

Shrub facilitation of tree establishment varies with ontogenetic stage across environmental gradients

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Summary

- Plant–plant interactions are important drivers of ecosystem structure and function, yet predicting interaction outcomes across environmental gradients remains challenging. Understanding how interactions are affected by ontogenetic shifts in plant characteristics can provide insight into the drivers of interactions and improve our ability to anticipate ecosystem responses to environmental change.
- We developed a conceptual framework of nurse shrub facilitation of tree establishment. We used a combination of field experiments and environmental measurements to test the framework with a shrub (*Artemisia tridentata*) and a tree (*Pinus monophylla*), two foundation species in a semiarid environment.
- Shrub microsites allowed trees to overcome an early population bottleneck and successfully establish in areas without tree cover. Shrubs facilitated trees at multiple ontogenetic stages, but the net outcome of the interaction shifted from strongly positive to neutral after the transition of *P. monophylla* from juvenile to adult foliage. Microhabitat conditions varied across a broad elevational gradient, but interaction outcomes were not strongly related to elevation.
- Favorable microsites provided by *A. tridentata* cover are crucial for *P. monophylla* recovery after stand-replacing disturbance. Models of vegetation response to rapid global environmental change should incorporate the critically important role of nurse shrub interactions for ameliorating population bottlenecks in tree establishment.

Introduction

Interactions among plant species are important for the structure and functioning of ecological communities (Brooker *et al.*, 2008; Soliveres *et al.*, 2015). These interactions can range from positive to negative, with the net outcome depending on the characteristics of the underlying abiotic environment (Bertness & Callaway, 1994), plant modification of that environment (Schöb *et al.*, 2013), and plant traits related to resource requirements or physiological tolerances (Liancourt *et al.*, 2005). Numerous theoretical and empirical studies have focused on predicting changes in the outcome of interactions across environmental gradients, yet generalizable patterns are elusive, and some argue that plant characteristics are more important than environmental conditions for determining the outcome of pairwise interactions between species (Soliveres *et al.*, 2015). Because plant characteristics shift with ontogeny, considering how interactions change throughout the life cycle of a plant species could help clarify this issue. Additionally, knowledge of how plant–plant interactions vary in relation to ontogeny and environmental gradients may provide insights into how plant communities and associated taxa will respond to climate change, disturbance, and species invasions.

Several syntheses have highlighted the importance of studying plant–plant interactions across multiple life-history (ontogenetic) stages because the balance between the positive and negative components of interactions often shifts as plants age (i.e. ontogenetic shift) (Callaway & Walker, 1997; Brooker *et al.*, 2008; Filazzola & Lortie, 2014; Soliveres *et al.*, 2015). Many studies show that the effect of neighboring plants transitions from net positive (i.e. facilitative) to neutral or net negative (i.e. competitive) as a plant progresses through life-history stages (Miriti, 2006; Reisman-Berman, 2007; Valiente-Banuet & Verdú, 2008; Armas & Pugnaire, 2009; Paterno *et al.*, 2016). This pattern likely reflects the fact that larger plants typically have greater resource requirements and lower sensitivity to abiotic extremes (Grubb, 1977; Niinemets, 2010), reducing the benefits of environmental stress amelioration by neighboring plants and increasing their competitive effects.

Ontogenetic shifts in plant tolerances and water and nutrient requirements likely interact with spatial variability in the underlying abiotic environment to determine the net outcome of plant–plant interactions. The abiotic environment determines resource availability and the type and magnitude of stress, thus affecting the interactions among neighboring plants. Despite much scientific attention (Bertness & Callaway, 1994; Maestre *et al.*, 2009;

He *et al.*, 2013; Michalet *et al.*, 2014a), our ability to predict plant–plant interactions in response to environmental gradients requires additional understanding (Brooker *et al.*, 2008). Some of the uncertainty stems from the fact that the environmental conditions relevant to plant–plant interactions depend on the ecological adaptations of the particular species involved (He *et al.*, 2016; Zhang & Tielbörger, 2019). Considering ontogenetic shifts within beneficiary species, in which plant traits change gradually among life-history stages, can provide insight into the relative importance of species characteristics and environmental conditions on the outcome of interactions (Schiffers & Tielbörger, 2006; Davis *et al.*, 2007; Soliveres *et al.*, 2010; Le Roux *et al.*, 2013; Wright *et al.*, 2014).

In dryland ecosystems, shrub facilitation of tree establishment is a widespread example of plant–plant interactions, with major implications for community dynamics. Large, long-lived perennials such as trees experience profound changes in environmental tolerance and resource needs with age, and the requirements for seedlings and juveniles are much narrower than the range of conditions in which adults can persist (Niinemets, 2010). Therefore, tree recruitment in dry forests is often limited to favorable microsites where abiotic stress is reduced (Redmond & Barger, 2013). Shrub modification of the under-canopy environment can provide favorable conditions for tree establishment, and ‘nurse shrub’ facilitation of tree seedlings has been observed in drylands across the globe (McAuliffe, 1986; Kitzberger *et al.*, 2000; Chambers, 2001; Gómez-Aparicio *et al.*, 2005; Smit *et al.*, 2007; Sthultz *et al.*, 2007; Ziffer-Berger *et al.*, 2017). Multiple mechanisms may explain why shrub cover often facilitates regenerating trees, and relevant mechanisms may change throughout the life cycle of a beneficiary tree (Filazzola & Lortie, 2014). Shrubs ameliorate abiotic stress by reducing solar radiation, wind velocity, and soil temperatures, leading to decreased plant water loss from transpiration and soil water loss through evaporation (Kitzberger *et al.*, 2000; Lortie & Cushman, 2007; Holmgren *et al.*, 2012). Shrubs also modify under-canopy resources, although increases in soil fertility and moisture retention from litter and reduced evaporation (Walker *et al.*, 2001) can be offset by the shrub’s use of soil water and nutrients (Maestre *et al.*, 2003). Additionally, shrubs can indirectly facilitate tree seedlings through protection from herbivory (Chambers, 2001; Gómez-Aparicio *et al.*, 2008).

Here, we propose a conceptual framework of nurse shrub facilitation of tree establishment (Fig. 1) and test the framework using a widespread shrub–tree interaction. We expect the strongest facilitative effects for early life-history stages in which tree seedlings are highly sensitive to abiotic extremes, with a shift toward net neutral effects for late juvenile stages (Fig. 1a). We hypothesize that shrub effects on tree performance will follow this ontogenetic pattern across broad environmental gradients, but that the underlying abiotic environment will influence interaction outcomes by constraining the microhabitat conditions available to young trees (Fig. 1b). We test our hypothesis for two foundation species in a semiarid environment: a shrub (big sagebrush; *Artemisia tridentata*) and a tree (singleleaf pinyon pine; *Pinus monophylla*). These species co-dominate across broad environmental gradients and provide a model system for studying

interactions between nurse shrubs and young trees in dryland ecosystems. Prior research has shown that *A. tridentata* facilitates *P. monophylla* seedlings (Callaway *et al.*, 1996; Chambers, 2001). *P. monophylla* individuals that reach maturity eventually outsize and outlive their *A. tridentata* nurse plants. We use controlled seeding and shrub removal experiments at three sites located along an elevation gradient to ask: (1) does the interaction between *A. tridentata* and *P. monophylla* shift with *P. monophylla* ontogeny; and (2) how does the abiotic environment influence the interaction between *A. tridentata* and *P. monophylla* within and among *P. monophylla* ontogenetic stages?

Materials and Methods

Study area

This study took place in Barrett Canyon (39°05′37″ N, 117°29′38″ W), an east-to-west oriented drainage on the eastern slope of the Shoshone Mountains in Nye County, Nevada, USA. The study canyon was chosen for its uniformity in relation to potentially confounding factors – aspect, canyon bottom width, and the density and composition of woody plant communities. We conducted our experiments along an elevational gradient that ranged from hot and dry at the low elevation to cooler and wetter at the high elevation (Chambers *et al.*, 2007). Lithology of the Shoshone Mountains consists of welded and non-welded silica ash flow tuff, and alluvial fan soils are classified as coarse loamy mixed frigid Typic Haploxerolls (Rau *et al.*, 2005). A weather station from the adjacent Desatoya Mountains (Western Regional Climate Center, 2018) recorded a drought in recent years (2012–2013) but above-average precipitation for the study period (2016–2017; Supporting Information Fig. S1). Woodlands within Barrett Canyon are dominated by *Pinus monophylla* Torr. & Frem., with lesser cover of *Juniperus osteosperma* (Utah juniper). *P. monophylla* foliage is dimorphic, transitioning from juvenile to adult (needle) foliage with plant growth stage. Pinyon–juniper woodlands within the study canyon are dissected by several large alluvial fans, which are dominated by *Artemisia tridentata* Nutt., a non-sprouting shrub. *A. tridentata* is also present in tree interspaces within the woodland belt and in areas above and below the current extent of pinyon–juniper woodlands. Subspecies *A. tridentata* ssp. *wyomingensis* occurs at the lower end of the canyon, *A. tridentata* ssp. *vaseyana* occurs at the upper end, and the two subspecies overlap and likely hybridize in the middle portions of the canyon. Our experiments were conducted on *A. tridentata*-dominated alluvial fans where the interaction between shrubs and young trees could be isolated.

Experimental design

Three study sites were spaced along an elevational gradient (2100, 2250, and 2450 m) and encompass the full elevational range of *P. monophylla* in the Barrett Canyon watershed. At each site, we tested the effects of microhabitat treatments on abiotic environmental conditions and on *P. monophylla* performance at multiple ontogenetic stages of establishment. Three microhabitat

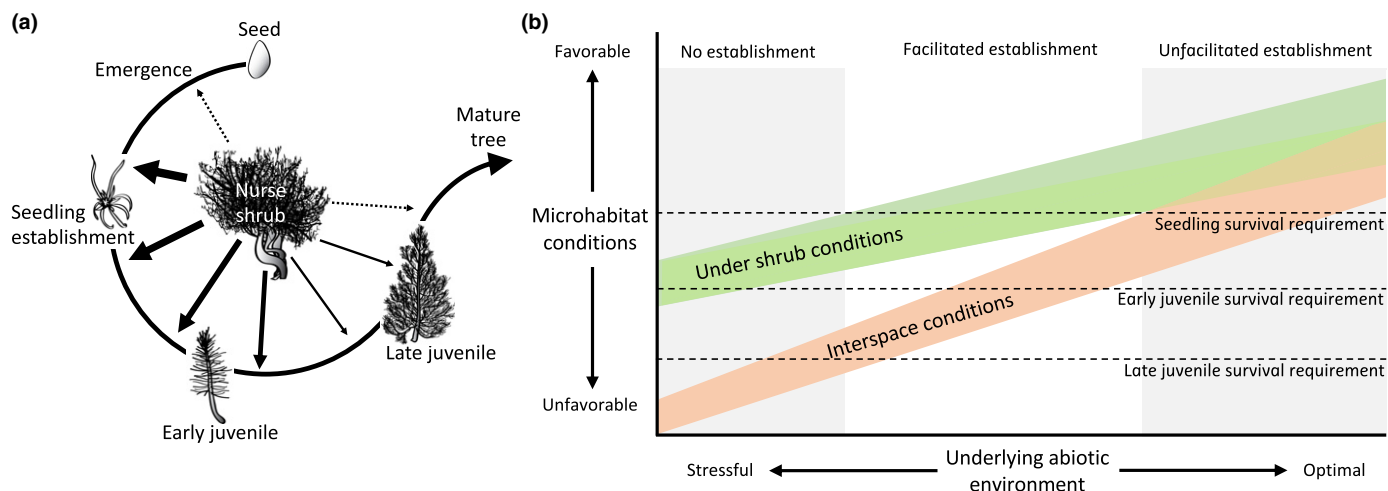


Fig. 1 A conceptual model of nurse shrub facilitation of tree establishment. (a) Nurse shrub effects across tree ontogenetic stages. Net facilitation is strongest (thicker arrows) for early ontogenetic stages of the beneficiary species, and interactions become net neutral (dashed arrows) at later ontogenetic stages. All interactions likely include some combination of competitive and facilitative effects. (b) Hypothesized relationship between the underlying abiotic environment, shrub cover effects on microhabitat conditions, and tree ontogeny. Green and orange bars represent the range of conditions that are available to young trees given the interaction between the underlying abiotic environment and microhabitat. Dashed lines represent simplified minimum requirements for tree survival at three ontogenetic stages, assuming that microhabitat conditions drive survival at a given position on the underlying environmental gradient. In extremely stressful environments, both interspace and under-shrub microhabitats are unfavorable for seedling survival, and establishment cannot occur. At intermediate levels of abiotic stress, seedling and early juvenile survival is facilitated by shrub cover, and facilitation is strongest for early life-history stages with more sensitive requirements for survival. In optimal abiotic environments, interspace environments become more favorable and shrub modification of microhabitat conditions is less important for seedling and early juvenile survival.

treatments were used: under the canopy of an *A. tridentata* plant ('under-shrub'), next to the stem of a removed *A. tridentata* plant ('shrub-removal'), and in the sparsely vegetated area between shrubs ('interspace'). In the shrub-removal treatment, *A. tridentata* stems were cut at the ground surface using a reciprocating saw and all aboveground plant parts were removed from the site. We randomly assigned *A. tridentata* to the shrub-removal or under-shrub treatments; *A. tridentata* size did not differ among treatment groups (height: $P=0.918$; cross-sectional canopy area: $P=0.847$).

Environmental measurements We measured abiotic environmental conditions from three microhabitat treatments: under-shrub, shrub-removal, and interspace (Table S1). Abiotic measurements were collected from a central location at each study site and from microhabitat locations that were not used for *P. monophylla* establishment experiments.

We measured soil temperature and soil moisture from May to October of 2017. At each site, three sensor arrays were installed in each microhabitat treatment. For under-shrub and shrub-removal microhabitats, the sensor arrays were located 15 cm to the north of the *A. tridentata* stem, corresponding to an average of 41 cm inside the drip line. Soil temperatures were recorded every 2 h by five Maxim Thermochron iButton temperature data loggers in each sensor array: two were placed just under the soil surface, two were buried 3-cm deep, and one was buried 10-cm deep. For each sensor, we calculated minimum and maximum temperature for each day, which were then summarized over the May–October measurement period. We also derived frost

days (total days for which minimum soil surface temperature was $<0^{\circ}\text{C}$) and growing season length (total days between latest spring frost and earliest fall frost) for each sensor. We assigned a maximum growing season length of 132 d for sensors that did not record a frost event during the May–October study period ($n=6$, out of 54 total).

Volumetric water content was measured in each sensor array with one EC-5 soil moisture sensor (Meter, Pullman, WA, USA). The sensor measured integrated soil moisture in the top 10 cm of the mineral soil for a 0.2-l measurement volume. EM50 data loggers (Meter) were programmed to read sensors every minute and to store hourly averages. Soil samples were collected with a 5 cm-by-15 cm slide hammer (AMS, American Falls, ID, USA) from three shrub interspaces at each site, dried at 105°C until constant mass, sieved to 2 mm, and weighed to derive soil rock fraction and fine-soil bulk density. The soil particle size distribution was measured using a sedimentation method with a hydrometer (Gee & Bauder, 1986). We developed soil water release curves for each site, using a WP4C water potential meter (Meter), to measure soil matric potential for at least 10 fine soil (<2 mm) subsamples across a range of known gravimetric water contents. Recorded volumetric water content was converted to gravimetric water content using fine-soil bulk density, and gravimetric water content was converted to soil water potential using the soil water release curves derived for each site. We derived total growing season wet days for each sensor, defined as the number of growing season days (derived as described above) with soil water content above the permanent wilting point (total hours 24 h^{-1} when hourly soil water matric potential was $>-1.5\text{ MPa}$). We measured soil depth in nine shrub interspace locations in each site by driving a 90 cm

metal rod into the soil using a sledgehammer until the rod was either stopped by rock or clay hardpan, or the entire length of rod was driven into the soil.

We measured photosynthetically active radiation (PAR) at noon on 26 June 2017. We measured PAR once per minute for 20 min at three locations in each microhabitat treatment at each site. There was no cloud cover during our measurement period. PAR was measured using QSO-S PAR photon flux sensors (Meter), and raw data were converted to photosynthetic photon flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) using the manufacturer-provided conversion.

We measured plant-available pools of soil inorganic nitrogen (N) seasonally at four locations in each microhabitat treatment at each site. Soil samples were collected in May 2016, August 2016, October 2016, and May 2017. We used a 2-cm diameter soil probe to collect soil samples to 10 cm mineral soil depth. For under-shrub and shrub-removal treatment microhabitats, soil samples were collected from the eastern *A. tridentata* drip line. Soil ammonium and nitrate were extracted from 8-g soil samples with 40 ml 1 M potassium chloride. After shaking for 1 h, the slurry was filtered through Whatman no. 1 paper and the elutant was analyzed on a flow injection analyzer (Lachat QuickChem 8500; Hach Co., Loveland, CO, USA).

Seeding experiment: *P. monophylla* seedling emergence and first-year survival To examine the effect of *A. tridentata* cover on *P. monophylla* first-year seedlings, we planted *P. monophylla* seeds into shrub microhabitats using a randomized blocked design. Seeds were collected in September 2016 from a nearby mountain range at approximately the same elevation as our mid-elevation study site. All seeds were visually inspected and float-tested (Mueller *et al.*, 2005), and only seeds that were both structurally sound and filled were used. Microhabitat treatment blocks were established at a central location at each study site. At each of the three sites, we established five replicate blocks, separated by at least 5 m. Three microhabitat treatments were applied in each block: under-shrub, shrub-removal, and interspace (Table S1). For each experimental block, two *A. tridentata* plants were chosen with a canopy diameter of > 50 cm for under-shrub and shrub-removal treatments, and the interspace treatment was located centrally between them.

Nine *P. monophylla* seeds were planted within each microhabitat treatment location. In November 2016, seeds were planted 3-cm deep (Chambers, 2001) into gridded arrays with 7-cm spacing within each microhabitat treatment location. For the under-shrub and shrub-removal treatments, seed arrays were planted in a central location under the nurse shrub canopy. To minimize rodent predation, the seed arrays were covered with hardware cloth cages that were 30-cm wide and 10-cm high. The cage walls were buried 1–2 cm and staked down with U-pins. In the under-shrub and shrub-removal microhabitat treatments, surface litter was removed before planting and then evenly applied on top of the planted seed array. No litter was present in the interspace microhabitat treatments. Planting locations were monitored for seedling emergence and survival at 1–2 wk intervals from April to July 2017 and then monthly until October 2017.

Nurse shrub removal experiment: *P. monophylla* juvenile survival and growth To examine the effect of *A. tridentata* cover on *P. monophylla* juveniles, we experimentally manipulated naturally-occurring associations between adult *A. tridentata* and young *P. monophylla* in a randomized control design. In May of 2016, we surveyed the alluvial fans that comprised the three study sites for *P. monophylla* juveniles shorter than 1.5 m in height. At each site (ranging from 4 to 17 ha), we located 30–32 *P. monophylla* early juveniles (with juvenile foliage only) and 50–56 late juveniles (with adult foliage in at least one previous year and < 1.5 m in height) that were growing in the canopy of an adult *A. tridentata*. For our experimental treatments, we only used clear associations between *P. monophylla* juveniles and healthy adult *A. tridentata* plants. Each plant was randomly assigned to one of two microhabitat treatments: under-shrub (no alteration of *A. tridentata*–*P. monophylla* association) and shrub-removal (full removal of the *A. tridentata* nurse shrub). Few *P. monophylla* juveniles were found in shrub interspaces, and all of these had evidence that they had once grown in association with a now-deceased *A. tridentata* plant (e.g. wood remnants near their base), so we were not able to include an ‘interspace’ group for the juvenile experiment. We used high-precision GPS to map the locations of the study plants so they could be reliably relocated.

Before implementing experimental treatments, we measured the following for each *A. tridentata*–*P. monophylla* association: *A. tridentata* height and canopy area, *P. monophylla* height, and the distance and cardinal direction from the *A. tridentata* stem to the *P. monophylla* juvenile. For late juvenile *P. monophylla*, we used bud scars to measure annual terminal leader growth for the 5 yr before treatment (2011–2015) and for 2 yr after treatment (2016–2017). All study plants were monitored for survival every 2–4 wk during the 2016 growing season and every 4–8 wk during the 2017 growing season.

Statistical analyses

To test how treatments affected the abiotic environment, we used linear regression models of abiotic variables (mean daily temperature minimum and maximum; frost days; growing season length; growing season wet days; and photosynthetically active radiation) as a function of microhabitat, elevation, and their interaction. We modeled daily mean gravimetric water content (square-root-transformed) as a function of microhabitat, elevation, and their interaction, with random intercepts for sensor ID and day of year to account for repeated measures and seasonal trends. We used linear mixed models of total available N as a three-way interaction between microhabitat, elevation, and sampling date (factor), with a random intercept for sample ID to account for repeated measures at the same locations. Elevational differences in pre-treatment plant characteristics and general abiotic environmental characteristics were analyzed using ANOVA models. Plant characteristics included *A. tridentata* height and canopy cross-sectional area, *P. monophylla* 5-yr average late juvenile stem growth, and *P. monophylla* juvenile direction from the *A. tridentata* nurse

shrub (transformed into a northeastness index ranging from -1 to $+1$). General abiotic characteristics included soil depth, soil rock fraction, and soil bulk density.

Regression models were used to analyze *P. monophylla* outcomes as a function of microhabitat treatment, elevation, and their interaction for each ontogenetic stage. Linear regressions with Type-2 ANOVA statistics were used for models with a normally-distributed response variable (emergence, growth), and logistic regressions with Chi-squared ANOVA statistics were used for models with a categorical response (survival). For all models, both microhabitat treatment ('under-shrub', 'shrub-removal', and 'interspace' (emergence and seedling survival only)) and elevation (low, mid, and high) were categorical variables. *P. monophylla* emergence was represented by the percentage of seeds in each array that emerged during one growing season. Seedling survival was calculated at the end of the first growing season, late juvenile survival was calculated at the end of the second growing season, and early juvenile survival was calculated at three time periods: the end of the first growing season, the end of the first winter, and the end of the second growing season. Some models were unable to estimate the microhabitat–elevation interaction effect due to fitted probabilities of exactly 0 or 1 (either 0% or 100% survival in some treatment–elevation combinations). In these cases, we modeled the effect of elevation on survival within specific treatment types: seedling survival in 'under-shrub' treatment only, and early juvenile survival in 'shrub-removal' treatment only. To test whether shrub removal effects were dependent on plant size, we modeled final survival of early juveniles from the 'shrub-removal' treatment as a function of the interaction of elevation and early juvenile height at the start of the study.

To directly compare the strength of ecological interactions among *P. monophylla* ontogenetic stages, we used the relative interaction index (RII; Armas *et al.*, 2004). RII is calculated as: $RII = (P_{+N} - P_{-N}) / (P_{+N} + P_{-N})$ where P_{+N} represents *P. monophylla* performance with nurse shrubs and P_{-N} represents

performance without nurse shrubs. RII ranges from -1 to 1 , with positive values indicating net facilitation, negative values indicating competition, and larger absolute values indicating stronger interaction intensity. RII was calculated for each site and *P. monophylla* ontogenetic stage by comparing outcomes for nearby pairs of under-shrub and shrub-removal individuals (except for emergence, which was compared between pairs of seed arrays to account for spatial clustering of seeds). We used a linear regression with Type-2 ANOVA statistics to model RII as a function of the interaction between ontogenetic stage and elevation. All analyses were performed in R v.3.3.3 (R Core Team, 2015).

Results

Abiotic and biotic characteristics of the study sites

Before treatment implementation, plant characteristics varied across the elevational gradient (Table 1). *A. tridentata* size had a non-linear relationship with elevation: plants were both tallest ($P < 0.001$) and had the largest canopy cross-sectional area ($P = 0.003$) at the middle elevation. For the 5-yr period before treatment, *P. monophylla* late juvenile terminal leader growth was positively related to elevation ($P = 0.02$), and annual growth at the high elevation site averaged 125% of growth at the low elevation (Table 1). At low and mid elevations, *P. monophylla* juveniles tended to be located to the northeast of their *A. tridentata* nurse shrub, whereas *P. monophylla* juveniles at the high elevation varied in their position within the *A. tridentata* canopy ($P = 0.01$; Table 1; Fig. S2).

Soils were classified as sandy loams at all elevations, although the low elevation site had a smaller fraction of sand (Table 1). High elevation soil had lower fine soil (< 2 mm) bulk density and nearly double the rock fraction than the low elevation soil (Table 1). Soil depth did not differ significantly among elevations ($P = 0.43$; Table 1).

Table 1 Abiotic and biotic characteristics of the three study sites.

	Low elevation	Mid elevation	High elevation	P^2
Soil characteristics				
Soil texture ¹ (% sand–silt–clay)	Sandy loam (46–49–5)	Sandy loam (62–34–4)	Sandy loam (56–40–4)	
Soil rock fraction (proportion; $n = 9$)	0.37 ± 0.06	0.56 ± 0.02	0.62 ± 0.03	0.01
Fine-soil bulk density (g cm^{-3} ; $n = 9$)	0.95 ± 0.03	0.65 ± 0.02	0.48 ± 0.04	< 0.001
Total bulk density (g cm^{-3} ; $n = 9$)	1.53 ± 0.11	1.48 ± 0.03	1.25 ± 0.03	0.04
Soil depth (cm; $n = 27$)	28.7 ± 3.7	32.8 ± 4.6	40.0 ± 8.8	0.43
Pre-treatment plant characteristics				
<i>Pinus monophylla</i> juvenile pre-treatment direction from <i>Artemisia tridentata</i> stem (northeastness index (-1 to 1); $n = 241$)	0.36 ± 0.07	0.36 ± 0.07	0.08 ± 0.09	0.01
<i>P. monophylla</i> late juvenile 5-yr pre-treatment average stem growth (cm; $n = 156$)	3.2 ± 0.16	3.8 ± 0.25	4.0 ± 0.18	0.02
<i>A. tridentata</i> pre-treatment height (cm; $n = 248$)	51.3 ± 1.7	86.4 ± 2.5	73.7 ± 2.5	< 0.001
<i>A. tridentata</i> pre-treatment canopy area (m^2 ; $n = 248$)	0.64 ± 0.04	0.93 ± 0.07	0.78 ± 0.06	0.003

Site names (column headings) correspond to their relative position on an elevational gradient. Values are mean $\pm$ SE.

¹United States Department of Agriculture classification.

²From analysis of variance testing differences among sites (elevations).

Artemisia tridentata microhabitat effects on environmental characteristics across a gradient of elevation

At all measured depths (0, 3, and 10 cm), soil temperature was related to both microhabitat treatment ($P < 0.001$) and elevation ($P < 0.01$), but there was no interaction between treatment and elevation ($P > 0.06$). For all soil depths and at all elevations, maximum daily temperature was lower and minimum daily temperature was higher in the under-shrub treatment compared to the shrub-removal or interspace treatments (Figs 2a,b, S3, S4). Maximum and minimum daily soil temperatures at all depths were highest at the low elevation site. Frost days were more frequent at the middle and high elevation ($P = 0.002$), and were, on average, twice as frequent in the interspace or shrub removal microhabitats than under shrubs ($P < 0.001$). Growing season was shorter at the middle and high elevations than at the low elevation ($P = 0.012$) and was 4–5 d longer in the under-shrub treatment compared to interspaces or shrub-removals ($P = 0.002$; Fig. S5).

Photosynthetically active radiation (PAR) differed among treatments ($P < 0.001$) and elevations ($P < 0.001$), but there was no interaction between treatment and elevation ($P = 0.38$). Among treatment types, PAR varied by more than an order of magnitude. In the under-shrub treatments, PAR averaged $160 \mu\text{mol m}^{-2} \text{s}^{-1}$, compared to $3738 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the shrub-removal and interspace treatments (Fig. 2c). For all treatment types, PAR was lowest at the high elevation.

Plant-available soil inorganic N varied through time, and all treatments showed a pulse of available N after the growing season ended (date; $P < 0.001$; Fig. 2d). Across elevations, the late-season pulse of N was nearly three times higher in the shrub removal treatment than in the interspace or under-shrub treatments (treatment by date interaction; $P < 0.001$). Available N did not differ significantly by elevation ($P = 0.06$; Fig. S6).

Soil gravimetric water content (GWC) differed among elevations ($P < 0.001$; Fig. 2e) and was generally higher at the high elevation site during both relatively wet and dry periods. Soil GWC did not differ among treatments ($P = 0.35$), and there was no interaction between treatment and elevation effects on GWC ($P = 0.52$). Total wet days (number of growing season days for which soil matric potential was above wilting point) was not related to treatment ($P = 0.50$), elevation ($P = 0.74$), or their interaction ($P = 0.42$; Fig. S7).

Elevation and *A. tridentata* microhabitat effects on *P. monophylla* at multiple stages

From 405 planted *P. monophylla* seeds, a total of 77 (19%) emerged (Fig. 3a). Emergence varied by elevation of the study site ($P = 0.003$): 20% at the low elevation, 26% at the mid-elevation, and 11% at the high elevation. Emergence did not differ among *A. tridentata* microhabitat treatments ($P = 0.58$), nor by the interaction between microhabitat treatment and elevation ($P = 0.40$).

Pinus monophylla seedling survival during the first growing season was strongly dependent on *A. tridentata* microhabitat treatment (Fig. 3b; $P < 0.001$). Half of all seedlings located under *A. tridentata* survived the first growing season, whereas all

seedlings located in the interspace or shrub-removal habitats died. Among seedlings in the under-shrub treatment, survival was positively related to elevation ($P = 0.04$).

Pinus monophylla early juvenile survival was greatly reduced by removal of *A. tridentata* (Fig. 4a; $P < 0.001$ for all time intervals). At the end of the second growing season, only 35% of all early juveniles in the shrub-removal treatment were still alive, compared to 90% in the under-shrub treatment. The interaction between *A. tridentata* microhabitat treatments and elevation shifted through time (Fig. 4a). At the low elevation, shrub removal induced a rapid mortality response, and at the end of the first growing season, survival in the shrub-removal treatment was lowest at the low elevation site ($P = 0.02$). At mid and especially high elevation, the response to shrub removal was more delayed. There was no difference in survival among elevations after the first winter ($P = 0.41$) or at the end of the second growing season ($P = 0.71$). Taller early juveniles were more likely to survive shrub removal ($P < 0.001$), and there was an interaction with elevation ($P = 0.002$) such that there was a threshold relationship between height and survival at the low elevation and a more continuous relationship at the high elevation site (Fig. 4b).

The survival of late juvenile *P. monophylla* did not differ by *A. tridentata* microhabitat treatment, elevation, or their interaction, and only two late juveniles (1%) died over the course of our study (Fig. 5a). Consistent with pre-treatment measurements, leader growth was positively related to elevation in both the first ($P = 0.002$) and second ($P = 0.01$) years after treatment (Fig. 5b, c). In the first year after treatment (Fig. 5b), late juvenile terminal leader growth was not affected by treatment ($P = 0.87$). However, shrub removal decreased late juvenile growth in the year following treatment ($P = 0.02$; Fig. 5c) consistently for all elevations (treatment by elevation interaction; $P = 0.79$).

Interaction intensity (RII) varied by *P. monophylla* ontogenetic stage ($P < 0.0001$), with the strongest facilitative effects on seedling survival and early juvenile survival (Fig. 6). Overall interaction intensity did not differ among elevations ($P = 0.92$). There was not a significant interaction between elevation and ontogenetic stage ($P = 0.08$), although interaction strength peaked at a later ontogenetic stage at the low and mid elevation sites (early juvenile survival) than at the high elevation site (first-year seedling survival).

Discussion

The results of our study suggest that plant ontogeny is a primary driver of the outcome of plant–plant interactions and that environmental conditions have a lesser, modulating influence. We found that interaction outcomes were largely explained by ontogenetic shifts in juvenile tree characteristics, even across a broad elevational gradient that encompassed vastly different abiotic environmental conditions. Tree seedling and early juvenile stages were the primary bottlenecks for successful establishment. Microclimate amelioration under shrubs facilitated the survival of the smallest and most vulnerable individuals and greatly eased the establishment bottleneck. The underlying abiotic environment constrained microhabitat conditions, yet consistent interaction

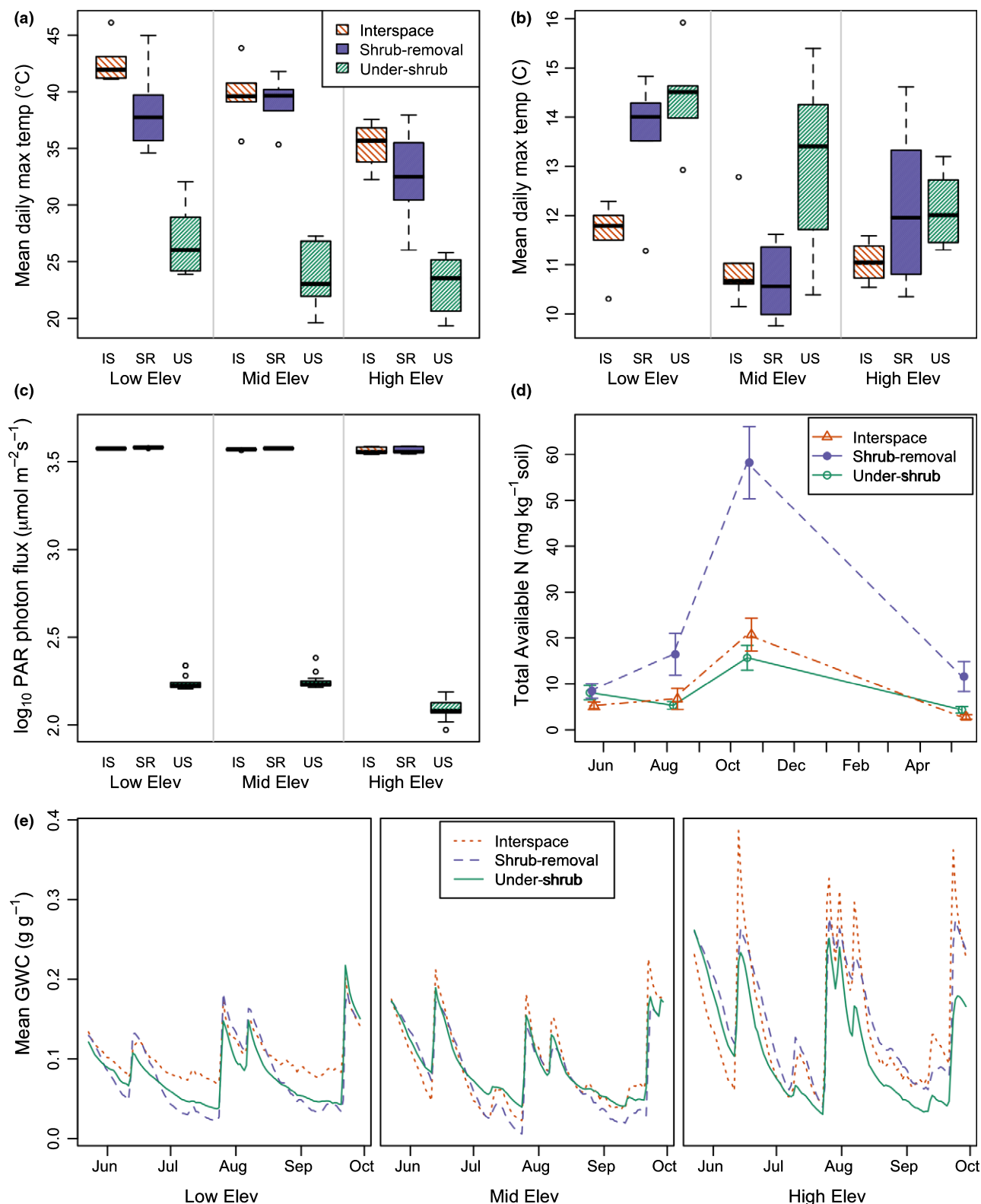


Fig. 2 Abiotic environmental measurements from three *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient. (a) Daily maximum soil temperature (3-cm depth), averaged for May–October 2017 ($n = 54$). (b) Daily minimum soil temperature (3-cm depth), averaged for May–October 2017 ($n = 54$). (c) Midday photosynthetically active radiation (PAR) at the soil surface on 26 June 2017 (shown in $\log_{10}$ scale; $n = 27$). (a–c) Boxplots: bold line is the median, box margins show the interquartile range, whiskers are farthest points within 1.5 of the interquartile range, and points are outliers. (d) Total inorganic nitrogen (0–10-cm depth) from four sampling dates in 2016 and 2017 ($n = 36$ for each observation date). There was not a significant relationship between total inorganic nitrogen and elevation, so samples from all elevations (sites) are pooled for each microhabitat treatment (mean ± 1 SE). (e) Daily mean gravimetric soil water content (GWC; 0–10-cm depth) in 2017 ($n = 27$ for each observation date). IS, interspace; SR, shrub-removal; US, under shrub.

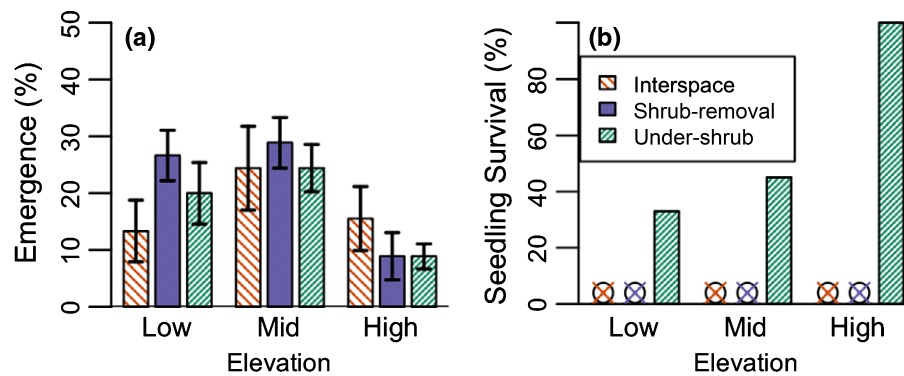


Fig. 3 Fate of *Pinus monophylla* seeds planted in three *Artemisia tridentata* shrub microhabitat treatments at three study sites along an elevational gradient. (a) Percentage of seeds ($n = 405$) that emerged (mean $\pm$ 1 SE). (b) Percentage of emerged seedlings ($n = 77$) that survived the first growing season.

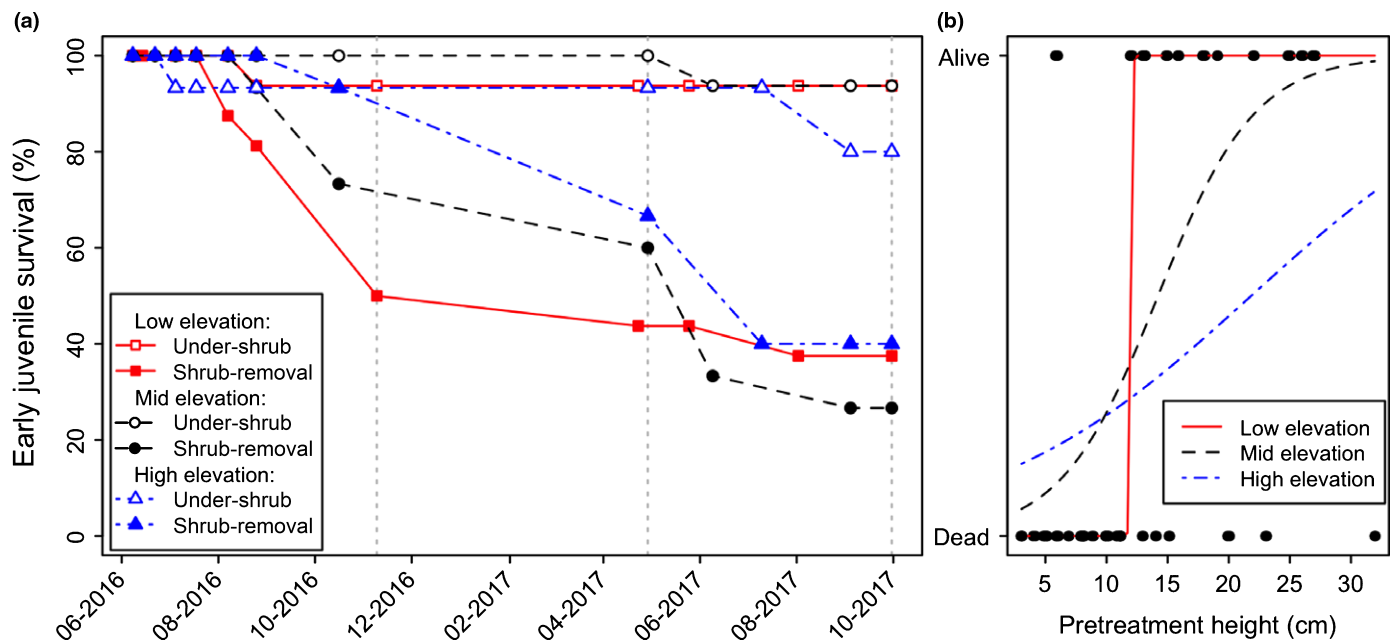


Fig. 4 *Pinus monophylla* early juvenile survival in two *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient. Treatment occurred in early May 2016. (a) Early juvenile survival (%) for two growing seasons after treatment, for 'under-shrub' (open symbols) and 'shrub-removal' (closed symbols) treatments ($n = 93$). Temporal differences in pinyon early juvenile mortality were tested on three dates represented by vertical dotted lines. (b) Status at the end of the second post-treatment growing season for all early juveniles in the shrub-removal treatment ($n = 46$) as a function of pretreatment height, overlaid with logistic regression prediction curves for each elevation.

outcomes across the elevational gradient suggests that the presence of nurse shrubs reduced the influence of abiotic stress on tree establishment. Our results help inform a general hypothesis of nurse shrub facilitation of tree establishment.

Ontogenetic shift in plant–plant interactions

Our study shows that a widespread shrub–tree interaction in the semiarid Great Basin is dependent on tree life-history stage, following a pattern that may be generalizable across many dryland ecosystems (Fig. 1a). Species traits have important effects on plant–plant interactions (Callaway, 1998; Schöb *et al.*, 2013, 2017; Rolhauser & Pucheta, 2016), and ontogenetic shifts in physiology related to stress tolerance or resource acquisition can regulate the interactions between neighboring plants (Armas & Pugnaire, 2009; Soliveres *et al.*, 2010; Le

Roux *et al.*, 2013). We found that *A. tridentata* directly facilitated *P. monophylla* at multiple stages of regeneration, but that the effect of *A. tridentata* on *P. monophylla* shifted from positive to neutral with changes in *P. monophylla* ontogeny. Under-shrub environments differed substantially from interspaces, but our measurements of the abiotic environment suggest that shrub facilitation of tree establishment likely results primarily from shrub amelioration of abiotic stress, rather than from increased resource availability.

In arid ecosystems, modification of abiotic factors associated with both non-resource stress (e.g. heat, cold) and resource availability (e.g. soil water or nutrients) by shrubs results in complex effects on nearby plants (Michalet, 2006; Maestre *et al.*, 2009; Graff & Aguiar, 2017). The shading structure provided by *A. tridentata* cover in our study reduced irradiance and was associated with lower maximum daily temperatures. These effects

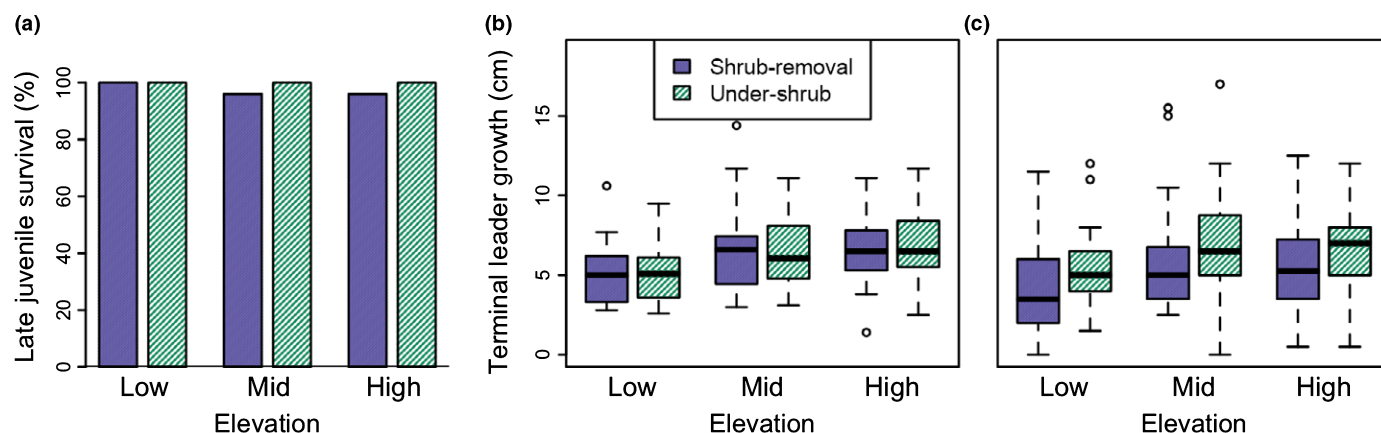


Fig. 5 *Pinus monophylla* late juvenile survival and growth in two *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient ($n = 156$). (a) Percentage of late juveniles that were alive at the end of the second post-treatment growing season. Terminal leader growth at the end of (b) the first year after treatment (2016) and (c) the second year after treatment (2017). (b–c) Boxplots: bold line is the median, box margins show the interquartile range, whiskers are farthest points within 1.5 of the interquartile range, and points are outliers.

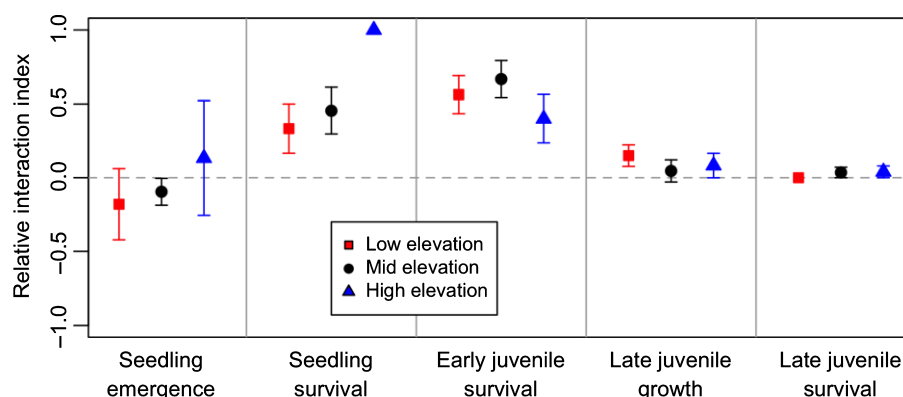


Fig. 6 Ontogenetic shifts in interaction intensity between *Artemisia tridentata* and *Pinus monophylla* for three sites along an elevational gradient. Relative interaction index (RII; shown as mean $\pm$ 1 SE), a measure of interaction intensity, where positive values indicate facilitation and negative values indicate competition, is shown for each life stage of *P. monophylla*. Seedling emergence and survival were quantified at the end of the first growing season after treatment, and early and late juvenile survival and growth were quantified at the end of the second growing season.

likely benefit *P. monophylla* juveniles by reducing cumulative atmospheric water demand (Kitzberger *et al.*, 2000; Lortie & Cushman, 2007; Holmgren *et al.*, 2012), although our data do not allow us to identify specific mechanisms of facilitation. Under-shrub environments also had longer growing seasons and higher minimum temperatures, suggesting that *P. monophylla* juveniles growing in association with *A. tridentata* are less exposed to frost damage early and late in the growing season (Carlsson & Callaghan, 1991; Callaway *et al.*, 2002). In a previous study, *A. tridentata* cover reduced overwinter mortality of *P. monophylla* seedlings (Chambers, 2001).

In contrast to the strong effects of shrub cover on non-resource stress, we found little evidence that shrubs influenced resource availability. We found no net effects of *A. tridentata* cover on near-surface soil moisture, suggesting that the positive effects of shading (Holmgren *et al.*, 2012), increased litter and organic matter (Pugnaire *et al.*, 2004), and hydraulic redistribution (Prieto *et al.*, 2011) were outweighed by shrub water uptake (Armas & Pugnaire, 2009) and canopy interception (Dunkerley, 2000). Overall, the neutral effects of *A. tridentata* cover on soil moisture

suggest that facilitation of *P. monophylla* is not likely due to increased water availability. Additionally, we did not observe the elevated levels of soil fertility under shrubs that have been observed in many dryland ecosystems (Charley & West, 1975; Chambers, 2001; Mudrak *et al.*, 2014). The substantial increase in soil available N concentrations after shrub removal could be the result of several factors, including N mineralized during the decomposition of dead roots. However, given the generally low N concentrations in these soils and the high C : N ratio of shrub roots, microbes would likely have immobilized N after shrub removal. If microbial immobilization approximately offsets N mineralized from dead shrub roots, the increase of soil available N after shrub removal represents mineralized N that might otherwise have been taken up by *A. tridentata*. This is supported by similar net soil available N concentrations under live *A. tridentata* and in interspaces. *A. tridentata* shrubs likely had higher gross N mineralization rates from the relatively larger soil organic matter pool associated with shrubs than interspaces (Schlesinger *et al.*, 1996), but plant uptake kept soil available N concentrations low. Higher gross rates of N mineralization under shrubs

would represent increased opportunities for shrub-associated plants to compete for shrub-derived N (Miller *et al.*, 2007).

Even though we did not observe net negative effects of *A. tridentata* on any tested stage of *P. monophylla* regeneration, our finding that the interaction shifted from strongly positive to neutral with *P. monophylla* ontogeny indicates weaker positive effects and/or stronger negative effects over time (Armas *et al.*, 2013; Michalet *et al.*, 2014b). We found that shrub facilitative effects declined in association with a physiological transition in *P. monophylla* from juvenile to adult foliage, a pattern that may apply to other dryland tree species. The physiological importance of the shift from juvenile to adult foliage has not been investigated for *P. monophylla*, but for other dryland conifers, such as *Juniperus* spp., the transition from juvenile to adult foliage is associated with a reduction in maximum daily carbon assimilation and stomatal conductance, and a corresponding reduction in water lost to transpiration (Miller, 1995; Nowak *et al.*, 1999). Juvenile foliage thus tends to have more negative water potentials than adult foliage for most of the growing season (Miller, 1995), which may partially explain why stress amelioration associated with *A. tridentata* shading was less important once juveniles developed adult foliage. Additionally, resource requirements increase with plant size, so *P. monophylla* ontogeny also likely corresponds to an increase in competitive interactions with *A. tridentata* for limited resources (Miriti, 2006).

Nurse shrub facilitation of tree establishment across a gradient of elevation

We found that abiotic environmental conditions, represented by sites along a gradient of elevation, did not consistently affect the outcome of the shrub–tree interaction after accounting for the influence of tree ontogenetic stage. Previous work has found that nurse plant facilitation becomes more important with greater distance from the ecological optimum of a beneficiary plant, where distance from the optimum is represented by individual performance measures such as growth rate or abundance (Lortie & Callaway, 2006; Gross *et al.*, 2010; He *et al.*, 2013, 2016). These studies did not explicitly account for life-history stage, however, and incorporating ontogenetic shifts in the beneficiary species may allow for a better understanding of the relationship between abiotic stress and interaction outcomes. In our study, elevation was positively correlated with performance (seedling survival and juvenile growth rate) of the beneficiary species *P. monophylla*, and both under-shrub and interspace microhabitats varied with elevation. Nonetheless, we did not find evidence that facilitation was stronger at lower elevation sites that are presumably more stressful (Pugnaire & Luque, 2001; Sthultz *et al.*, 2007). Also, the timing of the ontogenetic shift in the shrub–tree interaction did not differ significantly across the elevational gradient (Fig. 6; Le Roux *et al.*, 2013).

What accounts for the lack of elevational differences in interaction outcomes? One potential explanation is that elevation integrates multiple gradients of stress, which may be oriented differently in relation to the gradient of elevation (Fig. 7) and have different effects on the overall outcome of pairwise interactions (Baumeister & Callaway, 2006; Kawai & Tokeshi, 2007). Other studies in dry

mountainous environments have found complex spatial patterns of plant–plant interactions (Tielbörger & Kadmon, 2000; Callaway *et al.*, 2002; Maestre & Cortina, 2004; Cavieres *et al.*, 2006), which may be related to the presence of multiple stressors across a measured environmental gradient. Our low elevation site had higher mean and maximum temperatures, higher levels of irradiance, and lower soil moisture than the high elevation site, exposing *P. monophylla* juveniles to greater stress from increased atmospheric water demand. However, the higher elevation sites had lower minimum temperatures and more frost events, potentially exposing early stages of *P. monophylla* to frost damage in the absence of shrub cover. Additionally, more favorable precipitation amounts may be offset by variation in soil water storage, as surface soils at the mid and high elevations had larger particle sizes and a higher rock fraction and thus likely had lower water storage capacity than the low elevation site. Unmeasured sources of biotic stress, including herbivory from insects and mammals (Callaway *et al.*, 1996; Chambers, 2001; Smit *et al.*, 2007; Gómez-Aparicio *et al.*, 2008), also likely varied across the elevational gradient. Disentangling the effects of multiple stress gradients in dry, mountainous regions will require more mechanistic studies that explicitly incorporate spatial and temporal variation to define stress in ways that are functional for the focal species (Malkinson & Tielbörger, 2010).

An additional explanation for the weak relationship between elevation and interaction outcomes is that profound differences in microhabitat environments may have reduced the influence of the underlying abiotic environment on *P. monophylla* performance (Bruno *et al.*, 2003). Consistent shrub treatment effects across elevations suggests that, for the elevational range included in our study, microhabitat conditions were driven more by nurse shrub effects than by the underlying abiotic environment. Nonetheless, our results suggest that abiotic conditions are generally less favorable for *P. monophylla* at lower elevations, where under-shrub seedling survival was reduced, shrub removal induced rapid mortality of small juveniles, and juveniles had slower growth rates. This pattern can be seen at intermediate levels of abiotic stress in Fig. 1(b), where, across a broad range of abiotic conditions, interspace environments are inadequate for seedling survival while under-shrub environments are relatively favorable. At very high levels of environmental stress (left side of Fig. 1b), there likely exists a threshold beyond which shrubs cannot alleviate environmental stress to a degree needed for *P. monophylla* establishment, and seedlings cannot establish in either microhabitat (Kitzberger *et al.*, 2000). At the other extreme, in environments that are near the *P. monophylla* ecological optimum (right side of Fig. 1b), interspace environments may be favorable enough for *P. monophylla* seedling survival, and shrub cover may no longer increase establishment (Gross *et al.*, 2010). Such conditions may exist in the wettest portions of the *P. monophylla* range, where *P. monophylla* juveniles have been observed to be just as common in interspaces as under *A. tridentata* (Ziffer-Berger *et al.*, 2014). The relationship between abiotic stress and shrub modification of the microhabitat is likely non-linear (Malkinson & Tielbörger, 2010), and future research should focus on describing the shape of these relationships and identifying the abiotic thresholds associated with shrub facilitation (Soliveres *et al.*, 2015). Such knowledge would allow the

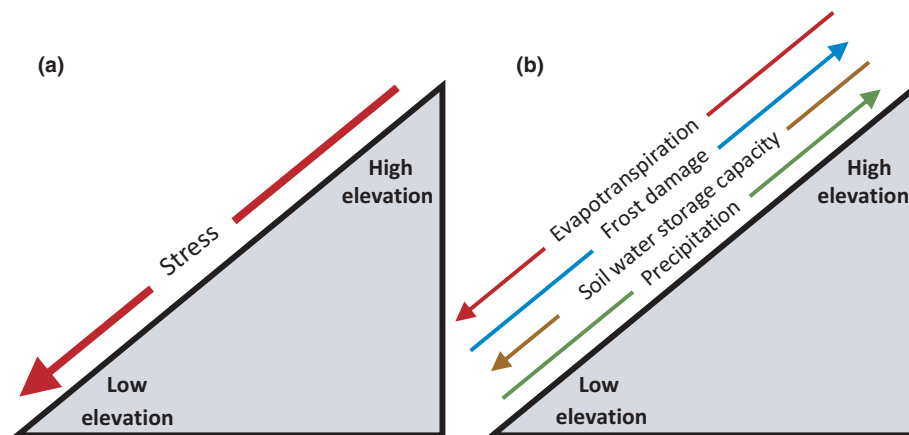


Fig. 7 Alternative models of elevational stress gradients for arid and semiarid ecosystems in which productivity increases with elevation. (a) Classical model, where stress is assumed to be inversely related to overall ecosystem productivity. (b) Multiple stressor model, where multiple gradients of stress occur simultaneously and may be oriented differently in relation to elevation.

mechanisms underlying plant–plant interactions to be incorporated into models predicting species responses to global change processes (Wisn *et al.*, 2013).

Conservation implications

Pinus monophylla and *A. tridentata* are dominant components of some of the most widespread vegetation types of the western US (US Geological Survey, 2011) and are a common example of shrub–tree interactions in arid and semiarid ecosystems worldwide. Both of these species are considered to be foundation species because they influence the distribution and abundance of many dependent community members, and their interaction is particularly important in the context of rapid global environmental change. In the western US, climate change is expected to increase the frequency and severity of disturbance events, including fire (van Mantgem *et al.*, 2013) and drought-induced tree mortality (van Mantgem *et al.*, 2009). First-year survival of *P. monophylla* seedlings represents a population bottleneck, as all seedlings in interspace microhabitats died before the end of their first growing season. Relatively high seedling survival under *A. tridentata* reaffirms the importance of shrub cover as a major factor allowing *P. monophylla* to overcome this bottleneck and successfully establish in open areas (Callaway *et al.*, 1996; Chambers, 2001). These results also indicate that favorable microsites provided by *A. tridentata* cover are crucial for *P. monophylla* recovery after stand-replacing disturbance. In consequence, *P. monophylla* responses to disturbance are tightly linked to those of *A. tridentata*, which are also highly variable and dependent on seed availability and favorable weather conditions (Ziegenhagen & Miller, 2009; Nelson *et al.*, 2014). Other shrub species may also facilitate *P. monophylla* establishment, but the spatial association of *P. monophylla* with other shrub species is much weaker than with *A. tridentata* (A. K. Urza, unpublished). Others have hypothesized that with increasing aridity, tree regeneration may become increasingly dependent on the availability of cooler, wetter microsites for successful seedling establishment (Stultz *et al.*, 2007; Redmond & Barger, 2013). We did not observe consistent differences in interaction outcomes across broad spatial gradients

of climate, but our results suggest that under more arid conditions, shrub cover may be less effective at allowing *P. monophylla* to overcome an early population bottleneck and successfully establish in areas without tree cover. Our study contributes to the growing body of evidence that species interactions are important drivers of plant community dynamics (Brown *et al.*, 2001) and should be incorporated into models of vegetation responses to global change (Araújo & Luoto, 2007; Malanson *et al.*, 2017).

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Author contributions

AKU, PJW, JCC and BWS conceived of and designed the study. AKU and PJW completed the fieldwork and collected the data. AKU and BWS conducted the soils laboratory work. AKU analyzed the data and wrote the manuscript. All authors were involved in the interpretation of the results and contributed substantially to the manuscript at all stages.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Annual precipitation totals for the cold season (October to March) and warm season (April to September) before and during the study period.

Fig. S2 Frequency distribution of the location of *Pinus monophylla* juveniles in relation to the stem of associated *Artemisia tridentata* plants.

Fig. S3 Soil temperatures at 0-cm depth from three *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient.

Fig. S4 Soil temperatures at 10-cm depth from three *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient.

Fig. S5 Surface frost occurrence in three *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient.

Fig. S6 Total inorganic nitrogen from 2016 and 2017 from three *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient.

Fig. S7 Growing season wet days from three *Artemisia tridentata* shrub microhabitat treatments at three sites along an elevational gradient.

Table S1 Overview of experimental design and environmental measurements collected across treatment types.

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