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The benefits of woody plant stem photosynthesis extend to hydraulic function and drought survival in *Parkinsonia florida*

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As climate change exacerbates drought stress in many parts of the world, understanding plant physiological mechanisms for drought survival is critical to predicting ecosystem responses. Stem net photosynthesis, which is common in arid environments, may be a drought survival trait, but whether the additional carbon fixed by stems contributes to plant hydraulic function and drought survival in arid land plants is untested. We conducted a stem light-exclusion experiment on saplings of a widespread North American desert tree species, *Parkinsonia florida* L., and after shading acclimation, we then subjected half of the plants to a drought treatment to test the interaction between light exclusion and water limitation on growth, leaf and stem photosynthetic gas exchange, xylem embolism assessed with micro-computed tomography and gravimetric techniques, and survival. Growth, stem photosynthetic gas exchange, hydraulic function and survival all showed expected reductions in response to light exclusion. However, stem photosynthesis mitigated the drought-induced reductions in gas exchange, xylem embolism (percent loss of conductivity, PLC) and mortality. The highest mortality was in the combined light exclusion and drought treatment, and was related to stem PLC and native sapwood-specific hydraulic conductivity. This research highlights the integration of carbon economy and water transport. Our results show that additional carbon income by photosynthetic stems has an important role in the growth and survival of a widespread desert tree species during drought. This shift in function under conditions of increasing stress underscores the importance of considering stem photosynthesis for predicting drought-induced mortality not only for the additional supply of carbon, but also for its extended benefits for hydraulic function.

Keywords: drought, green stem, mortality, *Parkinsonia florida*, photosynthetic stem, stem net photosynthesis.

Introduction

As climate change exacerbates drought stress in many parts of the world, understanding plant physiological mechanisms for drought survival is critical to predicting ecosystem responses. A recent framework of drought survival strategies included stem photosynthesis as one of the traits that can aid in plant survival during drought (Pivovarov et al. 2016, Santiago et al. 2016). Evidence for stem photosynthesis has been found in at least 93 families (Berry et al. 2021), and it is typical in plants from seasonally dry ecosystems (Gibson 1983, Nilsen 1995, Tinoco-Ojanguren 2008, Ávila et al. 2014, Ávila-Lovera and Tezara 2018). Desert trees and shrubs in particular rely on stem photosynthesis for carbon gain both in periods of water availability and water deficit (Ávila-Lovera et al. 2017, 2019), but the contribution during water deficit is greater as many species with photosynthetic stems are drought deciduous as well. Therefore, evaluating the role of stem photosynthesis during drought can bring about insights into how desert plants will respond to more frequent, more severe and prolonged global-change-type droughts (Breshears et al. 2005).

There are two types of stem photosynthesis, stem net photosynthesis (SNP), where stems assimilate CO₂ from the atmosphere, and stem recycling photosynthesis (SRP), where stems re-assimilate internal CO₂ that comes from respiration from the stem and other organs (Ávila et al. 2014). Advantages of stem net photosynthesis include extra carbon gain (Osmond 1987, Nilsen et al. 1989, Nilsen and Bao 1990) and increased whole-plant water-use efficiency (WUE) (Nilsen and Sharifi 1997, Ávila-Lovera and Tezara 2018), whereas SRP advantages include reduction of carbon respiratory losses (Pfnz et al. 2002, Berveiller et al. 2007, De Roo et al. 2020c) and near zero water loss (Wittmann and Pfnz 2008, Cernusak and Cheesman 2015). So far, all experiments where light exclusion has been imposed on photosynthetic stems of plants have been on species performing SRP, and they have shown that SRP affects stem growth and production of new buds after defoliation in chaparral shrub species (Saveyn et al. 2010). Furthermore, carbon derived from SRP that contributes to stem growth has been estimated to be 11% in *Eucalyptus miniata* (Cernusak and Hutley 2011) and up

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to 24% in *Populus nigra* (Bloemen et al. 2013). In plants with SNP, the benefits are expected to be greater, but to our knowledge, no light-exclusion experiment has been performed in species with green stems that can perform SNP. For SNP, the advantages are also expected to become even more important for plant performance in the face of climate change, as leaf area and canopy size decline along aridity gradients leading many plants to increasingly rely on stem photosynthetic activity (Gibson 1983, Comstock and Ehleringer 1992, Smith et al. 2004).

Stem photosynthesis in woody plants is well represented among plants in arid ecosystems, suggesting its adaptive importance in this environment (Nilsen and Sharifi 1994, Ávila et al. 2014, Natale et al. 2023). Desert plants that remain leafless during summer and fall months, typically for ~6 months in normal years or longer in drought years (Ávila-Lovera et al. 2019), benefit from having green stems that continue photosynthetic carbon assimilation. Green stems maintain and sometimes increase their net photosynthetic rate (A_{net}) in dry months (Ávila-Lovera et al. 2017, 2019), with increases thought to be caused by higher light availability in the dry season because of lower cloud cover and lower leaf canopy shading. The gained carbon can then be used in maintenance respiration of stems, and any surplus can be used for growth (De Roo et al. 2020a) or reserved as non-structural carbohydrates (NSC) (Natale et al. 2023).

More recently, stem photosynthesis has been reported to help maintain stem hydraulic functioning, as reviewed by Vandegehuchte et al. (2015) and Cernusak and Cheesman (2015). For example, chloroplasts in stem xylem and bark have an important role in xylem embolism repair in mangroves (Schmitz et al. 2012). Furthermore, in *Populus nigra*, greater xylem vulnerability to embolism formation, evaluated as the water potential at which half of hydraulic conductivity is lost (P_{50}), is found in stems of light-excluded plants compared with control plants, indicating that SRP has a role in xylem resistance to embolism formation through the accumulation of NSC (De Baerdemaeker et al. 2017). SRP also promotes the accumulation of NSC in the bark of *Salix matsudana* stems, but in this case the mechanistic link between NSC accumulation and refilling of embolized vessels was shown through xylem water uptake (Liu et al. 2019). More recent work on *Populus tremula* has also shown that SRP delays drought stress by maintaining stem starch concentration levels (De Roo et al. 2020b). Thus, there is a growing body of literature now connecting stem photosynthesis, NSC and hydraulic function. This focus on stem photosynthesis is an important addition to the active research area linking NSC and drought (McDowell et al. 2008, Pratt et al. 2021).

Despite the accumulating literature on the role of SRP in plant hydraulics, the combined effect of light exclusion and drought on the physiology of trees with green stems, those that perform SNP, has not been tested. To address this gap, our study examined light exclusion on stems using green-stemmed *Parkinsonia florida* L. (Blue palo verde) saplings to test how stem photosynthesis may affect growth and hydraulic functioning, and mediate plant responses to drought and survival. We addressed the following questions. (i) What is the role of stem photosynthesis in stem growth of *P. florida*? (ii) What is the effect of stem light exclusion on xylem vulnerability to embolism? (iii) Does stem photosynthesis mediate plant survival when facing drought? We hypothesized that

stem light exclusion would negatively influence stem growth and hydraulic conductivity, with further consequences for the ability of plants to survive drought.

Materials and methods

Plant material and lathhouse conditions

Parkinsonia florida L. (Fabaceae), commonly known as Blue palo verde, is a shrub or small tree native to the Sonoran Desert in North America, and one of the dominant species in the ecosystem. It has bright green cortex covered by an epidermis with stomata, through which it conducts stem net photosynthesis (Ávila-Lovera et al. 2017). Seedlings were collected from the Botanic Gardens Desert Collection at the University of California Riverside (33.97, -117.32) (UCR) in July (27 seedlings) and November (16 seedlings) of 2015. Seedlings were transplanted to 3.79-L pots filled with UC soil mix #2 (a combination of plaster sand, bark, peat moss, limestone flour and nutrients, with high porosity and water holding capacity) and watered daily. Twenty-two surviving seedlings were used for this experiment. Many species with a single tap root are susceptible to transplant, and the seedling stage of most species is the most susceptible stage. The plants grew little during spring 2016 and the main stems were too thin for measurements of hydraulic conductivity (K_h) and vulnerability curves (VC). For this reason, the plants were transplanted to larger pots of 7.57 L in April 2016. The plants grew over summer 2016 and attained heights between 79.19 cm (seedlings collected in November 2015) and 104.58 cm (seedlings collected in July 2015) by April 2017. The plants were watered with a dilute nutrient solution (21-5-20, NPK) according to the UCR Botanic Garden watering regime, which depends on the climate of the different seasons, watering the pots to full capacity every 2 days during summer, and every 3–4 days during the rest of the year (fall to spring).

Sampling was done two to three times per week during the 16-week experiment. We measured microclimatic conditions such as air temperature and RH above the plants at ~110 cm using a digital hygrometer/thermometer (11-661-9; Fisher Scientific, Waltham, MA, USA) at the beginning and end of each sampling day. At the beginning of each sampling day, we also took point measurements of soil volumetric water content (VWC) near the center of the pot using a soil moisture probe (12-cm-long probe; Hydrosense, Campbell, Logan, UT, USA), and measured light interception above each plant ($\text{PPFD}_{\text{canopy}}$) and by the main stem on radial ($\text{PPFD}_{\text{stem, radial}}$) and axial ($\text{PPFD}_{\text{stem, axial}}$) directions using a light sensor connected to a light meter (LI-250A; LI-COR, Lincoln, NE, USA). These measurements were taken to ensure that the microclimatic and environmental conditions did not differ among treatments.

Experimental design

The experiment started in April 2017 and ended in August 2017 (Figure S1 available as Supplementary data at *Tree Physiology* Online). The surviving 22 two-year-old *P. florida* saplings were randomly assigned to two groups of 11 plants each: group 1 ('wrapped') consisted of plants with their stems loosely covered with aluminum foil that effectively blocked light transmission (100% light exclusion) but allowed gas

exchange, and group 2 ('control') consisted of plants without light exclusion (Figure S2 available as Supplementary data at *Tree Physiology* Online). Stem surface temperature is relatively lower and relative humidity (RH) higher under the aluminum foil than in control plants, but this does not affect gas exchange values (Valverdi et al. 2023). We started wrapping the stems in the 'wrapped' group on 25 April 2017 (day of year, DOY, 115) and ended on 10 May 2017 (DOY 130) because of the extensive branching of the plants that required detailed wrapping. Light exclusion lasted ~16 weeks until 28 August 2017 (DOY 240).

After allowing the plants in the 'wrapped' group to acclimate to stem light exclusion for 6 weeks, on 21 June 2017 (DOY 172) we started a drought treatment in six plants each within the 'wrapped' and 'control' groups, yielding a complete 2 × 2 factorial design from this point in time onwards: control droughted, control non-droughted, wrapped droughted and wrapped non-droughted. Soil VWC in the non-droughted plants was kept at 20–30%. In the droughted plants, water was withheld for three consecutive weeks until soil VWC reached values of ~5% (on 26 July 2017, DOY 207). We then watered the plants and allowed the soil to dry once again, and repeated this three more times to test for mortality. On 28 August 2017 (DOY 240), we finished the experiment and removed the aluminum foil from all plants.

Before starting each treatment of wrapping the stems and the application of drought, we measured total plant height as the length from the base of the stem, at the soil surface, to the apical meristem of the tallest branch. We found no differences in height between 'wrapped' and 'control' plants at the beginning of the light exclusion ($t_{21} = 0.19$, $P = 0.850$) or at the beginning of the drought treatment ($t_{21} = 0.60$, $P = 0.553$). Furthermore, we measured the basal diameter of plants at the beginning of each sampling day (two times per week) using a Vernier caliper.

Gas exchange

We measured gas exchange three times per week during the duration of the experiment. During the light-exclusion period, we only measured gas exchange in leaves and non-wrapped stems. We then measured gas exchange in non-wrapped stems and leaves, if they were present, and in wrapped stems by momentarily unwrapping twigs, which were randomly selected in each sampling day during the light exclusion + drought period. We measured a subset of five individuals per treatment in each sampling day. We used an infrared gas analyzer (LI-6400; LI-COR, Lincoln, NE, USA) in open mode to measure net CO₂ assimilation rate (A_{net}) and transpiration rate (E), and to estimate stomatal conductance (g_s) and intercellular CO₂ concentration (C_i). We also calculated intrinsic water-use efficiency (IWUE) as the ratio between positive A_{net} and g_s . Measurements were taken at ambient temperature, which varied from 24 °C in April 2017 to 40 °C in August 2017, ambient air RH, which varied from 27% in April 2017 to 60% in August 2017, vapor pressure deficit of 0.99–5.28 kPa, CO₂ concentration of 400 μmol mol⁻¹, and 1500 μmol m⁻² s⁻¹ of photosynthetic photon flux density (PPFD) and between 10 a.m. and 2 p.m. to ensure saturating rates. We used 1500 μmol m⁻² s⁻¹ as previous work in desert plants showed this to be the value of PPFD at which electron transport rate saturates (Ávila-Lovera et al. 2017). The measured stems were between 1.5

and 2.5 cm in diameter. To measure stems, we followed the protocol in Ávila-Lovera et al. (2017). Briefly, we made gaskets with modeling clay that were fitted to the leaf chamber and allowed for an adequate seal. This seal was tested by blowing air around the gasket and noting no change in the CO₂S parameter.

X-ray micro-computed tomography scanning and native PLC

On 18 August 2017 (DOY 230), we took a subset of plants, two from each light × drought treatment (for a total of eight plants), to be scanned at the 3D Imaging Center at California State University Bakersfield, USA. We first bagged a leaf shoot from each plant to allow stem and leaf water potential to equilibrate for at least 1 h. We then cut this leafy twig and measured stem xylem water potential (Ψ_x) using a pressure chamber (Model 1000; PMS Instrument Co., Albany, OR, USA). In a subsection of samples, we also measured water potential using a dew point potential meter (WP4; Decagon Services, Pullman, WA, USA). After this, we measured native stem hydraulic conductivity in a separate twig before scanning it ($K_{h, \text{pre-scan}}$). We sampled stems that were 2.7–8.0 mm in diameter. For this, the stem sample was cut under water and recut twice from each cut end until a segment of 13.6–14 cm in length was obtained (this is the size of the centrifuge rotor, see below). We measured K_h gravimetrically using a de-gassed and ultra-filtered (0.1 μm pore filter) 20 mM KCl solution, and a pressure head of 1.3–1.7 kPa. Stem samples were always kept underwater before measurement and cut ends were debarked and shaved underwater. The proximal cut end was connected to tubing connected to a solution reservoir, and the distal cut end was connected to tubing that ended in a bottle on a digital balance. The flow of water through the stem was recorded every 10 s in an Excel spreadsheet interfaced with the balance, and once steady state was reached, we averaged the last six values. Stem hydraulic conductivity was calculated as follows:

$$K_h = \frac{F}{\Delta\Psi_x/L} \quad (1)$$

where F is the solution flow rate (kg s⁻¹) and $\Delta\Psi_x/L$ is the pressure gradient (MPa m⁻¹), where $\Delta\Psi_x$ is the pressure driving flow (MPa) and L is the stem length (m) used (Tyree and Ewers 1991). Measurements were corrected for background flow at a pressure head of 0 kPa, which can occur due to passive water uptake by dehydrated stems (Hacke et al. 2000).

After measuring $K_{h, \text{pre-scan}}$, we removed the stem sample from the conductivity apparatus and the proximal cut end was placed inside a microcentrifuge tube filled with deionized water to maintain hydration during micro-computed tomography (microCT) scanning. The stem samples were scanned vertically using a microCT system (Model 2211; Bruker Corporation, SkyScan, Billerica, MA, USA). Scans were performed at 60 kV and 500-μA energy and at a resolution of 1.5–2.9 μm. We ran post-scan K_h measurements ($K_{h, \text{post-scan}}$) to determine if scanning might trigger embolism. The $K_{h, \text{post-scan}}$ values were not statistically different from the $K_{h, \text{pre-scan}}$ values ($K_{h, \text{post-scan}} = 1.60 \pm 1.04$ (SE), $K_{h, \text{pre-scan}} = 1.87 \pm 0.71$ (SE), Student's paired t test, $t_{11} = 0.22$, $P = 0.83$); therefore, pre-scan values were used for calculating native percent loss of conductivity (PLC). This test also suggests that the observed emboli were stable over the course of our measurements and that significant refilling was not occurring when we were

measuring samples in the microCT (Trifilò et al. 2014, Venturas et al. 2015). We flushed stem samples for >30 min with de-gassed ultra-filtered 20 mM KCl solution at 100 kPa to remove embolism, and measured conductivity again to obtain $K_{h, \max}$ and calculate PLC as follows:

$$\text{PLC} = \frac{(K_{h, \max} - K_{h, \text{pre-scan}})}{K_{h, \max}} \times 100 \quad (2)$$

From the microCT images, we also estimated the amount of embolism in the stems using three metrics. First, we measured the amount of gas area in the stem sapwood per unit sapwood area (%); second, we measured the amount of gas area in vessels per unit sapwood area (%); and third, we counted the number of embolized vessels and total number of vessels to calculate the proportion that were embolized. All of these estimates were strongly and positively correlated ($r \geq 0.84$); thus, we focused on the percentage of gas per sapwood area for simplicity, as embolized sapwood area is strongly correlated to native PLC (Secchi et al. 2021).

Vulnerability curves

At the end of the experiment, on 28 August 2017 (DOY 240), we cut the main stem of the 22 plants under water to examine hydraulic vulnerability. We measured initial stem hydraulic conductivity ($K_{h, \text{ini}}$) in *c.* 14-cm-long stems using the gravimetric method described above. We then vacuum infiltrated the stem samples using a partially de-gassed 20 mM KCl solution for *c.* 12 h to remove embolism, and measured K_h again ($K_{h, \max}$). We then used the centrifuge method (Alder et al. 1997) to induce a range of xylem tensions and measured K_h at each of those tensions to generate vulnerability curves (VCs), which show the relationship between PLC (calculated as above) and Ψ_x as the stems dehydrate. The centrifuge method was deemed to be appropriate for determining xylem vulnerability to embolism given that *Parkinsonia praecox*, a close relative of *P. florida*, has short vessels that range from 0.3 to 3 cm (Ávila-Lovera and Tezara 2018), shorter than the length of the stem sample used, and open-vessel artifacts are unlikely (Cochard et al. 2010, 2013). The VCs were corrected to account for unrealistic maximum K_h values at water potentials above -0.25 MPa, hence we took the value of K_h at -0.25 MPa as $K_{h, \max}$. Curves were then fitted using a sigmoidal exponential function that allowed for estimation of two important parameters: the Ψ_x at which 50% of conductivity is lost (P50) and the slope of the curve (*a*):

$$\text{PLC} = 100 - \left(\frac{100}{1 + e^{a \times (\Psi_x - b)}} \right) \quad (3)$$

where *b* is P50 (MPa) and *a* is the slope of the curve at P50 (% MPa⁻¹).

With the VC curves, we also estimated P12, the water potential at which 12% of hydraulic conductivity is lost (also known as air-entry threshold, P_e ; Meinzer et al. 2009).

After performing the VCs, we measured xylem diameter in the distal end of the stem segment to calculate sapwood area (SA; cm²). We calculated sapwood-specific hydraulic conductivity (K_s) as K_h divided by SA. We also measured wood density (WD; g cm⁻³) using the water displacement method (De Guzman et al. 2017).

Survival

We determined survival (as a binary trait for each plant) at the end of the experiment, 28 August 2017 (DOY 240). Plants showed signs of stress including: the progression of leaf loss, yellowing of branches and dieback, but mortality was eventually determined by assessing PLC, which indicated complete loss of hydraulic function even after multiple watering events, and extremely low water potential measurements (-84 MPa) in one case.

Statistical analyses

We performed multiple analyses of covariance (ANCOVA) to test for changes in air temperature and relative humidity between morning and midday, and as a function of DOY. All of our models included main treatment effects and all possible interactions as specified below. We tested for temporal autocorrelation and in cases where there was no autocorrelation, we show results of regular ANCOVAs. We also performed multiple ANCOVAs to test the effect of light exclusion and drought treatment on the hydraulic traits measured at the end of the experiment. For these analyses, we tested the interactive effect of light and drought treatment on K_s and PLC only, because we could not estimate P50 and P12 for the dead individuals. ANCOVAs were run using the ‘lm’ function in R v.4.0.2. We used linear mixed-effect models (LMM) when analyzing repeated measures designs to test for the effect of light exclusion (fixed effect), drought treatment (fixed effect) and DOY (fixed effect after testing for autocorrelation), as well as all interactive effects, on soil VWC, plant light interception (PPFD_{canopy}, PPFD_{axial} and PPFD_{radial}) and basal diameter, using plant ID as a random effect. We also used LMM to test the effect of light exclusion (fixed effect), drought treatment (fixed effect), organ (fixed effect) and DOY (fixed effect after testing for autocorrelation), as well as all interactive effects, on gas exchange traits, using plant ID as a random effect. We kept DOY as a main effect after testing for autocorrelation to account for the variation given by the change in season. For these analyses, we used the function ‘lme’ from the ‘nlme’ package in R. To test the relationships among hydraulic traits at the end of the experiment, we ran correlation analyses using the ‘rcorr’ function of the ‘corrplot’ package in R. We tested significance at $P < 0.05$.

Results

Abiotic conditions by treatment

Results from linear models showed that air temperature increased from morning to midday ($F_{1,53} = 83.73$, $P = 2.921 \times 10^{-12}$), and as a function of DOY as expected by the progression of the warm season ($F_{1,53} = 11.05$, $P = 1.666 \times 10^{-3}$; Figure S3a available as Supplementary data at *Tree Physiology* Online), with no time of day \times DOY effect. Values of RH decreased from morning to midday ($F_{1,53} = 70.40$, $P = 4.167 \times 10^{-11}$) but did not change as a function of DOY ($F_{1,53} = 3.64$, $P = 0.062$; Figure S3b available as Supplementary data at *Tree Physiology* Online), with no time of day \times DOY effect. Linear mixed models with a correlated structured defined by DOY showed that there was a significant effect of light exclusion and drought treatment on soil VWC ($F_{5,593} = 4.14$, $P = 0.016$; Figure S4 available as Supplementary data at *Tree Physiology* Online), with no differences in the droughted plants between the

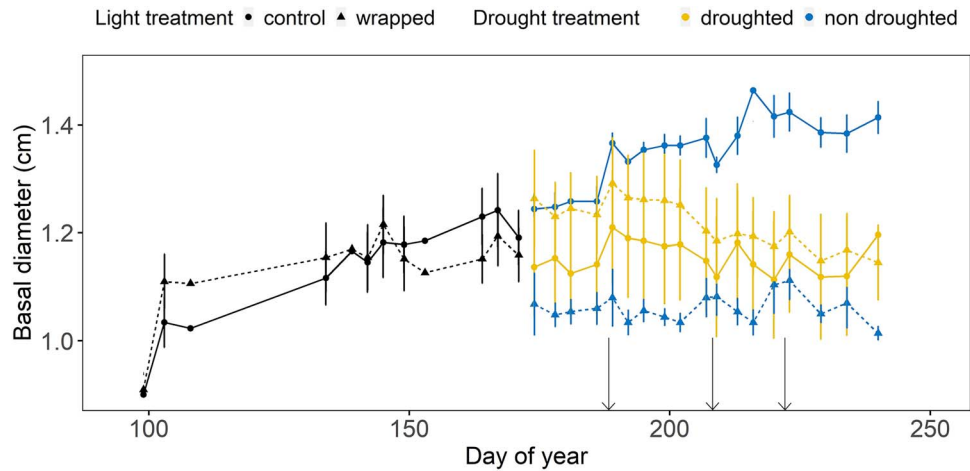


Figure 1. Basal diameter as a function of day of year (DOY). Each symbol is the mean of the individuals in the different treatments \pm standard error in each sampling date. Light treatment ('control' and 'wrapped') and drought treatment ('droughted' and 'non-droughted') are represented as different symbols, line types and colors. Arrows indicate the watering events after the onset of the drought treatment (three events).

control and the wrapped plants, and higher soil VWC in the wrapped non-droughted plants than in the control non-droughted plants, likely because of decreased transpiration in the wrapped plants. Light intercepted by the canopy of the plants changed as a function of DOY ($F_{1,582} = 5.45$, $P = 0.020$; Figure S5 available as Supplementary data at *Tree Physiology Online*), and as a function of drought treatment ($F_{2,582} = 29.86$, $P < 0.0001$), with no effect of light exclusion or interaction among factors. Similarly, light intercepted axially changed as a function of DOY ($F_{1,582} = 12.11$, $P = 5.000 \times 10^{-4}$) and drought treatment ($F_{2,582} = 24.09$, $P < 0.0001$), with an interactive effect between drought treatment and DOY ($F_{2,582} = 16.40$, $P < 0.0001$; Figure S6 available as Supplementary data at *Tree Physiology Online*). Light intercepted radially by the stem was only affected by DOY ($F_{1,582} = 17.29$, $P < 0.0001$; Figure S7 available as Supplementary data at *Tree Physiology Online*).

Stem growth

Before starting the drought treatment, there was no effect of light exclusion on basal diameter ($F_{1,20} = 0.002$, $P = 0.965$) but there was an effect of DOY ($F_{1,222} = 147.08$, $P < 0.0001$) as plants grew during the warm season. After the onset of the drought treatment, stem light-exclusion and drought had a statistically significant interactive effect on radial stem growth ($F_{1,18} = 6.42$, $P = 0.021$), with final basal diameter being greatest in the non-wrapped non-droughted plants, and smallest in the wrapped non-droughted plants (Figure 1), and no significant main effects of each of the treatments. The interactive effect of light and drought treatment was evidenced by wrapped plants subjected to drought having thicker stems than wrapped plants not subjected to drought (Figure 1), which may have resulted by chance from the assignment of plants to the droughted and non-droughted treatments.

To determine if stem photosynthesis was indeed decreased in the light-exclusion treatment, we monitored gas exchange during the duration of the experiment. We ran separate linear models for gas exchange variables in leaves and stems. For leaves, before the start of the drought treatment, there was no significant effect of DOY ($F_{1,143} = 3.04$, $P = 0.083$) or

light exclusion ($F_{1,18} = 0.000$, $P = 0.987$) on A_{net} . After the onset of the drought treatment, there was a significant main effect of drought treatment and DOY on leaf A_{net} (Table S2 available as Supplementary data at *Tree Physiology Online*, Figure 2a), with values being higher in the non-droughted than in the droughted plants from the wrapped group, and no difference between drought treatments in the plants from the control group, but there was no interaction effect between light and drought treatments or any other factors (Table S2 available as Supplementary data at *Tree Physiology Online*). Similarly, drought treatment had a significant effect on leaf g_s , as well as a light \times drought treatment interaction (Table S2 available as Supplementary data at *Tree Physiology Online*); however, the post hoc test only showed differences between the pairs droughted–non-droughted for each of the levels of the light treatment (Figure 2c). In stems, we did not measure gas exchange during the light-exclusion period to allow for shade acclimation. After the onset of the drought treatment, main effects of DOY and drought treatment were similar to those of leaves (Table S3 available as Supplementary data at *Tree Physiology Online*, Figure 2b), and there was also a main effect of light exclusion, with lower overall A_{net} values in the stems of wrapped plants, as well as a negative effect of drought in both control and wrapped plants. For stem g_s , we found significant effects of both light and drought treatments and a light \times drought treatment interaction (Table S3 available as Supplementary data at *Tree Physiology Online*, Figure 2d), with lower g_s in control-droughted plants than in control non-droughted plants, and similar g_s values in wrapped droughted and non-droughted plants.

Xylem vulnerability to embolism

At the end of the experiment, light exclusion did not influence native K_S ($F_{1,18} = 0.38$, $P = 0.55$), but there was a strong effect of drought treatment ($F_{1,18} = 86.80$, $P = 2.62 \times 10^{-8}$), with lower native K_S values in the plants experiencing drought regardless of light exclusion (Figure 3a), and no significant interactive effect ($F_{1,18} = 0.81$, $P = 0.38$). On the other hand, native PLC was affected by both light exclusion ($F_{1,18} = 5.70$, $P = 0.03$) and drought treatment ($F_{1,18} = 28.70$, $P = 4.31 \times 10^{-5}$), with higher overall native PLC values

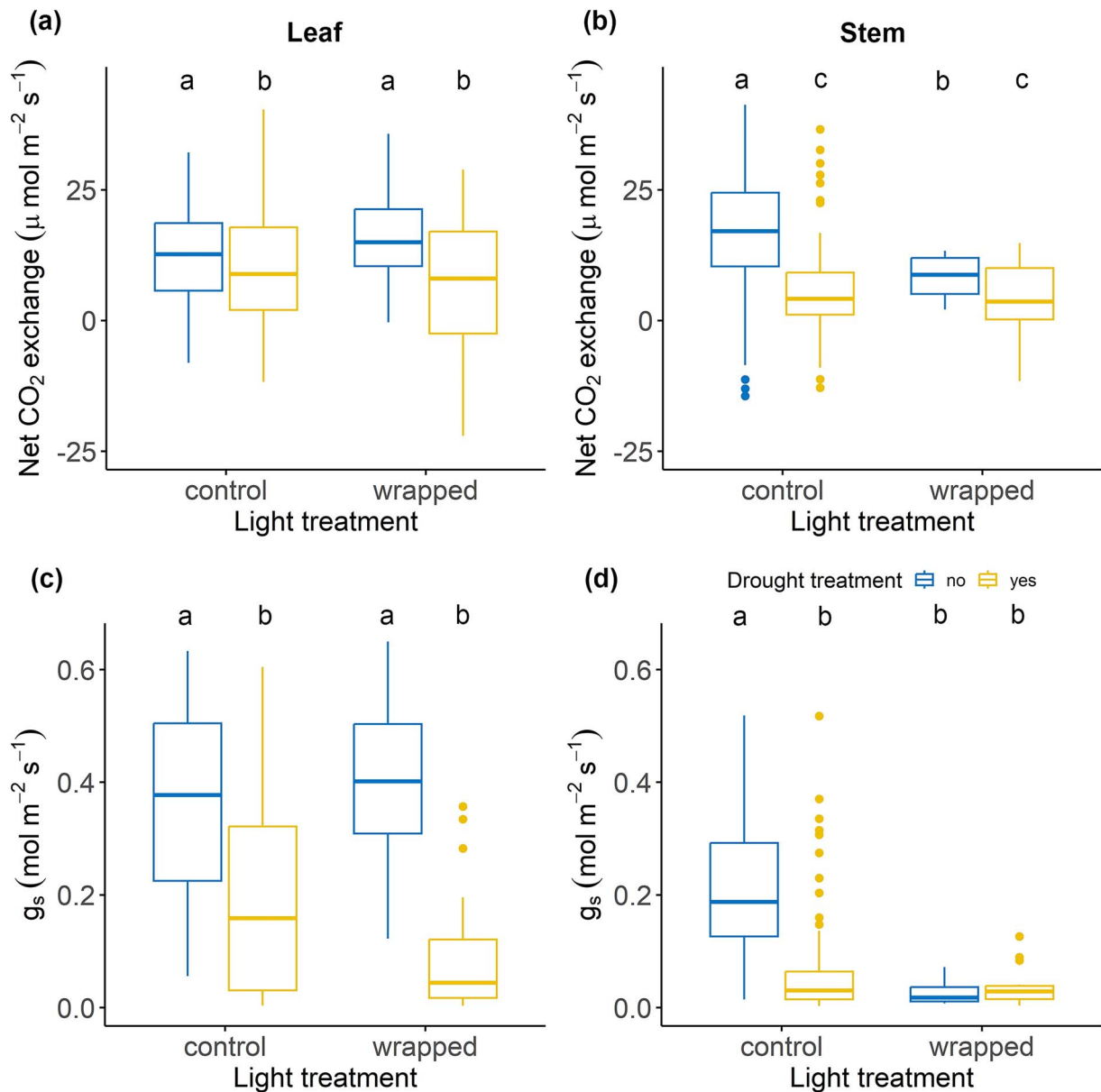


Figure 2. Gas exchange traits of leaves and stems of 22 *P. florida* plants. Net CO₂ exchange rate of (a) leaves and (b) stems, and stomatal conductance (g_s) of (c) leaves and (d) stems. Measurements were performed at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux density in five individuals per treatment during the light exclusion + drought treatment phase. Letters come from the results of the post hoc test of the linear models including main fixed and interactive effects when present (at $P < 0.05$). See also Tables S2 and S3 available as Supplementary data at *Tree Physiology Online*.

in wrapped stems than in control plants, and higher PLC values in droughted plants (Figure 3b), with no interactive effect ($F_{1,18} = 4.29$, $P = 0.05$). For P50 and P12, there are no values for plants with wrapped stems and subjected to drought because they were dead at the end of the experiment (Figure 3c and d). Values of P50 did not differ between the levels of the light exclusion ($F_{1,11} = 2.75$, $P = 0.13$), or between droughted and non-droughted plants ($F_{1,11} = 3.81$, $P = 0.08$; Figure 3c). We found similar results for P12, with no difference between light-exclusion levels ($F_{1,11} = 1.53$, $P = 0.24$), or between droughted and non-droughted plants ($F_{1,11} = 4.23$, $P = 0.06$; Figure 3d). Interactive effects between light and drought treatment were not able to be tested for P50 and P12 given the lack of data in the light-excluded droughted group (because of mortality, see below). In addition, we found coordination among some of the hydraulic traits, including

a negative relationship between maximum K_S and P12 (Figure 4a, $r = -0.56$, $P = 0.039$), and a negative relationship between native K_S and native PLC (Figure 4b, $r = -0.79$, $P < 0.001$).

Survival

Survival was affected by both drought and light exclusion, with the highest mortality (100%) in plants with wrapped stems and subjected to drought, followed by 16.67% mortality in droughted plants not subjected to light exclusion, and 100% survival in well-watered plants (Figure 5). Furthermore, analyzing microCT images (Figure 6) revealed that percent of gas area in the sapwood was positively related to native PLC measured gravimetrically ($r = 0.744$, $P = 0.034$). Values of PLC and sapwood gas area (Table 1) indicate that hydraulic failure, the loss of hydraulic function that cannot be

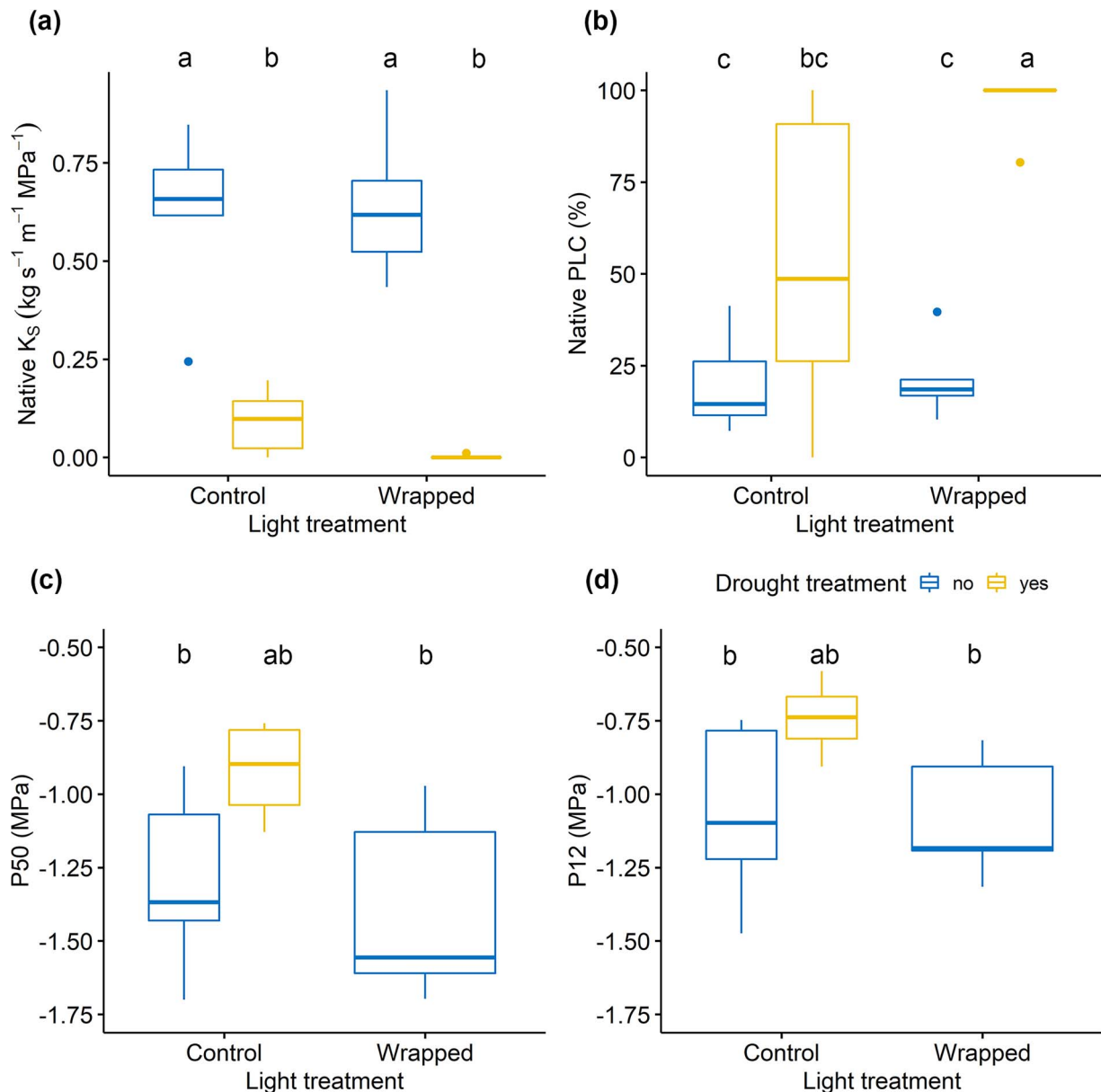


Figure 3. Effect of light exclusion and drought treatment on hydraulic traits of plants at the end of the experiment: (a) native sapwood-specific hydraulic conductivity (native K_S), (b) native percent loss of conductivity (native PLC), (c) xylem vulnerability to embolism (P50) and (d) water potential threshold for air-entry and embolism formation (P12). Note that for P50 and P12, there are no values for plants with wrapped stems and subjected to drought because they were dead at the end of the experiment. Sample size for the plots in (a) and (b) are six for droughted plants in both the control and wrapped treatments, and five for non-droughted plants in both the control and wrapped treatments. Sample sizes for (c) and (d) are four for control-droughted, five for control non-droughted and four for wrapped non-droughted. Letters come from the results of the post hoc test of the linear models including main fixed and interactive effects when present (at $P < 0.05$).

recovered after removing water stress, was likely the mortality mechanism.

Discussion

We studied Blue palo verde to understand the role of stem photosynthesis on growth, hydraulic functioning and survival in response to drought. Excluding light from the green stems of palo verde saplings for 16 weeks in combination with drought slowed growth, suggesting that both treatments, independently and together, negatively impacted stem carbon balance. In response to drought, individuals with shaded stems

suffered 100% mortality, compared with 16.67% in non-wrapped plants, suggesting that stem photosynthesis and the carbon it generates contribute to drought resistance. Whereas previous studies have been able to demonstrate that stem recycling photosynthesis contributes to maintaining hydraulic function (Schmitz et al. 2012, De Baerdemaeker et al. 2017, Liu et al. 2019), to our knowledge, we are the first to examine evidence that the combined effect of light exclusion and drought on the physiology of trees with green stems that perform stem net photosynthesis leads to mortality. These results point toward the importance of stem photosynthesis in palo verde beyond growth and hydraulic function.

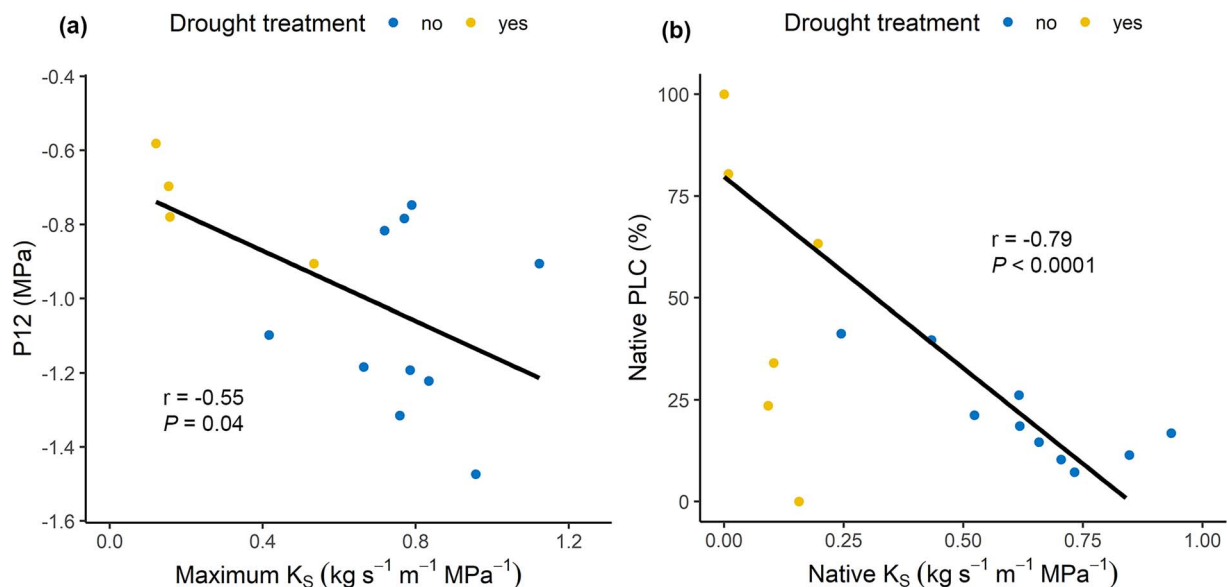


Figure 4. Relationships among hydraulic traits: (a) P12 and maximum sapwood-specific hydraulic conductivity (maximum K_S), and (b) native PLC and native K_S in surviving plants. Each symbol represents one individual. Symbols are color coded by drought treatment level.

Table 1. Hydraulic traits measured in the eight saplings that were scanned at the 3D imaging Center at CSU Bakersfield, California, USA. Native water potential (Ψ_x), native and maximum sapwood-specific hydraulic conductivity (K_S), and native percent loss of conductivity (PLC). Images were analyzed for sapwood gas area (%) as well. We did not perform statistical analyses with these data given the low n value per treatment (2).

Sample ID	Light treatment	Drought treatment	Survival	Native Ψ_x (MPa)	Native K_S ($\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$)	Max. K_S ($\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$)	Native PLC (%)	Sapwood gas area (%)
12	Control	No	1	-0.908	0.323	0.458	29.503	0.648
21	Control	No	1	-0.669	0.273	0.361	24.459	2.943
2	Wrapped	No	1	-0.783	0.666	0.574	0.000	0.434
8	Wrapped	No	1	-0.683	0.207	0.281	26.459	0.283
1	Control	Yes	1	-1.566	0.405	0.530	23.685	6.441
19	Control	Yes	0	-19.785 ¹	0.000	0.000	100.000	5.197
11	Wrapped	Yes	0	-86.765 ¹	0.009	0.046	80.409	7.573
20	Wrapped	Yes	0	-12.710 ¹	0.000	0.000	100.000	8.156

¹These very negative water potential values were measured following recent mortality.

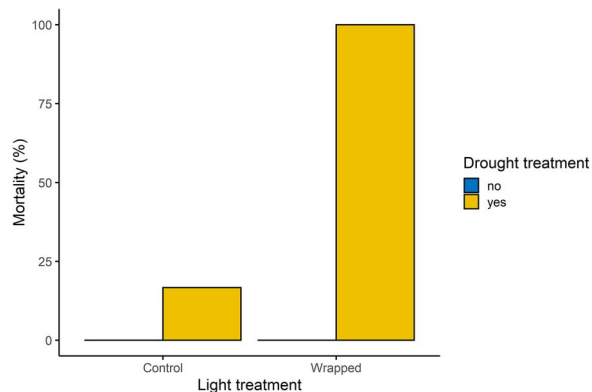


Figure 5. Percent mortality as a function of both light-exclusion treatment and drought treatment.

Research on desert species has shown that green stems are less susceptible to water deficit, as shown by smaller reductions in stem A_{net} compared with leaf A_{net} during drought (Nilsen and Bao 1990, Tinoco-Ojanguren 2008), and that they have higher WUE (Ehleringer et al. 1987, Osmond et al. 1987, Nilsen and Sharifi 1997, Ávila-Lovera and Tezara

2018), both of which are traits of paramount importance for success in seasonally dry and hot environments. In our study, long-term light exclusion from stems reduced stem radial growth, suggesting that the carbon derived from stem photosynthesis is used in situ for growth (Saveyn et al. 2010, Bloemen et al. 2013, De Roo et al. 2020b). These results support the idea that, despite the presence of a large leaf canopy in some desert species, stem contribution to whole-plant carbon gain is significant (Comstock et al. 1988).

Light exclusion increased native PLC values with no differences in native K_S between control and wrapped plants; furthermore, the difference in PLC between wrapped and control plants could not be attributed to differences in P50 or P12. Xylem vulnerability to embolism formation (P50) was similar between wrapped and control plants, which contrast with results in *P. nigra* where long-term light exclusion increased P50 from -1.45 to -1.00 MPa (De Baerdemaeker et al. 2017). For *P. nigra* with relatively modest P50 values, a change in 0.45 MPa is biologically important. The threshold for air-entry and initiation of embolism (P12) is usually positively correlated with P50 (Meinzer et al. 2009), but has been less studied as a measure of xylem vulnerability to embolism.

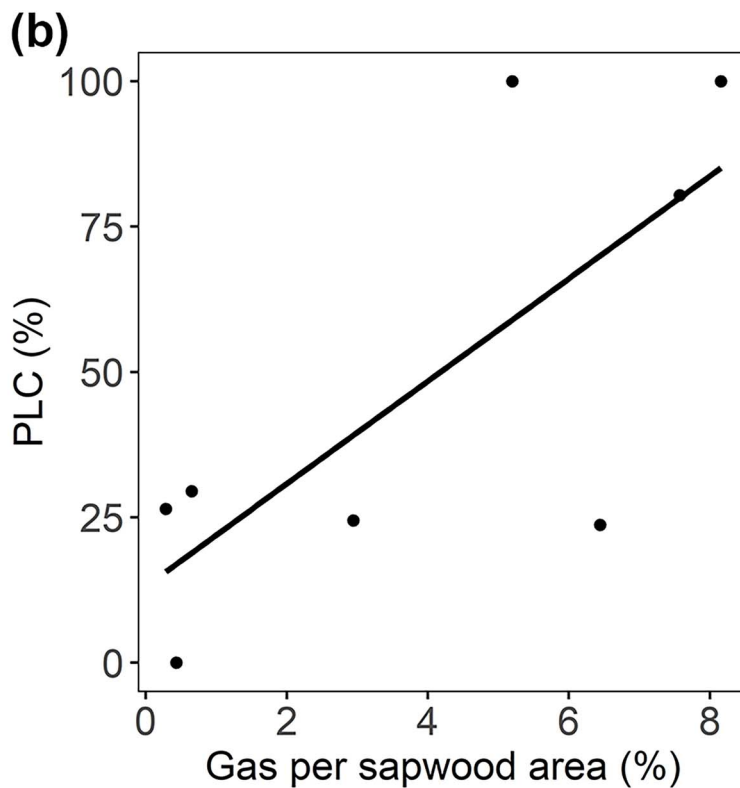
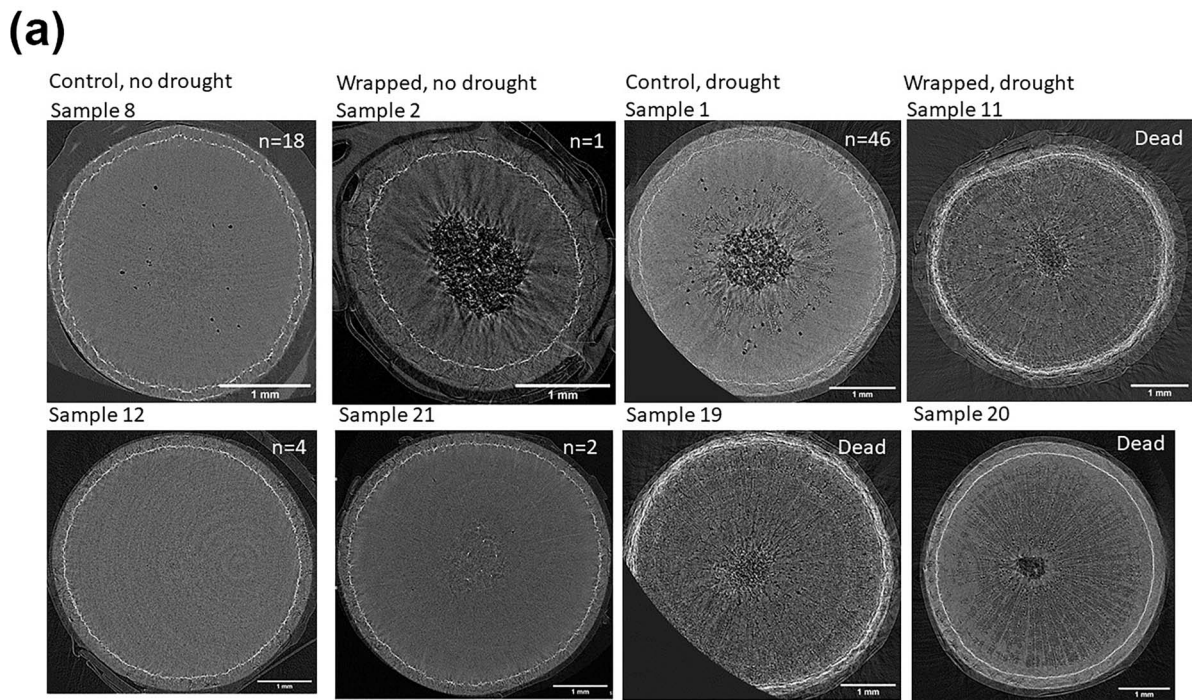


Figure 6. (a) MicroCT images of the main stem of selected *P. florida* plants. The values included in the pictures refer to the number of embolized vessels (n). Scale bars are all 1 mm long. (b) Relationship between the percentage of gas per sapwood area (calculated from images) and percent loss of conductivity measured gravimetrically.

Taken together, the increase in native PLC in wrapped stems without a change in P50 and P12 suggests that what differs between light treatments is not xylem resistance to embolism, but that embolism formation could be potentially repaired in the control plants and not in the wrapped plants, that osmoregulation in the control plants could mediate delay

in water potential drop (Ozturk et al. 2021), and/or that there could be a delay in embolism formation mediated by surfactants (Schenk et al. 2017). The hypothesis of embolism repair is supported by recent findings where stem photosynthesis has been mechanistically linked to xylem refilling (Liu et al. 2019). However, we note that the 100% mortality of the

plants subjected to both light exclusion and drought may have affected our results, as the values of P50 and P12 reported here are from surviving plants only, and dead plants may have had different values.

In this study, we show that net photosynthesis occurring in woody green stems has a key role in plant survival during drought. The role of stem photosynthesis in plant survival during drought was clear in that all plants in the wrapped and droughted group died by the end of the experiment in contrast to only 16.67% of plants that died in the control (non-wrapped) droughted group. Light exclusion is expected to affect photosynthesis and carbon balance in the stems, with a reduced capacity for stems to perform metabolic functions such as to osmoregulate their water potential, or use photosynthates to refill xylem after embolism (Holbrook and Zwieniecki 1999). The exact point of mortality is difficult to pinpoint, but gas exchange data show that g_s of stems was near zero after the onset of the drought treatment and did not recover after four re-watering events. That the leaves did recover g_s indicates that stems may take longer to fully hydrate and allow for stomatal opening. Another explanation may be that the leaves measured later in the season were produced during the dry-down phase of the drought treatment and had morphological and anatomical traits that allow them to recover g_s faster after re-watering (Gorai et al. 2015). Despite low g_s in stems, which could have helped prevent stem dehydration, at the end of the experiment plants in the wrapped group had greater native PLC than control plants. We could not follow hydraulic traits during the course of the experiment because of their destructive nature and our relatively small sample size; nevertheless, elevated levels of embolism in the wrapped droughted treatment suggest hydraulic failure as the ultimate cause of death (McDowell et al. 2008).

We conclude that stem photosynthesis has an important role in the growth and survival of the desert plant *P. florida* during early stages of growth and during drought. Stem photosynthesis is very common in arid ecosystems and the fact that these ecosystems in North America are predicted to experience more frequent, more severe and prolonged droughts (IPCC 2023) means that, all else being equal, plants that can photosynthesize with their stems will be favored and will be more likely to survive, which will have significant effects on the structure of desert communities. Furthermore, most of what we know about stem net photosynthesis comes from studies on desert plants, and open questions remain regarding how widespread green stems are, or how common stem recycling photosynthesis is in plants without obvious green stems in other seasonal or aseasonal ecosystems. Future research should address these questions across a broader range of species to determine the extent of influence of stem photosynthesis on drought survival and eventually incorporate such processes into models that predict mortality to drought.

Supplementary data

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

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Authors' contributions

E.A.-L. and L.S.S. designed the research; E.A.-L., R.H., M.C., A.A.-R. and R.B.P. performed the research; E.A.-L. and R.B.P. contributed new analytical and computational tools; E.A.-L. and R.B.P. analyzed the data; E.A.L. wrote the paper with significant contributions from all authors.

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Data availability statement

Datasets used in this study will be published as Table S1 available as Supplementary data at *Tree Physiology* Online.

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