



Tree Physiology 00, 1–10
doi:10.1093/treephys/tpr030



Research paper

Rooting depth explains $[CO_2] \times$ drought interaction in *Eucalyptus saligna*

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Received December 7, 2010; accepted March 16, 2011; handling Editor Nathan Phillips

Elevated atmospheric $[CO_2]$ (eC_a) often decreases stomatal conductance, which may delay the start of drought, as well as alleviate the effect of dry soil on plant water use and carbon uptake. We studied the interaction between drought and eC_a in a whole-tree chamber experiment with *Eucalyptus saligna*. Trees were grown for 18 months in their C_a treatments before a 4-month dry-down. Trees grown in eC_a were smaller than those grown in ambient C_a (aC_a) due to an early growth setback that was maintained throughout the duration of the experiment. Pre-dawn leaf water potentials were not different between C_a treatments, but were lower in the drought treatment than the irrigated control. Counter to expectations, the drought treatment caused a larger reduction in canopy-average transpiration rates for trees in the eC_a treatment compared with aC_a . Total tree transpiration over the dry-down was positively correlated with the decrease in soil water storage, measured in the top 1.5 m, over the drying cycle; however, we could not close the water budget especially for the larger trees, suggesting soil water uptake below 1.5 m depth. Using neutron probe soil water measurements, we estimated fractional water uptake to a depth of 4.5 m and found that larger trees were able to extract more water from deep soil layers. These results highlight the interaction between rooting depth and response of tree water use to drought. The responses of tree water use to eC_a involve interactions between tree size, root distribution and soil moisture availability that may override the expected direct effects of eC_a . It is essential that these interactions be considered when interpreting experimental results.

Keywords: forest hydrology, global climate change, plant water relations, root allocation, Sydney blue gum.

Introduction

Atmospheric CO_2 concentration (C_a) continues to rise as a result of anthropogenic emissions (e.g., Le Quéré et al. 2009). The effect of rising C_a on vegetation is likely to be profound, particularly on productivity and water use (Ainsworth and Long 2005). The effect of C_a on vegetation is, however, modified by a number of environmental factors, such as nutrient status, temperature and water availability (e.g., Chaves and Pereira 1992, Kimball et al. 1993, Saxe et al. 2001, Norby and Luo 2004). Because water availability frequently limits plant productivity, there is a need to increase understanding of this

potentially important interaction with C_a . It has long been recognized that elevated C_a may have a relatively large effect on plant biomass increment during periods of water stress (Gifford 1979, Sionit et al. 1980, Tolley and Strain 1984, Conroy et al. 1988, Rogers et al. 1994). However, the mechanisms underlying this interaction are complex and may be difficult to unravel (Wullschleger et al. 2002).

In particular, it is important to distinguish how C_a affects whole-plant water relations from the effects on water use at the leaf level. In most species, elevated C_a (eC_a) leads to lower stomatal conductance and hence water use per unit leaf area

(Eamus 1991, Medlyn et al. 2001, Ainsworth and Rogers 2007). If this leaf-level response translates to a reduction in water use per unit ground area (e.g., Wullschlegel and Norby 2001, Hungate et al. 2002), then soil water content should be higher in eC_a , affecting various hydrological processes (Warren et al. 2010). If this saved water is in fact available for uptake (i.e., it does not drain below the rooting zone), then eC_a would lead to a delay in the onset of drought. In studies with potted plants, reduced leaf-level water use is often counteracted, in varying degrees, by an increase in plant leaf area under eC_a (Samarakoon and Gifford 1995, Roden and Ball 1996, Centritto et al. 1999, 2002), so that this drought delay effect is not usually observed. Studies that control the confounding effect of plant leaf area by maintaining a set soil water content sometimes do find a larger enhancement of biomass production under drought due to eC_a (Atwell et al. 2007). Field-based studies in crops and grasslands often find lower water use per unit ground area and an increase in soil water content, as well as a delay in the drought effect on water use (Field et al. 1997, Niklaus et al. 1998, Owensby et al. 1999, Morgan et al. 2004, Nelson et al. 2004). In field-grown trees however, evidence for each of these three components of eC_a -induced delay in drought is scarce and ambiguous.

Current free-air CO_2 enrichment (FACE) studies in forests have not been designed to test drought interactions, and as such rely on comparing eC_a effects across years with varying rainfall or brief periods of drought. These comparisons have not confirmed a clear soil water saving effect (Gunderson et al. 2002, Nowak et al. 2004, Uddling et al. 2008). In a deciduous beech forest, Leuzinger and Körner (2007) reported increased soil water content in an eC_a patch, but soil water was only measured at 10 cm depth and the impacts of this water savings on drought occurrence were not explicitly studied. During an episodic drought in an open-top chamber experiment with field-grown scrub oak, Li et al. (2007) found a smaller reduction in net ecosystem exchange (NEE) in the eC_a compared with the aC_a treatment. However, there was no evidence for a water saving effect because soil water content did not differ between the C_a treatments. In the same experiment, Hymus et al. (2003) found that the eC_a enhancement of NEE increased with soil water content, contrary to the expected response. The reasons for these mixed results are not clear, but interactions with other environmental factors are likely important.

Long-term studies on the interaction between drought and eC_a are complicated by a number of feedbacks that may occur (Beerling et al. 1996), which may help explain the varied and confusing results of $C_a \times$ drought experiments. One important feedback is plant size, which can affect the $C_a \times$ drought interaction in at least two important ways. First, larger size at eC_a leads to greater water use and hence less water saving at a given water supply. Second, in field studies, larger plants can be expected to have deeper roots to tap additional water

sources (Prior and Eamus 1999). Moore and Field (2006) found in a grassland species that growth at eC_a stimulated root biomass and root depth, and resulted in water uptake at greater depth. Other studies have confirmed greater fine root mass under eC_a , often also at greater depth (Day et al. 1996, Iversen et al. 2008, Iversen 2010). Feedback on plant water relations through enhanced root biomass at eC_a would alter the $C_a \times$ drought effects observed in field studies.

Here, we studied the effect of a prolonged drought on water use in Sydney blue gum (*Eucalyptus saligna* Sm.) growing at ambient and elevated (ambient + 240 ppm) C_a . The experiment consisted of 12 whole-tree chambers that were operated as large cuvettes, allowing detailed analysis of whole-tree water use (Barton et al. 2010). Trees were grown for 19 months in well-watered conditions in the C_a treatments, resulting in a wide range of final tree sizes. Somewhat unexpectedly, final tree size was smaller at eC_a than aC_a , which was attributed to an early setback in growth in the trees growing in eC_a . After this setback, which occurred during a time span of a few weeks during early spring (Sept.–Oct. in the southern hemisphere), size-corrected growth rates were similar between C_a treatments, due to photosynthetic downregulation in the eC_a trees (D.S. Ellsworth et al. unpublished data). We do not know the reason for the setback in the eC_a treatment, but have the following hypotheses: (i) more severe frost damage in the eC_a treatment in the 2007 winter (cf. Barker et al. 2005), (ii) growth reduction due to an insect attack that may have been more severe in the eC_a treatment, and (iii) low replication and high genetic variation. For half of the trees, water was withheld for 5 months, resulting in a clear decrease in soil water content. We tested the hypothesis that the smaller trees in eC_a would be less susceptible to drought through lower water use and hence water saving, amplified by the expectation that eC_a reduces water use per unit leaf area.

Methods

Whole-tree chamber experiment

A detailed description of the experiment is available in Barton et al. (2010). Twelve whole-tree chambers were established at the Hawkesbury Forest Experiment (HFE) in 2006, and a single Sydney blue gum (*E. saligna* Sm.) sapling was planted in each chamber in April 2007. Final harvest occurred in March 2009. The experiment is a crossed $C_a \times$ drought design with three replicate chambers in each of four treatments. The C_a treatments commenced at the time of planting and were ambient (~380 ppm; aC_a) and ambient + 240 ppm (eC_a). The chambers are climate controlled; excellent control of temperature and, to a lesser extent, relative humidity was achieved (Barton et al. 2010). All chambers were fitted with a root enclosure barrier that extended to 1 m depth, which prevented roots from the chamber tree growing beyond the chamber in the topsoil, and prevented those from neighbouring trees from

growing into the chamber. Below 1 m, roots were able to grow freely. All chambers were well-watered until 27 October 2008 when 6 of the 12 chambers were subjected to a dry-down, imposed by completely withholding water. The remaining six chambers were kept well-watered for the entire period. The drought lasted until 13 February 2009 when heavy rainfall resulted in a cessation of the drought effect.

Whole-tree water flux

The whole-tree chambers operate as large gas exchange cuvettes, thereby allowing measurement of CO₂ and H₂O fluxes at high temporal resolution (every 14th minute) (see Barton et al. (2010) for details). Here, we report only on the water fluxes during the dry-down. Whole-tree water flux was expressed on a per unit leaf area basis (E_L) or expressed in equivalent depth (mm) for comparison with soil water storage; this could be accomplished because the ground surface area (the area enclosed by the root barrier) of each chamber was 10 m² and during the drought period tree crowns occupied the entire horizontal surface area of the chambers. Leaf area estimates were based on two accurate measurements (one based on leaf counting in April 2008, and the other from the final harvest in March 2009), and interpolation using height growth and litter fall rates. We averaged all E_L data by C_a and irrigation treatment into 2-hourly bins. We used generalized additive models (package *mgcv* in R 2.12.0; R Development Core Team 2010) (Wood 2006) to visualize the trends in E_L with vapour pressure deficit (VPD) and the differences between treatments. To test the interaction between C_a and irrigation treatments on E_L , we used a linear mixed-effects model (package *nlme* in R) with chamber as the random effect (i.e., for this analysis E_L was not averaged by treatment). In this analysis, VPD was used as a covariate (as well as the quadratic term of VPD due to a non-linear response of E_L to VPD).

Soil measurements

The soil type is in the Clarendon Formation (Chromosol; Isbell 1996), an alluvial formation of low-fertility sandy loam soils (top 70 cm) with low organic matter content (0.7%). There is a partially cemented hard layer with manganese nodules (70–100 cm), and a clay layer (below 100 cm). In each chamber, volumetric soil water content was monitored on an hourly basis using Sentek TriSCAN[®] sensors (Sentek Technologies, Stepney, SA, Australia). The probes were configured to measure volumetric soil water content at four depths (0.3, 0.7, 1.1 and 1.5 m). Theta probes (Delta T Instruments) measured volumetric soil water content at 10 cm depth within the chambers throughout the study period. In addition, a neutron probe (503DR Hydroprobe[®], Instrotek, NC, USA) was used to measure soil water content to a depth of 4.25 m (in 0.25 or 0.5 m steps) at approximately bi-weekly intervals. Calibration of the Sentek sensors was difficult due to variation in bulk density across the

site, and sensitivity to the geometry of the access tubes. We rescaled the soil water content measurements so that the maximum value matched that measured by the Theta probe in the topsoil. This method should yield reliable absolute soil water content measurement, because all chambers were irrigated very heavily post-drought, and soil water saturation was observed (resulting in temporary ponding of water at the soil surface due to low hydraulic conductivity of the hard deep soil layer). The maximum water content varied little by chamber (35.7%, SE = 0.7), and compared well with estimates of porosity from bulk density in two soil pits dug at the site.

Pre-dawn leaf water potentials

A Scholander-style pressure chamber (Model 600; PMS Instrument Company, Corvallis, OR, USA) was used to measure pre-dawn leaf water potential (Ψ_{pd}) at regular intervals in each whole-tree chamber. Leaves were sampled from the middle to upper canopy and immediately sealed in a foil-laminate bag prior to measurement.

Root water uptake

We inferred root water uptake from vertical profiles of soil moisture measured by the neutron probes (Rambal 1984, Dye 1996, Calder et al. 1997). We did not correct for possible gravitational drainage of water, which could be mistaken for root water uptake, because soil water content in the clay layer (below 1 m depth) was constant in all chambers up to the drought period. It is therefore unlikely that drainage plays a large role in the soil water dynamics in this system. It is possible that some of the water extraction at depth constitutes hydraulic lift rather than direct uptake (Dawson 1996), but this is not important because hydraulic lift also implies that roots were present at that depth. Fractional water uptake at depth z (F_z) over the time interval t_1 to t_2 was calculated with Eq. (1):

$$F_z = \frac{(W_{z,t2} - W_{z,t1})}{\int_0^{Z_{\max}} (W_{z,t2} - W_{z,t1}) dz} \quad (1)$$

where $W_{z,t}$ is the soil water content (% volumetric) at depth z at time t and Z_{\max} is the maximum depth of sensors (4.25 m). F_z integrates to unity up to the maximum soil depth of the sensors (4.25 m).

Results

There was substantial variation between chambers in the amount of soil water stored in the top 1.5 m of soil (Figure 1), and no clear effect of C_a treatment on soil water storage was observed during the dry-down. Soil water storage declined rapidly in the early stages of the drought, but the rate of decline slowed as the drought progressed.

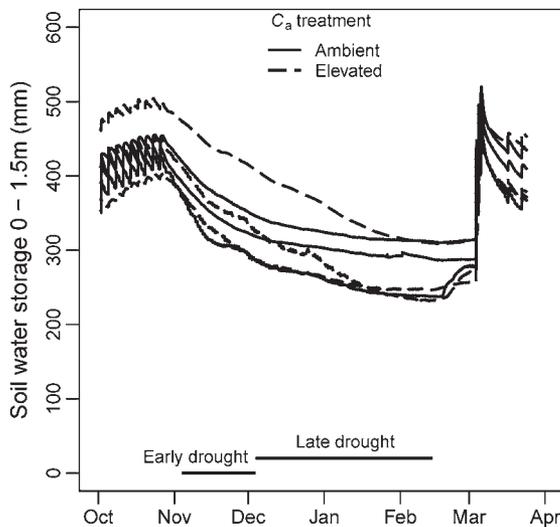


Figure 1. Soil water storage in the top 1.5 m for the six droughted chambers. The early drought was defined as the period just after the drought started when there was no discernable effect on either pre-dawn leaf water potential or whole-tree H_2O flux. The late drought extends up to a period of heavy rain in February 2009, which affected soil water storage in spite of the root closures.

The drought treatment applied was effective in reducing the pre-dawn leaf water potential (Ψ_{pd}) (Figure 2, and see Zeppel et al. 2011). Counter to our expectations, Ψ_{pd} did not differ between C_a treatments, either at the peak of the drought, when the lowest Ψ_{pd} values were reached (t -test, $P=0.13$), or when considered over the entire period (see Zeppel et al. 2011). The two lowest Ψ_{pd} values, however, were reached for two eC_a trees (-1.23 and -1.1 MPa com-

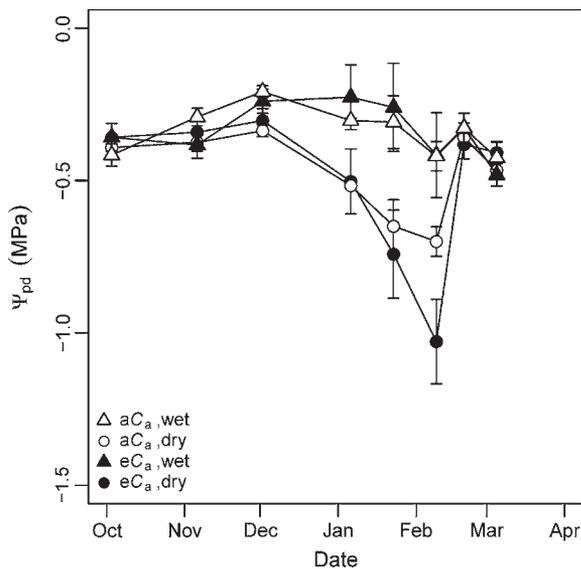


Figure 2. Pre-dawn leaf water potentials (Ψ_{pd}) averaged by C_a and water treatment. Error bars denote one standard error. Each value is the average of three chambers.

pared with -0.61 to -0.76 for the four other droughted trees).

The whole-tree H_2O flux expressed on a per unit leaf area basis (E_L) was strongly controlled by VPD (Figure 3). Because there were small differences in VPD between C_a treatments arising from differences in tree size, we analysed the effects of the drought treatment on E_L with VPD as a covariate. In the well-watered treatments, E_L was lower in the eC_a treatment especially at low VPD. There was only a small effect of the drought treatment on E_L during the early drought period (Figure 3a and b), but a very strong reduction in E_L at a given VPD during the later stages of the drought (Figure 3c and d). The reduction in E_L due to drought was larger in the eC_a treatment (Figure 3d), and this interaction was especially clear when VPD was between 1.5 and 3 kPa. Results from the linear mixed-effects model showed strong significant effects of VPD and irrigation treatment on 2-hourly E_L (Table 1), as well as a significant interaction between C_a and irrigation treatment when accounting for VPD (the $C_a \times \text{water} \times \text{VPD}$ interaction was significant at $P < 0.001$). Taken together, these results demonstrated that the drought treatment reduced E_L at a given VPD, and E_L was more reduced due to drought in the eC_a treatment.

Total tree water use (E_T ; litres per day) during the dry-down was strongly and linearly related to tree leaf area (A_L) (Figure 4). Leaf area differed threefold among the droughted trees (and four-fold among the irrigated trees), and leaf area was lower for the eC_a trees compared with the aC_a trees. We compared cumulative E_T of droughted trees with the total drawdown in soil water storage and found that they were related ($P=0.03$, $R^2=0.65$), but with a substantial 'missing source'; that is, the total amount of water used in the drought period was much larger (by 27–88%) than the decline in soil water storage for the upper 1.5 m of the soil profile (Figure 5a). This difference (the residual in Figure 5a) was smaller for the eC_a trees (t -test, $P=0.053$), and was also weakly related to tree leaf area ($P=0.065$, $R^2=0.52$).

These results suggest that a substantial volume of water was taken up from depths below 1.5 m, and that this uptake may depend on tree size. We were able to further test this conclusion using neutron-probe measurements to a depth of 4.25 m, which were recorded eight times during the dry-down. An example of the pattern of water extraction with depth is shown for one aC_a chamber during the dry-down, distinguishing between the early and late drought periods (Figure 6). We calculated the relative water uptake with depth for the two periods of the drought from the relations shown in Figure 6b for all six droughted chambers. For the early drought, we found a clear difference between the C_a treatments in the pattern of relative water uptake with soil depth (Figure 7a). During this period, the proportion of soil water taken up by roots from 0 to 1.5 m soil depth was 0.68–0.84 for the aC_a treatment, and 0.95–1.0 for the eC_a trees. This difference between the C_a treatments is

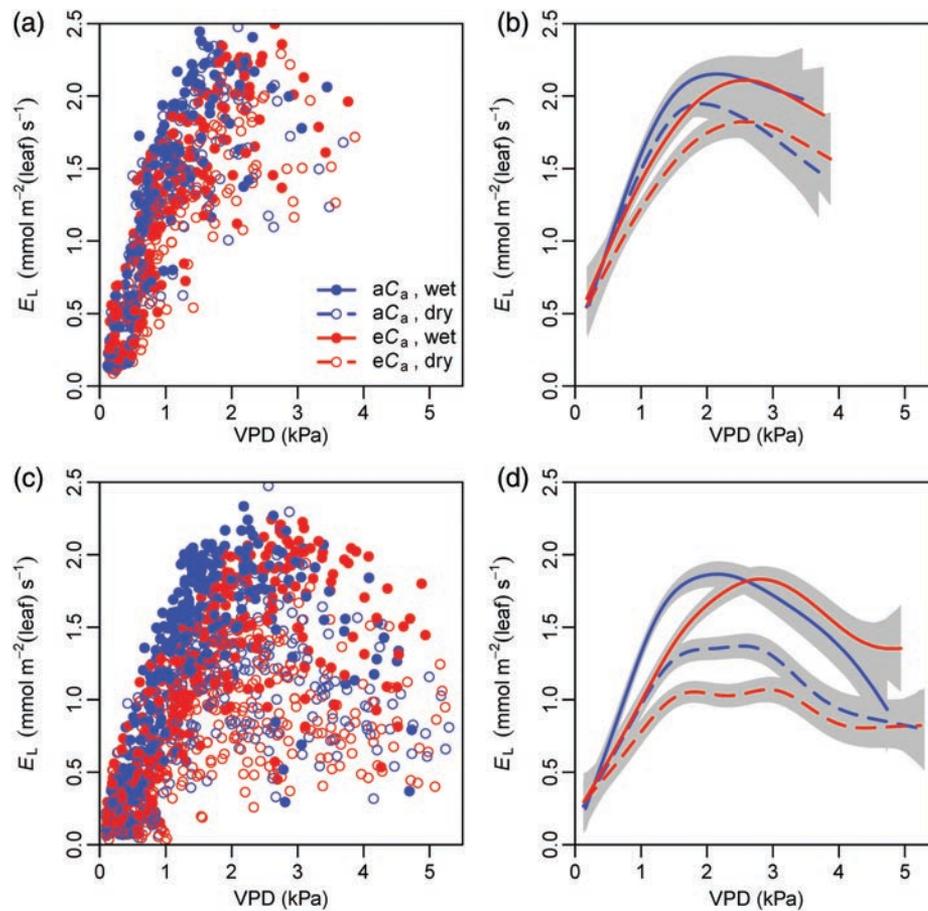


Figure 3. Canopy-average transpiration rate (E_L , expressed on a leaf area basis) as a function of VPD for the early drought (a, b) and late drought (c, d) periods. Panels a and c show 2-hourly averages of whole-tree fluxes averaged by treatment (each symbol is the mean for three chambers). Panels b and d show smoothed regressions with ~95% confidence intervals (grey area) (see Methods).

Table 1. Linear mixed-effects model results for whole-tree H₂O flux expressed on a per unit leaf area basis (E_L) during the late drought period. Num DF and Den DF are numerator and denominator degrees of freedom for the F -tests.

	Num DF	Den DF	F value	P value
Intercept	1	3941	391.52	<0.001
VPD	1	3941	2892.15	<0.001
VPD ²	1	3941	2836.49	<0.001
C_a	1	8	2.65	0.14
Water	1	8	14.07	0.01
VPD × C_a	1	3941	1.6	0.21
VPD ² × C_a	1	3941	82.14	<0.001
VPD ² × water	1	3941	70.4	<0.001
VPD × water	1	3941	322.75	<0.001
C_a × water	1	8	0.03	0.86
VPD × C_a × water	1	3941	26.45	<0.001

qualitatively consistent with the smaller difference between transpiration and soil water storage decrease for the eC_a trees (Figure 5). As the drought progressed, soil water uptake was greater from soil depths below 1.5 m in all trees (Figure 7b), but was consistently from greater depths for aC_a trees.

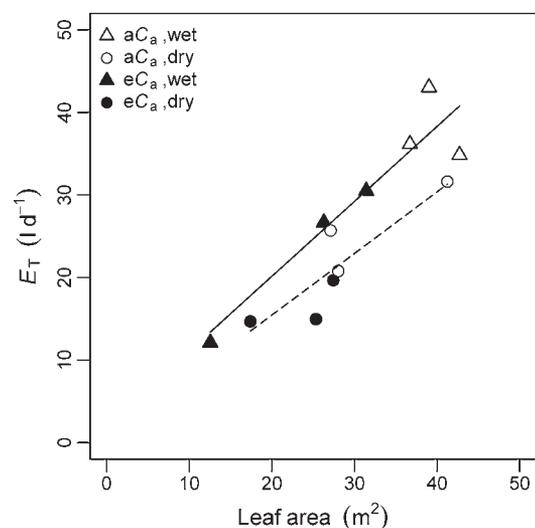


Figure 4. Total tree water use (E_T , in litres per day) during the dry-down (early and late drought combined, see Figure 1) is related to average tree leaf area (A_L). Solid line: regression line for well-watered trees ($E_T = 1.98 + 0.91 \times A_L$, $P = 0.006$, $R^2 = 0.84$), dashed line for droughted trees ($E_T = 0.52 + 0.75 \times A_L$, $P = 0.02$, $R^2 = 0.72$). Note the lower leaf area and water use for eC_a trees, and the substantially lower water use for the droughted trees over this period.

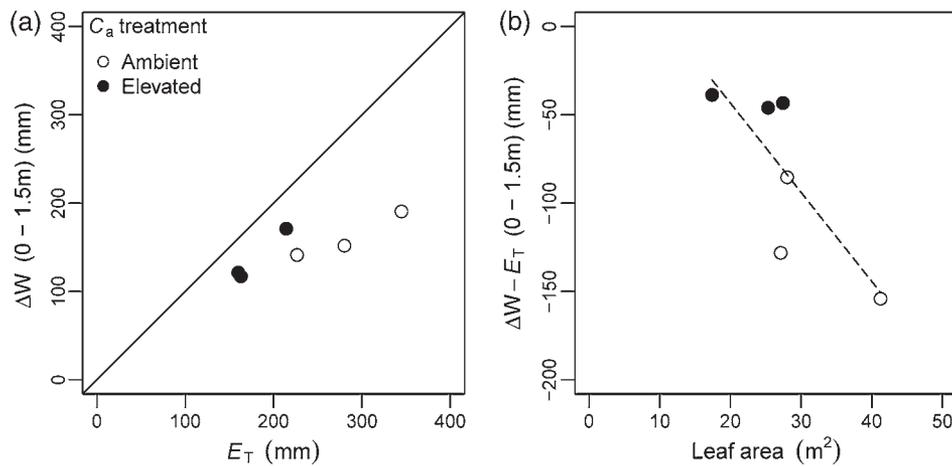


Figure 5. (a) Total decrease in soil water storage in the top 1.5 m of soil (ΔW) for the six droughted trees over the whole drought period (from Figure 1) compared with the total tree H_2O flux over the same period (E_T). The solid line is a 1:1 line. (b) The residual in panel a as a function of average tree leaf area for the same period. The regression (dashed line) was marginally significant ($P = 0.06$), and the residual was smaller for eC_a trees than aC_a trees (t -test, $P = 0.053$).

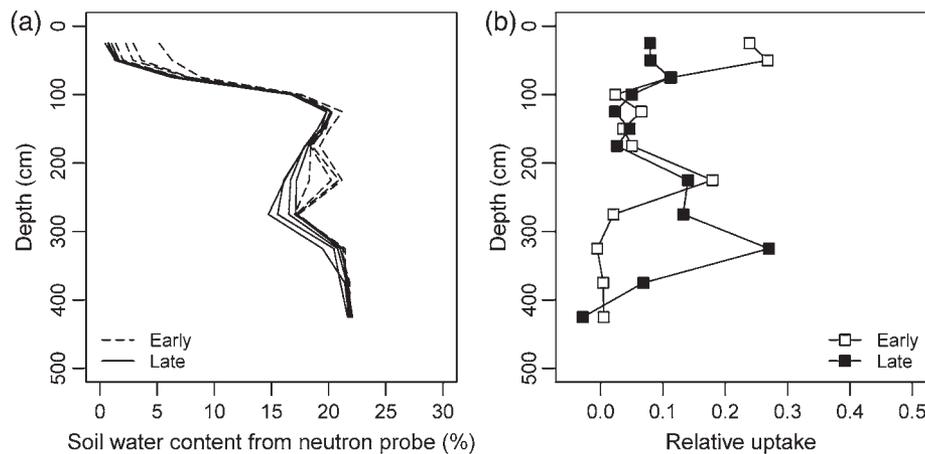


Figure 6. (a) Example of the decrease in soil water content from neutron-probe measurements during the drought for one aC_a chamber. The progression of the drought is from right to left (early and late drought periods are defined in Figure 1). Note the decrease in soil water between 200 and 350 cm depth. (b) Based on panel a, the relative water uptake (normalized to add up to unity, Eq. (1)) was estimated as a function of depth for the early and late drought periods. Note the shift in relative water uptake to deeper soil layers when the drought progressed.

Discussion

Contrary to expectations, the smaller trees in the eC_a treatment were not less susceptible to drought stress than were the aC_a trees. Although trees in the eC_a treatment used substantially less water than aC_a trees because they had lower leaf area, there was no delay in the effect of drought stress on tree water use. The minimum Ψ_{pd} reached during the drought cycle was similar in the C_a treatments and the relative effect of withholding water on canopy-average transpiration rate was greater in the eC_a trees. This result can be explained in terms of rooting depth. We found that the larger trees in the aC_a treatment accessed soil water to greater depths. Consequently, smaller trees in the eC_a treatment could not substantially access deep soil water and therefore experienced

greater drought stress than aC_a trees, as evidenced by a greater reduction in water use compared with the irrigated treatment

Our finding that smaller trees experience increased water stress is consistent with several previous studies. Prior and Eamus (1999) showed that Ψ_{pd} increased with sapling size in a savannah, and that this relationship was explained by differences in rooting depth. Dawson (1996) also showed that larger trees had access to groundwater while smaller trees did not. For an annual forb species, eC_a led to increased root biomass and earlier extraction of water from deeper soil layers than the aC_a controls (Moore and Field 2006). Our results show that it is vital that $C_a \times$ drought experiments provide observations of soil water content over the entire root profile, because plant

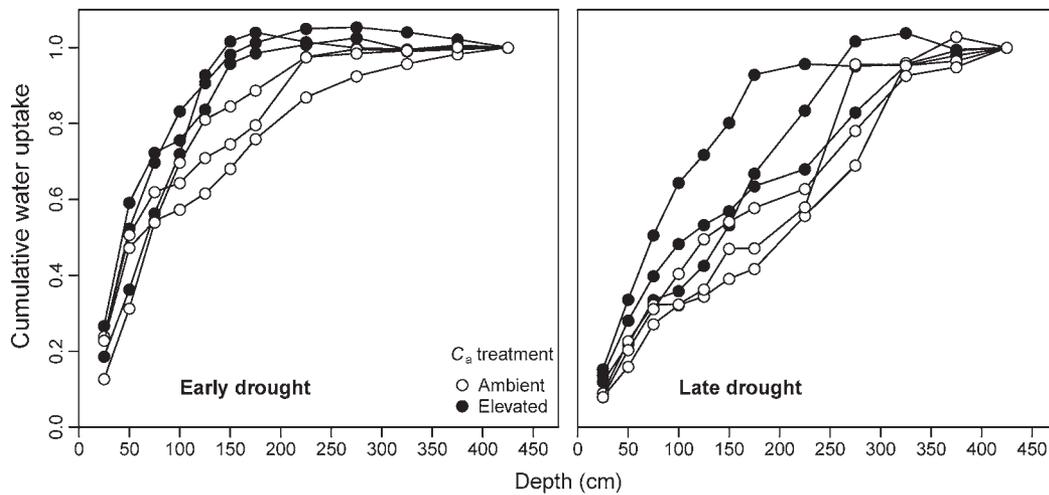


Figure 7. Cumulative relative water uptake for the six droughted trees for the early drought period (left panel) and the late drought period (right panel). See Figure 1 for definition of early and late drought periods. Water extraction was normalized to be equal to unity at 4.25 m depth. Note the difference in depth of water extraction between C_a treatments, and the shift to deeper soil layers as the drought progressed.

size may affect the depth at which water is taken up, and hence the response to drought.

Root responses to eC_a have received much attention (Morison 1993, Norby 1994, Iversen et al. 2008, Iversen 2010), but usually with the focus on soil cycling of nitrogen and carbon (Norby 1994, Iversen et al. 2008, Franklin et al. 2009), rather than whole-plant water relations. Many studies on crop and grassland species have shown that root biomass is more responsive to eC_a than other plant parts (Rogers et al. 1994). At the Oak-Ridge FACE site within a plantation of *Liquidambar styraciflua* L. most of the biomass response to eC_a was due to greater root biomass (Norby et al. 2004), which was more pronounced at greater depths in the soil (30–60 cm) than at shallower depths (Iversen et al. 2008, Iversen 2010). The implications of increased root biomass for water relations are not clear because there is no direct relationship between root biomass and total volume of water available for root uptake. An increase in root biomass can mean higher fine root density, deeper rooting depth or both, but these two possibilities have profoundly different implications for plant water relations. In *E. saligna*, we found that the difference in root water uptake between the C_a treatments was greatest at depth (ca. 1–2.5 m). The vast majority of studies on root responses to varying environmental conditions focus on the upper soil profile (up to usually 50–100 cm depth at the most), presumably because the majority of root biomass is in the upper 1 m of soil (Jackson et al. 1996). However, deep roots have a large influence on plant water relations (Eamus et al. 2006) and therefore, particularly in deeply rooted species such as *Eucalyptus*, responses of root biomass to eC_a need to be studied in much greater detail.

As the drought progressed at the HFE, water was extracted from deeper soil layers (Figures 6 and 7), which is consistent

with previous studies (Rambal 1984, Bréda et al. 1995, Romero-Saltos et al. 2005). During the late drought period, five of the six droughted trees extracted ~40–60% of their water from soil depths below 2 m. Such relatively deep roots pose a significant problem for modelling the soil water balance, soil water extraction patterns and overall tree water use in drought. For example, Dye (1996) found only moderate water stress in *Eucalyptus grandis* W. Hill ex Maiden even though rainfall was excluded for >7 months because root water uptake occurred predominantly below 8 m depth. Similarly, Calder et al. (1997) found prolonged decoupling of water use from precipitation by *Eucalyptus camaldulensis* Dehnh. because roots extended deeper than the soil measurement limit of 7.5 m. More recently, for a native *Eucalyptus* woodland, Zeppel et al. (2008) observed that tree water use was independent of soil moisture content in the top 80 cm of the soil profile, despite the majority of root biomass occurring in this zone. Clearly, the connection between tree size and rooting depth is important in developing a deeper mechanistic understanding of whole-tree water use, particularly for *Eucalyptus* species, which are often deep-rooted.

Three potential mechanisms might explain the common observation that plant growth is stimulated more by elevated C_a during drought (Gifford 1979, Sionit et al. 1980, Tolley and Strain 1984, Conroy et al. 1988, Rogers et al. 1994). The first mechanism is a delay in the onset of drought due to lower water use in eC_a , which we have ruled out in our experiment. It can also be expected that lower stomatal conductance (g_s) in eC_a should lead to higher mid-day leaf water potentials (Ψ_L), thereby slowing the decline in Ψ_L resulting from a drying soil. Many studies have found an increase in Ψ_L in plants grown under eC_a in crop or grassland species (Nelson et al. 2004) and in potted tree seedlings (Roden and Ball 1996, Polley et al.

2002, Atwell et al. 2007), and for field-grown tree seedlings (Eamus et al. 1995), but not for loblolly pine in a FACE experiment (Domec et al. 2009). However, it is not clear how much relief a slightly less negative Ψ_L offers during a severe soil drought. In particular, the response of plant water use to soil water potential is relatively insensitive to Ψ_L (Sinclair 2005), because the soil-to-root pathway becomes increasingly limiting as a drought progresses (Sperry et al. 1998, Williams et al. 2001, Fisher et al. 2006, Duursma et al. 2008), and Ψ_L has no control on the resistance in that pathway. In any case, more detailed modelling will be required to quantify the components of plant water relations that are altered under eC_a (Wullschlegel et al. 2002).

A third mechanism that may lead to enhanced stimulation of plant growth by eC_a under drought stress is the relative enhancement of photosynthesis. Because of the saturating response of leaf CO_2 assimilation (A) to intercellular $[CO_2]$ (c_i), the relative response of A to C_a is higher at low g_s (Grossman-Clarke et al. 2001, McMurtrie et al. 2008). Thus, a reduction in g_s during drought should lead to a relatively larger response of A to C_a . This mechanism has not been clearly demonstrated with measurements of g_s and A during a dry-down. Some experiments do seem consistent with this expectation (Picon-Cochard and Guehl 1999), but in some studies the C_a treatment effect on A disappears during a drought rather than being enhanced (re-analysis of data in Damesin et al. 1996, Gunderson et al. 2002, Johnson et al. 2002).

In summary, eC_a reduced leaf-level water use and smaller trees in the eC_a treatment used less water overall, but drought stress was more severe due to more shallow rooting depth compared with larger trees in the aC_a treatment. An implication is that the response of vegetation to eC_a cannot be easily predicted from the direct leaf-level physiological responses. Other studies have confirmed that, in long-term field experiments, many feedbacks alter the initial response of vegetation to eC_a (Chaves and Pereira 1992, Niklaus et al. 1998). As such, we should not prematurely conclude that, on a global scale, vegetation in a CO_2 -enriched atmosphere will be less susceptible to drought, because whole-plant and ecosystem-level feedbacks may well alter or even reverse this response. A better understanding of these feedbacks and interactions is needed to explain conflicting results in different experiments.

Acknowledgments

We thank Burhan Amiji for outstanding technical support.

Funding

This research was supported by the NSW Government Climate Action Grant (NSW T07/CAG/016), and funding by the

Australian Government's Department of Climate Change for the Hawkesbury Forest Experiment.

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