



Tree Physiology 35, 453–469  
doi:10.1093/treephys/tpv016



## Research paper

# Contrasting drought-response strategies in California redwoods

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Received April 3, 2014; accepted January 30, 2015; published online March 17, 2015; handling Editor David Whitehead

We compared the physiology and growth of seedlings originating from different *Sequoia sempervirens* (D. Don.) Endl. (coast redwood) and *Sequoiadendron giganteum* (Lindl.) Buchh. (giant sequoia) populations subjected to progressive drought followed by a recovery period in a controlled greenhouse experiment. Our objective was to examine how multiple plant traits interact to influence the response of seedlings of each species and seed population to a single drought and recovery cycle. We measured soil and plant water status, leaf gas exchange, stem embolism and growth of control (well-watered) and drought-stressed (water withheld) seedlings from each population at the beginning, middle and end of a 6-week drought period and again 2 weeks after re-watering. The drought had a significant effect on many aspects of seedling performance, but water-stressed seedlings regained most physiological functioning by the end of the recovery period. *Sequoiadendron* seedlings exhibited a greater degree of isohydry (water status regulation), lower levels of stem embolism, higher biomass allocation to roots and lower sensitivity of growth to drought compared with *Sequoia*. Only minor intra-specific differences were observed among populations. Our results show that seedlings of the two redwood species exhibit contrasting drought-response strategies that align with the environmental conditions these trees experience in their native habitats, and demonstrate trade-offs and coordination among traits affecting plant water use, carbon gain and growth under drought.

**Keywords:** anisohydric, biomass allocation, climate change, embolism, isohydric, *Sequoia sempervirens*, *Sequoiadendron giganteum*.

## Introduction

Drought is one of the primary environmental factors limiting the growth, distribution and survival of trees worldwide (Boyer 1982, Allen and Breshears 1998, Engelbrecht et al. 2007). Global climatic changes are expected to lead to increases in the frequency, severity and duration of droughts in many regions of the world, with profound implications for ecosystem structure and function as well as biosphere–atmosphere interactions (Malhi et al. 2002, Solomon et al. 2007, Anderegg et al. 2012). Understanding how different tree species respond to drought is therefore critical for modeling and predicting the fate of forest and woodland ecosystems under future climate conditions (Sitch et al. 2008, Jump et al. 2010).

To remain physiologically active, plants must be able to supply sufficient water to their leaves to replace that lost through transpiration. As soil moisture levels decline, increasing tension

leads to an increasing risk of xylem cavitation, leading to a loss of hydraulic conductance and a further decline in xylem water potential (Zimmermann 1983, Tyree and Sperry 1989). When the supply of water declines under drought, plants typically close their stomata to reduce transpiration rates and maintain the integrity of the root-to-leaf water transport pathway (Sperry et al. 1998). The degree of stomatal closure has been linked with the critical water potential causing hydraulic failure, demonstrating close coordination between stomatal behavior and xylem hydraulic traits (Sperry et al. 2002, Meinzer et al. 2009). However, stomatal closure may not be sufficient to prevent xylem cavitation under severe drought conditions, potentially leading to complete hydraulic failure, crown dieback and even plant death (Brodribb and Cochard 2009, Choat et al. 2012).

The general terms 'isohydric' and 'anisohydric' have been used to describe opposite ends of a broad continuum of water-use strategies displayed by various species (Tardieu and Simonneau

1998, West et al. 2012). Isohydric plants generally exhibit strong stomatal control of transpiration in response to drought, thereby maintaining relatively high minimum leaf water potentials and avoiding severe hydraulic failure due to xylem cavitation. Anisohydric plants in contrast typically exhibit lower stomatal sensitivity (responsiveness) under drought conditions, maintaining relatively high transpiration rates but allowing minimum leaf water potentials to decline to more negative values compared with isohydric plants, consequently increasing the possibility for hydraulic failure.

The difference between the minimum xylem water potential a plant experiences and the water potential at which it would suffer a critical loss of hydraulic conductivity is referred to as the 'hydraulic safety margin' and indicates how well a species is buffered from hydraulic failure (Alder et al. 1996, Sperry 2000, Meinzer et al. 2009, Johnson et al. 2012). Species that have high vulnerability to cavitation tend to have smaller hydraulic safety margins and are likely to experience some degree of native embolism even under non-drought conditions (Pockman and Sperry 2000, Meinzer et al. 2009). Several studies in recent decades have concluded that plants can refill emboli in xylem conduits even when nearby functional conduits are still under considerable tension (Zwieniecki and Holbrook 1998, McCully 1999, Holbrook et al. 2001, Bucci et al. 2003, Brodersen et al. 2010, Brodersen and McElrone 2013). Species with small safety margins and high cavitation vulnerability have shown greater capacity to recover from embolism (Preston et al. 2006, Markesteijn et al. 2011, Choat et al. 2012, Ogasa et al. 2013). Xylem hydraulic traits have also been linked with stomatal behavior and photosynthetic rate (Santiago et al. 2004), leaf structure (Ambrose et al. 2009) and biomass allocation and growth (Poorter et al. 2010). Thus, the interaction of functional traits affecting water uptake and transport, carbon assimilation, mechanical strength and growth appear to play a critical role in determining plant vulnerability to and potential recovery from drought. However, our understanding of how different trait combinations determine a species' general drought-response strategy remains limited.

In this experiment, we examined the effects of soil drought and subsequent recovery on the physiology and growth of 1-year-old seedlings representing northern, central and southern populations of California's two monotypic redwoods, *Sequoia sempervirens* (D. Don.) Endl. (coast redwood) and *Sequoiadendron giganteum* (Lindl.) Buchh. (giant sequoia). Our primary objective was to examine how multiple plant traits interact to influence the response of seedlings of each species and population to a single drought and recovery cycle. We measured soil and plant water status, leaf gas exchange, stem embolism and growth of control (well-watered) and drought-stressed (water withheld) seedlings from each population at the beginning, middle and end of a progressive 6-week drought period and again 2 weeks after re-watering. Because *Sequoia* grows in

a relatively mild coastal environment while *Sequoiadendron* grows in a more arid mountain environment, a comparison of the two redwoods provides an opportunity to evaluate the influence of contrasting climates on tree drought-response strategies in closely related species. Moreover, temperature and precipitation vary throughout the geographic distribution of both species, allowing us to further investigate potential effects of climate on intra-specific variation in drought responses.

## Materials and methods

### Plant material and experimental design

The experiment was conducted on 1-year-old potted *Sequoia* and *Sequoiadendron* seedlings from February through April 2012. We obtained seeds from trees growing in three different regional populations across a latitudinal precipitation gradient spanning much of the geographic range of each species. *Sequoia* seeds were from Del Norte (north), Mendocino (central) and Santa Cruz (south) counties, while *Sequoiadendron* seeds were from Calaveras (north), Redwood Mountain (central) and Mountain Home (south) groves. Seeds were germinated and seedlings nursery-grown in 0.164 L containers for 1 year by the Green Diamond Resource Company in Humboldt Co., and then transported to the Oxford Tract greenhouse facility at the University of California, Berkeley (lat 37.875182°, long -122.266999°), where they were immediately transplanted into 7.6 l pots filled with commercial organic potting soil and left to acclimate to greenhouse conditions for 1 month before starting the experiment. During the acclimation period all seedlings were irrigated 3–4 times per week using automatic drip-line systems and fertilized biweekly with quarter-strength Hoagland's solution.

At the beginning of the experiment, *Sequoia* seedlings were 273–529 mm tall, 3.1–6.2 mm diameter at the stem base and 3.8–14.7 g total dry mass, while *Sequoiadendron* seedlings were 147–206 mm tall, 3.6–6.2 mm diameter at the stem base and 2.0–14.1 g total dry mass. A total of 330 seedlings of both species were used in the experiment, and were randomly assigned to either control or drought-stress treatments in one of three different experimental groups. The first group consisted of 240 seedlings ( $n = 20$  per treatment from each population) in which soil water content, shoot water potential, leaf gas exchange and seedling size were measured after acclimation but prior to imposing drought (baseline period), 3 weeks after imposing drought (mild-drought period), 6 weeks after imposing drought (severe drought period), and finally 2 weeks after re-watering the drought-stressed plants (recovery period). Of these, 60 seedlings ( $n = 5$  per treatment from each population) were harvested at the final recovery period for measurement of basal trunk embolism and whole-plant biomass. The second experimental group consisted of 30 seedlings ( $n = 5$  per population) in which soil water content, shoot water potential, size and whole-plant biomass were measured at the baseline period

prior to imposing drought. The third experimental group consisted of 60 seedlings ( $n = 5$  per treatment from each population) in which soil water content, shoot water potential and size were measured at the baseline, mild-drought and severe drought periods, and were harvested at the severe drought period for measurement of basal trunk embolism and whole-plant biomass. Measurements were made over the course of 4 days during each measurement period (60 plants per day), with plants from all treatment groups randomly assigned to one of the four days in each period.

Baseline measurements were obtained on all seedlings prior to imposing the drought treatment. Control plants were maintained under well-watered conditions throughout the experiment using automatic drip-lines, while water was withheld from drought-stressed plants between the baseline and recovery periods. No additional fertilizer was added to either control or drought plants after the baseline measurement period ended. Pilot tests conducted prior to the experiment indicated that redwood seedlings showed signs of severe drought stress ~6 weeks after withholding water, including shoot water potentials lower than observed in mature trees in the field (approximately  $-2.8$  MPa mid-day values), complete cessation of leaf gas exchange and signs of physical damage (i.e., severe wilting or crown dieback). Because we were interested in examining the recovery of drought-stressed seedlings after re-watering, we decided to limit the experimental drought period to this 6-week time frame in order to avoid killing the seedlings. We therefore defined the 'mild-drought' period as the mid-point of this time frame (i.e., 3 weeks) and the 'severe drought' period as 6 weeks after withholding water. After completion of the severe drought period measurements, all drought-stressed plants were thoroughly re-watered until the entire soil column was saturated and then maintained under well-watered conditions using automatic drip-lines until the end of the experiment.

Air temperature and relative humidity were monitored every 30 min using 14 sensors installed at plant height throughout the greenhouse (EL-USB-2+, Lascar Electronics, Ltd, Erie, PA, USA). Vapor pressure deficit (VPD) was calculated using air temperature and relative humidity data (Buck 1981). Daily (24-h) mean  $\pm 1$  SD (min–max) temperature during the experiment was  $20.7 \pm 3.8$  °C ( $12.0$ – $35.0$  °C), relative humidity was  $50.8 \pm 14.8\%$  ( $15.2$ – $87.2\%$ ) and VPD was  $1.3 \pm 0.6$  kPa ( $0.3$ – $3.5$  kPa). The greenhouse roof reduced photosynthetic photon flux density (PPFD) by ~50% and mean PPFD measured inside the greenhouse at mid-day on a typical sunny day during the experiment was  $\sim 750 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

### Soil and plant water status

Soil volumetric water content (VWC) was measured on each plant at each measurement period using a portable time-domain reflectometry (TDR) soil moisture measurement system (Hydro-Sense CD-CS620, Campbell Scientific, Inc., Logan, UT, USA).

TDR probes were inserted into each pot at an angle so that the entire probe length would be in close contact with the soil and span the entire rooting zone of each plant. Pre-dawn shoot water potential ( $\Psi_{\text{PD}}$ ) and daytime shoot water potential ( $\Psi_{\text{DT}}$ ) was measured on each plant at each measurement period using a Scholander pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Daytime water potential measurements were made on a shoot adjacent to the one used for simultaneous leaf gas exchange measurements.

### Leaf gas exchange

Leaf gas exchange was measured using a portable open gas exchange system (Li-6400, Licor, Inc., Lincoln, NE, USA) on one shoot per plant during mid-day (10:00–14:00 h) at each measurement period. Light-saturated gas exchange rates were measured on mature shoots with a standard leaf chamber equipped with a blue-red light source at PPFD of  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  and an ambient  $\text{CO}_2$  concentration of  $400 \mu\text{mol mol}^{-1}$ . Air temperature inside the chamber was maintained between  $19.6$  and  $22.6$  °C and leaf-to-air VPD between  $1.2$  and  $2.0$  kPa. Following measurement, each shoot within the chamber was removed and digitally scanned for determination of shoot area and leaf area via image analysis (ImageJ, National Institute of Mental Health, Bethesda, MD, USA). Shoots were oven-dried at  $60$  °C for 3 days and then weighed. Leaf area and dry mass were used to calculate projected area- and mass-based net photosynthesis ( $A_{\text{area}}$  and  $A_{\text{mass}}$ ) and stomatal conductance ( $g_{\text{area}}$  and  $g_{\text{mass}}$ ). Instantaneous intrinsic water-use efficiency was calculated as the ratio of net photosynthesis and stomatal conductance ( $A/g$ ). Mass-based leaf-level net photosynthesis, stomatal conductance, and transpiration rates were multiplied by total leaf mass to estimate instantaneous daytime whole-plant gas exchange.

### Trunk xylem embolism

The degree of basal trunk xylem embolism experienced by control and drought-stressed seedlings at the severe drought and recovery measurement periods was measured using a tracer dye method following Brodribb et al. (2010). After measurements of soil VWC,  $\Psi_{\text{PD}}$ ,  $\Psi_{\text{DT}}$  and size (between 10:00 and 12:00 h), plants from the third experimental group ( $n = 5$  per treatment from each population; 60 seedlings total) were cut at soil level, immediately re-cut under water, shaved with a razor, and transferred underwater to a filtered solution of the fluorescent dye sulphorhodamine G. Plants were left to transpire for 15–30 min in order to ensure sufficient time for the dye to be absorbed and transported through the base of the trunk before they were recut  $\sim 75$  mm above the original cut. A 1 mm-thick section of the trunk in the center of this segment was then excised. Tests conducted prior to the experiment demonstrated that this was a sufficient time to allow full dye uptake and transport beyond the furthest cutting point, and during the experiment all stem samples showed evidence of dye uptake throughout the sample

stem segment. These sections were photographed under visible and UV light at 25× magnification (DM 2000, Leica Microsystems, Inc., Wetzlar, Germany) and the images used to quantify the amount of xylem tissue that was actively transporting water at the time of harvest.

### Seedling size and growth

Plant height and trunk diameter at three different trunk locations (basal, middle and upper) were recorded at each measurement period. A fourth location (top) was measured at the final measurement period to account for new height growth. Height was measured parallel to the primary axis of the trunk (i.e., trunk path length) to account for additional path length in leaning plants. Height and diameter data were used to calculate total trunk volume using a conic frustum model. At the baseline and recovery measurement periods a total of 90 seedlings were harvested after obtaining height and diameter measurements. Each harvested plant was dissected and separated into photosynthetic shoot, woody stem and root components and the dry mass of each component was recorded after oven drying at 60 °C for 3 days. Allometric relationships between height, diameter, trunk volume and dry mass of the harvested plants were then used to predict each dry mass component for the remaining un-harvested plants in the experiment at each measurement period (Table S1 available as Supplementary Data at [Tree Physiology](#) Online). Relative growth rate (RGR) was calculated for height, basal diameter and total above-ground dry mass for each plant for the baseline to mild drought, mild drought to severe drought and severe drought to recovery time intervals as:  $RGR = (\ln X_2 - \ln X_1) / (t_2 - t_1)$ , where  $X$  is either plant height, diameter or dry mass, and  $t$  is the number of days (Fisher 1920). The subscripts 1 and 2 refer to the first and last measurement period within each time interval (e.g., baseline and mild drought, respectively).

### Statistics

Differences in soil VWC,  $\Psi_{PD}$ ,  $\Psi_{DT}$ ,  $A$ ,  $g$ ,  $A/g$  and RGR among provenances and water regimes were tested for the entire experiment using three-way repeated-measures analysis of variance (ANOVA). Each species was analyzed separately. Water regime, provenance and their interaction were analyzed as between-subjects factors and measurement period, water regime, provenance and their interactions as within-subjects factors. Values of each variable were log-transformed to meet assumptions of normality and homogeneity of variances. However, we present untransformed data in all figures and tables for ease of interpretation. We tested the assumption of sphericity using Mauchley's criterion. In most cases, the compound symmetry of the covariance matrix did not meet the assumptions of repeated-measures ANOVA, so we report Huynh–Feldt corrected significance levels for all within-subjects effects (Potvin et al. 1990). To further examine the effects of drought and subsequent recovery on

seedling responses, differences in each response variable among provenances and water regimes were tested for each measurement period using two-way ANOVA, analyzing each species separately. We compared the effects of drought on shoot water potential and xylem embolism among provenances and between species, and compared component mass fractions between the species, using one-way ANOVA. When differences were significant we compared means using Tukey's Honestly Significant Difference orthogonal contrast post hoc tests. Relationships between soil VWC and shoot water potential, between shoot water potential and leaf gas exchange, and between mean leaf gas exchange and total growth rates were examined using ordinary least-squares regression. Analysis of covariance (ANCOVA) on log-transformed data was used to test for species differences in the trends of these relationships. Differences in total dry mass between species and treatments were examined using two-way ANOVA at each measurement period. Three-way repeated-measures ANOVA were performed using SPSS v21 (IBM Corporation, Armonk, NY, USA). All other analyses were performed using JMP Pro v10 (SAS Institute, Cary, NC, USA).

## Results

### Effects on soil and plant water status

There was a significant effect of water regime and also an interaction of measurement period with water regime on soil VWC, shoot  $\Psi_{PD}$  and shoot  $\Psi_{DT}$  in both *Sequoia* and *Sequoiadendron* (Figure 1, Table 1; Table S2 available as Supplementary Data at [Tree Physiology](#) Online). Control seedlings had significantly higher soil VWC and shoot  $\Psi_{DT}$  than drought-stressed seedlings at the mild and severe drought periods as well as higher shoot  $\Psi_{PD}$  at the severe drought period in both species. Two weeks after re-watering, soil VWC, shoot  $\Psi_{PD}$  and shoot  $\Psi_{DT}$  in drought-stressed seedlings returned to levels either equal to or greater than control seedlings of both species. There were no significant differences in soil VWC, shoot  $\Psi_{PD}$  or shoot  $\Psi_{DT}$  between control and drought-stressed seedlings or among populations within each species at the baseline period prior to the experimental drought (Figure 1).

Control *Sequoia* maintained mean ( $\pm$ SD) soil VWC of  $28.3 \pm 4.5\%$  and control *Sequoiadendron* maintained soil VWC of  $31.4 \pm 4.2\%$  for the entire 53-day experiment, while soil VWC decreased to  $3.9 \pm 1.1\%$  in drought-stressed *Sequoia* and  $4.9 \pm 0.2\%$  in drought-stressed *Sequoiadendron* by the end of the severe drought period (Figure 1). Despite similar levels of soil water deficit, drought-stressed *Sequoia* exhibited substantially lower shoot water potentials than drought-stressed *Sequoiadendron*, with mean ( $\pm$ SD) shoot  $\Psi_{PD}$  declining to  $-2.35 \pm 0.26$  MPa in *Sequoia* compared with  $-0.69 \pm 0.08$  MPa in *Sequoiadendron*, and shoot  $\Psi_{DT}$  declining to  $-2.81 \pm 0.31$  in *Sequoia* compared with  $-1.39 \pm 0.11$  MPa in *Sequoiadendron* (Figure 1).



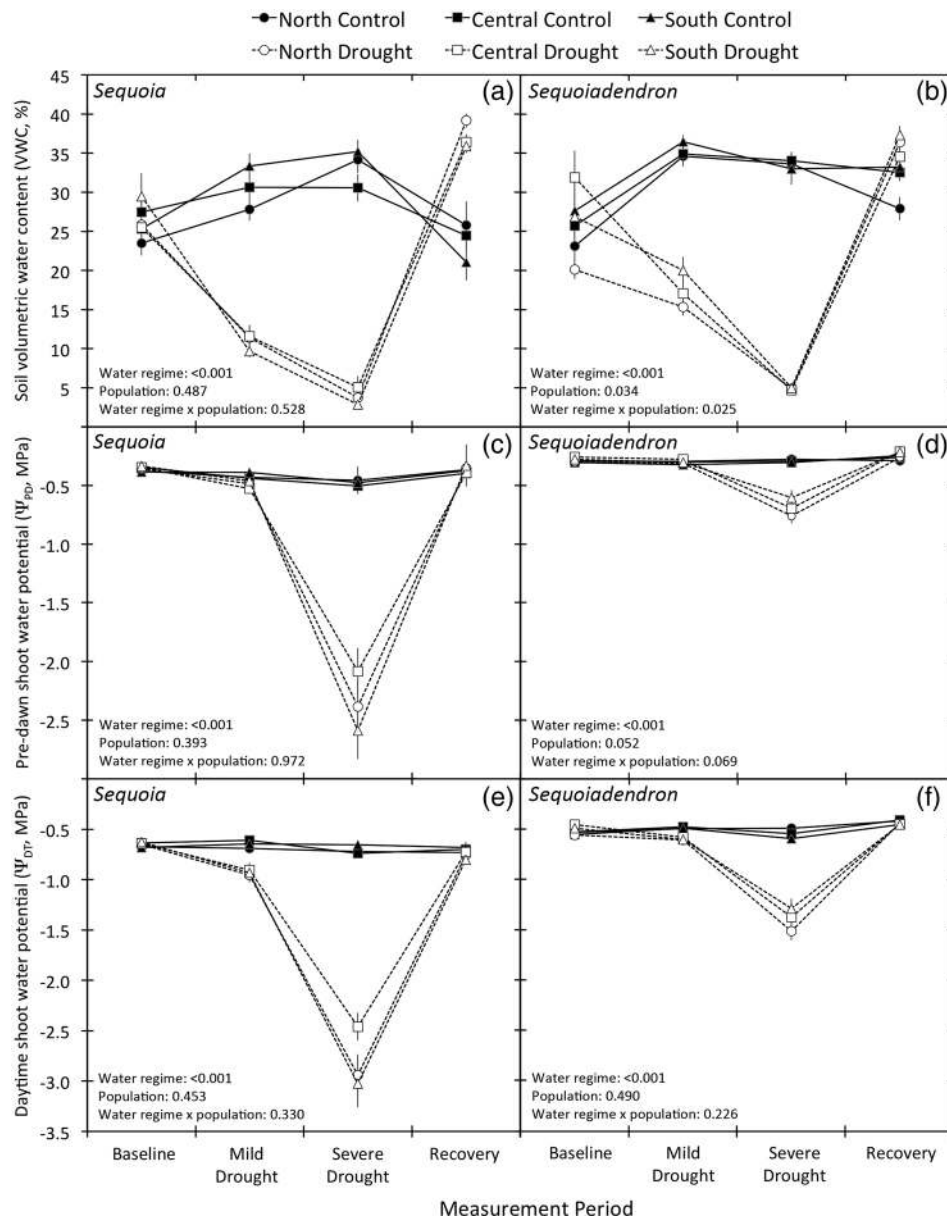


Figure 1. Mean ( $\pm 1$  SE) (a, b) soil VWC, (c, d) pre-dawn shoot water potential, and (e, f) daytime shoot water potential of *Sequoia* and *Sequoiadendron* seedlings representing six different populations subjected to control (well-watered) and drought (no water) water regimes at four different measurement periods. Note the large difference in shoot water potential between the two species at the time of severe drought despite relatively similar soil water contents, highlighting more isohydric behavior in *Sequoiadendron* and more anisohydric behavior in *Sequoia*. Summaries of between-subject three-way repeated-measures ANOVA *P*-values are listed for each source of variation.

Shoot water potentials responded non-linearly to declining soil VWC, with very little change in shoot  $\Psi_{PD}$  or shoot  $\Psi_{DT}$  at soil VWC levels above  $\sim 10\%$ , followed by a transition zone between  $\sim 5$  and  $10\%$ , and finally a steep decline in water potential below  $\sim 5\%$  (Figure 2). *Sequoia* seedlings experienced significantly lower shoot water potentials at a given soil VWC, exhibited a steeper decline in water potential with changing soil VWC, and reached a more negative minimum water potential than *Sequoiadendron* seedlings (Figure 2, Table 2). At soil VWC  $> 10\%$ , mean ( $\pm 1$  SE)  $\Psi_{PD}$  was  $-0.41$  ( $\pm 0.01$ ) MPa in *Sequoia* compared with  $-0.27$  ( $\pm 0.01$ ) MPa in *Sequoiadendron*, while

$\Psi_{DT}$  was  $-0.71$  ( $\pm 0.01$ ) MPa in *Sequoia* compared with  $-0.50$  ( $\pm 0.01$ ) MPa in *Sequoiadendron*. At soil VWC  $\leq 10\%$ , mean ( $\pm 1$  SE)  $\Psi_{PD}$  was  $-0.75$  ( $\pm 0.04$ ) MPa in *Sequoia* compared with  $-0.35$  ( $\pm 0.01$ ) MPa in *Sequoiadendron*, while  $\Psi_{DT}$  was  $-1.10$  ( $\pm 0.05$ ) MPa in *Sequoia* compared with  $-0.65$  ( $\pm 0.02$ ) MPa in *Sequoiadendron* (Figure 2).

#### Effects on trunk embolism

Both species showed a negative relationship between shoot water potential and percent embolism (Figure 3). Drought-stressed seedlings had significantly more embolism than control

Table 1. Summary of three-way repeated-measures ANOVA *P*-values for treatment effects on soil and plant water status, leaf gas exchange and RGRs in *Sequoia* and *Sequoiadendron* seedlings. Soil VWC, soil volumetric water content;  $\Psi_{PD}$ , pre-dawn shoot water potential;  $\Psi_{DT}$ , daytime shoot water potential;  $A_{mass}$ , mass-based net photosynthesis;  $g_{mass}$ , mass-based stomatal conductance;  $A/g$ , intrinsic water-use efficiency; Height RGR, height relative growth rate; Diameter RGR, basal diameter relative growth rate; Mass RGR, above-ground dry mass relative growth rate. Huynh–Feldt corrected *P*-values are provided for all within-subject terms. Absolute values of all data were log-transformed prior to analysis. *P*-values in bold are significant ( $\alpha = 0.05$ ). Each species was analyzed separately.

Species	Source of variation	Soil VWC	$\Psi_{PD}$	$\Psi_{DT}$	$A_{mass}$	$g_{mass}$	$A/g$	Height RGR	Diameter RGR	Mass RGR
<i>Sequoia</i>	Within-subject									
	Period	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Period × water regime	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.033</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Period × population	0.616	0.253	0.987	<b>0.007</b>	0.074	<b>&lt;0.001</b>	0.930	0.869	0.779
	Period × water regime × population	<b>0.019</b>	0.451	0.211	0.060	0.798	<b>&lt;0.001</b>	0.491	<b>0.023</b>	0.140
	Between-subject									
	Water regime	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Population	0.487	0.393	0.453	0.114	0.147	0.163	0.734	0.999	0.514
	Water regime × population	0.528	0.972	0.330	0.178	0.195	0.421	0.679	0.597	0.070
<i>Sequoiadendron</i>	Within-subject									
	Period	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Period × water regime	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Period × population	<b>0.031</b>	0.517	0.357	0.647	0.743	0.768	0.595	0.092	0.603
	Period × water regime × population	<b>0.018</b>	0.514	0.230	0.075	<b>0.052</b>	0.460	0.578	0.249	0.313
	Between-subject									
	Water regime	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.789	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	Population	<b>0.034</b>	<b>0.052</b>	0.490	0.264	0.357	<b>0.045</b>	0.081	0.999	0.271
	Water regime × population	<b>0.025</b>	0.069	0.226	<b>0.005</b>	0.289	0.432	0.859	0.181	0.255

seedlings in both species at the severe drought period but not the recovery period, and *Sequoia* had significantly more embolism than *Sequoiadendron* at both severe drought and recovery periods. Both species showed a similar hydraulic recovery when expressed as a percentage of the maximum during severe drought (mean = 49.5% in *Sequoia* versus 49.3% in *Sequoiadendron*). However, *Sequoia* showed a greater hydraulic recovery when expressed as the absolute change in embolism after severe drought (mean = 17.5% in *Sequoia* versus 6.9% in *Sequoiadendron*). No significant differences in embolism were detected among populations of either species.

### Effects on leaf gas exchange

There was a significant effect of water regime and the interaction of measurement period with water regime on  $A_{mass}$ ,  $g_{mass}$  and  $A/g$  (Figure 4, Table 1). No significant differences were observed in  $A_{mass}$ ,  $g_{mass}$  or  $A/g$  between water treatment groups or among populations within each species at the baseline period prior to the experimental drought, and only a minor yet statistically significant difference in  $g_{mass}$  was observed between control and drought-stressed *Sequoia* at the mild-drought period (Figure 4, Tables S3 and S4 available as Supplementary Data at *Tree Physiology* Online). Control seedlings had significantly

higher  $A_{mass}$  and  $g_{mass}$  and significantly lower  $A/g$  than drought-stressed seedlings at the severe drought period in both species. Two weeks after rehydration,  $A_{mass}$  in drought-stressed seedlings recovered to control levels in all *Sequoiadendron* populations as well as northern and central *Sequoia* populations,  $g_{mass}$  recovered in northern and central *Sequoia* populations but not in any *Sequoiadendron* population, and  $A/g$  recovered only in the central *Sequoiadendron* population but not in any *Sequoia* population (Figure 4).

Control *Sequoiadendron* exhibited higher  $A$  and  $g$  rates and lower  $A/g$  than control *Sequoia* throughout the experiment, with a mean ( $\pm$ SD)  $A_{mass} = 215.6 \pm 36.1$  nmol g<sup>-1</sup> s<sup>-1</sup> in *Sequoiadendron* versus 146.5  $\pm$  19.5 nmol g<sup>-1</sup> s<sup>-1</sup> in *Sequoia* ( $A_{area} = 21.3 \pm 2.2$  versus 10.2  $\pm$  1.4  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>),  $g_{mass} = 4.83 \pm 0.84$  mmol g<sup>-1</sup> s<sup>-1</sup> in *Sequoiadendron* versus 2.05  $\pm$  0.22 mmol g<sup>-1</sup> s<sup>-1</sup> in *Sequoia* ( $g_{area} = 0.48 \pm 0.08$  versus 0.14  $\pm$  0.01 mol m<sup>-2</sup> s<sup>-1</sup>), and  $A/g = 49.3 \pm 8.5$  nmol mmol<sup>-1</sup> in *Sequoiadendron* versus 79.8  $\pm$  10.1 nmol mmol<sup>-1</sup> in *Sequoia*. However, drought-stressed *Sequoiadendron* showed a greater decrease in  $A$  and  $g$  and a greater increase in  $A/g$  compared with controls than drought-stressed *Sequoia* during the severe drought period, with a mean  $A_{mass}$  difference between drought-stressed and control seedlings of 138.2 nmol g<sup>-1</sup> s<sup>-1</sup> in *Sequoiadendron* versus 119.8 nmol g<sup>-1</sup> s<sup>-1</sup> in

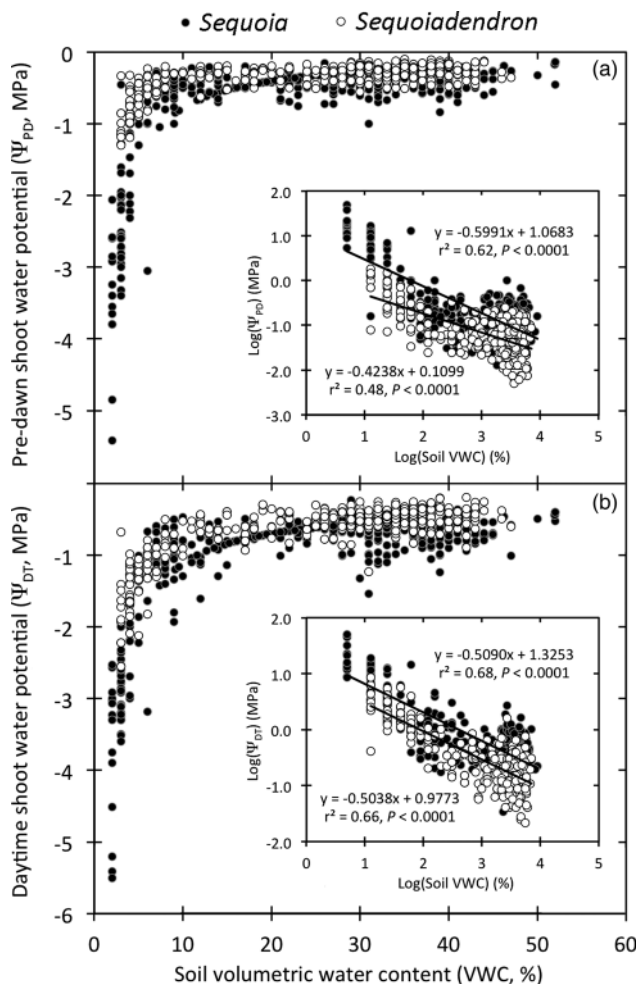


Figure 2. (a) Pre-dawn and (b) daytime shoot water potential as a function of soil VWC for *Sequoia* and *Sequoiadendron* seedlings. *Sequoia* seedlings generally exhibit a more negative shoot water potential at a given soil VWC compared with *Sequoiadendron*. Measurements from all populations and both water regimes (control and drought) are pooled for each species. Each circle represents one individual measurement. Inset figures show log–log plots using the absolute value of water potential and best-fit linear regression models, coefficients of determination and *P*-values for each variable and species.

*Sequoia* ( $A_{\text{area}} = 12.1$  versus  $8.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), a mean  $g_{\text{mass}}$  difference of  $4.28 \text{ mmol g}^{-1} \text{s}^{-1}$  in *Sequoiadendron* versus  $1.93 \text{ mmol g}^{-1} \text{s}^{-1}$  in *Sequoia* ( $g_{\text{area}} = 0.38$  versus  $0.13 \text{ mol m}^{-2} \text{s}^{-1}$ ) and a mean  $A/g$  difference of  $95.2 \text{ nmol mmol}^{-1}$  in *Sequoiadendron* versus  $68.7 \text{ nmol mmol}^{-1}$  in *Sequoia* (Figure 4).

Leaf gas exchange responded non-linearly to changes in shoot water potential in both species, with a steep decline in  $A_{\text{mass}}$  and  $g_{\text{mass}}$  at  $\Psi_{\text{PD}}$  from 0 to  $-0.5$  MPa, a transition zone at  $\Psi_{\text{PD}}$  from  $-0.5$  to  $-1.5$  MPa, and relatively little change at  $\Psi_{\text{PD}}$  lower than  $-1.5$  MPa (Figure 5, Table 2). *Sequoiadendron* maintained significantly higher gas exchange than *Sequoia* at high  $\Psi_{\text{PD}}$  but experienced a steeper decline and complete cessation of gas exchange as  $\Psi_{\text{PD}}$  decreased below  $-0.5$  MPa, while *Sequoia* experienced more negative  $\Psi_{\text{PD}}$  at low gas exchange

rates (Figure 5, Table 2). At  $\Psi_{\text{PD}} \geq -1.0$  MPa, mean ( $\pm 1$  SE)  $A_{\text{mass}}$  was  $133.59 (\pm 2.71) \text{ nmol g}^{-1} \text{s}^{-1}$  in *Sequoia* compared with  $206.73 (\pm 3.80) \text{ nmol g}^{-1} \text{s}^{-1}$  in *Sequoiadendron*, while  $g_{\text{mass}}$  was  $1.70 (\pm 0.05) \text{ mmol g}^{-1} \text{s}^{-1}$  in *Sequoia* compared with  $4.09 (\pm 0.12) \text{ mmol g}^{-1} \text{s}^{-1}$  in *Sequoiadendron*. At  $\Psi_{\text{DT}} \geq -1.0$  MPa, mean ( $\pm 1$  SE)  $A_{\text{mass}}$  was  $143.20 (\pm 2.32) \text{ nmol g}^{-1} \text{s}^{-1}$  in *Sequoia* compared with  $208.37 (\pm 2.85) \text{ nmol g}^{-1} \text{s}^{-1}$  in *Sequoiadendron*, while  $g_{\text{mass}}$  was  $1.92 (\pm 0.05) \text{ mmol g}^{-1} \text{s}^{-1}$  in *Sequoia* compared with  $4.34 (\pm 0.09) \text{ mmol g}^{-1} \text{s}^{-1}$  in *Sequoiadendron*.

### Effects on growth and biomass allocation

There was a significant effect of water regime on height RGRs in *Sequoia* and a significant effect of water regime and the interaction of water regime with measurement period on basal diameter and above-ground mass RGRs in both species (Figure 6, Table 1). No significant effect of population or the interaction of population with either water regime or measurement period on any RGR was observed (Table 1). At the baseline-to-mild-drought time interval, there were no significant differences in height, diameter or above-ground mass RGRs between control versus drought-stressed seedlings or among populations within each species (Figure 6, Table S5 available as Supplementary Data at *Tree Physiology* Online). At the mild to severe drought time interval, control seedlings had significantly greater height RGRs than drought-stressed seedlings in *Sequoia*, and control seedlings had significantly greater basal diameter and above-ground mass RGRs than drought-stressed seedlings in both species (Figure 6). And at the severe drought to recovery time interval, there were either no or only minor significant differences in height, basal diameter or above-ground mass RGRs between control and drought-stressed seedlings of both species (Figure 6).

Despite having lower leaf gas exchange, control *Sequoia* had 50% greater height, 38% greater basal diameter, and 33% greater above-ground mass RGRs than control *Sequoiadendron* (Figure 6). However, *Sequoia* growth rates were more sensitive to drought than *Sequoiadendron*, with drought-stressed *Sequoia* experiencing 41% less height, 56% less basal diameter and 49% less above-ground mass RGRs than control *Sequoia*, compared with drought-stressed *Sequoiadendron* experiencing 11% less height, 20% less basal diameter and 15% less above-ground mass RGRs than control *Sequoiadendron* (Figure 6).

Although all seedlings were the same age, the total dry mass of *Sequoia* seedlings was significantly greater than *Sequoiadendron* seedlings, with the difference between species increasing throughout the experiment due to greater growth rates in *Sequoia* (Figure 7). Differences between control and drought-stressed seedlings were greater in *Sequoia* than in *Sequoiadendron*, with these differences increasing over the course of the experiment. *Sequoiadendron* had a significantly greater fraction of total plant dry mass allocated to

Table 2. Summary of analysis of covariance effects tests for bivariate fits shown in inset Figures 2 and 5. Soil VWC, soil volumetric water content;  $\Psi_{PD}$ , pre-dawn shoot water potential,  $\Psi_{DT}$ , daytime shoot water potential;  $A_{mass}$ , mass-based net photosynthesis;  $g_{mass}$ , mass-based stomatal conductance.

Covariate	Response variable	Source of variation	df	Sum of squares	F-ratio	Prob > F
Log(soil VWC)	Log( $\Psi_{PD}$ )	Species	1	34.1837	223.6176	<0.0001
		Log(soil VWC)	1	133.04512	870.3336	<0.0001
		Species $\times$ log(soil VWC)	1	3.90892	25.5708	<0.0001
	Log( $\Psi_{DT}$ )	Species	1	19.31468	209.3892	<0.0001
		Log(soil VWC)	1	130.43596	1414.048	<0.0001
		Species $\times$ log(soil VWC)	1	0.00351	0.038	0.8455
Log( $\Psi_{PD}$ )	Log( $A_{mass}$ )	Species	1	5.40313	21.7259	<0.0001
		Log( $\Psi_{PD}$ )	1	131.65955	529.4009	<0.0001
		Species $\times$ log( $\Psi_{PD}$ )	1	0.28184	1.1333	0.2874
	Log( $g_{mass}$ )	Species	1	23.43738	50.2841	<0.0001
		Log( $\Psi_{PD}$ )	1	307.86843	660.5207	<0.0001
		Species $\times$ log( $\Psi_{PD}$ )	1	4.74887	10.1885	0.0015
Log( $\Psi_{DT}$ )	Log( $A_{mass}$ )	Species	1	5.58418	29.1565	<0.0001
		Log( $\Psi_{DT}$ )	1	158.13814	825.6812	<0.0001
		Species $\times$ log( $\Psi_{DT}$ )	1	6.96182	36.3495	<0.0001
	Log( $g_{mass}$ )	Species	1	34.55874	110.5734	<0.0001
		Log( $\Psi_{DT}$ )	1	418.85798	1340.169	<0.0001
		Species $\times$ log( $\Psi_{DT}$ )	1	0.23474	0.7511	0.3864

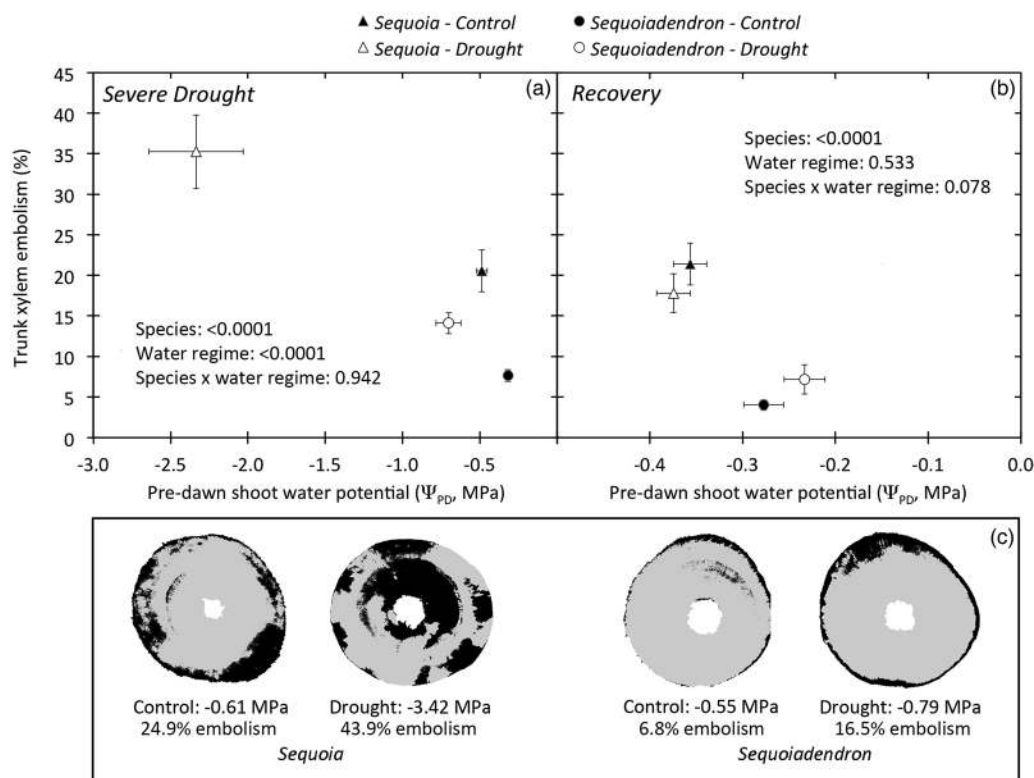


Figure 3. Trunk xylem embolism (%) as a function of pre-dawn shoot water potential in *Sequoia* and *Sequoiadendron* seedlings subjected to control (well-watered) and drought (no water) water regimes during (a) severe drought and (b) recovery measurement periods, with (c) stem cross-section images showing example spatial patterns and degrees of embolism (dark areas) for each species and water regime during the severe drought measurement period but not the recovery period, while *Sequoia* experienced significantly greater embolism than *Sequoiadendron* seedlings during both measurement periods.



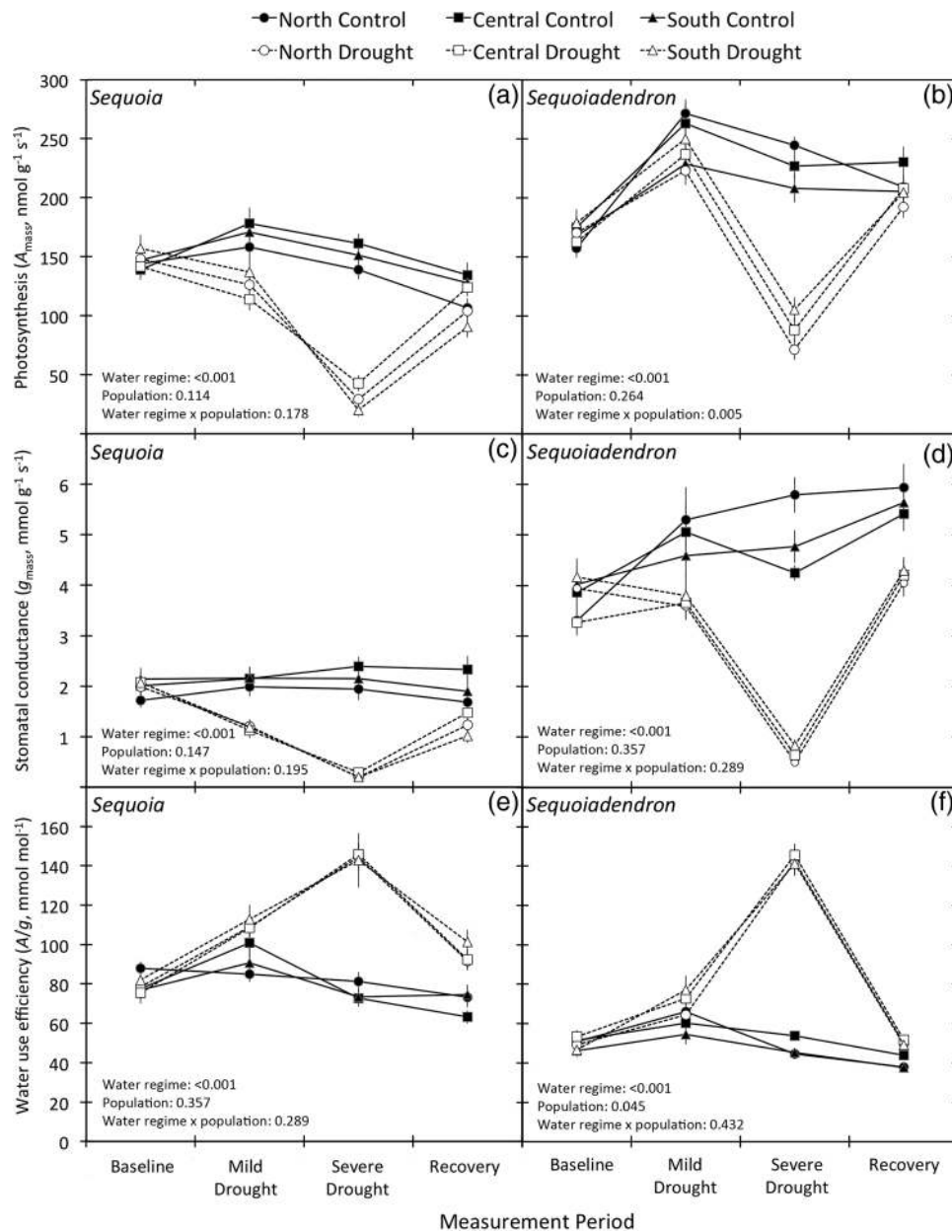


Figure 4. Mean ( $\pm 1$  SE) (a, b) leaf net photosynthesis, (c, d) stomatal conductance and (e, f) intrinsic water-use efficiency of *Sequoia* and *Sequoiadendron* seedlings representing six different populations subjected to control (well-watered) and drought (no water) water regimes at four different measurement periods. *Sequoiadendron* generally exhibit higher photosynthesis and stomatal conductance and lower intrinsic water-use efficiency compared with *Sequoia* seedlings under well-watered conditions, but also show a greater absolute change in these rates in response to severe drought. Summaries of between-subject three-way repeated-measures ANOVA  $P$ -values are listed for each source of variation.

roots but a significantly lower fraction allocated to woody stems than *Sequoia*, while both species had similar fractions allocated to photosynthetic shoots (Figure 7). No significant drought or population effect on dry mass allocation was observed in either species.

#### Links between gas exchange and growth

There were distinct differences in the relationship between mean leaf gas exchange rates and total growth rates in *Sequoia* compared with *Sequoiadendron*. Both species showed a linear

increase in total height, basal diameter and above-ground dry mass growth with increasing mean leaf gas exchange rate, but there was a significantly greater increase in growth per unit increase in  $A$  and  $g$  in *Sequoia* than in *Sequoiadendron* (Figure 8). Although *Sequoia* seedlings generally possessed more shoots than *Sequoiadendron* seedlings, greater leaf-level gas exchange in *Sequoiadendron* compensated for these differences when scaled to the entire plant, with *Sequoiadendron* seedlings exhibiting either comparable or significantly greater whole-plant instantaneous net photosynthesis, stomatal

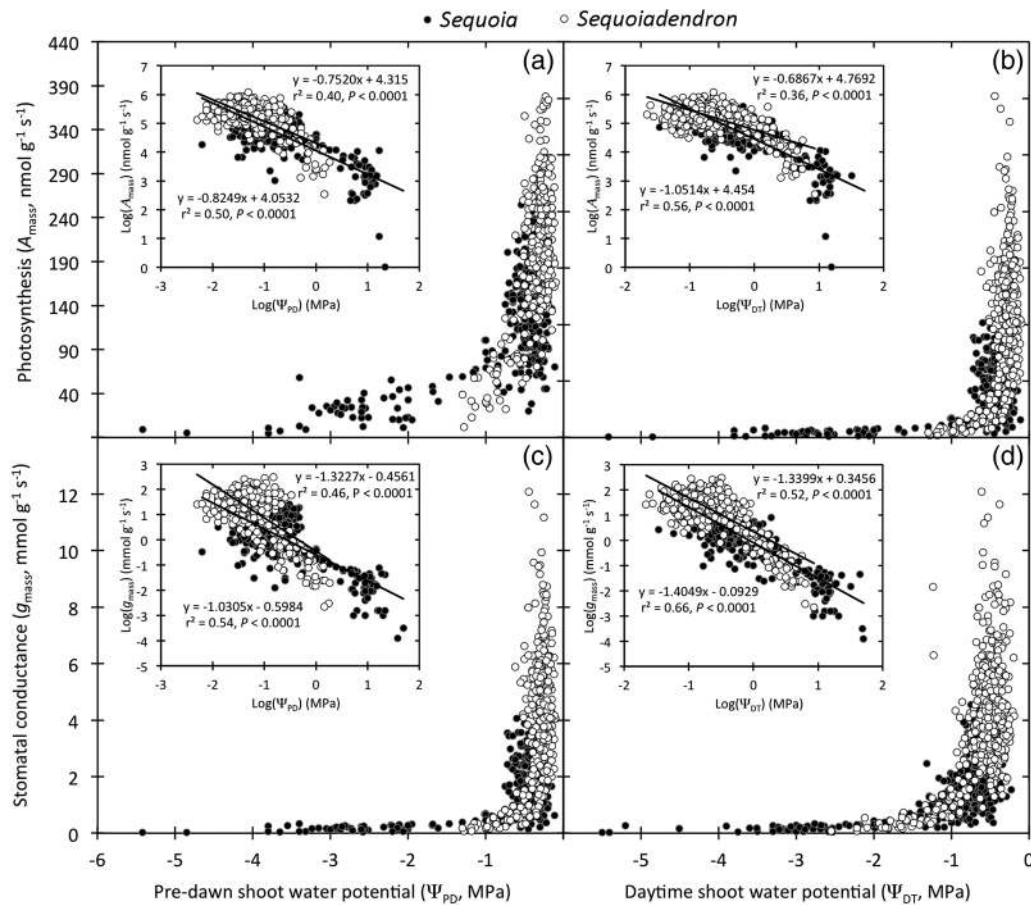


Figure 5. (a, b) Leaf net photosynthesis and (c, d) stomatal conductance as a function of pre-dawn and daytime shoot water potential for *Sequoia* and *Sequoiadendron* seedlings. Both species exhibited relatively similar leaf gas exchange responses to changing shoot water potential, although *Sequoiadendron* generally maintained higher gas exchange rates at high water potential and ceased gas exchange at less negative water potential compared with *Sequoia* seedlings. Measurements from all populations and both water regimes (control and drought) are pooled for each species. Each circle represents one individual measurement. Inset figures show log–log plots and best-fit linear regression models, coefficients of determination and  $P$ -values for each variable and species.

conductance and transpiration than *Sequoia* seedlings within a given watering treatment (Table 3).

## Discussion

### Contrasting drought-response strategies

Our study revealed that *Sequoia* and *Sequoiadendron* possess contrasting drought-response strategies that likely reflect adaptations to the divergent environmental conditions each species experiences in its native habitat. *Sequoiadendron* seedlings had greater leaf gas exchange rates than *Sequoia* seedlings when soil water was abundant, but also showed a much greater leaf-level response to declining soil moisture, closing their stomata to maintain favorable plant water status, increase intrinsic water-use efficiency and minimize trunk xylem embolism. In contrast, *Sequoia* seedlings had lower leaf gas exchange rates and higher intrinsic water-use efficiency when soil moisture was high, but did not close their stomata as much when soil water declined, leading to substantially lower shoot

water potential and greater levels of xylem embolism than drought-stressed *Sequoiadendron* seedlings. *Sequoia* seedlings also had greater RGRs than *Sequoiadendron* seedlings when soil moisture was abundant, but experienced a greater decline in RGRs when exposed to drought. While both species invested similar amounts of biomass into green photosynthetic shoots, *Sequoia* seedlings invested proportionally more biomass into above-ground woody stem growth whereas *Sequoiadendron* invested more into below-ground root growth. Substantial root investments are critical for successful establishment and survival of *Sequoiadendron* seedlings in their relatively warm and dry native habitat, as desiccation has been identified as the primary cause of *Sequoiadendron* seedling mortality in the field (Stark 1968, Rundel 1972, Harvey et al. 1980). In contrast, investment in woody trunk growth that facilitates rapid increases in height contributes to the competitive success of *Sequoia* seedlings and trees in the relatively moist, humid and closed canopy conditions of their coastal habitat (Sawyer et al. 2000).

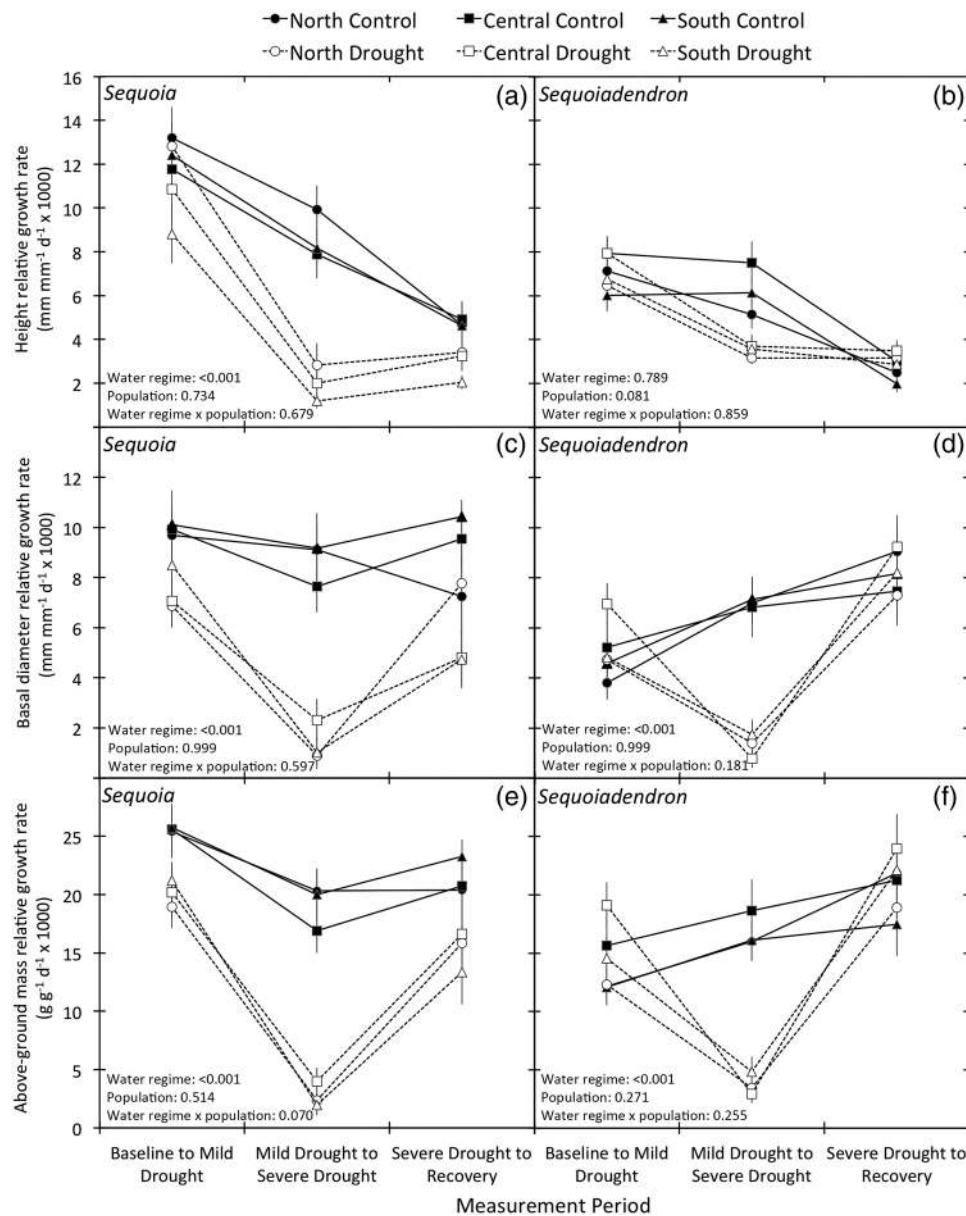


Figure 6. Mean ( $\pm 1$  SE) (a, b) relative height growth rate, (c, d) relative basal diameter growth rate and (e, f) relative above-ground mass growth rate of *Sequoia* and *Sequoiadendron* seedlings representing six different populations subjected to control (well-watered) and drought (no water) water regimes at three different measurement period intervals. *Sequoia* generally exhibit greater RGRs compared with *Sequoiadendron* seedlings under well-watered conditions, but also show a greater absolute decline in growth rates in response to severe drought. Summaries of between-subject three-way repeated-measures ANOVA *P*-values are listed for each source of variation.

### Isohydry versus anisohydry

Both species showed relatively similar stomatal responses to mild-drought conditions but showed divergent responses when challenged by severe drought, with greater stomatal closure in *Sequoiadendron* preventing daytime shoot water potential from going below  $-2.5$  MPa while *Sequoia* daytime values reached less than  $-5.3$  MPa under similar soil moisture conditions. These results highlight the importance of considering both the shape of the stomatal response curve to changing plant water status as well as the absolute minimum water potential experienced by a

plant when comparing water-use behavior and degree of isohydry among species (e.g., Figures 1, 2 and 5). Because there is a continuum between isohydry and anisohydry, species will fall somewhere along a gradient describing the control of plant water status under changing soil moisture and evaporative demand. In some cases, such as in piñon pine–juniper woodlands of the American southwest, there are clear differences in both the shape of the stomatal response to changing plant water status as well as in the minimum water potential reached by the two species (McDowell et al. 2008). In other cases, such as in

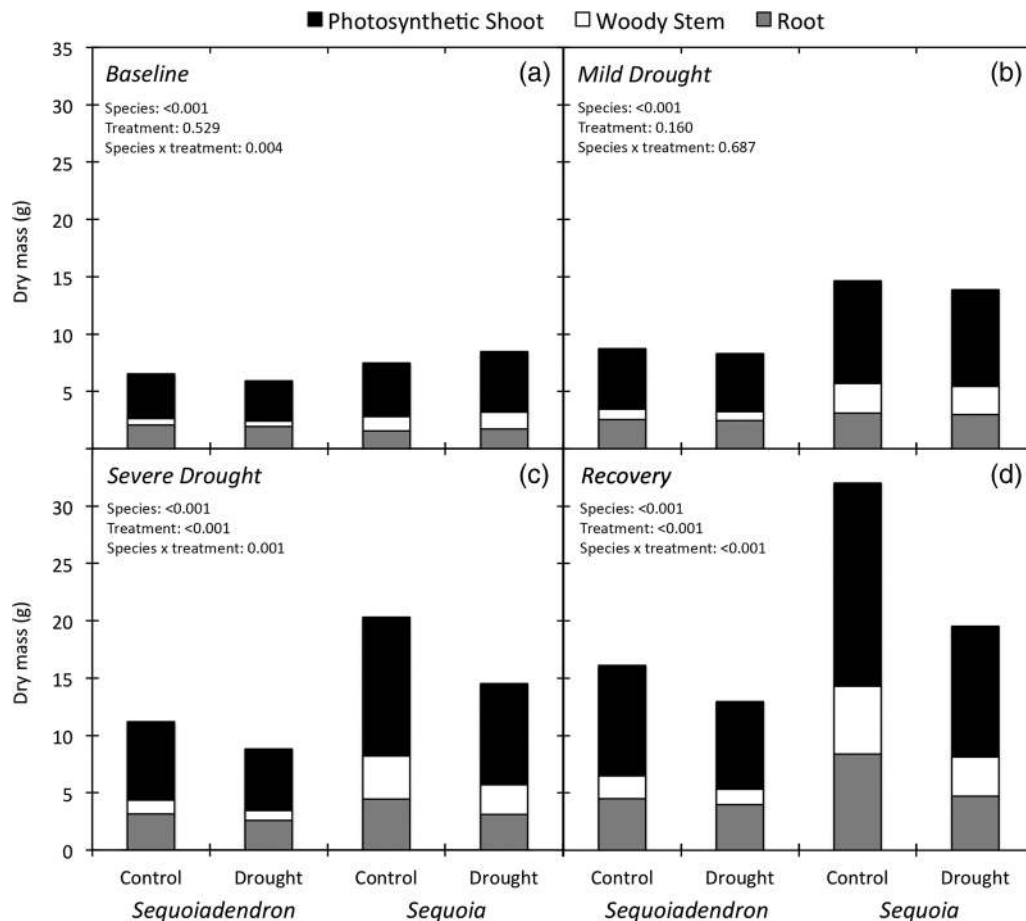


Figure 7. Whole-plant dry mass of photosynthetic shoots, woody stems and roots in control and drought-stressed *Sequoia* and *Sequoiadendron* seedlings at (a) baseline, (b) mild drought, (c) severe drought, and (d) recovery measurement periods. Photosynthetic shoots include all green tissue, woody stems include all brown non-photosynthetic above-ground tissue covered by bark, and roots include all below-ground tissue. Relative to total plant size, *Sequoia* seedlings invested significantly more dry mass in woody stems while *Sequoiadendron* seedlings invested significantly more dry mass in roots at all measurement periods ( $P < 0.0001$  for both components). No significant difference in the photosynthetic shoot fraction between species was observed. Summaries of two-way ANOVA  $P$ -values are listed for each source of variation.

the fynbos ecosystem of South Africa, the shape of the stomatal response to changing water status is relatively similar among both iso- and anisohydric species under well-watered conditions but the minimum water potential differs, with anisohydric species reaching significantly lower minimum shoot water potential than isohydric species under severe drought conditions (West et al. 2012). We conclude that even though *Sequoia* and *Sequoiadendron* share similar stomatal responses to changing water potential under well-watered and mild-drought conditions, the large difference in minimum shoot water potential experienced by the two species as well as the water potential at which stomatal closure occurs under severe water deficit warrant describing *Sequoiadendron* as relatively isohydric and *Sequoia* as relatively anisohydric.

Differences in plant size and allocation patterns (i.e., root-to-shoot ratios) may have also contributed to some of the differences in the observed water-use patterns exhibited by the two species. Greater total leaf area in *Sequoia* seedlings

could potentially increase total water loss via transpiration, leading to a greater drawdown of soil moisture. However, greater leaf-level stomatal conductance and transpiration in *Sequoiadendron* compensated for lower leaf area, resulting in similar or even greater water use at the whole-plant scale. The lower shoot water potential experienced by *Sequoia* at a given soil water content may be potentially explained by higher nighttime transpiration rates in *Sequoia*, leading to dis-equilibrium between soil water potential and pre-dawn shoot water potential (Donovan et al. 2001). We have previously documented large nighttime transpiration rates in *Sequoia*, attributed in part to asymmetrical guard cells and the presence of fungal endophytes which prevent full stomatal closure, as well as relatively poor stomatal control in response to increasing VPD (Burgess and Dawson 2004, Dawson et al. 2007). While we do not know the extent or magnitude of nighttime transpiration in *Sequoiadendron*, this species does not have asymmetrical guard cells, appears to have lower levels of fungal



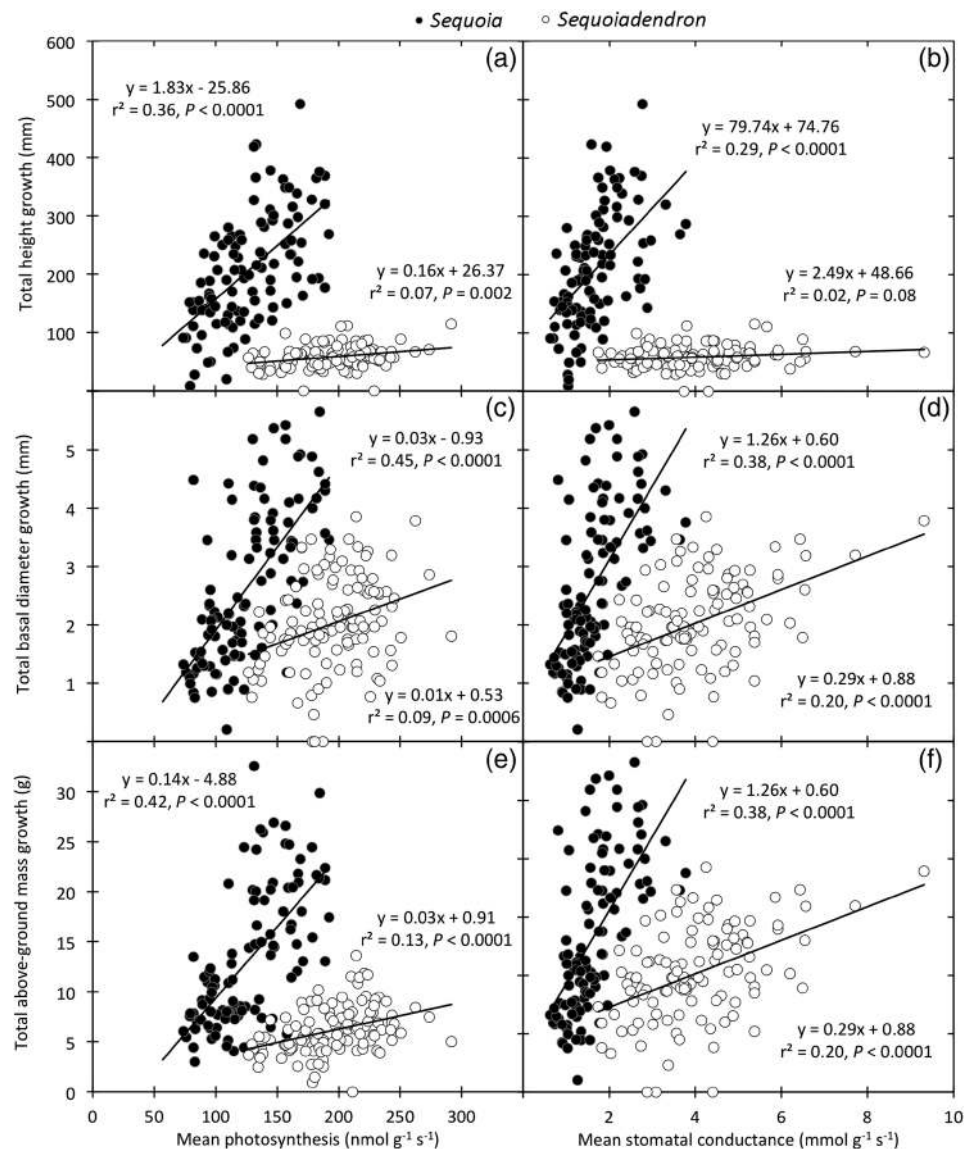


Figure 8. Total (a, b) height growth, (c, d) basal diameter growth and (e, f) above-ground mass growth as a function of mean leaf net photosynthesis and stomatal conductance in *Sequoia* and *Sequoiadendron* seedlings. *Sequoia* generally experienced greater total growth at a given photosynthesis or stomatal conductance but also exhibited greater sensitivity to changes in leaf gas exchange compared with *Sequoiadendron* seedlings. Each circle represents one individual plant over the entire experiment. Summaries of linear regressions with coefficients of determination and *P*-values are listed for each variable and species.

endophytes, and has greater stomatal sensitivity to VPD compared with *Sequoia* (A.R. Ambrose, unpublished data), suggesting that nighttime water loss is likely lower in *Sequoiadendron*. *Sequoia* also lacks root hairs which likely reduces the functional root surface area available for soil water absorption (Olson et al. 1990) and further contributes to lower shoot water potential compared with *Sequoiadendron*. Finally, even though *Sequoiadendron* exhibited comparable or greater instantaneous whole-plant water use, greater levels of stomatal closure may have resulted in lower total integrated daily water use compared with *Sequoia*. These results suggest that plant size, biomass allocation and other morphological traits should be considered in addition to stomatal behavior

when characterizing the drought-response strategies of plant species.

### Links between gas exchange and growth

*Sequoiadendron* showed a greater sensitivity of leaf gas exchange to severe drought, whereas *Sequoia* showed a greater sensitivity of growth, resulting in contrasting relationships between leaf gas exchange and RGRs in the two species. *Sequoiadendron* seedlings had greater light-saturated photosynthetic rates than *Sequoia*, yet *Sequoia* had greater RGRs at a given mean photosynthetic rate than *Sequoiadendron*, and experienced a much greater change in RGR for a given change in mean photosynthetic rate. We speculate that these divergent patterns may be

Table 3. Summary of whole-plant mass-based gas exchange of *Sequoia* and *Sequoiadendron* seedlings subjected to two different water regimes at four measurement periods.  $A_{\text{mass}}$ , mass-based net photosynthesis;  $g_{\text{mass}}$ , mass-based stomatal conductance;  $E_{\text{mass}}$ , mass-based transpiration. Values are means  $\pm$  1 SE. Significant differences (2-way ANOVA) among treatment groups within each measurement period are distinguished by different letters (Tukey-Kramer HSD,  $\alpha = 0.05$ ).

Measurement period	Species	Water regime	$A_{\text{mass}}$ ( $\mu\text{mol s}^{-1}$ )	$g_{\text{mass}}$ ( $\text{mol s}^{-1}$ )	$E_{\text{mass}}$ ( $\text{mmol s}^{-1}$ )
Baseline	<i>Sequoia</i>	Control	672.8 $\pm$ 31.9 <sup>ab</sup>	9.31 $\pm$ 0.80 <sup>c</sup>	148.3 $\pm$ 10.4 <sup>b</sup>
		Drought	765.9 $\pm$ 38.2 <sup>a</sup>	10.61 $\pm$ 0.76 <sup>bc</sup>	166.0 $\pm$ 10.1 <sup>ab</sup>
	<i>Sequoiadendron</i>	Control	630.1 $\pm$ 32.1 <sup>b</sup>	13.99 $\pm$ 0.81 <sup>a</sup>	196.3 $\pm$ 10.5 <sup>a</sup>
		Drought	588.6 $\pm$ 37.2 <sup>b</sup>	12.71 $\pm$ 0.74 <sup>ab</sup>	179.2 $\pm$ 9.8 <sup>ab</sup>
Mild Drought	<i>Sequoia</i>	Control	1550.6 $\pm$ 81.0 <sup>a</sup>	19.08 $\pm$ 1.57 <sup>b</sup>	293.7 $\pm$ 20.2 <sup>ab</sup>
		Drought	1050.3 $\pm$ 60.6 <sup>c</sup>	9.66 $\pm$ 1.06 <sup>c</sup>	154.9 $\pm$ 14.1 <sup>c</sup>
	<i>Sequoiadendron</i>	Control	1334.8 $\pm$ 80.3 <sup>ab</sup>	25.96 $\pm$ 1.56 <sup>a</sup>	352.1 $\pm$ 20.0 <sup>a</sup>
		Drought	1195.8 $\pm$ 58.9 <sup>bc</sup>	18.52 $\pm$ 1.03 <sup>b</sup>	260.7 $\pm$ 13.7 <sup>b</sup>
Severe Drought	<i>Sequoia</i>	Control	1843.9 $\pm$ 79.0 <sup>a</sup>	27.02 $\pm$ 1.88 <sup>b</sup>	399.2 $\pm$ 25.0 <sup>a</sup>
		Drought	276.4 $\pm$ 31.5 <sup>c</sup>	2.02 $\pm$ 0.25 <sup>c</sup>	32.9 $\pm$ 3.7 <sup>b</sup>
	<i>Sequoiadendron</i>	Control	1550.2 $\pm$ 79.7 <sup>b</sup>	34.35 $\pm$ 1.89 <sup>a</sup>	460.4 $\pm$ 25.2 <sup>a</sup>
		Drought	471.5 $\pm$ 30.6 <sup>c</sup>	3.49 $\pm$ 0.24 <sup>c</sup>	53.9 $\pm$ 3.6 <sup>b</sup>
Recovery	<i>Sequoia</i>	Control	2183.0 $\pm$ 107.9 <sup>a</sup>	34.93 $\pm$ 2.91 <sup>b</sup>	532.2 $\pm$ 36.8 <sup>b</sup>
		Drought	1257.8 $\pm$ 57.7 <sup>b</sup>	14.78 $\pm$ 1.30 <sup>c</sup>	231.3 $\pm$ 16.4 <sup>c</sup>
	<i>Sequoiadendron</i>	Control	2073.7 $\pm$ 107.9 <sup>a</sup>	55.53 $\pm$ 2.91 <sup>a</sup>	740.9 $\pm$ 36.8 <sup>a</sup>
		Drought	1549.3 $\pm$ 55.5 <sup>b</sup>	32.64 $\pm$ 1.25 <sup>b</sup>	441.5 $\pm$ 15.8 <sup>b</sup>

associated with differences in leaf morphology and stomatal sensitivity to drought. Both species allocated similar proportions of their total biomass to foliage. However, significantly greater leaf mass per area (LMA) in *Sequoiadendron* seedlings imply greater construction costs per unit leaf area, resulting in less total leaf surface area available for light interception and hence photosynthetic carbon gain for a given plant size compared with *Sequoia* (Poorter and Remkes 1990). Greater LMA values in *Sequoiadendron* also are likely to be associated with greater respiration and carbon losses compared with *Sequoia* (Reich et al. 1998, Mullin et al. 2009), possibly further contributing to species-level differences in RGRs. Finally, even though *Sequoiadendron* had higher maximum light-saturated photosynthetic rates, greater stomatal closure under severe drought likely led to lower total integrated daily carbon gain, further contributing to lower growth rates compared with *Sequoia*.

### Hydraulic failure and recovery

Previous studies show that *Sequoia* has similar or slightly lower vulnerability to xylem embolism than *Sequoiadendron* (Ambrose et al. 2009, Pittermann et al. 2010, Jansen et al. 2012). We found that trunk embolism during severe drought was significantly greater in *Sequoia* than *Sequoiadendron*, indicating that the lower minimum shoot water potential in *Sequoia* resulted in a smaller trunk hydraulic safety margin compared with the more isohydric *Sequoiadendron*. Anisohydric behavior has previously been considered as an adaptation to more drought-prone habitats because plants in these areas tend to possess more cavitation-resistant xylem (Oren et al. 1999, Brodribb and Holbrook 2004a, McDowell et al. 2008, West et al. 2012). However, our results support other studies suggesting that anisohydric

species can be more vulnerable than isohydric species to hydraulic failure (Vilagrosa et al. 2003, Quero et al. 2011, Kumagai and Porporato 2012), and highlight the need to consider both xylem cavitation resistance as well as stomatal behavior when evaluating species vulnerability to drought.

Control seedlings of both redwood species had 4–21% average loss of hydraulic conducting area, a range consistent with other studies showing up to 30% native embolism even under well-watered conditions (Sperry et al. 1998, Pockman and Sperry 2000, Cobb et al. 2007, Lovisolo et al. 2008). Low levels of native embolism may represent a group of vulnerable conduits that the plant is unable to refill (e.g., perhaps due to compromised structural integrity) but may not strongly influence daily gas exchange (Martorell et al. 2014). The patterns we observed are consistent with previous studies suggesting that some degree of embolism may enhance productivity because the loss of trunk hydraulic function may be reversible over short time scales (Jones and Sutherland 1991, Brodribb and Holbrook 2004b, Meinzer et al. 2009).

We found that the degree of embolism in drought-stressed seedlings of both species returned to similar levels as control seedlings 2 weeks after re-watering. Drought-stressed *Sequoia* showed an average 11.6% increase in basal diameter between the severe drought and recovery time periods while drought-stressed *Sequoiadendron* showed 15.9% increase, corresponding to an average 17.5% decrease in embolism in *Sequoia* and 6.9% decrease in *Sequoiadendron*. This suggests that hydraulic recovery at least partially resulted from the growth of new functional xylem (Brodribb and Cochard 2009, Brodribb et al. 2010), but also that some degree of embolism refilling might have played a role. Although the details of the

repair mechanism are still under debate, sugars released from the phloem and transported via ray parenchyma cells may generate an osmotic gradient that drives water movement into embolized conduits (Salleo et al. 2009, Brodersen et al. 2010, Nardini et al. 2011, Secchi and Zwieniecki 2012). While likely fewer in number than angiosperms, both *Sequoia* and *Sequoiadendron* possess relatively abundant ray parenchyma cells that might facilitate embolism refilling (Mitchell 1936, Panshin and de Zeeuw 1980). In addition, the small height of the seedlings in this study might have allowed some degree of capillary refilling even in the absence of metabolic processes. Angiosperms are generally considered to have lower hydraulic safety margins but also a greater capacity to recover from drought-induced embolism than conifers (Meinzer et al. 2009, Choat et al. 2012, Johnson et al. 2012). Nevertheless, evidence of embolism repair has been observed in several conifers (Borghetti et al. 1991, Edwards et al. 1994, McCulloh et al. 2011), including *Sequoia* (Litvak et al. 2011), indicating that traits influencing embolism avoidance and repair may not be strictly limited by phylogeny.

#### Limited intra-specific variation in redwood drought response

Large genetic variation among populations of long-lived and sessile organisms such as trees provides an important evolutionary capacity for adapting to changing environmental conditions (Hamrick and Godt 1996). Significant intra-specific variation in drought response and associated physiological traits has been reported in a large number of tree species, including members of *Picea* (Kapeller et al. 2012), *Pinus* (Taeger et al. 2013), *Pseudotsuga* (Chen et al. 2010), *Betula* (Aspelmeier and Leuschner 2006), *Fagus* (Peuke et al. 2002) and *Populus* (Schreiber et al. 2011). Contrary to expectations, we found no significant differences in water potential, hydraulic function or growth and only minor differences in leaf gas exchange among *Sequoia* and *Sequoiadendron* populations. It is possible that larger differences exist among other populations we did not examine, that differences might become apparent over a longer experimental time frame, that differences might exist in other functional traits, or that differences might become apparent with changes in tree size or age. For example, significant population-level differences have been observed previously in *Sequoia* metabolic response to temperature (Anekonda et al. 1994) and *Sequoiadendron* frost resistance and growth (Guinon et al. 1982). Although further research is needed on other important plant traits and in other populations not sampled here, our results indicate that functional drought response may not substantially vary among populations of either redwood species.

#### Effects of tree size on drought vulnerability

Tree seedlings are likely to be the most vulnerable life history stage to drought due to their small stature and limited rooting

depth. Our study examined seedlings under controlled greenhouse conditions, allowing us to better understand detailed physiological mechanisms of drought response. We believe that the results of this study provide important insights into general response strategies in redwood seedlings, such as degree of isohydry versus anisohydry and biomass allocation patterns. Nevertheless, the artificial conditions inherent in any greenhouse experiment limit our ability to extend fully the results to seedlings growing in the field. Our results also cannot be extrapolated to mature trees. Drought responses in seedlings and mature trees growing under natural conditions should vary due to differences in both environmental and physiological factors such as rooting depth, carbon and water storage capacity, stomatal behavior and hydraulic architecture, including xylem conductivity and cavitation vulnerability (Zimmermann 1983, Meinzer et al. 2001, Ryan et al. 2006, McDowell et al. 2008). Further research is needed on potential impacts of water deficit on redwood seedlings in the field and how these impacts scale with tree size and age.

#### Concluding remarks

The results of this study reveal contrasting drought-response strategies in the two California redwoods that reflect species-specific adaptations to their native ecological settings, and demonstrate trade-offs and coordination between stomatal regulation of gas exchange, xylem hydraulic properties, growth and biomass allocation patterns. Although both *Sequoia* and *Sequoiadendron* experienced some hydraulic limitation of leaf gas exchange and growth at soil VWC <10%, the severity and type of impact differed between the two species. These differences highlight the need to consider plant traits that influence both the resistance to water stress as well as the capacity to recover from that stress when evaluating species vulnerability to drought (West et al. 2012, O'Grady et al. 2013).

#### Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

#### Acknowledgments

We gratefully acknowledge Rob York at the UC Berkeley Center for Forestry and Teri Griffiths at the CALFIRE seed bank for assistance with seed acquisition and seed viability testing, Green Diamond Resource Company for growing the seedlings used in this experiment, Barbara Rotz and the staff of the UC Berkeley Oxford Tract Greenhouse facility for logistical support, Tara Najim and Cameron Musser for help with measurements and sample processing, and David Whitehead, Ram Oren and two anonymous reviewers for comments and suggestions which greatly improved the manuscript.

## Conflict of interest

None declared.

## Funding

The Save-the-Redwoods League funded this study through the Redwoods and Climate Change Initiative.

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