Elevated CO₂ enhances leaf senescence during extreme drought in a temperate forest

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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\textsubscript{CO₂}) or ambient (A\textsubscript{CO₂}) CO₂ treatments. Stem sap velocities were analyzed to assess plant response to potential interactions between CO₂ and these weather extremes. Canopy conductance and net carbon assimilation (A\textsubscript{net}) were modeled based on patterns of sap velocity to estimate indirect impacts of observed reductions in transpiration under E\textsubscript{CO₂} on premature leaf senescence. Elevated CO₂ 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\textsubscript{CO₂} plots during this period, thereby directly reducing carbon gain at a greater rate than in A\textsubscript{CO₂} plots. Indeed, pre-drought canopy A\textsubscript{net} was similar across treatment plots, but declined to ~40% less than A\textsubscript{net} in A\textsubscript{CO₂} 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\textsubscript{CO₂}. While E\textsubscript{CO₂} can reduce leaf-level water use under droughty conditions, acute drought may induce excessive stomatal closure that could offset benefits of E\textsubscript{CO₂} 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 CO₂ concentrations [elevated CO₂ (E\textsubscript{CO₂})]. Plants exposed to moderate temperature increases or drought may benefit under future E\textsubscript{CO₂} conditions through CO₂ 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\textsubscript{3} plants stomatal conductance (g\textsubscript{s}) is reduced under E\textsubscript{CO₂} (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\textsubscript{CO₂} 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 CO₂ 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\textsubscript{CO₂}
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 $T_s$; 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 hypothesized 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 ($L.~syracilua$ L.) tree plantation established in Oak Ridge National Environmental Research Park in eastern Tennessee, USA ($35^\circ 54'N; 84^\circ 20'W$). 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 (cm²) was linearly related to BA (including bark) for all 70 sample trees:

\[ SA = 0.792 \times BA - 5.18 \quad (R^2 = 0.99) \quad (1) \]

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

**Sap flow analysis**

Sap velocity (or sap flow 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 Eco2 and two \( A_{CO2} \) 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 (\( \Delta V \)) within the probe set and can be related to sap velocity (\( v_s \) \( \text{m}^2 \text{m}^{-2} \text{s}^{-1} \)) based on the empirical relationship between \( \Delta V \) and the heat dissipation attributable to sap flow near the heated probe (Granier 1985, Lu et al. 2004):

\[ v_s = 118.99 \times 10^{-06} \times \left( \frac{\Delta V_{\text{max}} - \Delta V}{\Delta V} \right)^{1.231} \quad (2) \]

where \( \Delta V_{\text{max}} \) 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 \( \Delta V_{\text{max}} \) values that are lower than actual \( \Delta V_{\text{max}} \), 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 \( \Delta V_{\text{max}} \), between the heated and unheated probes (Lu et al. 2004). Therefore, we modeled \( \Delta V_{\text{max}} \) for each sensor based on linear regression of the multi-day maximum value of \( \Delta V_{\text{max}} \) 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 \( \Delta V_{\text{max}} \) associated with water deficit and increasing trends in \( \Delta V_{\text{max}} \) 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 \( \Delta V \) 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; m³ 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:

\[ Q = \Sigma (v_i \times SA), \]

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 \( A_{\text{net}} \)**

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):

\[ g_c = \frac{\gamma E_i g_a}{s(R_{\text{net}} - G) + pc_D Dg_s - \lambda(s + \gamma)E_i} \]  

where \( \gamma \) is the psychrometer constant (kPa °C⁻¹), \( \lambda \) is latent heat of vaporization (\( \text{kJ kg}^{-1} \)), \( E_i \) 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 \( T_{\text{air}} \) adjusted by elevation (230 m). \( R_{\text{net}} \) (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 \( T_{\text{soil surface}} \) (modeled from measured \( T_{\text{soil}} 10cm; T_{\text{air}} 2m \)), and horizontal leaf display in full sun in the upper canopy. \( G \) is soil heat flux (assumed to be 10% of \( R_{\text{net}} \)) based on maximal summer values at a nearby site; Wilson and Baldocchi 2000), \( p \) 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). \( g_s \) 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, \( g_{\text{ref}} \) (\( g_c \) at \( D = 1.0 \) kPa), where \( g_c = -m \ln(D) - g_{\text{ref}} \) (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:

\[ A_{\text{net}} = g_c C_{\text{a}} \left( 1 - \frac{C_i}{C_{\text{a}}} \right) \]  

where diffusive carbon uptake through stomata is approximately equivalent to diffusive water loss from stomata divided by their relative diffusivity (1.6), \( C_{\text{a}} \) is atmospheric CO₂ concentration (measured above the canopy) and \( C_i/C_{\text{a}} \) is the ratio of intercellular to atmospheric CO₂ concentrations based on A–C 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 \( C_i/C_{\text{a}} \) in late summer, so a single relationship was used: \( C_i/C_{\text{a}} = 0.631 + 0.267g_c \) (\( n = 19; R^2 = 0.47 \)). We computed \( g_c \) on an hourly time step when PAR >50 \( \mu \)mol 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, \( C_{\text{a}} \) and \( C_i/C_{\text{a}} \).

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 CO₂-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 \( A_{\text{net}} \) (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 ($A_{\text{max}}$) 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 ($k_{s,\text{max}}$) 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 $E_{\text{CO2}}$ and $n = 3$ for $A_{\text{CO2}}$). 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 $E_{\text{CO2}}$ plots was 72% of transpiration in $A_{\text{CO2}}$ 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 ($Q = aS\text{A} + b$) 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 $E_{\text{CO2}}$ compared with $A_{\text{CO2}}$. The intercept ($b$) was generally higher for $A_{\text{CO2}}$, which resulted in greater sap flow in $A_{\text{CO2}}$ small trees but less flow in $A_{\text{CO2}}$ large trees, compared with $E_{\text{CO2}}$ 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 $E_{\text{CO2}}$ trees used only 55% that of $A_{\text{CO2}}$ 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 $E_{\text{CO2}}$ plots. Elevated CO$_2$ sap flow was lower across tree size, and flux was more depressed by drought than $A_{\text{CO2}}$ 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](image.png)

**Figure 2.** Relative sap flow decline in relation to tree size (as sapwood radius) in individual $A_{\text{CO2}}$ or $E_{\text{CO2}}$ 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 $A_{\text{CO2}}$ tree (circled) peaked at DOY 216 and then remained constant throughout the drought, and was not used in regressions.
plots for PAR levels during the late drought (DOY 228–239; $t = 2.8, P = 0.008$), with a loss of

**Canopy conductance and $A_{\text{net}}$**

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 $A_{\text{net}}$ during the moderate drought period (DOY 119–210) as compared with A$_{CO_2}$ plots (E$_{CO_2}$: A$_{CO_2}$ $A_{\text{net}}$ ~1.00). $A_{\text{net}}$ increased as leaves expanded in spring, reaching up to ~15 g C m$^{-2}$ day$^{-1}$ for some days during mid-summer (Figure 5). $A_{\text{net}}$ 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 $A_{\text{net}}$ 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 $A_{\text{net}}$ for E$_{CO_2}$ plots relative to A$_{CO_2}$ plots (August). The relative benefit of E$_{CO_2}$ to A$_{CO_2}$ $A_{\text{net}}$ returned a week after a significant rainfall event ended the drought, exhibited by E$_{CO_2}$: A$_{CO_2}$ $A_{\text{net}}$ ~0.96 (September), similar to the pre-drought ratio (0.99; July), although absolute values of $A_{\text{net}}$ 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...
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 \( E_{CO_2} \) \( (10.0–10.6 \text{ cm}^2 \text{ tree}^{-1}) \) and \( A_{CO_2} \) \( (9.4–10.0 \text{ cm}^2 \text{ tree}^{-1}) \) 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 \((P < 0.01, n = 12–31 \text{ per treatment per year})\).
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\text{CO}_2 rings compared with the A\text{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\text{CO}_2 rings compared with A\text{CO}_2 rings as the drought progressed (\(P = 0.14\)). Sixty percent of E\text{CO}_2 minirhizotron tubes displayed evidence of root mortality during this extreme drought period as compared with just 15% of A\text{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\text{CO}_2 trees (Figure 7). Photosynthesis declined by 50% from peak values as \(T_{\text{leaf}}\) 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\text{CO}_2) or 15% (\(P = 0.10\); E\text{CO}_2) lower than \(k_{s,\text{max}}\). One-year-old E\text{CO}_2 branches displayed greater potential loss of conductivity under drying conditions as measured by P50 based on sigmoid regressions (\(P = 0.016\); E\text{CO}_2 \(P50 = 5.15–6.43\); A\text{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\textsubscript{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\textsubscript{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\text{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\text{CO}_2.
Sap flow, g_{c} and A_{net}

The reduction in transpiration under E_{CO2} in the current investigation was primarily dependent upon reduced g_{c}. 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_{c} under E_{CO2} 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 Liquidambar 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_{CO2} plots (Warren et al. 2010).

Transpiration declined at a greater rate for E_{CO2} trees than for A_{CO2} trees during the severe drought period, such that by the end of the drought E_{CO2} 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_{CO2} treatments during moderate drought in July 1997 and 1998, but substantially lower (~25%) in E_{CO2} 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_{CO2} sap flux density, regardless of exposure to moderate drought.

In our study under severe drought we hypothesized that E_{CO2} reduced g_{c} led to increased soil moisture that would maintain stomatal aperture and allow E_{CO2} leaves to maintain greater C uptake than A_{CO2} leaves. This hypothesis was rejected as E_{CO2} 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_{CO2} as compared with A_{CO2} plots, which was directly correlated to the enhanced E_{CO2} leaf loss. Drought-induced ABA may provide a mechanistic link to reduced g_{c}, as it has the potential to enhance stomatal response to E_{CO2} (Leymarie et al. 1999), consistent with the greater reductions in E_{CO2} g_{c}, 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_{CO2} 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-watered 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 $T_{leaf}$ 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 CO₂ did not enhance any measure of tree biomass production during the drought relative to trees in ACO₂ 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 ECO₂ trees relative to ACO₂ trees. Elevated CO₂ enhanced loss of LAI and C uptake during each of the drought years, and thereby also likely reduced overwinter carbon storage and thus carbon available for spring growth. As a result in 2003, following the 2002 drought, ECO₂ 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 minimal NPP enhancement (Norby et al. 2010). The reduction in ECO₂ : ACO₂ LAI and productivity during drought years, and particularly during acute drought periods, highlights the potential negative consequences of ECO₂ trees in response to prolonged drought or heat events. Simpler 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 ECO₂ on foliar loss (McCarthy et al. 2007).

**Conclusions**

Elevated CO₂ 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 ECO₂ should buffer trees against droughty conditions and thereby reduce potential growth limitations. Despite these apparent benefits, large reductions in gₛ lead directly to large reductions in assimilation that can result in a negative leaf carbon balance and ultimately leaf loss. The reductions in gₛ may also reduce latent heat loss and thereby elevate leaf temperatures, both reducing photosynthesis and increasing respiration, which could further exacerbate impacts of the direct effects of gₛ on assimilation. In addition, through anatomical changes, ECO₂ may reduce xylem hydraulic resistance to cavitation in ephemeral tissues that could compromise hydraulic capacity under drought. In this manner, ECO₂ may exacerbate, rather than reduce, the apparent stress response of foliage to extreme conditions, which is consistent 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 confounded by the balance between positive and negative effects of increased atmospheric CO₂ 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 environmental drivers.

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