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Research paper

Testing the effects of species interactions and water limitation on tree seedling biomass allocation and physiology

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Species interactions mediate tree responses to water limitation because competition and/or facilitation alter plant physiology and growth. However, because it is difficult to isolate the effects of plant–plant interactions and water limitation from other environmental factors, the mechanisms underlying tree physiology and growth in coexisting plants under drought are poorly understood. We investigated how species interactions and water limitation impact the physiology and growth of trembling aspen (*Populus tremuloides*), narrowleaf cottonwood (*Populus angustifolia*) and ponderosa pine (*Pinus ponderosa*) seedlings in a controlled environment growth chamber, using aspen as a focal species. Seedlings were grown in pots alone or with a con- or hetero-specific seedling, and were subjected to a water limitation treatment. Growth, water status and physiological traits were measured before, during and after the treatment. Under well-watered conditions, the presence of another seedling affected growth or biomass allocation in all species, but did not impact the physiological traits we measured. Under water limitation, the presence of a competing seedling had a marginal impact on seedling growth and physiological traits in all species. Throughout the study, the magnitude and direction of seedling responses were complex and often species-specific. Our study serves as an important step toward testing how species' interactions mediate physiological responses and growth in well-watered and water-limited periods.

Keywords: biomass allocation, competition, forest, growth, growth chamber, physiology, seedlings, water limitation, water-use strategy.

Introduction

The mechanisms that underlie tree physiology and growth for coexisting species (e.g., water draw down, increased height for sunlight and gene regulation in sensing a neighbor) are also critical regulators of physiology and growth during periods of water stress (Pierik et al. 2013). From this physiological basis, the question naturally arises: are plant–plant interactions important mediators of tree resilience to water limitation? Although tree species can have positive or facilitative interactions that increase resilience during water limitation (Brooker et al. 2008), facilitation tends to decline under increasing aridity and water scarcity (Soliveres and Maestre 2014). In the absence of facilitation, adjustments in resource-acquisition strategies (e.g., increasing root surface area and growth) and physiological traits (e.g.,

increasing hydraulic conductivity) can strongly impact a tree's ability to coexist with competitors when water is limited (Ballaré et al. 1994, Dawson 1996, Casper and Jackson 1997, Franklin 2008, Farrior et al. 2013). However, increased depletion of soil water during periods of water stress because of competition can lead to enough physiological stress to overcome these adjustments and ultimately result in tree mortality (Wang et al. 2012, Adams et al. 2017). Tree species are therefore under strong evolutionary pressure to balance water-use in order to simultaneously avoid mortality from water limitation, yet remain competitive with their neighboring trees to maximize fitness (Piutti and Cescatti 1997, Anderegg et al. 2018).

A wide variety of biomass allocation (e.g., growth) patterns, physiological traits and water-use strategies help tree species

exist in their natural environments during periods of lower water availability (Shiple and Meziane 2002, Poorter et al. 2012b). Adjustments in biomass allocation patterns, such as increases in the ratio of root to shoot tissue or decreases in leaf area, can improve the uptake of water and limit water spending to help maintain plant function (Mencuccini and Grace 1995, DeLucia et al. 2000, Piñol and Sala 2000, Fotelli et al. 2001, Gersani et al. 2001, Chaves et al. 2003, Martínez-Vilalta et al. 2009, Mencuccini and Bonosi 2011, Farrior et al. 2013, Brunner et al. 2015, Rosas et al. 2019, Trugman et al. 2019). In addition, trees can rapidly (on the order of minutes) close stomata to prevent water loss and minimize risk of hydraulic damage, or can adjust leaf turgor loss point (at longer time scales—days to months) to cope with and acclimate to drier conditions (Hsiao 1973, Chaves 1991, Forrester 2014, Fotelli et al. 2001, Bartlett et al. 2014, Forrester et al. 2016). Tree water-use strategies span a spectrum that ranges from water spenders to water savers, integrating a wide diversity of species' growth and physiological traits (Bloom and Mooney 1985). Although these strategies typically reflect a species' life history (e.g., trees native to arid environments tend to be water savers), changes in physiology and growth can result in plastic changes to a tree's water-use strategy and its ability to withstand periods of water stress (Schwendenmann et al. 2015).

The role of species interactions in plant responses during water limitation has been extensively studied for a number of forest tree species (Forrester 2014, 2015, Clark et al. 2016, Forrester et al. 2016, Goisser et al. 2016, Buechling et al. 2017, Rötzer et al. 2017, Grossiord et al. 2018, Vitali et al. 2018). However, results from these investigations report a wide range of tree responses and susceptibility to water stress when growing under competition. Furthermore, although these studies are a critical component of our understanding of forest responses to water limitation, they were not able to isolate the effects of plant–plant interactions and water stress from numerous other environmental factors. Studies on crops and other nonwoody species have utilized more controlled experiments (see Fotelli et al. 2001) to tease apart the effects of plant–plant interactions like competition and water limitation (see Donald 1958, Percy et al. 1981, Griffin et al. 1989, Liebman and Robichaux 1990, Gersani et al. 2001 for examples). Yet these experiments can not necessarily be used to infer responses of naturally occurring woody species because crop species have different physiology and competitive pressures. Thus, controlled experiments on trees are needed to carefully isolate and control environmental factors that affect tree physiology and growth, thereby making it possible to study the separate and joint effects of plant–plant interactions and water stress on tree responses.

Here, we report results from a controlled environment growth chamber study that examined the effects of plant–plant interactions and water limitation on physiology and growth of tree seedlings for three species with different water-use

strategies that co-occur throughout the Intermountain West of the USA: trembling aspen (*Populus tremuloides* Michx.), narrowleaf cottonwood (*Populus angustifolia* James) and ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) (Glenn et al. 1998, Wullschlegel et al. 1998, Anderegg and HilleRisLambers 2016). We tested the following hypotheses: (i) plant–plant interactions in the absence of water stress will increase aboveground growth and photosynthetic performance, but will not affect plant water status, as the coexisting seedling will sense its neighbor but will not be water-limited (WW), (ii) plants competing with other plants for scarce water resources will experience increased water stress and allocation of root tissues relative to seedlings planted alone; (iii) growth and physiological responses to plant–plant interactions and water limitation will depend on the focal seedlings' water-use strategy (defined here as mean midday stomatal conductance (g_s) under well-watered (WW) conditions) as well as the water-use strategy of proximal species.

Material and Methods

Experimental design

Trembling aspen and ponderosa pine seedlings were obtained from the University of Idaho Pitkin Forest Nursery and narrowleaf cottonwood seedlings were obtained from the Colorado State Forest Service Seedling Tree Nursery. Hereafter, these species will be referred to as aspen, pine and cottonwood. All seedlings were ~1-year-old and 25 cm in height upon arrival to the University of Utah. These species were selected because they coexist in natural ecosystems that are routinely water stressed and represent a spectrum of water-use strategies ranging from water spenders (cottonwood, WW $g_s > 0.2 \text{ mol m}^{-2} \text{ s}^{-1}$), to intermediate water-users (aspen, WW g_s between 0.15 and $0.2 \text{ mol m}^{-2} \text{ s}^{-1}$) and water savers (pine, WW $g_s < 0.15 \text{ mol m}^{-2} \text{ s}^{-1}$) (Wullschlegel et al. 1998).

Seedlings ($n = 59$ aspen, 24 cottonwood and 25 pine in total) were transplanted into plastic pots with soil (Metro Mix 900, Sunagro Horticulture, Agawam, MA, USA) and set in a greenhouse on the University of Utah campus (40.4554°N , 111.5055°W) on 13 November 2017. Seedlings were planted either alone (solo treatment) into 18-l (30×30 cm) pots with 15 l of soil, or with a con- or hetero-specific other seedling (competition treatment) 36-l (30×60 cm) pots with 30 l of soil. Therefore, every seedling was transplanted into the same volume (15 l) of soil. We selected seedlings of comparable size to be planted together (see Figure S1 for photographs of seedlings). Pot size was selected to aim for the guideline that plant biomass did not exceed 1 g l^{-1} (Poorter et al. 2012a). Greenhouse environmental conditions during daylight hours (06:00–19:15 h) were as follows: temperature 25°C , relative humidity (RH) 74%, photosynthetically active radiation (PAR) $1050 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and vapor-pressure deficit

(VPD) 0.824 kPa (Table S1 available as Supplementary data at *Tree Physiology* Online). Seedlings were watered daily to field capacity, and received water soluble fertilizer (Nutriculture Cal-Mag Special 16-3-16, Plant Marvel, Chicago Heights, IL, USA) once a week in the greenhouse. Treatments that included two trees grown in the same pot aim to test the importance of species interactions. Because water limitation was imposed for much of the experiment, the primary mode of interaction is highly likely to be competition for a scarce resource between the seedlings. Thus, we refer to this treatment as 'competition' for shorthand, although not all interactions might inherently be competitive during nonresource-limiting periods.

Aspen is one of the most widespread species across North America and has been shown to be susceptible to drought-induced mortality (Anderegg et al. 2012, Worrall et al. 2013). Given aspen's ecological importance, and space constraints in the growth chamber, we designed our study to focus on aspen. In total, our study consisted of eight planting groups in the following combinations: aspen alone (A), aspen with another aspen (A × A), aspen with a cottonwood (A × C), aspen with a pine (A × P), cottonwood alone (C), cottonwood with an aspen (C × A), pine alone (P) and pine with an aspen (P × A). Aspen received a differing experimental design whereby aspen was planted in every possible planting combination: alone, with another aspen, with a cottonwood and with a pine. The cottonwood and pine seedlings only received two planting combinations: alone, and with an aspen (due to growth chamber space constraints). For pots containing two plants, both seedlings were used for all applicable measurements.

We allowed 25 days for seedlings to recover from transplanting shock. Subsequently, a subset of pots was randomly selected and placed into a Percival PR-915 (Percival Scientific, Perry, IA, USA) controlled environment growth chamber. Pots were arranged into six replicates in the growth chamber. Each replicate had the same layout of pots, which consisted of two pots of aspen alone (A) and one pot of every other planting group (Figure S2 available as Supplementary data at *Tree Physiology* Online). Pots were arranged to limit canopy overlap and prevent shading between pots. Growth chamber environmental conditions during daylight hours (06:00–19:15 h) were set to mimic the greenhouse and were recorded as follows: temperature 25 °C, RH 76%, PAR 1150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and VPD 0.761 kPa (Table S1 available as Supplementary data at *Tree Physiology* Online). Remaining pots were kept in the greenhouse under the same environmental conditions as described above.

Here, we summarize our study timeline and then in the following paragraphs we elaborate in depth about each stage. Our study consisted of three main time periods: the WW, WL and recovery (R) periods (Figure S4 available as Supplementary data at *Tree Physiology* Online illustrates the experimental timeline). Although the overall duration of this study (13 weeks)

is shorter than for naturally growing and developing seedlings, other controlled chamber ecophysiological seedling studies have used similar timelines (see Kolb and Robberecht 1996, Duan et al. 2015 for examples).

After relocation to the growth chamber, seedlings were given a 5-day acclimation period. Subsequently, all growth chamber pots were weighed for four consecutive days to determine water loss due to evaporation using a bench scale (CPWplus 200, Adam Equipment Inc., Oxford, CT, USA). This calibration was repeated 1 month later to account for increased seedling growth and water-use. Water loss, as estimated by pot weight, was averaged for each planting group and used for determining watering regimes throughout the study (e.g. all A pots received the same amount of water based on the average weight of A pots, whereas all A × A pots received the same amount of water based on the average weight of the A × A pots, etc.). Soil moisture sensors (Models CS655 and CS616, Campbell Scientific Inc., Logan, UT, USA) were installed at a depth of 15 cm across two replicates of pots to measure the volumetric water content of the soil throughout the study. In the growth chamber, seedlings were watered daily to field capacity, and received water soluble fertilizer (Nutriculture Cal-Mag Special 16-3-16, Plant Marvel, Chicago Heights, IL, USA) once a week for 7 weeks. The 7-week period ensured that seedlings had enough time to (i) respond to the presence of another seedling (if applicable), (ii) adjust to the growth chamber and (iii) flush new leaves. This period was our well-watered (WW) period.

During the WL period, seedlings in the growth chamber experienced three stages of water limitation. These stages of water limitation were designed to stress the seedlings without inducing mortality. The first stage consisted of a reduced soil moisture treatment, whereby pots received a 50% reduction of daily water. The second stage consisted of an elevated VPD treatment, whereby pots were watered to field capacity but RH in the growth chamber was reduced from 75% to ~45%, a RH frequently experienced by forests in the Intermountain West. The third stage consisted of a combination treatment whereby pots received both a 75% reduction of daily water and a decrease in RH from 75% to ~45%, which increased VPD to 1.870 kPa (Table S1 available as Supplementary data at *Tree Physiology* Online only reflects environmental conditions during the third stage). Each stage lasted 5 days and was followed by a 3-day window where growth chamber environmental conditions were returned to those used in the WW period and pots were watered to field capacity. Results from the first two stages are the subject of Zenes et al. 2020, which focused on modeling stomatal behavior. Here, we focus on physiological and growth responses to the third stage because it was the most stressful stage for the seedlings as verified by physiological metrics (i.e., predawn leaf water potential), and integrated any physiological stress responses that were not recoverable from the duration of the water limitation treatment. Following the WL period, the

growth chamber pots entered the R period, which lasted for 1.5 weeks. During the R period, growth chamber environmental conditions were returned to those used in the WW period and pots were watered to field capacity.

During Weeks 4 and 5 of the WW period, a subset of pots from both the growth chamber and the greenhouse were randomly selected for nondestructive and destructive measurements. We used pots from both locations in order to simultaneously obtain larger sample sizes and ensure there were pots remaining in the growth chamber for the subsequent WL treatment. We did not find any significant differences in seedling size between pots from the growth chamber and greenhouse during the WW period measurements (Figure S3; see Data analysis section for more information regarding these tests). Because the greenhouse seedlings were not significantly different in size, we randomly selected and moved pots from the greenhouse into the growth chamber to replace the pots that were destructively sampled for WW measurements. All remaining pots in the growth chamber underwent nondestructive and destructive measurements during the R period. Growth and physiological measurements were made on randomly selected pots throughout the study as described below. Sample sizes for the measurements are listed below, and are included in Table S2 available as Supplementary data at *Tree Physiology* Online.

Growth and plant water status

Height was measured on all plants during the WW period to the nearest 0.5 cm from the base of the stem to the apical meristem with a standard meter stick on 10–20 seedlings per planting group. Plants were harvested during the WW and R periods for destructive growth and physiological measurements. Harvested plants were separated into root, stem and leaf biomass tissue samples. Roots were washed to remove soil and debris, and gently squeezed to remove excess water. All tissue samples were placed into paper bags and dried at 65 °C until a stabilized dry weight was achieved (typically 3 days). Dry weights were recorded to the nearest 0.0001 g using a mass balance (Sartorius, Goettingen, Germany). The root to shoot ratio (R:S) was calculated as root mass divided by the shoot mass (stem mass + leaf mass). Root, stem and leaf biomass allocation (as a percent of whole-plant biomass) was calculated by dividing root, stem and leaf dry weights by total plant dry weight (root mass + stem mass + leaf mass) then multiplying by 100. To determine R:S and biomass allocation percentages, 2–8 seedlings per planting group were used during the WW period and 5–13 seedlings per planting group were used in the R period.

To assess plant water status and stress during water limitation, predawn leaf water potentials (Ψ_{PD}) were measured during all three periods. Pots were randomly selected for measurement, and Ψ_{PD} samples were collected and measured before the growth chamber lights turned on (between 05:00 and 06:00 h).

Water potentials were measured using a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA). Ψ_{PD} was measured on 3–6, 7–16 and 4–8 seedlings per planting group in the WW, WL and R periods, respectively.

Physiology and morphology

When a plant was harvested during the WW and R periods, a subsample of 18–24 leaves or needles were placed onto white paper and photographed using a digital camera (Nikon Inc., Japan) along with a linear scale. The imaged leaf samples were then placed into paper bags and dried at 65 °C until a stabilized dry weight was achieved. Dry weights were recorded to the nearest 0.0001 g using a mass balance (Sartorius, Tokyo, Japan). The digital images were processed using the freeware ImageJ (NIH, USA, <http://rsb.info.nih.gov/ij/>) by converting the images to binary, and counting the number of pixels in the leaves. Specific leaf area (SLA) was calculated by dividing leaf area by leaf mass. Specific leaf area was determined using 2–5 seedlings per planting group in the WW period and 4–11 seedlings per planting group in the R period.

Stem vulnerability curves were measured to determine the cavitation resistance of the seedling xylem using the centrifuge method (Auer et al. 1997) during the WW and R periods. Vulnerability curves relate xylem pressure and the percent loss of hydraulic conductivity (PLC) caused by cavitation and embolism in the xylem conduits. Stem segments for each species were cut under water and flushed of embolism via vacuum infiltration prior to conducting the vulnerability curve. After vacuum infiltration, maximum hydraulic conductivity (K_{max}) was measured using the standard pressure–flow method (Sperry et al. 1988). Stem segments were then spun in a centrifuge (Sorvall RC 5C Plus, Thermo Scientific, Waltham, Massachusetts, USA) to induce a known negative xylem pressure using the angular velocity and stem length. After sequential steps of centrifugation, the PLC was determined by measuring hydraulic conductivity until conductivity decreased to between 10% and 30% of K_{max} . Vulnerability curves were then used to compute the negative pressure at which 50% stem hydraulic conductivity was lost (P50). For all species, stem lengths far exceeded the known lengths of xylem vessels and tracheids for each species, so it is unlikely that artifacts were induced during the cavitation resistance measurements (Zimmermann and Jeje 1981, Sperry et al. 1994, Wheeler et al. 2005, Johnson et al. 2012, Skelton et al. 2019). P50 was determined using 1–7 seedlings per planting group in the WW period and 3–12 seedlings per planting group in the R period.

The pressure–volume (P–V) technique (Tyree and Hammel 1972) was used to determine leaf water potential at the turgor loss point (Ψ_{TLP}) during the WW and R periods. Leaf samples with attached petioles (aspens and cottonwoods) or intact fascicle bundles (pines) were excised, sealed in plastic bags, and transported to the laboratory where samples underwent an

overnight hydration treatment after recutting the bases under water. After rehydration, the portion of the petiole (or fascicle bundle) that had been under water was removed to minimize the impacts of oversaturation on the shape of the P–V curve, the influence of the ‘plateau effect’ (Parker and Pallardy 1987, Kubiske and Abrams 1990, Dichio et al. 2003). An initial leaf water potential (Ψ) and weight to the nearest 0.0001 g were determined rapidly using a Scholander-type pressure chamber and mass balance, then Ψ and weight were recorded periodically while samples dried on the laboratory bench. Regressions were fitted to the linear portions of plots of $1/\Psi$ versus relative water content until the coefficient of determination reached a maximum, and were used to estimate Ψ_{TLP} (Meinzer et al. 2009). To determine Ψ_{TLP} , 3–8 seedlings per planting group were used during the WW period and 2–11 seedlings per planting group were used during the R period.

Gas exchange measurements were made during the WW and R periods to construct photosynthetic CO_2 -reponse (A – C_i) for determination of maximum rate of carboxylation (V_{cmax}) using a portable open gas exchange system (Li-6400, LiCor, Lincoln, NE, USA) with a 6 cm^2 chamber aperture and a red–blue light source. Conditions in the Li-6400 chamber were set to match conditions in the growth chamber: leaf temperature at 25 °C, photosynthetic photon flux density at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, chamber RH was set to match growth chamber RH $\pm$ 5%, and ambient CO_2 at 400 p.p.m. Photosynthesis (A) was initially measured at ambient CO_2 . Then CO_2 concentration was gradually decreased stepwise to 50 p.p.m., brought back to ambient and was gradually increased stepwise until the curve reached a point where further increases in A appeared to be negligible ($\sim$ 1600 p.p.m.). At each step, stabilized readings of stomatal conductance to water vapor (g_s), and internal concentration of CO_2 (C_i) were recorded. For the pine samples, we laid six needles side-by-side forming a fan ‘needle mat’ to prevent shading. These needle mats did not cover the entire 6 cm^2 chamber aperture in the Li-6400. Therefore, we calculated the actual needle area used in the LiCor measurement to standardize the recorded measurements (Keri et al. 2015). To do so, we excised the portions of the needle mats that were in the Licor chamber, placed them onto white paper and photographed using a digital camera (Nikon Inc.,) along with a linear scale. The digital images were processed using the freeware ImageJ by converting the images to binary, and counting the number of pixels in the leaves. Fitted A – C_i curves were used to determine V_{cmax} using 2–5 seedlings per planting group in the WW period and 1–3 seedlings per planting group in the R period.

Gas exchange measurements were also made on a separate day from the V_{cmax} measurements, due to time constraints, to determine diurnal rates of A and g_s during all treatment periods. Diurnal A and g_s were measured using the Li-6400 as described above. Conditions in the Li-6400 chamber were also set as described above, but CO_2 was kept at ambient

(400 p.p.m.). Both A and g_s were measured every hour between 07:30 and 15:30 h, and stabilized readings of A , g_s and C_i were recorded. Measured leaf areas were again used to standardize the recorded measurements for the pine samples. A and g_s were measured on 1–4, 4–8 and 4–9 seedlings per planting group in the WW, WL and R periods, respectively.

Data analysis

Assumptions of normality for each statistical model used in this study were checked by examining plots of the residuals and with the Kruskal–Wallis test for normality. When the Kruskal–Wallis test failed, data were transformed with log (cottonwood V_{cmax}), Box–Cox (aspen stem diameter) and cubed (aspen Ψ_{PD} ; aspen A/g_s ; cotton A/g_s) transformations. Comparisons were never made between transformed and untransformed data. Further, statistical analyses were never made when the sample size for the measurement was low ($n < 3$). Results from measurements with $n < 3$ are provided in the supplementary information (Figures S6–S8 available as Supplementary data at *Tree Physiology Online*). When multiple comparisons were made, we used post-hoc Tukey–HSD adjusted pairwise comparisons to correct for the occurrence of false positives.

Due to the lack of significant differences between the different aspen competition planting groups ($A \times A$ versus $A \times C$ versus $A \times D$; see Figures S10 and S11 available as Supplementary data at *Tree Physiology Online* for more information), we pooled responses from these groups to form one group for aspens growing under competition. Two-factor ANOVAs were used to fit the relationships between treatment period (WW and R), planting group (solo and competition) and the mean values of response variables (height, R:S, biomass allocation, SLA, P50, Ψ_{TLP} , and V_{cmax}). Linear mixed-effects models, with seedling as a random effect to account for repeated measures, were used to fit the relationships between treatment period (WW, WL and R), planting group (solo and competition) and the mean values of response variables (Ψ_{PD} , A and g_s).

We also tested for significant differences in plant size between the growth chamber and greenhouse plants used during the WW period to ensure we could pool measurements taken on seedlings from both locations. Here we use total plant leaf area, total plant leaf count and plant height as proxies for seedling size. Paired t-tests between growth chamber and greenhouse aspen plants were used to fit the relationships between planting group and the mean values of response variables (total leaf area, total leaf count and height). Although sample sizes were low for these measurements in many planting groups, results suggest there were no significant differences in total leaf area, total leaf count or height between growth chamber and greenhouse aspen plants during the WW period (Figure S3 available as Supplementary data at *Tree Physiology Online*). Therefore, we feel confident in using both greenhouse and growth chamber plants for the WW period measurements.

Statistical analyses were conducted in R version 3.4.3 (R Development Core Team 2017). The 'car' package (version 2.1.6) was used to plot QQ normal lines with 95% confidence intervals (Fox et al. 2016). The 'lme4' (version 1.1–21), 'lmerTest' (version 3.1–0) and 'emmeans' (version 1.4) packages were used to fit and analyze mixed-effects models for repeated measures (Kuznetsova et al. 2014, Lenth et al. 2018, Bates et al. 2019). The 'fitplc' package (version 1.1.7) was used to fit vulnerability curves and determine P50 (Duursma and Choat 2017). In the 'fitplc' package, we corrected temperature for each within-species planting group to match the recorded leaf temperature of the Licor-6400, although leaf temperature measurements of conifer needles can have substantial uncertainty, and fit $A-C_i$ curves using the bilinear fit. No other parameters were altered. The 'plantecophys' package (version 1.3.2) was used to fit $A-C_i$ curves and determine V_{cmax} (Duursma 2015), although this package does not incorporate estimates of mesophyll conductance (g_m) and may underestimate V_{cmax} (Flexas et al. 2008). For all analyses, a significance level of $\alpha < 0.05$ was considered statistically significant.

Results

Responses to plant–plant interactions

Aspen seedlings were significantly taller ($P = 0.03$) when grown with another seedling, whereas pine seedlings were significantly taller ($P < 0.001$) when grown alone (Figure 1). Cottonwood seedlings showed no difference in height between solo and competition planting groups. We did not observe increased allocation to aboveground biomass (e.g., stems and leaves) for any species grown under competition during the WW period (Figure 2). Only cottonwood seedlings growing alone exhibited a significant increase ($P = 0.037$) in biomass allocation to leaves (Figure 2).

Plant–plant interactions did not have a significant effect on Ψ_{PD} between aspen or pine planting groups during the WW period (Figure 3a and b). Cottonwood seedlings growing alone exhibited marginally more negative Ψ_{PD} ($P = 0.062$) during the WW period (Figure 3b). Further, there were no significant differences in A (Figure 4a) and g_s (Figure 4b) between the aspen planting groups during the WW period. There were also no significant differences in V_{cmax} between the aspen planting groups during the WW period (Figure 5).

Responses to both plant–plant interactions and water limitation

All pots experienced a roughly 50% reduction in soil moisture during the WL period (Figure S5 available as Supplementary data at *Tree Physiology* Online). Ψ_{PD} was significantly more negative for all cottonwood seedlings ($P < 0.001$) during the WL period compared with the WW and R periods (Figure 3b). Ψ_{PD} was significantly more negative for all aspen seedlings under

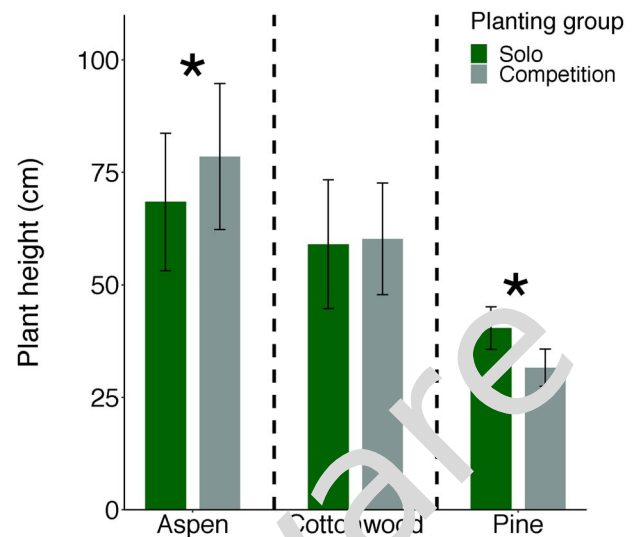


Figure 1. During the well-watered (WW) period, aspens grew taller under competition, pines grew taller when grown alone, and cottonwoods had similar growth when grown alone or with another seedling. Significant differences between planting groups are denoted with an asterisk. Bar heights represent mean plant height and error bars represent one standard deviation. Vertical dashed lines separate the three species. Planting groups include solo (green) and competition (gray) seedlings for each species.

competition ($P < 0.001$) during the WL period compared with the WW and R periods (Figure 3a). Ψ_{PD} was only significantly more negative for pine seedlings under competition ($P = 0.03$) during the WL period compared with the R period (Figure 3c).

A was significantly lower for all aspen planting groups during the WL period compared with both the WW ($P < 0.001$) and R ($P = 0.003$) periods (Figure 4a). Rates of g_s were also significantly lower for all aspen planting groups during the WL period compared with both the WW ($P = 0.03$) and R ($P = 0.03$) periods (Figure 4b). There were no significant differences in either A or g_s within any time period between aspen seedlings grown alone or with another seedling.

V_{cmax} was significantly lower for all aspen planting groups in the R period ($P < 0.001$) compared with the WW period (Figure S7 available as Supplementary data at *Tree Physiology* Online). However, there were no significant differences between the solo and competition planting groups during either time period. V_{cmax} was also significantly lower for cottonwoods growing alone ($P = 0.019$) in the R period compared with the WW period (Figure S7 available as Supplementary data at *Tree Physiology* Online).

No change in allocation to belowground biomass (e.g., higher R:S) for any species growing in the presence of a competitor seedling between the WW and R periods was observed (Figure 5). Only aspen ($P = 0.03$) and cottonwood ($P = 0.02$) seedlings growing alone exhibited a significant increase in R:S between the WW and R periods (Figure 5).

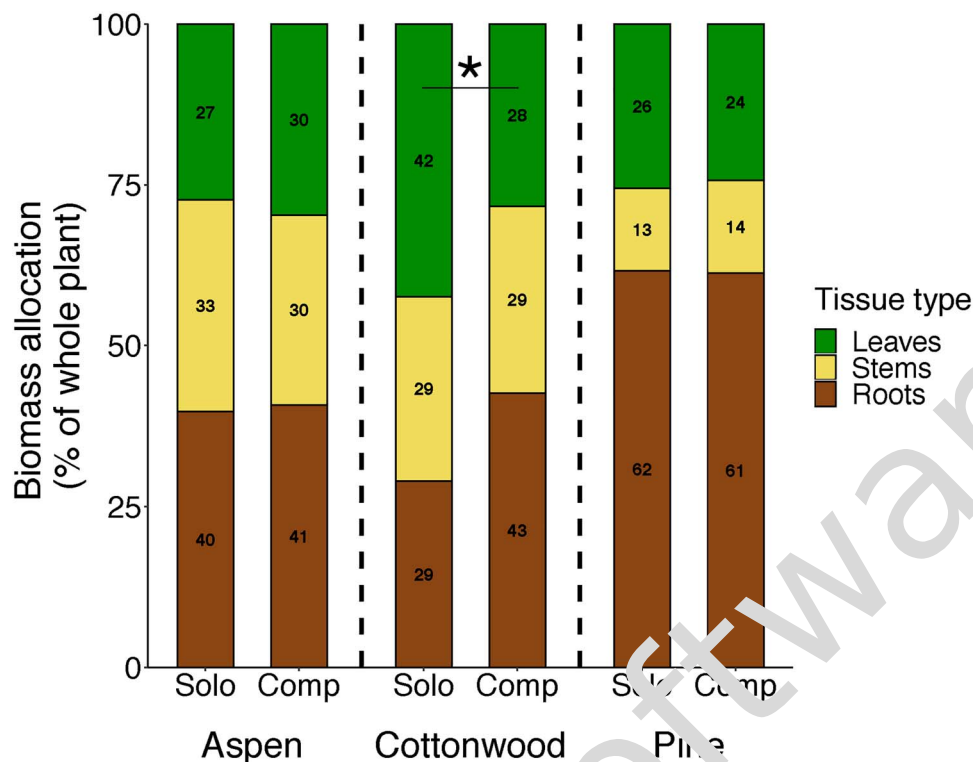


Figure 2. Only solo cottonwood seedlings showed differences in biomass allocation and significantly increased leaf biomass compared with competition cottonwood seedlings during the well-watered (WW) period. The numbers on stacked bars represent mean percent of biomass allocation of each tissue type. Significant differences between planting groups are denoted with an asterisk. Vertical dashed lines separate the three species. Planting groups include solo and competition (comp) seedlings for each species.

Ψ_{TLP} was significantly less negative in aspen seedlings growing with another seedling ($P = 0.03$) during the R period compared with the WW period (Figure 6). By contrast, Ψ_{TLP} was significantly more negative in cottonwood seedlings under competition ($P = 0.03$) during the R period compared with the WW period, and was marginally more negative in competition seedlings ($P = 0.068$) compared with solo seedlings in the R period (Figure 6). Ψ_{TLP} was significantly more negative in pine seedlings growing alone ($P = 0.01$) during the R period compared with the WW period, and was significantly less negative in pine seedlings growing with another seedling ($P = 0.002$) compared with solo seedlings in the R periods (Figure 6).

There were no significant differences in SLA (Figure S8) and P50 (Figure S9 available as Supplementary data at *Tree Physiology Online*) within planting groups and across time periods for all species.

Species-specific responses

We only observed differences in plant height and Ψ_{PD} between the different aspen competition groups. Aspen only grew significantly taller when grown with a pine seedling ($P = 0.001$) (Figure S10 available as Supplementary data at *Tree Physiology Online*). Aspen seedlings growing with either an aspen ($P = 0.007$) or with a cottonwood ($P = 0.004$) exhibited

significantly more negative Ψ_{PD} than aspen seedlings growing alone (Figure S11 available as Supplementary data at *Tree Physiology Online*). Aspen seedlings growing with a cottonwood also had significantly more negative Ψ_{PD} ($P = 0.03$) than aspen seedlings growing with a pine (Figure S11 available as Supplementary data at *Tree Physiology Online*).

Due to low sample sizes, we were unable to statistically test some response variables. We do not discuss results or comparisons for these response variables below, but see Figures S6–S9 available as Supplementary data at *Tree Physiology Online* for these results.

Discussion

In this study, we found that plant–plant interactions predominantly affected seedling growth or biomass allocation but not seedling water status or physiological traits, even during periods of water limitation. Overall, it appeared that the seedlings' water-use strategy mediated growth and physiological responses. However, we observed several null responses, potentially due to small sample sizes, which did not allow us to comprehensively test our hypotheses. Nevertheless, our investigation serves as an important step toward testing and understanding how species' interactions modify growth and physiological responses via the

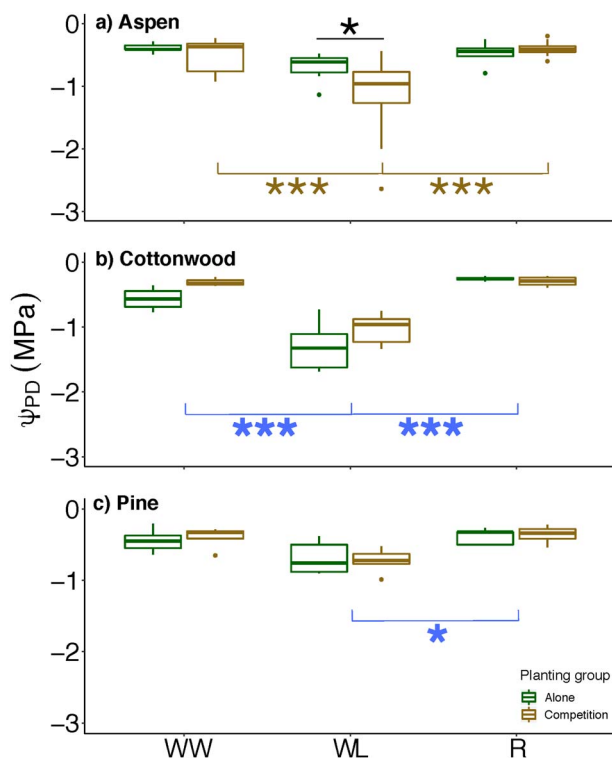


Figure 3. Predawn leaf water potential (Ψ_{PD}) decreased significantly for nearly all seedlings during the water-limited (WL) period. (a) Ψ_{PD} was also significantly lower in aspen competition seedlings compared with solo aspen seedlings during the WL period (denoted by the black asterisk). Within the aspen competition seedlings only, Ψ_{PD} was significantly lower during the WL period than the well-watered (WW) and recovery (R) periods (denoted by brown asterisks). (b) For all cottonwood seedlings, Ψ_{PD} was significantly lower during the WL period than the WW and R periods (denoted by blue asterisks). (c) Ψ_{PD} was significantly lower for all pine seedlings during the WL period than R period (denoted by blue asterisks). Boxplots represent the median Ψ_{PD} (center bar), interquartile range (IQR, edges of box), values at most $1.5 \times$ IQR from box edge (error bars) and outlier points (dots) for the well-watered (WW), water-limited (WL) and recovery (R) time periods. Asterisks reflect significant differences, where * is $P = 0.01–0.05$ and *** is $P < 0.001$. Planting groups include solo and competition seedlings for each species.

use of a controlled environment growth chamber to separately test the effects of plant–plant interactions and water limitation.

Responses to plant–plant interactions

During the WW period, the presence of another seedling did not affect plant water status (as measured through predawn water potential), which supports our first hypothesis that plant–plant interactions would not change plant water status in the absence of water limitation. However, contrary to our first hypothesis, we did not observe any increase in photosynthetic performance (A , g_s , V_{cmax}) or consistent increases in aboveground biomass allocation in seedlings grown under competition compared with seedlings growing alone.

Growth responses of aspen seedlings under competition were consistent with our expectations that competition stimulates

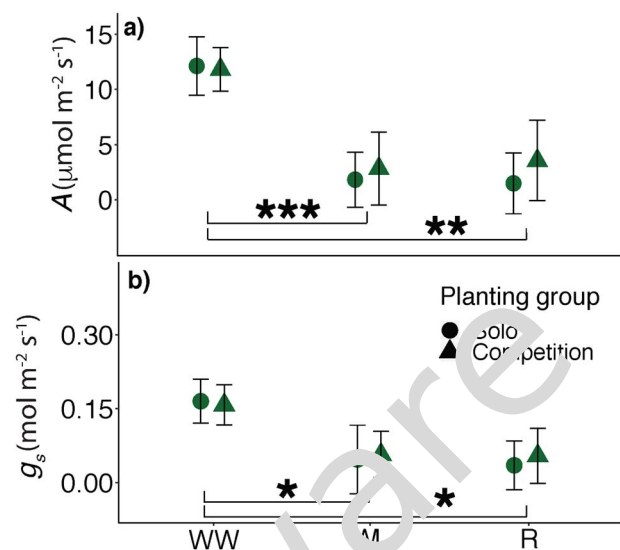


Figure 4. Photosynthetic assimilation (A , (a)) and stomatal conductance (g_s , (b)) decreased during the water-limited (WL) period for all aspen seedlings, indicating that gas exchange was negatively affected by the water limitation. Symbols represent mean A or g_s and error bars represent standard deviation for the well-watered (WW), WL and recovery (R) periods. Asterisks reflect significant differences, where * corresponds to $P = 0.01–0.05$, ** corresponds to $P = 0.001–0.01$ and *** corresponds to $P < 0.001$. Brackets with asterisks reflect comparisons made between all planting groups within different time periods. Planting groups include solo and competition seedlings.

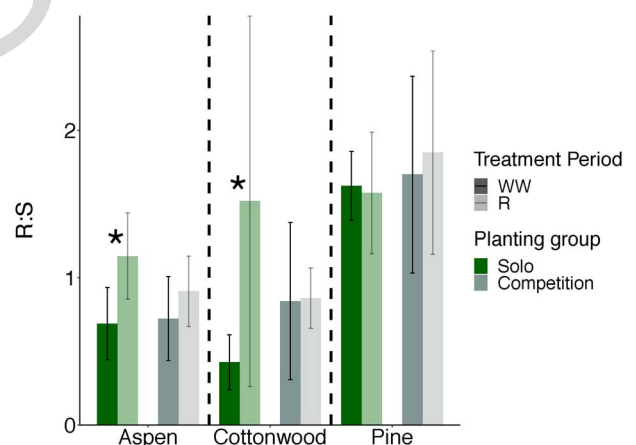


Figure 5. Solo aspen ($P = 0.029$) and cottonwood ($P = 0.019$) seedlings increased biomass allocation to root systems in response to the water limitation treatment, as reflected in significant differences between well-watered (WW) and recovery (R) root to shoot ratios (R:S, denoted by an asterisk). Bar heights represent mean R:S and error bars represent standard deviation for the well-watered (WW, dark) and recovery (R, light) periods. Vertical dashed lines separate the three species. Planting groups include solo and competition seedlings for each species.

height growth. The observed increase in plant height was likely driven by competition for light, a well-observed response to plant–plant interactions when water and nutrients are not limiting (Ballaré et al. 1994, Franklin 2008, Farrior et al. 2013). Aspen

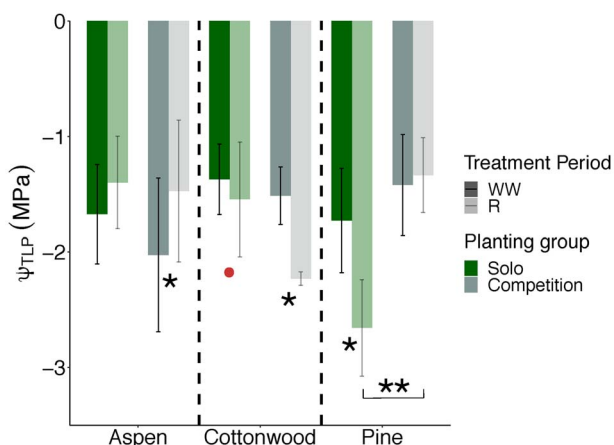


Figure 6. Leaf water potential at the turgor loss point (Ψ_{TLP}) differed significantly within-species but responses were varied. Ψ_{TLP} became significantly less negative for aspens grown under competition after the water limitation ($P = 0.032$, denoted with an asterisk). Ψ_{TLP} became significantly more negative for cottonwoods grown under competition after the water limitation ($P = 0.03$, denoted with an asterisk). Ψ_{TLP} became significantly more negative for pines grown alone after the water limitation ($P = 0.011$, denoted with an asterisk), and was more negative in solo pines compared with competition pines in the recovery (R) period ($P = 0.002$, denoted with two asterisks and brackets). Bar heights represent mean Ψ_{TLP} and error bars represent standard deviation for the well-watered (WW, dark) and recovery (R, light) periods. Vertical dashed lines separate the three species. Planting groups include solo and competition seedlings for each species. Note: low sample sizes prevented statistical testing within the solo cottonwood planting group as indicated by the red dot.

seedlings grown in the presence of another seedling maintained equivalent rates of photosynthesis (A) and stomatal conductance (g_s), and exhibited no difference in V_{cmax} , compared with aspen seedlings grown alone. The lack of stomatal and photosynthetic response suggests that aspen seedlings under competition may maintain high photosynthetic capacity to support increased aboveground growth when competing for light.

Growth and physiological responses to plant–plant interactions in cottonwood and pine seedlings differed from those of the aspen seedlings. Both cottonwood and pine showed reductions in aboveground biomass allocation in the presence of an aspen seedling, suggesting that aspen outcompeted these seedlings for resources, which resulted in decreased water-use in cottonwood and pine seedlings. Reduction in water-use likely prevented these seedlings from increasing resource-acquisition rates or V_{cmax} . Major changes in gas exchange or V_{cmax} in these species might require the production of a whole new canopy, which did not occur during this study.

Responses to both plant–plant interactions and water limitation

Water limitation affected growth, physiological traits and plant water status in all species regardless of competition. Further, we observed some differences in water potentials (Ψ_{PD} , Ψ_{TLP}),

and photosynthesis (A) between solo and competition seedlings during water limitation, which provides evidence for our second hypothesis that two seedlings competing for water can exacerbate stress from low water availability.

Most seedlings sensed and responded as expected to water stress, as reflected in increased root to shoot ratios ($R:S$), significantly more negative Ψ_{PD} , and lower rates of A and g_s during and following water stress. These are all responses to water stress that have been well documented in plants, including forest tree species (Chaves 1991, Chaves et al. 2003, Hamanishi and Campbell 2011). However, these responses were largely independent of competition, at least under the moderate levels of water stress induced during this study. We only observed within-species differences in Ψ_{PD} between solo and competition aspen seedlings, and in A and Ψ_{TLP} between solo and competition cottonwood seedlings when water availability was limited.

Aspen seedlings experienced significantly more negative Ψ_{PD} when grown in the presence of another seedling, specifically under competition with a water spender (e.g., another aspen or a cottonwood). This response is similar to results found in other studies. For example, Fotelli et al. (2001) found that Ψ_{PD} was significantly more negative in European beech (*Fagus sylvatica*) seedlings under both reduced and no irrigation treatments when grown with a fast-growing (i.e., water spender) competitor seedling, blackberry (*Rubus fruticosus*). Further, Robinson et al. (2011) discovered that differences in jack pine (*Pinus banksiana*) seedlings' water-use efficiency corresponded to available water, and that under competition with Canada blue-joint grass (*Calamagrostis canadensis*), a species that establishes and grows quickly, water availability was reduced. It is therefore likely that soil water dried down more rapidly when aspen was grown with another aspen or a cottonwood because these species are higher water spenders (mean midday $g_s > 0.15 \text{ mol m}^{-2} \text{ s}^{-1}$) than pine.

Cottonwood seedlings growing under competition exhibited growth and physiological responses to water limitation which likely gave them a competitive edge. First, these cottonwood seedlings had significantly more negative leaf water potentials at the turgor loss point (Ψ_{TLP}) following the water limitation treatment, suggesting a shift in drought tolerance as other studies have shown (Bartlett et al. 2014). One mechanism controlling a shift to more negative Ψ_{TLP} in these seedlings could be osmotic adjustment, the accumulation of solutes in response to water stress to promote turgor maintenance and plant growth during water stress (Hsiao 1973, Morgan 1984). Cottonwood seedlings growing under competition had higher rates of photosynthesis following the water limitation treatment, suggesting these seedlings may have generated the extra photosynthates needed for osmotic adjustment to help them tolerate the water limitation period and recover after its completion (Galiano et al. 2011, Sala et al. 2012).

Though all pots in the growth chamber were subjected to the decrease in RH during the WL period, comparisons between WW and WL or WW and R periods afforded us inferences as to the effects of water limitation in the absence of a contemporaneous control.

Species-specific responses

The species used in this study exhibited variation in responses to plant–plant interactions and water stress, which were likely due to the different life histories of each species that have resulted in their different water-use strategies, supporting our third hypothesis. Surprisingly, we found only moderate responses to competition in aspen, regardless of whether or not aspen was grown with a con- or hetero-specific competitor seedling. This may be because aspen is a clonal species and individual ramets coexist with and are often connected to neighboring ramets, complicating the typical notion of ‘competition’ (Barnes 1966, Mitton and Grant 2006, Baret and DesRochers 2011). However, previous ecophysiological studies of water limitation and mortality patterns in aspen are more consistent with individual ramets acting like individual trees (i.e., there is little evidence for facilitation and substantial resource sharing during drought) (Anderegg et al. 2012). The cottonwood seedlings rapidly transpired available water, resulting in some of the most negative leaf water potential values during the WL period. This behavior is indicative of riparian species that are water spenders and do not need to conserve water (Glenn et al. 1998, Scott et al. 2000, Farid et al. 2008). Because the water limitation treatment did not result in seedling mortality, the water-use strategy of the cottonwood seedlings also likely assisted in their recovery and ability to regain photosynthetic activity following the water limitation period.

The pine seedlings behaved like water savers when grown under competition, resulting in some of the least negative leaf water potentials during the WL period. The water savers strategy is indicative of species that live in more drought-prone habitats (Monson and Grant 1989, Wullschlegel et al. 1998). Pine seedlings growing under competition also seemed to decrease resource use and productivity compared with pine seedlings growing alone. Interestingly, the presence of another seedling seemed to buffer the pine seedlings to stress from the water limitation treatment as these seedlings maintained very consistent, low rates of A across the entire duration of the study (Figure S6 available as Supplementary data at *Tree Physiology* Online). This could be because both competition and water limitation elicit the same physiological responses in pine, or perhaps the pine seedlings benefitted from facilitative interactions with the aspen seedlings (Maestre et al. 2005). Another reason could be because these seedlings had already acclimated to the relatively lower water availability conditions as a result of the competitive environment before measurements were taken for the experiment.

The seedlings’ null responses found in this study may be due to a number of factors. First, sample sizes were small for certain measurements which prevented comprehensive statistical testing for some measurements and planting groups, warranting caution when interpreting results (Table S2 available as Supplementary data at *Tree Physiology* Online). Low sample sizes were due to space constraints in the growth chamber and the destructive nature of some measurements. Second, although the duration of our study is similar to other controlled chamber ecophysiological seedling studies (Kolb and Ebbrecht 1996, Duan et al. 2015), it is relatively short compared with the lifetime of a tree, and thus provides some limitations for observing certain growth and physiological responses. For example, changes in SLA and xylem vulnerability to cavitation rely on formation of new leaf and xylem tissues, respectively, both processes that require longer growing periods (months to years; Venturas et al. 2017). However, many of the growth and physiological responses reported here agree with other ecophysiological studies, so we believe our results reflect accurate short-term seedling responses to competition and water limitation. We also observed intertwined root systems during destructive measurements (personal observation), which indicates substantial belowground competition was likely occurring in the competition pots. Additionally, watering regimes were determined based on average per-seedling water-use within each species’ planting groups, providing the same ‘meteorological drought’ to each planting group but not to all seedlings. Our water limitation treatment therefore mimics a natural regional-type reduction in precipitation, but does not provide seedling-normalized or species-normalized water stress severities, which is inherently difficult to implement when multiple species share the same pot and might mask competition/facilitation effects. Finally, there may have been facilitative interactions between seedlings in the same pot, which could have dampened response signals that would otherwise be detectable under more stressful growing conditions (Maestre et al. 2005, Soliveres and Maestre 2014).

In conclusion, we documented complex effects of plant–plant interactions, water limitation stress and their combination on physiology and growth in tree seedlings. Observed responses were sometimes mediated by the water-use strategy of the seedling, indicating that plant responses to these co-occurring stresses are likely partially dependent on the diversity and species’ physiological trait compositions of forests (Forrester et al. 2016). Because seedlings and saplings likely interact differently with other plants compared with mature trees, given differences in growth patterns, physiology and resource-acquisition, results from this study may not be indicative of mature tree responses. Therefore, future opportunities abound to broaden study duration, drought severity, tree species, tree age and mechanisms underlying plant–plant interactions. The results reported here serve as an important step toward

testing and understanding how tree species interactions modify responses to water limitation.

Data availability

Data from this study have not been deposited into any online repository, but are available upon request.

Supplementary data

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

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Authors' contributions

The experiment was designed by all authors. Data collection and analyses were carried out by K.L.K. and N.Z., while data interpretation was carried out by all authors. The manuscript was written by K.L.K. with contributions from all authors.

Conflict of interest

None declared.

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References

- Adams HD, Zeppel MJB, Anderegg WRL et al. (2017) A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nat Ecol Evol* 1:1285–1291.
- Alder NN, Pockman WT, Sperry JS, Nuismer S (1997) Use of centrifugal force in the study of xylem cavitation. *J Exp Bot* 48:665–674.
- Anderegg LDL, HilleRisLambers J (2016) Drought stress limits the geographic ranges of two tree species via different physiological mechanisms. *Glob Chang Biol* 22:1029–1045.
- Anderegg WRL, Berry JA, Smith DD, Sperry JS, Anderegg LDL, Field CB (2012) The roles of hydraulic and carbon stress in widespread climate-induced forest die-off. *Proc Natl Acad Sci USA* 109:233–237.
- Anderegg WRL, Wolf A, Arango-Velez A et al. (2015) Woody plants optimise stomatal behaviour relative to hydraulic stress. *Ecol Lett* 21:968–977.
- Ballare CL, Scopel AL, Jordan ET, Viersma DD (1991) Signaling among neighboring plants and the development of size inequalities in plant populations. *Proc Natl Acad Sci USA* 88:10094–10098.
- Baret M, DesRochers A (2011) Root competition can trigger physiological responses to defoliation in non-defoliated aspen suckers. *Botany* 89:753–761.
- Barnes BV (1960) The cloning growth habit of American aspens. *Ecology* 47:439–447.
- Bartlett MK, Zhang K, Kreidler N, Sun S, Ardy R, Cao K, Sack L (2014) Global analysis of plasticity in turgor loss point, a key drought tolerance trait. *Ecol Lett* 17:1580–1590.
- Bates JM, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1. 1–21. Available at <http://CRAN.R-project.org/package=lme4>.
- Brooker AJ, Mooney HA (1985) Plants-an economic analogy. *Annu Rev Ecol Syst* 16:363–392.
- Brooker RW, Maestre FT, Callaway RM et al. (2008) Facilitation in plant communities: the past, the present, and the future. *J Ecol* 96:18–34.
- Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C (2015) How tree roots respond to drought. *Front Plant Sci* 6:1–16.
- Buechling A, Martin PH, Canham CD. 2017. Climate and competition effects on tree growth in Rocky Mountain forests. *J Ecol* 10.111:1–12.
- Casper BB, Jackson RB (1997) Plant competition underground. *Ann Rev Ecol Syst* 28:545–570.
- Chaves MM (1991) Effects of water deficits on carbon assimilation. *J Exp Bot* 42:1–16.
- Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought — from genes to the whole plant. *Funct Plant Biol* 30:239.
- Clark JS, Iverson L, Woodall CW et al. (2016) The impacts of increasing drought on forest dynamics, structure, and biodiversity in the United States. *Glob Chang Biol* 22:2329–2352.
- Dawson TE (1996) Determining water-use by trees and forests from isotopic, energy balance and transpiration analyses: the roles of tree size and hydraulic lift. *Tree Physiol* 16:263–272.
- DeLucia EH, Maherali H, Carey EV (2000) Climate-driven changes in biomass allocation in pines. *Glob Chang Biol* 6:587–593.
- Dichio B, Xiloyannis C, Angelopoulos K, Nuzzo V, Bufo SA, Celano G (2003) Drought-induced variations of water relations parameters in *Olea europaea*. *Plant Soil* 257:381–389.
- Donald C (1958) The interaction of competition for light and for nutrients. *Aust J Agr Res* 9:421–435.
- Duan H, O'Grady AP, Duursma RA, Choat B, Huang G, Smith RA, Jiang Y, Tissue DT (2015) Drought responses of two gymnosperm species with contrasting stomatal regulation strategies under elevated [CO₂] and temperature. *Tree Physiol* 35:756–770.
- Duursma RA (2015) Plantecophys - an R package for analysing and modelling leaf gas exchange data. *PLoS ONE* 10:1–13.

- Duursma R, Choat B (2017) Fitplc - an R package to fit hydraulic vulnerability curves. *J Plant Hydraulics* 4:1–14.
- Farid A, Goodrich DC, Bryant R, Sorooshian S (2008) Using airborne lidar to predict leaf area index in cottonwood trees and refine riparian water-use estimates. *J Arid Environ* 72:1–15.
- Farrior CE, Dybzinski R, Levin SA, Pacala SW (2013) Competition for water and light in closed-canopy forests: a tractable model of carbon allocation with implications for carbon sinks. *Am Nat* 181:314–330.
- Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H (2008) Mesophyll conductance to CO₂: current knowledge and future prospects. *Plant Cell Environ* 31:602–621.
- Forrester DI (2014) The spatial and temporal dynamics of species interactions in mixed-species forests: from pattern to process. *For Ecol Manage* 312:282–292.
- Forrester DI (2015) Transpiration and water-use efficiency in mixed-species forests versus monocultures: effects of tree size, stand density and season. *Tree Physiol* 35:289–304.
- Forrester DI, Bonal D, Dawud S, Gessler A, Granier A, Pollastrini M, Grossiord C (2016) Drought responses by individual tree species are not often correlated with tree species diversity in European forests. *J Appl Ecol* 53:1725–1734.
- Fotelli MN, Gessler A, Peuke AD, Rennenberg H (2001) Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus fruticosus*: responses of growth, water status and composition. *New Phytol* 151:427–435.
- Fox J, Weisberg S, Price B, Adler D et al. (2016) car: Companion to Applied Regression. R package version 2.1.6, Available at: <http://CRAN.R-project.org/package=car>.
- Franklin KA (2008) Shade avoidance. *New Phytol* 179:930–944.
- Galiano L, Martínez-Vilalta J, Lloret F (2011) Carbon reserves and canopy defoliation determine the recovery of Scots pine 4yr after drought episode. *New Phytol* 190:750–759.
- Gersani M, Brown JS, O'Brien EE, Maina GM, Abramsky Z (2001) Tragedy of the commons as a result of root competition. *J Ecol* 89:660–669.
- Glenn E, Tanner R, Mendez S, Kehret T, Moore D, Garcia J, Valdez C (1998) Growth rates, salt tolerance and water-use characteristics of native and invasive riparian plants from the delta of the Colorado River, Mexico. *J Arid Environ* 40:281–294.
- Goisser M, Geppert U, Rötzer T et al. (2016) Does belowground interaction with *Fagus sylvatica* increase drought susceptibility of photosynthesis and stem growth in *Picea abies*? *For Ecol Manage* 375:268–278.
- Griffin BS, Shilling DG, Bennett J, Cunniff W (1989) The influence of water stress on the physiology and competition of soybean (*Glycine max*) and Florida begonia (*Desmodium tortuosum*). *Weed Sci* 37:544–551.
- Grossiord C, Sevanto S, Bonal D, Borrego I, Dawson TE, Ryan M, Wang W, McDowell N (2018) Prolonged warming and drought modify belowground interaction for water among coexisting plants. *Tree Physiol* 39:55–63.
- Hamanishi ET, Campbell MM (2011) Genome-wide responses to drought in forest trees. *Forestry* 84:273–283.
- Hsiao TC (1973) Plant responses to water stress. *Ann Rev Plant Physiol* 24:519–570.
- Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC (2012) Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Sci* 195:48–53.
- Kerr KL, Meinzer FC, McCulloh KA, Woodruff DR, Marias DE (2015) Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. *Tree Physiol* 35:535–548.
- Kolb PF, Robberecht R (1996) High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. *Tree Physiol* 16:665–672.
- Kubiske ME, Abrams MD (1990) Pressure-volume relationships in non-rehydrated tissue at various water deficits. *Plant Cell Environ* 13:995–1000.
- Kuznetsova A, Brockhoff PB, Christensen RHB (2014) lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R package version 2.0–11. Available at: <http://CRAN.R-project.org/package=lmerTest>.
- Lenth R, Singmann H, Love J, Buerkner P, Herve M (2018) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4, Available at: <http://CRAN.R-project.org/package=emmeans>.
- Lieberman M, Robichaux RH (1990) Competition by barley and pea against mustard: effects on resource acquisition, photosynthesis and yield. *Agric Ecosyst Environ* 31:155–172.
- Maestre FT, Valladares F, Reynolds JF (2009) Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Ecol* 3:748–757.
- Martínez-Vilalta J, Cochard H, Mencuccini M et al. (2009) Hydraulic adjustment of Scots pine across Europe. *New Phytol* 184:353–364.
- Meinzer FC, Johnson DM, Jachewich L, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control, cavitation tension with hydraulic capacitance. *Funct Ecol* 23:922–930.
- Mencuccini M, Bonal D (2011) Leaf/sapwood area ratios in Scots pine show acclimation across Europe. *Can J For Res* 31:442–456.
- Mencuccini M, Grace J (1995) Climate influences the leaf area/sapwood ratio in Scots pine. *Tree Physiol* 15:1–10.
- Mittelman JB, Grant MC (2006) Genetic variation and the natural history of making spen. *Bioscience* 46:25–31.
- Monson RK, Grant MC (1989) Experimental studies of ponderosa pine. III. Differences in photosynthesis, stomatal conductance, and water-use efficiency between two genetic lines. *Am J Bot* 76:1041–1047.
- Morgan JM (1984) Osmoregulation and water stress in higher plants. *Annu Rev Plant Physiol* 35:299–319.
- Parker WC, Pallardy SG (1987) The influence of resaturation method and tissue type on pressure-volume analysis of *Quercus alba* L. seedlings. *J Exp Bot* 38:535–549.
- Pearcy RW, Tumosa N, Williams K (1981) Relationships between growth, photosynthesis and competitive interactions for a C₃ and a C₄ plant. *Oecologia* 48:371–376.
- Pierik R, Mommer L, Voisenek LA (2013) Molecular mechanisms of plant competition: neighbour detection and response strategies. *Funct Ecol* 27:841–853.
- Piñol J, Sala A (2000) Ecological implications of xylem cavitation for several Pinaceae in the Pacific northern USA. *Funct Ecol* 14:538–545.
- Piutti E, Cescatti A (1997) A quantitative analysis of the interactions between climatic response and intraspecific competition in European beech. *Can J For Res* 27:277–284.
- Poorter H, Bühler J, Van Dusschoten D, Climent J, Postma JA (2012a) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct Plant Biol* 39:839–850.
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012b) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol* 193:30–50.
- R Development Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Robinson DE, Wagner RG, Bell FW, Swanton CJ (2001) Photosynthesis, nitrogen-use efficiency, and water-use efficiency of jack pine seedlings in competition with four boreal forest plant species. *Can J For Res* 31:2014–2025.
- Rosas T, Mencuccini M, Barba J, Cochard H, Saura-Mas S, Martínez-Vilalta J (2019) Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient. *New Phytol* 223:632–646.

- Rötzer T, Häberle KH, Kallenbach C, Matyssek R, Schütze G, Pretzsch H (2017) Tree species and size drive water consumption of beech/spruce forests - a simulation study highlighting growth under water limitation. *Plant Soil* 418:1–20.
- Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? *Tree Physiol* 32:764–775.
- Schwendenmann L, Pendall E, Sanchez-Bragado R, Kunert N, Hölscher D (2015) Tree water uptake in a tropical plantation varying in tree diversity: interspecific differences, seasonal shifts and complementarity. *Ecohydrology* 8:1–12.
- Scott RL, James Shuttleworth W, Goodrich DC, Maddock T III (2000) The water-use of two dominant vegetation communities in a semiarid riparian ecosystem. *Agric For Meteorol* 105:241–256.
- Shipley B, Meziane D (2002) The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. *Funct Ecol* 16:326–331.
- Skelton RP, Anderegg LDL, Papper P, Reich E, Dawson TE, Kling M, Thompson SE, Diaz J, Ackerly DD (2019) No local adaptation in leaf or stem xylem vulnerability to embolism, but consistent vulnerability segmentation in a North American oak. *New Phytol* 223: 1296–1306.
- Soliveres S, Maestre FT (2014) Plant-plant interactions, environmental gradients and plant diversity: a global synthesis of community-level studies. *Perspect Plant Ecol Evol Syst* 16:154–163.
- Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11:35–40.
- Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* 75: 1736–1752.
- Trugman AT, Anderegg LDL, Wolfe BT, Birami B, Ruehr NK, Detto M, Bartlett MK, Anderegg WRL (2019) Climate and plant trait strategies determine tree carbon allocation to leaves and mediate future forest productivity. *Glob Chang Biol* 25:3395–3405.
- Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J Exp Bot* 23:267–282.
- Venturas MD, Sperry JS, Hacke UG (2017) Plant xylem hydraulics: what we understand, current research, and future challenges. *J Integr Plant Biol* 59:356–389.
- Vitali V, Forrester DI, Bauhus J (2018) Know your neighbours: drought response of Norway Spruce, Silver Fir and Douglas Fir in mixed forests depends on species identity and diversity of tree neighbourhoods. *Ecosystems* 21:1215–1229.
- Wang W, Peng C, Kneeshaw DD, Larocque GR, Luo Z (2012) Drought-induced tree mortality: ecological consequences, causes, and modeling. *Environ Rev* 20:109–124.
- Wheeler JK, Sperry JS, Hacke UG, Huang N (2005) Inter-vessel pitting and cavitation in woody Rosaceae and other vessel led plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell Environ* 28:800–812.
- Worrall JJ, Rehfeldt GE, Roman A, Hogg EH, Marchetti SB, Michaelian M, Gray LK (2017) Recent declines of *Populus tremuloides* in North America linked to climate. *For Ecol Manage* 299:35–51.
- Wullschlegel MJ, Meinzer FC, Vertessy RA (1998) A review of whole-plant water-use studies in trees. *Tree Physiol* 18:499–512.
- Zenes M, Trugman AT, Anderegg WRL (2020) Competition and drought alter optimal stomatal strategy in tree seedlings. *Front Plant Sci* 11:1–13.
- Zimmerman JWH, Jeje AA (1981) Vessel-length distribution in stems of some American woody plants. *Can J Bot* 59:1882–1892.