



Seedling responses to soil moisture amount versus pulse frequency in a successfully encroaching semi-arid shrub

Ashley R. Davis¹ · Kevin R. Hultine³ · Osvaldo E. Sala^{1,4,5} · Heather L. Throop^{1,2}

Received: 23 July 2021 / Accepted: 20 May 2022

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Rainfall timing, frequency, and quantity is rapidly changing in dryland regions, altering dryland plant communities. Understanding dryland plant responses to future rainfall scenarios is crucial for implementing proactive management strategies, particularly in light of land cover changes concurrent with climate change. One such change is woody plant encroachment, an increasing abundance of woody plants in areas formerly dominated by grasslands or savannas. Continued woody plant encroachment will depend, in part, on seedling capacity to establish and thrive under future climate conditions. Seedling performance is primarily impacted by soil moisture conditions governed by precipitation amount (quantity) and frequency. We hypothesized that (H1) seedling performance would be enhanced by both greater soil moisture and pulse frequency, such that seedlings with similar mean soil moisture would perform best under high pulse frequency. Alternatively, (H2) mean soil moisture would have greater influence than pulse frequency, such that a given pulse frequency would have little influence on seedling performance. The hypotheses were tested with *Prosopis velutina*, a shrub native to the United States that has encroached throughout its range and is invasive in other continents. Seedlings were grown in a greenhouse under two soil moisture treatments, each which was maintained by two pulse frequency treatments. Contrary to H1, mean soil moisture had greater impact than pulse frequency on seedling growth, photosynthetic gas exchange, leaf chemistry, and biomass allocation. These results indicate that *P. velutina* seedlings may be more responsive to rainfall amount than frequency, at least within the conditions seedlings experienced in this experimental manipulation.

Keywords Rainfall frequency · Gas exchange · Plant water relations · Dryland · Woody encroachment · *Prosopis*

Introduction

Plant responses to environmental stress, particularly at the seedling stage, may influence seedling performance and plant community composition (Grime and Hillier 2000). Environmental resource limitations are projected to be exacerbated in many locations due to climate change, with altered amount and timing of precipitation potentially impacting seedling performance and ultimately affecting plant community composition or distribution. Global mean surface temperatures are likely to increase by 1.5 °C from 2021–2040 compared to 1850–1990 (IPCC 2021). Seedlings may be particularly affected by climate change in arid and semi-arid ecosystems (hereafter referred to as ‘drylands’), given that resource limitations already frequently push plants beyond their physiological thresholds in these systems (de Graaff et al. 2014). Dryland seedling responses to water stress may influence ongoing land cover change in these systems

Communicated by Louis Stephen Santiago.

✉ Heather L. Throop
heather.throop@asu.edu

¹ School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

² School of Earth and Space Exploration, Arizona State University, Tempe, AZ 85287, USA

³ Department of Research, Conservation and Collections, Desert Botanical Garden, Phoenix, AZ 85008, USA

⁴ School of Sustainability, Arizona State University, Tempe, AZ 85287, USA

⁵ Global Drylands Center, Arizona State University, Tempe, AZ 85287, USA

and understanding of these responses is needed to enable informed management and restoration decisions.

Drylands cover roughly 45% of the global surface area and support more than one third of the human population (Maestre et al. 2016; Právělie 2016), making future change in these systems critical. Drylands are characterized by high potential evapotranspiration coupled with low and typically episodic precipitation (Middleton and Thomas 1992). Infrequent precipitation can influence plant abundance and density, particularly in dryland ecosystems that are among the most impacted of all ecosystems by climate change (Smith et al. 2000; Maestre et al. 2005). This sensitivity can cause drylands to be at risk of declining productivity ('desertification'), biodiversity losses, and reduction of ecosystem services (El-Beltagy and Madkour 2012). The potential impacts of climate change on drylands are magnified by projections that global dryland area may increase with climate change (Huang et al. 2016; but see Berg and McColl 2021).

While seedlings are a highly vulnerable life history stage, critical questions remain regarding the impacts that the timing and amount of precipitation have on seedling establishment. Increases in mean soil moisture are likely to be beneficial to seedlings in water-limited systems, but the frequency of pulse delivery impacts the variation around mean soil moisture over time. One important factor affecting seedling stress is the minimum available soil moisture (Munson et al. 2021) experienced by seedlings independent of the mean delivered over a given period. Low pulse frequency could result in episodic soil moisture deficits that can limit seedling productivity (O'Brien et al. 2013). The same mean soil moisture might occur with more frequent delivery of small precipitation pulses or less frequent delivery of larger pulses. Biological responses to pulse frequency should vary based on the physiological ability of plants to effectively access soil moisture, as well as the ability to persist during inter-pulse periods.

The genus *Prosopis* consists of deeply-rooted woody plant species that are native to the dryland regions of North and South America, northern Africa and part of Asia. *Prosopis* species have been introduced widely to other regions for agricultural and economic purposes, such as in regions in Africa for shade and food for livestock (Wise et al. 2012; Shackleton et al. 2014). *Prosopis* species are prevalent in the lower deserts of North America, including the Sonoran and Chihuahuan Deserts. Over the last 150 years in the southwestern United States, *Prosopis velutina* has expanded from riparian areas into xeric grasslands and savannas despite the absence of plant-available groundwater (Brown and Archer 1988; McPherson et al. 1993; Nie et al. 2012). *Prosopis* seedlings can become abundant during years with sufficient rainfall to induce germination, although seedling establishment is not restricted to years with unusually high rainfall, at least at the higher-moisture portion of its range (Brown and

Archer 1999). Understanding what drives *Prosopis* seedling establishment and growth is important for predicting how dryland community structure may change in response to environmental stress from climate change. More information is needed to evaluate approaches for managing *Prosopis* populations in fragile dryland ecosystems.

We used a greenhouse experiment to analyze how mean soil moisture and moisture pulse frequency affect woody plant seedling gas exchange and growth (aboveground and belowground) in dryland ecosystems. We grew seedlings under two rainfall pulse frequencies (two or three times per week), modulating the amount of water delivered in the pulses to maintain two distinct mean soil moisture treatments. We hypothesized that seedling performance would be enhanced by both greater soil moisture and greater pulse frequency, such that seedlings with similar mean soil moisture and higher pulse frequency (thus lower soil moisture variability) would perform better than seedlings exposed to reduced frequency (Fig. 1a). Alternatively, mean soil moisture could have much greater influence than pulse frequency, leading to little difference in seedling performance with pulse frequency (Fig. 1b). We selected *Prosopis velutina* as a model species to study due to its prevalence and ecological importance within dryland ecosystems in the southwestern United States and relevance to management of these ecosystems.

Methods

Seed collection and germination

We sourced *P. velutina* seeds from trees growing on sandy loam Holocene soils at the Santa Rita Experimental Range (SRER) in southeastern Arizona in October 2018. Mean annual precipitation at this upland semi-desert grassland site is 475 mm (PRISM Climate Group 2021) which is distributed bimodally as winter rains and summer monsoons. During the summer monsoon season (01-Jul to 30-Sept) the historical (2011–2020) mean daily temperature was 26.5 ± 0.24 °C, the mean daily minimum temperature was 19.9 ± 0.18 °C, and the mean daily maximum temperature was 33.0 ± 0.33 °C (PRISM Climate Group 2021). We collected seed pods from eight *P. velutina* genotypes (trees that were separated by tens to hundreds of meters). Seed pods were dried in a 60 °C oven for 48 h. Seeds were then removed from the pods, scarified with sandpaper, and stored at room temperature.

Greenhouse set-up and planting

We grew seedlings in a roof-top air-conditioned glasshouse with natural light at Arizona State University. Seedlings

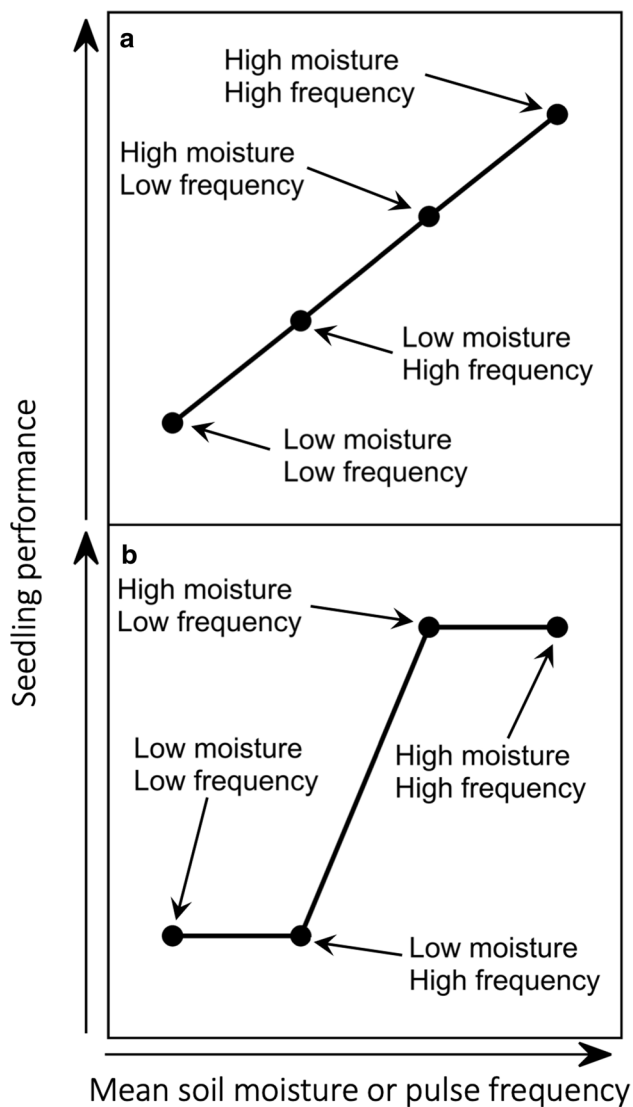


Fig. 1 Conceptual model for the two alternative hypotheses tested in the study. **a** Seedling growth and photosynthetic gas exchange is constrained by both pulse frequency and mean soil moisture conditions that a seedling experiences (H1). **b** Alternatively, seedling growth and photosynthetic gas exchange is primarily constrained by the mean amount of soil moisture available to a seedling (H2)

were grown in 36 cm deep pots (7 cm diameter, 983 mL volume; D60L Deepots, Stuewe & Sons, Inc., Tangent, OR, US) containing a 1:1 by volume mixture of potting soil (ScottsMiracle-Gro, The Scotts Company LLC) and sand. The potting soil contained 0.21% nitrogen, 0.11% phosphorus and 0.16% potassium by mass. We planted three *P. velutina* seeds per pot on 30 April 2019. On 15 May 2019, we selected the tallest seedling in each pot, removing other seedlings and non-germinated seeds. There were 256 seedlings at the start of the experiment ($N = 8$ genotypes \times 2 soil moisture treatments \times 2 rainfall frequencies \times 8 replicates per genotype by water combination). Genotypes had similar

seedling performance, so we pooled genotypes (treating each seedling as a distinct individual) for the experimental work. Seedling mortality prior to the start of the watering treatments was minimal. Nine seedlings across different assigned treatments died prior to the treatment start, resulting in 247 surviving plants at the onset of the watering treatments (see “Watering treatments” section, below). We used a stratified random design to assign each seedling to one of 16 trays, each of which held 16 seedlings. Each of the four rows per tray was designated for one of the four watering treatments. Genotypes were evenly distributed among the rows. To reduce edge effects and bias, we randomized the order of each treatment row and arrangement of individual plants within each treatment. We placed the trays on three benches in the greenhouse and rotated the placement of trays near the experiment mid-point to reduce the potential impact of spatial variation in abiotic factors, such as temperature, relative humidity (RH), and light.

Watering treatments

We watered seedlings with deionized water daily until they were established and then subjected seedlings to watering treatments from 01-Jul-2019 to 30-Aug-2019. The four watering treatments were a factorial combination of two soil moisture levels: low (L), and high (H) mean moisture and two pulse frequencies; infrequent (I) and frequent (F), which were delivered 2 and 3 times per week, respectively. When the watering treatments commenced there were no among-treatment differences in seedling height (mean \pm SE for all treatments = 22.5 ± 0.31 cm, $P > 0.05$ for main effects and interaction based on a 2-way ANOVA with mean moisture and pulse frequency treatments as main effects) or the number of leaves (28.5 ± 0.40 , $P > 0.05$ for main effects and interaction).

We altered the quantity of water per pulse throughout the experiment to maintain the desired soil moisture levels despite changes in ambient temperature and plant water uptake (Fig. 2; Table S1). We used Decagon ECH₂O EC-5 soil moisture probes (Decagon Inc., Pullman, WA, US) to monitor soil moisture every 30 min in a subset of 32 pots and used these data to determine when the pulse sizes needed to be adjusted in order to maintain the two distinct soil moisture levels. We aimed to keep the mean volumetric soil moisture content above $0.10 \text{ m}^3 \text{ m}^{-3}$ across each treatment for the first month of the experiment to minimize mortality. However, we then allowed the soil moisture content to decline for the low soil moisture treatments to further diverge the treatments. The moisture levels were selected to capture the range of moisture conditions experienced at the SRER during a summer monsoon season (Barron-Gafford et al. 2011). Soil moisture data indicated that these watering treatments led to mean soil moisture that was 21% greater in the high

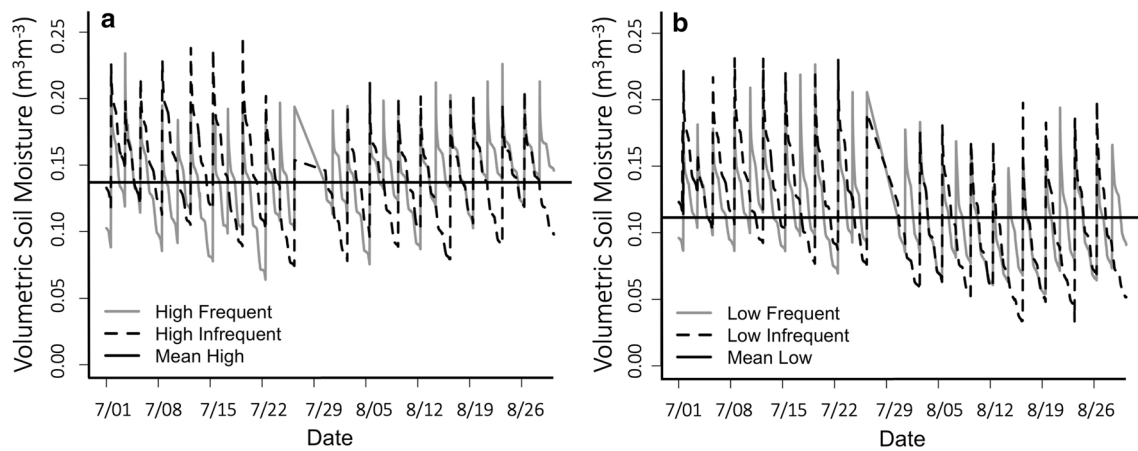


Fig. 2 Illustration of the four soil moisture treatments (volumetric soil moisture content, water m^3 soil m^{-3}). **a** The two soil moisture treatments with irrigation delivered at high frequency: high mean soil moisture delivered with high pulse frequency (HF, moderate water three times per week), low mean soil moisture delivered with high pulse frequency (LF, low water three times per week). **b** The two soil moisture treatments with irrigation delivered at low frequency: high mean soil moisture delivered with low pulse frequency (HI, moder-

ate water twice per week), and low mean soil moisture delivered at low pulse frequency (LI, low water twice per week). Data taken from 01-Jul-2019 to 30-Aug-2019. The solid horizontal lines represent the mean soil moisture, which did not differ with pulse frequency for either of the high or low mean soil moisture treatments. $N_{\text{HF}}=8$, $N_{\text{LF}}=6$, $N_{\text{HI}}=8$, $N_{\text{LI}}=10$, where N is the number of soil moisture probes for each watering treatment

than the low soil moisture pots (Fig. 2). However, mean soil moisture was not affected by the pulse frequency treatments.

Greenhouse conditions

We monitored the air temperature inside the greenhouse with iButtons (DS1921G-F5#, Maxim Integrated, San Jose, CA, US). Two iButtons were placed on each of the four corners of the greenhouse with one iButton placed on the bench and one suspended ~ 0.6 m above the trays in containers shielded from direct radiation. In total, there were eight iButtons used. The iButtons logged data every 30 min from 01-July-2019 to 27-Aug-2019. Periodically during August 2019, we measured RH at the same locations as the iButtons (Fisher Traceable Memory Hygrometer/Thermometer, Fisher Scientific, Pittsburgh, PA, US). Mean air temperature during the experiment was 27.4 ± 0.02 °C (range = 22.5–35.5 °C), with similar temperatures for the iButtons on the bench and suspended above the benches (27.3 ± 0.02 °C and 27.6 ± 0.02 °C for bench and suspended positions, respectively). While temperatures did not differ among horizontal positions for bench top or suspended locations, we rotated the plant trays during the experiment in case of any abiotic differences. The greenhouse mean temperature was slightly above the field site mean temperature (26.5 ± 0.24 °C, see “Seed collection and germination” section), although the temperature range in the greenhouse was somewhat more moderate than the typical field temperature range (13.5–39.1 °C mean range for 1 July to 30 September from 2011 to 2020, PRISM Climate Group 2021). The mean RH during the experiment was

$30.4 \pm 0.47\%$, with similar RH among greenhouse monitoring locations ($30.6 \pm 0.70\%$ and $30.2 \pm 0.65\%$ for the bench and suspended measurements, respectively).

Growth response variables

We measured seedling mortality and growth throughout the experiment. We collected data on the stem height and the number of leaves per plant just prior to initiating watering treatments and four times during the experiment (01-Jul, 26-Jul, 16-Aug, and 05-Sep-2019). We harvested the plants on 05-Sep-2019, partitioning the aboveground biomass into leaf and stem material before drying at 50 °C. The belowground biomass was obtained by gently washing soil from the roots. Mortality was low, with only three seedlings succumbing during the water treatment period.

Physiological response variables

Leaf nitrogen (N) content was analyzed on a subset of 24 harvested plants (six per watering treatment). We collected all the leaves from each of these plants, removed the rachises, and ground the leaflets into fine powder using a ball mill. We analyzed two replicates per plant on an elemental analyzer (ECS 4010, Costech Analytical Technologies, Inc., Valencia, CA, US).

Instantaneous leaf gas exchange, including photosynthesis (A_{net}) and stomatal conductance (g_s), was measured every 2 weeks on a subset of 16 plants (four per watering treatment) using a LiCor 6400 portable photosynthesis system

(LiCor Inc, Lincoln, NE, US). Environmental conditions were held constant among plants (photosynthetic photon flux density = $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, leaf temperature = 28°C , sample $\text{CO}_2 = 410 \text{ ppm}$, $\text{RH} = 30\text{--}60\%$). Since the leaves were small and filled only a fraction of the chamber, gas exchange measurements were conducted with two leaves in the chamber. Leaves were arranged in the chamber to avoid any leaf overlap and mean values of A_{net} and g_s were calculated from three consecutive measurements on each plant. Since the entire area of the chamber was not filled with the compound *P. velutina* leaves, we took photos of the leaves after measurements, estimated leaf area inside the chamber with ImageJ v. 1.5 (National Institutes of Health, Bethesda, MD), and corrected the gas exchange values based on the measured leaf area.

We measured leaf chlorophyll fluorescence ($F_v:F_m$; unitless) to evaluate the maximum quantum yield of PSII (Maxwell and Johnson 2000). Specifically, $F_v:F_m$ was calculated as $(F_v:F_0):F_m$, where F_m is maximum fluorescence yield and F_0 is the yield of fluorescence in the absence of photosynthetic active radiation. To determine $F_v:F_m$, leaves were exposed to an initial $30 \mu\text{S}$ ($0.027 \mu\text{mol m}^{-2}$) light pulse to determine F_0 followed by a $2400 \mu\text{mol m}^{-2} \text{s}^{-1}$ saturating light pulse to determine F_m . We used a FluorPen FP 100 portable fluorometer (Photon Systems Instruments, Drasov, Czech Republic) to measure $F_v:F_m$ on a subset of 64 plants. Since a FluorPen measures light emitted in a dark-adapted state, the measurements were taken at night using headlamps with the greenhouse lights turned off. Mean plant $F_v:F_m$ values were calculated from measurements conducted on two healthy, mature leaves per plant. We measured $F_v:F_m$ prior to the start of the treatments (pre-treatment; 28-Jun-2019) and 5 days after the last watering treatment (post-treatment; 04-Sept-2019).

Statistical analysis

Soil moisture data were analyzed with a two-way analysis of variance (ANOVA) with main effects of soil moisture treatment and pulse frequency.

We used linear modeling with mixed effects (R package lme4 version 1.1.21; Bates et al. 2015) to analyze the impact of the watering treatments on response variables that were measured multiple times on the same plant (A_{net} , g_s , number of leaves). Plant ID was considered a random effect (modeled as a random intercept fitted for each individual) to account for repeated measurements on the same individual. To account for variability in soil moisture through the course of the experiment, we considered antecedent soil moisture a continuous variable. We determined the mean soil moisture for the 2 weeks prior to measurement and applied that moisture value to all replicates within a watering treatment. The complete models were formatted as:

$$\text{RV} = \beta_0 + \beta_1 \text{mean}_{\text{sw}} + \beta_2 \text{CV}_{\text{sw}} \quad (1)$$

where RV is the response variable, β_0 is the intercept, mean_{sw} is the mean soil moisture for the 2-week interval prior to the measurement (weeks 0–2, weeks 2–4, weeks 4–6, weeks 6–8 or weeks 7–9), CV_{sw} is the soil moisture CV for the given 2-week interval, and β_1 and β_2 are the regression coefficients. We ran all possible combinations of models by sequentially excluding explanatory variables and selected the best-fit model based on AIC values. The marginal and conditional r^2 values were obtained using R package MuMIn version 1.43.15 (Bartoń 2009). Marginal r^2 refers to the observed variance that is explained by the fixed factor (soil moisture) and conditional r^2 refers to that explained by the fixed and random factors (soil moisture and plant ID) (Nakagawa and Schielzeth 2012).

For each of the plant response variables (height, number of leaves, aboveground biomass, belowground biomass, belowground:aboveground biomass, leaf C, leaf N, A_{net} , g_s , and the pre- and post-treatment $F_v:F_m$), we considered two main effects categories (soil moisture and pulse frequency) and their interaction in a two-way ANOVA.

All analyses were done with R (R Core Team 2020). We used an alpha of 0.05 to determine significance for statistical tests. Data were tested for departures from the assumption of normality prior to analysis. If necessary, data were transformed by Tukey's Ladder of Powers. Plant mortality led to slightly unbalanced experimental design for some response variables, we used Type II sums of squares for ANOVAs in these cases (Langsrud 2003).

Results

Growth response variables

At the harvest, seedlings in the low soil moisture treatments were 11% shorter than those in the high soil moisture treatments (Fig. 3a). The height at harvest was impacted by soil moisture treatment ($F_{1,240} = 24.94$; $P < 0.0001$) but there was not a significant soil moisture \times pulse frequency interaction ($F_{1,240} = 2.57$; $P = 0.11$) or pulse frequency main effect ($F_{1,240} = 0.03$; $P = 0.87$).

The number of green leaves per plant was similar across treatments at the start of the experiment (28 ± 0.40 ; mean \pm SE), but the soil moisture treatments led to different trajectories in leaf number over time. The number of leaves in the low soil moisture treatments had decreased by the post-treatment period (24.0 ± 0.66 leaves for the low, frequent treatment 25.1 ± 0.59 leaves for the low, infrequent treatment) (Fig. 3b). The high soil moisture treatments increased in leaf number until week 7 (36.7 ± 1.42 ; mean \pm SE), but declined to the post-treatment period

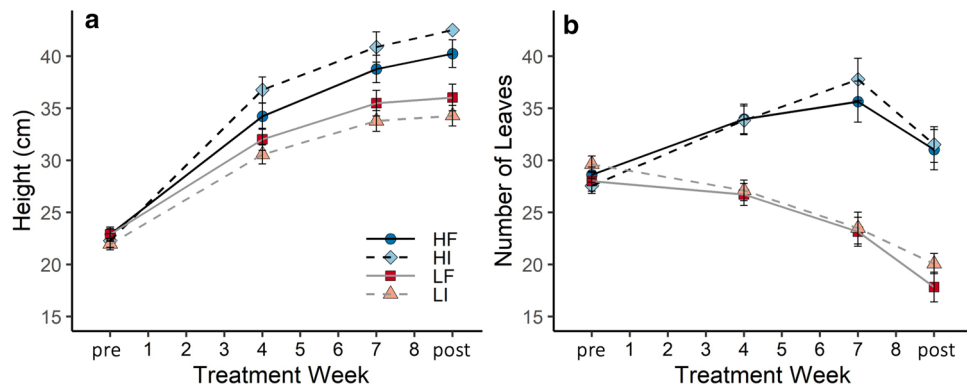


Fig. 3 Growth responses in *P. velutina* seedlings over time following commencement of the greenhouse watering experiment. Data are mean (\pm SE) values for **a** height (cm) and **b** number of leaves for the four treatments. Symbols and line types represent the treatments, as described in Fig. 2. For the treatment week, “pre” represents the

pre-treatment measurements taken the same day before the first treatment watering started, and “post” is the post-treatment where measurements were taken 5 days after the last treatment watering ended. $N=244\text{--}247$ for each treatment week

(post-treatment was 32.5 ± 0.80 leaves for high, frequent treatment and 32.7 ± 0.80 leaves for the high, infrequent treatments). A linear model predicting leaf number that included the entire experiment period showed a positive influence of antecedent soil moisture and a negative influence of soil moisture CV (Online Resource 1, Fig. S1a and Fig. S1b: Leaves = $1.67 + 60.12 \cdot \text{mean}_{\text{sw}} - 4.21 \cdot \text{CV}_{\text{sw}}$, marginal $r^2 = 0.11$, conditional $r^2 = 0.24$).

Plant biomass was affected by the soil moisture treatments. The total aboveground biomass was greater in the high than low soil moisture treatments ($F_{1, 240} = 78.94$; $P < 0.0001$), but was not impacted by pulse frequency ($F_{1, 240} = 0.98$, $P = 0.32$) or the interaction between soil moisture and pulse frequency ($F_{1, 240} = 0.001$, $P = 0.97$). Leaf biomass was impacted by soil moisture ($F_{1, 240} = 80.68$, $P < 0.0001$), with the high soil moisture treatments having 40% greater leaf biomass than low soil moisture treatments. However, there was no effect of pulse frequency or interaction between soil moisture and pulse frequency (Fig. 4a; pulse frequency: $F_{1, 240} = 0.059$, $P = 0.81$; interaction: $F_{1, 240} = 0.50$, $P = 0.48$). Stem biomass responded positively to soil moisture treatment ($F_{1, 240} = 59.01$; $P < 0.0001$) but not to pulse frequency or the interaction (pulse frequency: $F_{1, 240} = 1.31$, $P = 0.25$; interaction: $F_{1, 240} = 0.10$, $P = 0.76$). Stem biomass was 31% lower for the low than high soil moisture treatments (Fig. 4b). Similarly, the belowground biomass was 20% greater in the high than low soil moisture treatments ($F_{1, 240} = 42.78$; $P < 0.0001$), but neither pulse frequency nor the interaction between soil moisture and pulse frequency had an impact on belowground biomass (Fig. 4c; pulse frequency: $F_{1, 240} = 2.41$, $P = 0.12$; interaction: $F_{1, 240} = 0.66$, $P = 0.42$). The belowground:aboveground biomass in the high soil moisture treatments was 16% lower than in the low soil moisture treatments ($F_{1, 240} = 25.28$; $P < 0.0001$), but again neither pulse frequency nor the

interaction between soil moisture and pulse frequency had an effect (Fig. 4d; pulse frequency: $F_{1, 240} = 0.02$, $P = 0.90$; interaction: $F_{1, 240} = 1.84$, $P = 0.18$).

Physiological trait responses

Leaf N concentration responded to soil moisture treatment ($F_{1, 20} = 8.43$, $P < 0.01$), with lower leaf N in the low soil moisture treatment ($N = 2.12 \pm 0.06$ mg N g⁻¹ leaf) than the high soil moisture treatment (2.59 ± 0.10 mg N g⁻¹ leaf). There was no significant interaction between soil moisture and pulse frequency (interaction: $F_{1, 20} = 2.90$, $P = 0.10$) or main effect of pulse frequency (pulse frequency: $F_{1, 20} = 1.30$, $P = 0.27$) on leaf N.

Mean A_{net} across all treatments and sampling periods was 9.8 ± 0.31 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. From pre-treatment to week 8, the A_{net} decreased in all treatments, ranging from a 27% decline in the high, frequent treatment to a 66% decline in the low, frequent treatment (Fig. 5a). Linear model analysis showed a positive response of A_{net} to soil moisture ~ 24 h prior to the photosynthesis measurement ($A_{\text{net}} = 2.59 + 23.7 \text{sw}_{24}$; marginal $r^2 = 0.11$, conditional $r^2 = 0.55$) (Fig. 6a). The linear models using the antecedent moisture for the 2-week interval prior to measurements also showed that A_{net} responded positively to mean soil moisture and negatively to the soil moisture CV (Online Resource 1, Fig. S3a and Fig. S3b).

The overall mean g_s across all treatments was 0.093 ± 0.004 mol H₂O m⁻² s⁻¹. From pre-treatment to week 8 there was a general, but inconsistent, decline for all treatments (Fig. 5b). At the post-treatment measurement, which was made after the seedlings had not received a rainfall treatment for 6 days, g_s of the low soil moisture treatments fell to near zero (Fig. 5b). There was a positive linear relationship between g_s and A_{net} (Fig. 5c). The results of the linear models

Fig. 4 Boxplots for biomass per treatment from the harvest of the seedlings grown in a greenhouse, including **a** leaf biomass, which is the sum of all leaves per plant (including rachises), **b** stem biomass, **c** belowground biomass, the biomass of the cleaned and dried roots, and **d** the belowground:aboveground biomass ratio (unitless). Colored bars represent the treatments, as described in Fig. 2. The box plots represent medians (center horizontal line), first and third quartiles (bottom and top box edges, respectively), variability outside the first and third quartiles (whiskers), and outliers (dots). Different letters above boxes indicate significant differences among treatments ($P < 0.05$)

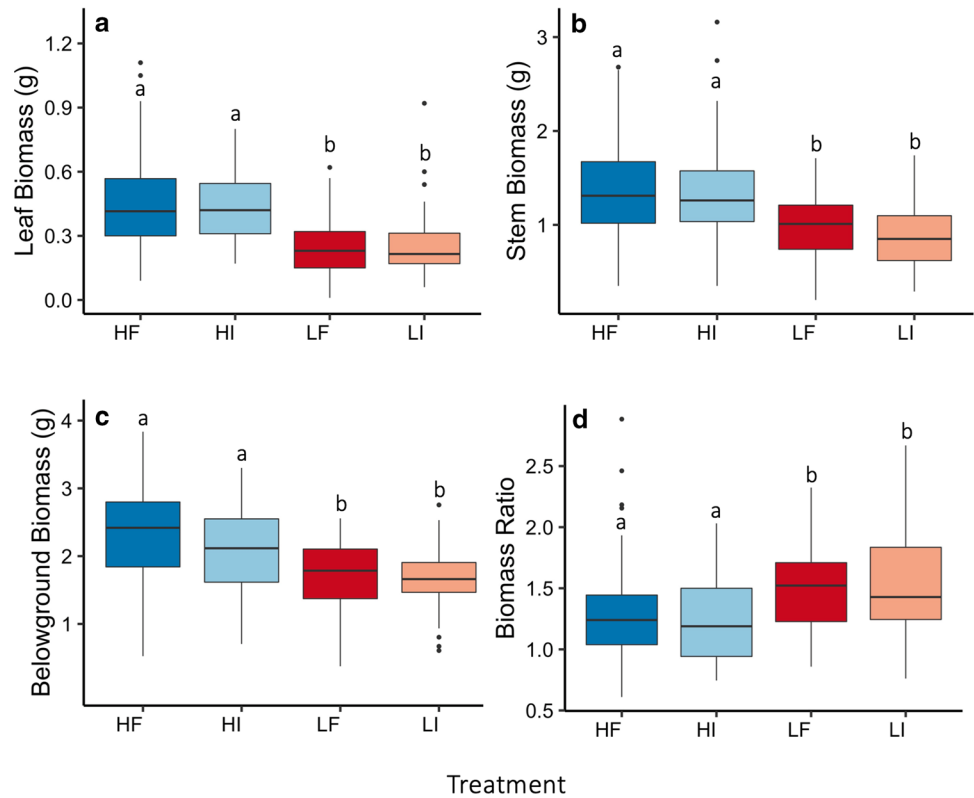
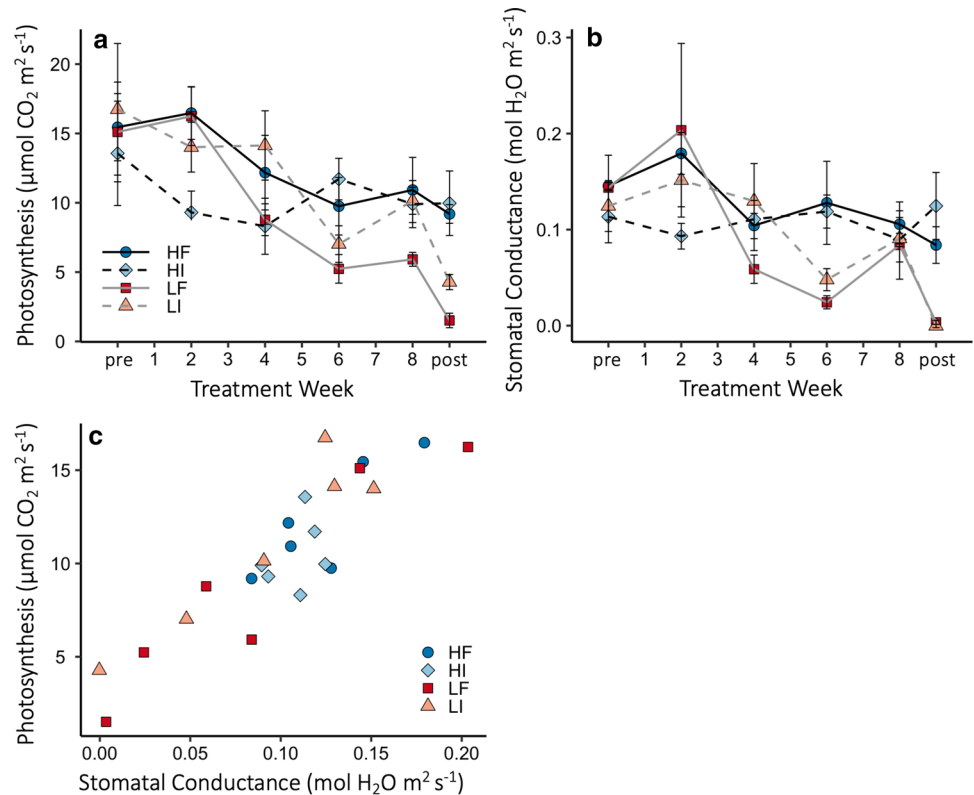


Fig. 5 Photosynthetic gas exchange responses in seedlings over time following commencement of the greenhouse watering experiment. Data are **a** mean (\pm SE) photosynthetic rate and **b** stomatal conductance and **c** photosynthetic rate versus stomatal conductance. Symbols and line types represent the treatments, as described in Fig. 2. Physiological data were collected on four replicate plants per treatment for each set of measurements. For the treatment week, “pre” represents the pre-treatment measurements taken the day before first treatment watering started, and “post” is the post-treatment where measurements were taken 5 days after last treatment watering ended



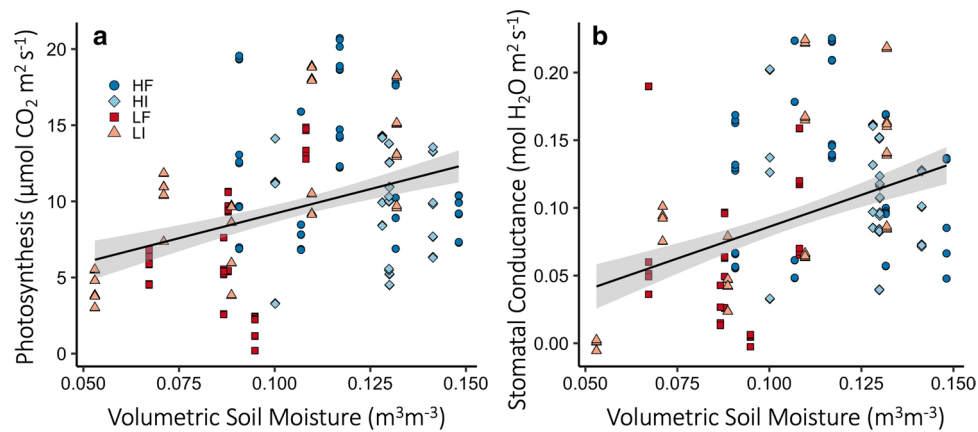


Fig. 6 Gas exchange of seedlings in response to volumetric soil moisture, including **a** photosynthetic rate and **b** stomatal conductance. Symbols represent the treatments, as described in Fig. 2. Photosynthesis = $2.59 + 23.7 \times (\text{soil moisture 24 h before measurement})$;

r^2 (marginal)=0.11, r^2 (conditional)=0.55. Stomatal Conductance = $0.01 + 0.80 \times (\text{soil moisture 24 h before measurement})$; r^2 (marginal)=0.12, r^2 (conditional)=0.51. Bold lines represent trend lines. Gray bands represent $\pm 95\%$ confidence intervals

showed similar patterns to photosynthesis. There was a positive relationship between g_s and soil moisture at ~24 h prior to the measurement (Fig. 6b; $g_s = 0.01 + 0.80sw_{24}$; marginal $r^2 = 0.12$, conditional $r^2 = 0.51$). The linear models using the 2-week antecedent moisture also showed that g_s responded positively to mean soil moisture and negatively to the soil moisture CV (Online Resource 1, Fig. S3c and Fig. S3d).

The pre-treatment $F_v:F_m$ values did not differ significantly with soil moisture treatment, pulse frequency or their interaction (across treatment mean = 0.77 ± 0.007). However, differences were detected in $F_v:F_m$ among treatments in the post-treatment measurement period. Specifically, soil moisture treatment had a significant impact on the $F_v:F_m$ ($F_{1,60} = 6.96$; $P = 0.01$), with greater $F_v:F_m$ for the high soil moisture treatments than the low soil moisture treatments (0.68 ± 0.01 and 0.57 ± 0.02 , respectively). Neither pulse frequency nor the interaction between soil moisture and pulse frequency had a significant impact on $F_v:F_m$ (pulse frequency: $F_{1,60} = 3.72$; $P = 0.06$; interaction: $F_{1,60} = 0.31$; $P = 0.58$).

Discussion

Seedling success post germination in dryland regions is governed largely by precipitation inputs that can vary in amount, intensity, duration, and seasonality (Potts et al. 2019). The interaction among these varying precipitation characteristics ultimately controls the availability of soil moisture for seedlings to support survival, growth, and gas exchange. In this study, we posed two inter-related questions: does the rainfall pulse frequency substantially affect *Prosopis* seedling performance? Or, does the mean soil moisture alone play the key role in regulating seedling performance? Our results

indicate that growth and photosynthetic gas exchange of *P. velutina* seedlings under our experimental manipulations were largely governed by mean soil moisture conditions. While seedling performance was not strongly affected by the pulse frequencies used in this experiment, small negative responses to antecedent soil moisture CV for leaf number, A_{net} , and g_s indicate some sensitivity to soil moisture variability. Very low soil moisture availability would ultimately result in seedling mortality depending on the duration between rainfall events, independent of rainfall accumulation. However, evaluating relationships between precipitation patterns and drought-induced mortality was beyond the scope of this study, and instead we focused on precipitation inputs that maintained seedling performance.

We hypothesized that seedling performance would be enhanced by both greater soil moisture and greater rainfall frequency, such that seedlings with similar mean soil moisture but higher pulse frequency would perform better (Fig. 1a). Alternatively, mean soil moisture could have greater influence than pulse frequency, leading to little difference in seedling performance with rainfall pulse frequency (Fig. 1b). We detected little evidence to support the hypothesis that higher pulse frequency would positively affect seedling performance (Fig. 1a), and instead found considerable evidence to support our alternative hypothesis that mean soil moisture availability would predominantly impact plant performance independent of the rainfall pulse frequency experienced by seedlings (Fig. 1b). On one hand, there is evidence that seedlings of woody taxa can be sensitive to pulse frequency. For example, a 90-day greenhouse study on eight Dipterocarpaceae species in Borneo found that height growth was greater in seedlings that received daily watering relative to seedlings given the same cumulative water but at 6-day intervals (O'Brien et al. 2013).

Conversely, a 14-month greenhouse watering experiment on seedlings of seven Mediterranean shrub species found that reduced watering frequency (two versus four water pulses a week) resulted in increased root biomass, but had no impact on aboveground growth (Padilla et al. 2009). One possibility is that among-study differences in pulse frequencies play an important role in the observed responses. As with Padilla et al. (2009), the pulse frequencies in the present study were selected to explore growth rather than survival responses. Had we used less frequent pulses, such as those used by O'Brien et al. (2013), we may have found a stronger response to frequency with greater difference in frequency treatments. Alternatively, the response of some woody seedlings to reductions in watering frequencies while others do not respond may be related to species-specific rooting patterns. Seedlings that rapidly produce deep roots tend to be less susceptible to the effects of soil water depletion than more shallowly rooted seedlings (Padilla and Pugnaire 2007). *Prosopis* seedlings can achieve root elongation rates that exceed 24 mm day^{-1} (Brock 1986) and as a consequence can quickly forage for water in deeper soils that dry much more slowly from evaporation than shallow soils. The rapid root elongation of *Prosopis* seedlings may therefore explain why in the present study seedlings were largely insensitive to pulse frequency relative to mean soil moisture. However, it is important to note that the slight negative influence of soil moisture CV on number of leaves (Online Resource 1, Fig. S1b), photosynthesis (Online Resource 1, Fig. S3b) and stomatal conductance (Online Resource 1, Fig. S3d) also suggest that *Prosopis* seedlings are not entirely insensitive to pulse frequency. Further assessment of the relative controls of these variables under different manipulative combinations is warranted.

Allocation to belowground biomass was favored in the low mean soil moisture treatments compared to the high mean soil moisture treatments. By prioritizing root elongation over aboveground growth, plants under water-limited conditions can reduce plant water potential gradients and increase resilience to declining soil moisture. A glass-house study on early *P. velutina* and *Senegalia greggii* (catclaw acacia) seedling development found that *P. velutina* taproots were significantly longer than *S. greggii* taproots when the seedlings were given an initial water pulse of 10 mm day^{-1} for 5 days followed by 5 mm day^{-1} for 17 days (Woods et al. 2014). Another study found that the taproots of *Prosopis glandulosa* (honey mesquite) seedlings had reached deeper than 40 cm in the soil 4 months after germination and that their elongated taproots enabled access to water deeper in the soil than the grass, *Chloris cucullate* (Brown and Archer 1990). This pattern of *P. velutina* seedlings responding to precipitation by advancing taproot growth may be a driving factor to their spread in precipitation-limited environments. Ansley et al. (2014)

examined the root response of 12 large *P. glandulosa* trees to long-term rainfall manipulation in a northern Texas field site and found that root growth into deeper soil was favored after a period of drought. The observed differences in the present study in belowground biomass between the low and high soil moisture accumulation treatments could have impacts on the long-term growth and physiological performance of *P. velutina* seedlings exposed to water stress. However, it is possible that the full extent of the impact of water stress on whole-plant biomass allocation may need to be evaluated over multiple seasons as older plants would have different physiological constraints than seedlings.

Controlled environment studies present a particular challenge for assessing responses of plants with capability for rapid root elongation. It is important to note that the increased proportional belowground allocation in the low soil moisture treatments may have led plants in these treatments to restrain growth due to pot size limitations more quickly than plants in the high mean soil moisture treatments. Results from a meta-analysis indicate that greenhouse-grown plants are frequently limited by pot volume, with small pot volumes associated with diminished overall plant growth via reduced photosynthesis per unit leaf area (Poorter et al. 2012). A further potential complication with interpreting greenhouse results is the possibility that soil moisture was not consistent across the soil profile. Our soil moisture probes integrated soil moisture conditions across the pot depth, so we do not know how soil moisture varied with depth. However, shrub root allocation can vary along the soil profile in response to moisture availability (Ansley et al. 2014), with strong links between shrub seedling ability to access moist soil layers and seedling survival (Padilla and Pugnaire 2007).

At the conclusion of the experiment, A_{net} , g_s and $F_v:F_m$ were significantly greater in the high than the low soil moisture treatments, indicating that not only were high soil moisture plants acquiring more photosynthates, but these plants were also maintaining a higher photosynthetic capacity. Enhanced leaf N in the high moisture treatments may have facilitated greater plant photosynthetic capacity, as N provides the building blocks for chlorophyll and photosynthetic enzymes (Hikosaka 2004; Hikosaka and Shigeno 2009). In turn, a higher photosynthetic capacity in leaves could increase water use efficiency ($A_{\text{net}}:g_s$) when water is not limiting. Plants exposed to soil moisture deficits often have canopies with reduced $F_v:F_m$ (Hamerlynck and Huxman 2009). Under conditions of chronic drought stress, reductions in $F_v:F_m$ may be associated with the accumulation of photoprotective mechanisms such as the accumulation of chlorophyll-protein-xanthophyll complexes that dissipate excess light energy, resulting in photoinhibition (Adams et al. 1999).

Understanding seedling performance of *P. velutina* under variable soil moisture conditions is important for predicting future patterns of woody plant encroachment into grasslands and savannas. Understanding how precipitation amount and frequency impacts seedling performance has important implications for informed management practices. Prior manipulative experiments at the SRER found that *P. velutina* seedlings had the highest germination and seedling survival in sandy loam soils with high soil moisture (Resco de Dios et al. 2012). However, in a sandy loam site with low soil water availability, high grass mortality led to greater *P. velutina* seedling establishment (Resco de Dios et al. 2012). While the *P. velutina* seeds in our study had high germination success, the watering treatments did not commence until after germination. However, the low mortality observed throughout the study could reflect the ability of *P. velutina* to persist in environments with low soil moisture. Compared with the results from Resco de Dios et al. (2012), this study demonstrates that *P. velutina* can survive in environments with lower soil water availability via increased taproot depth allowing access to deep groundwater enabling *P. velutina* to outcompete grasses with shallow root systems for water.

The results of the present study demonstrate that *P. velutina* seedlings respond strongly to mean soil moisture but are relatively insensitive to variation in the pulse frequencies assessed in this study. While *Prosopis* spp. shrubs are influenced by current-year precipitation, survival and growth of established shrubs may be influenced by other factors, including precipitation legacies from previous years. Prior work in the Chihuahuan Desert found that precipitation legacies from prior years partially explained patterns of annual net primary productivity in *P. glandulosa* (Reichmann et al. 2013). A different study analyzing rainfall patterns on dryland vegetation found that infrequent rainfall events led to greater vegetation cover than frequent or continuous rainfall events when total rainfall accumulation was similar (Baudena et al. 2007). In the present study, the mean soil moisture treatments had stronger impacts on performance than did the pulse frequency treatments. These data suggest that changing precipitation patterns are likely to impact seedling performance, although specific responses to soil moisture and pulse frequency treatments are likely to differ with the specifics of the timing and amount of moisture treatments imposed. Careful manipulation of pulse frequency will be important for future experiments to gain a more nuanced consideration of how rainfall delivery affects seedling performance. Detailed information on how changes in the amount, frequency and seasonality of precipitation inputs will impact dryland plant communities and net primary productivity will be crucial for protecting ecosystem services in drylands.

Author contribution statement AD, KH, and HT conceived of and designed the study. AD carried out the study, conducted statistical analyses, and wrote the first draft. All authors contributed to revising the manuscript.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00442-022-05193-w>.

Acknowledgements We thank the Arizona State University School of Life Sciences for use of the greenhouse and the Desert Botanical Garden for the soil. We thank N. Hornslein, M. Farrell, A. Hayes, B. Monus, F. Kangombe, J. Hunter, K. Amari, and D. Koepke for laboratory and greenhouse assistance. We appreciate constructive comments from three anonymous reviewers on a prior version of this manuscript.

Funding No grant funds supported this work. Supplies and space were provided by Arizona State University and the Desert Botanical Garden.

Availability of data and materials Data are available in the Dryad repository: <https://doi.org/10.5061/dryad.tht76hf0d>.

Code availability R code used for this work are standard statistical analyses; no novel code was developed. Code is available upon request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent to participate This article does not contain any studies with human participants.

Consent for publication This article does not contain any studies with human participants.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Adams W III, Demmig-Adams B, Logan B, Barker D, Osmond C (1999) Rapid changes in xanthophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, *Stephania japonica* and *Smilax australis* growing in the understory of an open *Eucalyptus* forest. *Plant Cell Environ* 122:125–136
- Ansley RJ, Boutton TW, Jacoby PW (2014) Root biomass and distribution patterns in a semi-arid mesquite savanna: responses to long-term rainfall manipulation. *Rangeland Ecol Manag* 67:206–218
- Barron-Gafford GA, Scott RL, Jenerette GD, Huxman TE (2011) The relative controls of temperature, soil moisture, and plant functional group on soil CO₂ efflux at diel, seasonal, and annual scales. *J Geophys Res* 116:G01023
- Bartoń K (2009) MuMIn: multi-model inference. R package. <http://R-Forge.R-project.org/projects/mumin/>
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Baudena M, Boni G, Ferraris L, von Hardenberg J, Provenza L (2007) Vegetation response to rainfall intermittency in drylands:

- results from a simple ecohydrological box model. *Adv Water Resour* 30:1320–1328
- Berg A, McColl KA (2021) No projected global drylands expansion under greenhouse warming. *Nat Clim Change* 11:331–337
- Brock JH (1986) Velvet mesquite seedling development in three southwestern soils. *J Range Manage* 39:331–334
- Brown JR, Archer S (1988) Woody plant seed dispersal and gap formation in a North American subtropical savanna woodland: the role of domestic herbivores. *Vegetatio* 73:73–80
- Brown JR, Archer S (1990) Water relations of a perennial grassland seedling vs adult woody plants in a subtropical savanna, Texas. *Oikos* 57:366–374
- Brown JR, Archer S (1999) Shrub invasion of grassland: recruitment is continuous and not regulated by herbaceous biomass or density. *Ecology* 80:2385–2396
- de Graaff MA, Throop HL, Verburg PSJ, Arnone JA, Campos X (2014) A synthesis of climate and vegetation cover effects on biogeochemical cycling in shrub-dominated drylands. *Ecosystems* 17:931–945
- El-Beltagy A, Madkour M (2012) Impact of climate change on arid lands agriculture. *Agric Food Sec* 1:3
- Grime JP, Hillier SH (2000) The contribution of seedling regeneration to the structure and dynamics of plant communities, ecosystems and larger units of the landscape. In: Fenner N (ed) *Seeds: the ecology of regeneration in plant communities*, 2nd edn. CABI Publishing, New York, pp 361–374
- Hamerlynck E, Huxman T (2009) Ecophysiology of two Sonoran Desert evergreen shrubs during extreme drought. *J Arid Environ* 73:582–585
- Hikosaka K (2004) Interspecific difference in the photosynthesis–nitrogen relationship: patterns, physiological causes, and ecological importance. *J Plant Res* 117:481–494
- Hikosaka K, Shigeno A (2009) The role of Rubisco and cell walls in the interspecific variation in photosynthetic capacity. *Oecologia* 160:443–451
- Huang J, Yu H, Guan X, Wang G, Guo R (2016) Accelerated dryland expansion under climate change. *Nat Clim Change* 6:166–171
- IPCC (2021) *Climate change 2021: The Physical Science Basis*. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. In: Masson-Delmotte V et al (eds) Cambridge University Press, Cambridge
- Langsrud Ø (2003) ANOVA for unbalanced data: use type II instead of type III sums of squares. *Stat Comput* 13:163–167
- Maestre FT, Valladares F, Reynolds JF (2005) Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *J Ecol* 93:748–757
- Maestre FT et al (2016) Structure and functioning of dryland ecosystems in a changing world. *Annu Rev Ecol Syst* 47:215–237
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. *J Exp Bot* 51:659–668
- McPherson GR, Boutton TW, Midwood AJ (1993) Stable carbon isotope analysis of soil organic matter illustrates vegetation change at the grassland/woodland boundary in southeastern Arizona, USA. *Oecologia* 93:95–101
- Middleton N, Thomas DSG (eds) (1992) *World atlas of desertification* (United Nations Environment Programme), 2nd edn. Edward Arnold, London
- Munson SM, Bradford JB, Hultine KR (2021) An integrative ecological drought framework to span plant stress to ecosystem transformation. *Ecosystems* 24:739–754
- Nakagawa S, Schielzeth H (2012) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol Evol* 4:133–142
- Nie W, Yuan Y, Kepner W, Erickson C, Jackson M (2012) Hydrological impacts of mesquite encroachment in the upper San Pedro watershed. *J Arid Environ* 82:147–155
- O’Brien MJ, Philipson CD, Tay J, Hector A (2013) The influence of variable rainfall frequency on germination and early growth of shade-tolerant dipterocarp seedlings in Borneo. *PLoS ONE* 8:e70287
- Padilla FM, Pugnaire FI (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Funct Ecol* 21:489–495
- Padilla FM, Miranda JD, Jorquera MJ, Pugnaire FI (2009) Variability in amount and frequency of water supply affects roots but not growth of arid shrubs. *Plant Ecol* 204:261–270
- Poorter H, Bühler J, van Dusschoten D, Climent J, Postma JA (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct Plant Biol* 39:839–850
- Potts DL, Barron-Gafford GA, Butterfield BJ, Fay PA, Hultine KR (2019) Bloom and Bust: ecological consequences of precipitation variability in aridlands. *Plant Ecol* 220:135–139
- Prävalle R (2016) Drylands extent and environmental issues. *A Global Approach Earth-Sci Rev* 161:259–278
- PRISM Climate Group (2021) PRISM Data Explorer. Oregon State University. <http://prism.oregonstate.edu>
- R Core Team (2020) R: A language and environment for statistical computing. <https://www.R-project.org/>. R Foundation for Statistical Computing, Vienna
- Reichmann LG, Sala OE, Peters DPC (2013) Precipitation legacies in desert grassland primary production occur through previous-year tiller density. *Ecology* 94:435–443
- Resco de Dios V, Weltzin JF, Sun W, Huxman TE, Williams DG (2012) Windows of opportunity for *Prosopis velutina* seedling establishment and encroachment in a semiarid grassland. *Perspect Plant Ecol* 14:275–282
- Shackleton RT, Le Maitre DC, Pasiecznik NM, Richardson DM (2014) *Prosopis*: a global assessment of the biogeography, benefits, impacts and management of one of the world’s worst woody invasive plant taxa. *AoB PLANTS* 6:plu027
- Smith SD et al (2000) Elevated CO_2 increases productivity and invasive species success in an arid ecosystem. *Nature* 408:79–82
- Wise RM, van Wilgen BW, Le Maitre DC (2012) Costs, benefits and management options for an invasive alien tree species: the case of mesquite in the Northern Cape, South Africa. *J Arid Environ* 84:80–90
- Woods SR, Archer SR, Schwinning S (2014) Seedling responses to water pulses in shrubs with contrasting histories of grassland encroachment. *PLoS ONE* 9:e87278