smith. 2000. Effects of extreme high temperature, drought and elevated CO$_2$ on photosynthesis of the Mojave Desert evergreen shrub, *Larrea tridenta*. Plant Ecol. 148:183–193.

Hendrey, G.R., D.S. Ellsworth, K.F. Lewin and J. Nagy. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO$_2$. Global Change Biol. 5:293–309.

Herrick, J., H. Maherali and R. Thomas. 2004. Reduced stomatal conductance in sweetgum *Liquidambar styraciflua* sustained over long-term CO$_2$ enrichment. New Phytol. 162:387–396.

Hetherington, A.M. and F.I. Woodward. 2003. The role of stomata in sensing and driving environmental change. Nature 424:901–908.

Huxman, T.E., E.P. Hamerlynch, M.E. Loik and S.D. Smith. 1998. Gas exchange and chlorophyll fluorescence response of three southwestern Yucca species to elevated CO$_2$ and high temperature. Plant Cell Environ. 21:1275–1283.

Iversen, C.M., J. Ledford and R.J. Norby. 2008. CO$_2$ enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. New Phytol. 179:837–847.

Jentsch, A. and C. Beierkuhnlein. 2008. Research frontiers in climate change: effects of extreme meteorological events on ecosystems. Geoscience 240:621–628.

Keel, S.G., S. Pepin, S. Leuzinger and C. Körner C. 2007. Stomatal conductance in mature deciduous forest trees exposed to elevated CO$_2$. Trees 21:151–159.

Kim, H.-S., R. Oren and T.M. Hinckley. 2008. Actual and potential transpiration and carbon assimilation in an irrigated poplar plantation. Tree Physiol. 28:559–577.

Kimball, B.A. and C.J. Bernacchi. 2006. Evapotranspiration, canopy temperature, and plant water relations. *In* Managed Ecosystems and CO$_2$: Case Studies, Processes, and Perspectives. Ecological Studies, vol. 187. Eds. J. Nösberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendrey and H. Blum. Springer, Berlin, pp 311–324.

Leuzinger, S. and C. Körner. 2007*a*. Water savings in mature deciduous forest trees under elevated CO$_2$. Global Change Biol. 13:2498–2508.

Leuzinger, S. and C. Körner. 2007*b*. Tree species diversity affects canopy leaf temperatures in a mature temperate forest. Agric. For. Meteorol. 146:29–37.

Leymarie, J., G. Lascève and A. Vavasseur. 1999. Elevated CO$_2$ enhances stomatal responses to osmotic stress and abscisic acid in *Arabadopis thaliana*. Plant Cell Environ. 22:301–308.

Long, S.P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO$_2$ concentrations: has its importance been underestimated? Plant Cell Environ. 14:729–739.

Lu, P., L. Urban and Z. Ping. 2004. Granier's thermal dissipation probe TDP method for measuring sap flow in trees: theory and practice. Acta Bot. Sin. 46:631–646.

McCarthy, H.R., R. Oren, A. Finzi, D.S. Ellsworth, H.-S. Kim, K. Johnsen and B. Millar. 2007. Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO$_2$. Global Change Biol. 13:2479–2497.

Medlyn, B.E., C.V.M. Barton, M.S.J. Broadmeadow. et al. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO$_2$ concentration: a synthesis. New Phytol. 149:247–264.

Meehl, G.A., T.F. Stocker, W.D. Collins. et al. 2007. Global climate projections. *In* Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Norby, R.J. and M.L. Tharp. 2007. ORNL FACE Leaf Area Index LAI Data. Carbon Dioxide Information Analysis Center, http://cdiac.ornl.gov, US Department of Energy, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Norby, R.J., D.E. Todd, J. Fults and D.W. Johnson. 2001. Allometric determination of tree growth in a CO$_2$-enriched sweetgum stand. New Phytol. 150:477–487.

Norby, R.J., J.D. Sholtis, C.A. Gunderson and S.S. Jawdy. 2003. Leaf dynamics of a deciduous forest canopy: no response to elevated CO$_2$. Oecologia 136:574–584.

Norby, R.J., J. Ledford, C.D. Reilly, N.E. Miller and E.G. O'Neill. 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO$_2$ enrichment. Proc. Natl Acad. Sci. USA 101:9689–9693.

Norby, R.J., E.H. DeLucia, B. Gielen. et al. 2005. Forest response to elevated CO$_2$ is conserved across a broad range of productivity. Proc. Natl Acad. Sci. USA 102:18052–18056.

Norby, R.J., S.D. Wullschleger, P.J. Hanson, C.A. Gunderson, T.J. Tschaplinski and J.D. Jastrow. 2006. CO$_2$ enrichment of a deciduous forest: the Oak Ridge FACE experiment. *In* Managed Ecosystems and CO$_2$: Case Studies, Processes, and Perspectives. Ecological Studies, vol. 187. Eds. J. Nösberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendrey and H. Blum. Springer, Berlin, pp 231–251.

Norby, R.J., J.M. Warren, C.M. Iversen, B.E. Medlyn and R.E. McMurtrie. 2010. CO$_2$ enhancement of forest productivity constrained by limited nitrogen availability. Proc. Natl Acad. Sci. USA 107: 19368–19373.

Nowak, R.A., D.S. Ellsworth and S.D. Smith. 2004. Functional responses of plants to elevated atmospheric CO$_2$—do photosynthetic and productivity data from FACE experiments support early predictions? New Phytol. 162:253–280.

Oishi, A.C., R. Oren, K.A. Novick, S. Palmroth and G.G. Katul. 2010. Interannual invariability of forest evapotranspiration and its consequence to water flow downstream. Ecosystems 13:421–436.

Oren, R., N. Phillips, G. Katul, B.E. Ewers and D.E. Pataki. 1998. Scaling xylem sap flux and soil water balance and calculating variance: a method for partitioning water flux in forests. Ann. Sci. For. 55:191–216.

Oren, R., J.S. Sperry, G.G. Katul, D.E. Pataki, B.E. Ewers, N. Phillips and K.V.R. Schäfer. 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant Cell Environ. 22:1515–1526.

Overdieck D., D. Ziche, K. Bottcher-Jungclaus. 2007. Temperature responses of growth and wood anatomy in European beech saplings grown in different carbon dioxide concentrations. Tree Physiol. 27:261–268.

Pearcy, R.W., E.-D. Schulze and R. Zimmermann. 1989. Measurement of transpiration and leaf conductance. In Plant Physiological Ecology. Eds. R.W. Pearcy, J. Ehleringer, H.A. Mooney and P.W. Rundel. Chapman and Hall, London, UK, pp 137–160.

Radin, J., B. Kimball, D. Hendrix and J. Mauney. 1987. Photosynthesis of cotton plants exposed to elevated levels of carbon dioxide in the field. Photosynth. Res. 12:191–203.

Roden, J.S. and M.C. Ball. 1996. The effect of elevated [CO_2] on growth and photosynthesis of two eucalyptus species exposed to high temperature and water deficit. Plant Physiol. 111:909–919.

Salleo, S., A. Nardini, M.A. Lo Gullo and L.A. Ghirardelli. 2002. Changes in stem and leaf hydraulics preceding leaf shedding in Castanea sativa L. Biol. Plant. 45:227–234.

Schäfer, K.V.R., R. Oren and J.D. Tenhunen. 2000. The effect of tree height on crown level stomatal conductance. Plant Cell Environ. 23:365–375.

Schäfer, K., R. Oren, C. Lai and G. Katul. 2002. Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO_2 concentration. Global Change Biol. 8:895–911.

Schäfer, K., R. Oren, D. Ellsworth, C. Lai, J. Herrick, A. Finzi, D. Richter and G. Katul. 2003. Exposure to an enriched CO_2 atmosphere alters carbon assimilation and allocation in a pine forest ecosystem. Global Change Biol. 9:1378–1400.

Sperry, J.S. and N.Z. Saliendra. 1994. Intra- and inter-plant variation in xylem cavitation in Betula occidentalis. Plant Cell Environ. 17:1233–1241.

Steppe, K., D.J.W. De Paul, T.M. Doody and R.O. Teskey. 2010. A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods. Agric. For. Meteorol. 150:1046–1056.

Toole, E.R. and W.M. Broadfoot. 1959. Sweetgum blight as related to alluvial soils of the Mississippi river floodplain. For. Sci. 5: 2–9.

Tyree, M.T., S.D. Davis and H. Cochard. 1994. Biophysical perspectives of xylem evolution. Is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? IAWAJ 15:335–360.

Uddling, J., R.M. Teclaw, K.S. Pregitzer and D.S. Ellsworth. 2009. Leaf and canopy conductance in aspen and aspen-birch forests under free air enrichment of carbon dioxide and ozone. Tree Physiol. 29:1367–1380.

Valenzuela-Estrada, L.R., J.H. Richards, A. Diaz and D.M. Eissenstat. 2009. Patterns of nocturnal rehydration in root tissues of Vaccinium corymbosum L. under severe drought conditions. J. Exp. Bot. 60:1241–1247.

Warren, J.M., F.C. Meinzer, J.R. Brooks and J.C. Domec. 2005. Vertical stratification of soil water storage and release dynamics in Pacific Northwest coniferous forests. Agric. For. Meteorol. 130:39–58.

Warren, J.M., E. Pötzelsberger, S.D. Wullschleger, P.E. Thornton, H. Hasenauer and R.J. Norby. 2010. Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO_2. Ecohydrology. DOI: 10.1002/eco.173.

Wilson, K.B. and D.D. Baldocchi. 2000. Seasonal and interannual variability of energy fluxes over a broadleaved temperate deciduous forest in North America. Agric. For. Meteorol. 100:1–18.

Wullschleger, S. and R. Norby. 2001. Sap velocity and canopy transpiration in a sweetgum stand exposed to free-air CO_2 enrichment FACE. New Phytol. 150:489–798.

Wullschleger, S.D., C.A. Gunderson, P.J. Hanson, K.B. Wilson and R.J. Norby. 2002. Sensitivity of stomatal and canopy conductance to elevated CO_2 concentration—interacting variables and perspectives of scale. New Phytol. 153:485–496.