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To cite this article: Meredith D. Jabis , Matthew J. Germino & Lara M. Kueppers (2020): Colonisation of the alpine tundra by trees: alpine neighbours assist late-seral but not early-seral conifer seedlings, *Plant Ecology & Diversity*, DOI: [10.1080/17550874.2020.1762134](https://doi.org/10.1080/17550874.2020.1762134)

To link to this article: <https://doi.org/10.1080/17550874.2020.1762134>



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Published online: 19 Jun 2020.



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ARTICLE



Colonisation of the alpine tundra by trees: alpine neighbours assist late-seral but not early-seral conifer seedlings

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ABSTRACT

Background: Climate change is projected to alter the elevation and latitude of treeline globally. Seed germination and seedling survival are critical controls on treeline expansion. Neighbouring alpine plants, either through competition for resources or through altered microclimate, also affect seedlings emerging in the alpine zone. With warming, alpine plant species may interact with each other more or less strongly.

Aims: To determine whether establishing tree seedlings and an alpine herb are similarly sensitive to alpine plant neighbours under ambient and altered climate.

Methods: We imposed active heating, watering, and removed all plants adjacent to emerging conifer seedlings and an alpine herb.

Results: *Picea engelmannii* seedlings showed lower survival compared with *Pinus flexilis* 3 weeks following neighbour removal, and after 1 year only survived in watered plots. *Pinus* seedlings responded to neighbour removal by lowering the quantum yield of photosynthesis (Φ_{PSII}). Contrary to expectations from the stress gradient hypothesis, survival was reduced without neighbours near the low-elevation range limit of *Chionophila jamesii*.

Conclusions: *Pinus flexilis* has higher expansion potential into the alpine, while *Picea engelmannii* requires moist conditions that could be facilitated by neighbours to expand its range. This implies likely range expansion by *P. flexilis* with consequences for alpine plant diversity and ecosystem function.

ARTICLE HISTORY

Received 7 August 2019
Accepted 24 April 2020

KEYWORDS

Alpine tundra; *Chionophila jamesii*; climate change; competition; conifer; facilitation; plant neighbourhood; *Picea engelmannii*; *Pinus flexilis*; stress gradient hypothesis; tree colonisation

Introduction

Given the expected increase in global temperatures due to rising CO₂ levels, and the dependence of treeline position on temperature isoclines globally (Körner 1998, 2012; Paulsen et al. 2000; Körner and Paulsen 2004; Danby and Hik 2007), alpine and arctic treelines are expected to shift upward in elevation and latitude in the coming decades (Dullinger et al. 2004; Harsch et al. 2009; but see Davis and Gedalof 2018). This could result in substantial modification of current alpine plant community composition and diversity, which is primarily dominated by graminoids, forbs, succulents and low-stature woody species (Körner 1999). There is evidence of trees invading subalpine meadows over the twentieth and early twenty-first centuries (Haugo et al. 2011; Durak et al. 2015; Lubetkin et al. 2017), and of shrubs and trees beginning to expand into the alpine zone (Fortin and Pilote 2007; Myers-Smith 2007; Harsch et al. 2009; Formica et al. 2014). Expansion of trees into the alpine environment requires seed dispersal, germination and successful establishment (Malanson

et al. 2007). While temperature constraints may be primary for growth of mature trees, environmental constraints on seedling establishment may be different from those that maintain adults; microclimatic factors may be more important (Germino and Smith 1999; Maher et al. 2005; Smith et al. 2009).

Climatic conditions in the alpine zone such as low temperatures at night followed by high solar radiation the following day have been found to cause conifer seedling mortality (Germino and Smith 2000) in the alpine-treeline ecotone (hereafter referred to as treeline ecotone *sensu* Grabherr et al. 2003), and spring temperatures and soil moisture have emerged as key drivers for tree seedling establishment across several ecosystems (Weisberg and Baker 1995; Ibanez et al. 2007; Walck et al. 2011; Millar et al. 2015; Loranger et al. 2016). In the alpine zone, however, low-stature vegetation experiences less convective heat exchange and thus greater thermal decoupling from the free atmosphere. The mat-like structure of alpine herbs creates a boundary layer with a distinct climate

(Körner 1999). This layer can shade the soil surface from incoming solar radiation and thus moderate daytime high temperatures. Proximity to the ground, however, can also expose plants to cold air pooling. Greater radiative losses to a clear night sky may also expose plants to more frost events (Jordan and Smith 1994, 1995b). Thus, tree seedlings establishing away from forest, such as those in the treeline ecotone or in alpine vegetation, would experience a different microclimate than that of adult trees because of proximity to the ground and neighbouring alpine vegetation.

Assuming that viable seeds can arrive to the alpine zone from the forest edge or treeline ecotone, establishment and recruitment will likely depend on local topography-related microclimatic conditions that can be modified by the vegetation neighbourhood (Germino et al. 2002; Smith et al. 2003). Interactions between species such as competition and facilitation always co-occur, however depending on environmental conditions, one may predominate (Callaway and Walker 1997). The stress gradient hypothesis (SGH) states that under more extreme abiotic conditions, facilitation is more common between co-existing species. New tree seedlings may be competitively excluded by established plants (usually herbs) in alpine sites, presumably due to competition for resources (light, water, nutrients) with alpine neighbours (Moir et al. 1999; Tingstad et al. 2015). Alternately, the SGH predicts that, when establishing beyond their current high-elevation limit, new tree seedlings may be buffered from extreme environmental conditions such as strong and desiccating wind (Hadley and Smith 1987), temperature extremes, and intense solar radiation (Jordan and Smith 1995a; Cranston and Hermanutz 2013) by neighbouring alpine species (Germino et al. 2002; Smith et al. 2003; Malanson et al. 2007; Batllori et al. 2009; Grau et al. 2013; Neuschulz et al. 2017), and response may depend on ontogenetic stage (Loranger et al. 2017). Conversely, for alpine obligates where the treeline represents the low-elevation edge of species' distributional ranges, the hypothesis predicts that removal of neighbours would relieve competitive interactions near the treeline edge.

Consistent with the SGH, many studies in the treeline ecotone have found patterns of enhanced and often clustered tree seedling establishment near neighbouring vegetation such as beneath grass canopies (Germino et al. 2002), on the leeward side of tree islands (Hättenschwiler and Smith

1999) and boulders (Elliott 2011), or underneath abiotic shelters (Germino and Smith 1999) – all of which may moderate temperature and soil moisture (Maher et al. 2005). In addition, experimental shading and nocturnal warming, as would be experienced under the cover of alpine vegetation, had positive effects on photosynthesis in seedlings of *Picea engelmannii* and *Abies lasiocarpa* (Germino and Smith 1999). Under climate change, it is unclear how these microsite-related biotic and abiotic interactions may be modified (Spasojevic et al. 2013). It is possible that warming could eliminate any benefit of overhead shelter if, for example, warming eliminates low-temperature limitations and temperature benefits of shelter, or if warming exacerbates tree seedling water deficits such that the temperature benefits of shelter are offset (Moyes et al. 2015).

The expansion of the range of a species may place its pioneer individuals in an environment where their fitness is reduced relative to that in their original range. In addition to exposure to low temperature and radiation extremes, previous work has linked conifer seedling mortality with low volumetric soil water content (Reinhardt et al. 2015) and with drought stress evident in midday stem water potential, stomatal conductance, photosynthesis, and respiration (Moyes et al. 2013). Further, summer soil moisture has been found at least as important as temperature in limiting growing season carbon gain in conifers establishing in the treeline ecotone and within the alpine zone (Moyes et al. 2015).

The capacity to establish in a new environment depends, in part, on the life history traits and physiological properties of a species (Dullinger et al. 2004; Maher et al. 2005). Early successional tree species are expected to be adapted to high radiation and warmer temperatures, while late successional species may be more sensitive to fluctuating microclimates. Due to these differing ecological strategies, early or late seral species may demonstrate expectations of the SGH more or less strongly. In the treeline of the Colorado Front Range, *Picea engelmannii* Parry ex. Engelm (Engelmann spruce) and *Pinus flexilis* James (limber pine) are widely distributed conifers. *Pinus flexilis* is a shade-intolerant, early seral or pioneer species that can persist under conditions of high solar radiation and dry infertile soils (Letts et al. 2009), while *Picea engelmannii* is a later successional species tolerant of shade and adapted to higher moisture substrates (Rebertus et al. 1991). *P. flexilis* is adapted to low soil moisture, demonstrated by

higher intrinsic water use efficiency (WUE), a slower growth rate, and reliance on seed reserves as compared with *P. engelmannii* (Lazarus et al. 2017). Previous studies have found that survival and photosynthesis were reduced in *P. engelmannii* seedlings if there was no cover from neighbouring herbs, either naturally or following experimental removal of herb canopies, whereas fewer reductions in response to neighbours were observed in *Pinus albicaulis* (Maher et al. 2005; Bansal et al. 2011), which is closely related to *Pinus flexilis*. A key gap lies in determining whether climate warming will alter these important interactions between tree seedlings and neighbouring herbs as seedlings are establishing beyond current range margins.

We experimentally tested how neighbouring plants influenced the ability of *Picea engelmannii* and *Pinus flexilis* to establish above their elevation ranges in an undisturbed alpine meadow in the context of climate warming by using a climate change x common garden x neighbour removal experiment in the alpine zone at Niwot Ridge, Colorado. To test expectations of the SGH, we quantified the responses of an alpine-restricted herb (Weber 1976; Ackerfield 2015), Rocky Mountain snowlover (*Chionophila jamesii* Benth), to the same climate experiment, which was implemented in the lower alpine zone. We included the herb in the study to contrast a species that is presumably better adapted to the alpine abiotic environment with the two tree species. First, we asked how neighbouring alpine plants affect the survival and ecophysiology of the establishing tree seedlings and the alpine herb. We predicted that the removal of neighbouring alpine species would have a negative impact on seedling ecophysiology (measured as photosynthetic light- and water-use efficiency, ϕ_{PSII} and instantaneous WUE, respectively) in the short term, and lower survival in the longer term. Further, we expected *Picea engelmannii* to be more sensitive to neighbour removal due to its adaptation to moist, shaded conditions. Based on expectations from the SGH, we expected the alpine herb to benefit from neighbour removal (reduced WUE, and enhanced survival). Second, we were interested in how climate manipulations altered these biotic interactions. We expected warming to relieve low-temperature limitations but also to dry soils and thereby amplify the negative effects of neighbour removal for seedlings; we expected watering to offset negative effects of drier soils, thus, we expected warming and watering to amplify the benefits of neighbour removal for the alpine herb.

Materials and methods

Experimental design

We conducted our experiment at the alpine site within the Alpine Treeline Warming Experiment (ATWE) on Niwot Ridge in the Front Range of the Colorado Rocky Mountains (40°3' 14.84"N, 105° 35'37.71" W; 3540 m a.s.l.), on a shallow south-southeast facing slope. The site is 400 m above the elevation of established trees, primarily of *krummholtz* form, with thin and rocky soils. The majority of precipitation falls as snow, and snow depth is locally variable due to westerly winds and topography. Average annual precipitation and temperature are 966 mm and -2.15°C, respectively (Blanken et al. 2009). Our site is located 200 m above the lowest occurrence of the alpine herb, *Chionophila jamesii* (Ackerfield 2015).

Our fully crossed design included two arrays of 20 plots, each 3 m in diameter, assigned to four treatment groups: control (C), heated (H), watered (W), and heated + watered (HW; Figure 1). Six infrared (Mor Electric Heating, Comstock Park, MI, USA) heaters (1000 W each) were suspended 1.2 m above the ground in hexagonal arrays surrounding each heated plot (Kimball et al. 2007). The heating treatment delivered 215 W m⁻² under low wind conditions, but high wind speeds diminished heating efficiency (Kimball et al. 2007) and limited overall warming effects to +1.4°C (Kueppers et al. 2017). Once soil moisture dropped below ~0.2 m³ m⁻³ (2–3 weeks after snow-melt), we applied the equivalent of 2.5 mm of water (sourced primarily from melted snow) weekly to heated and watered plots to compensate for soil drying due to heating. Equal amounts of water were applied to (unheated) watered plots to study the impacts of increased growing season soil moisture. Annual water addition totalled ca. 30 mm, which is about 15–20% of mean June–September precipitation from 1951–2010 on Niwot Ridge (Greenland 1989; Kittel et al. 2015). See Castanha et al. (2012) and Winkler et al. (2016) for more details on the site and experimental design.

Each plot was divided into four 1 m x 1 m quadrats. One array of 20 plots was seeded in the autumn of 2014 with seed collected locally at 3370–3570 m elevation, within 8 km from the study site (Castanha et al. 2012). Although low-elevation seed was found to outperform high-elevation sources in the alpine (Kueppers et al. 2017), we chose high-elevation provenance seedlings for our study since this seed source was closest to the alpine site and most likely to arrive via natural dispersal. We sowed 280–1400

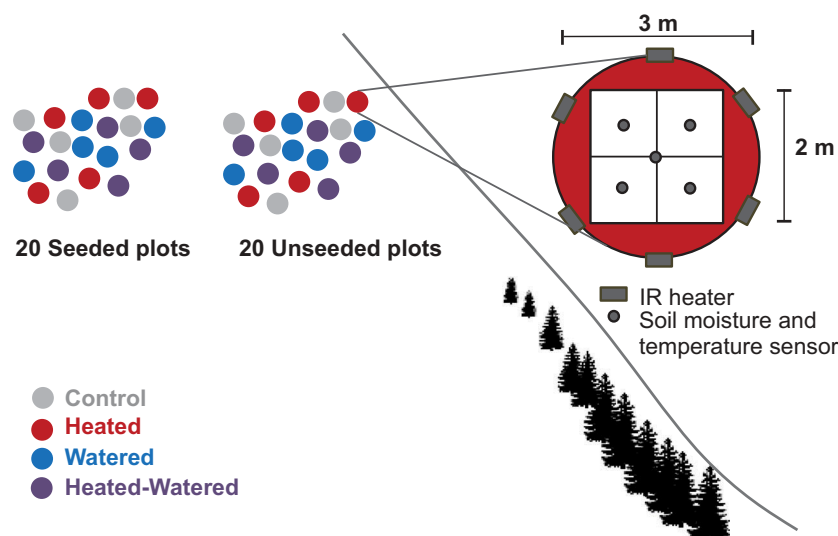


Figure 1. Experimental design for the portion of the Alpine Treeline Warming Experiment at Niwot Ridge, Colorado that is reported here, with two experimental arrays of 20 plots each. The first array was seeded with *Pinus flexilis* and *Picea engelmannii* and the second array was unseeded. Six infrared heaters suspended at 1.2 m on scaffolding directed infrared radiation into the plots. Watered plots received the equivalent of 2.5 mm extra rainfall per week during the growing season.

seeds per quadrat depending on seed availability and viability, determined by X-ray analysis (Kueppers et al. 2017). In 2015, within one of the four quadrats per plot, we selected two target individuals each of *Pinus flexilis* and *Picea engelmannii*, and removed all neighbouring alpine plants (graminoids, forbs, succulents, and cushion species) and spike moss (*Selaginella densa* Rydb.) within a 5-cm radius around each newly emerged target seedling. Additionally, we chose another two target individuals with neighbours intact ($N = 80$ per species). We chose cells with a single-germinated seedling wherever possible to minimise intraspecific interactions (although intraspecific interaction was probably low owing to the fact that seedlings were very small, usually <3 cm in height with a single thread-like taproot and a stem of 1–2 mm in diameter). Previous work at the site has revealed these young seedlings of the size and density used in our experiment had very little impact on soil resource availability or local microclimate, and thus little effect on neighbouring plants or seedling–seedling interactions (Germino, unpublished data). For *Chionophila jamesii* the neighbour removal protocol was the same, except we chose a single target and control individual within each plot of the second 20-plot experimental array ($N = 40$), which was not sown with tree seed. Survival assessments were conducted twice: at 3 weeks for seedlings and at 1 year following neighbour removal for both the alpine herb and tree seedlings to capture the short- and longer-term outcomes.

Physiology measurements

Instantaneous measurements of seedling physiology can indicate exposure to stress under recent or current environmental conditions. The efficiency of photosystem II photochemistry (ϕ_{PSII}) provides a measure of the rate of electron transport and can be used as an indication of overall photosynthetic function (Maxwell and Johnson 2000). Under conditions of greater abiotic stress, such as for plants experiencing temperature or water stress while in bright sunlight, more photons will be deflected from the photosynthetic apparatus and the efficiency of photosynthesis will decrease. Instantaneous photosynthetic water-use efficiency (WUE) is the ratio between carbon gain in photosynthesis and water loss during transpiration. It is measured as the ratio of mass of CO_2 fixed, to mass of water released into the atmosphere expressed as $\text{mg CO}_2\text{g}^{-1}\text{H}_2\text{O}$ (Nobel 1980) and provides a measure of water stress. Under low-stress conditions, the conductivity of the vascular system of a plant and the atmospheric water demand are the main constraints on water use (Lambers et al. 2008). However, when a plant is water stressed from heat, drought, or desiccation from wind (DeLucia and Schlesinger 1991) there can be more water lost per carbon gain, hence instantaneous WUE can be used to estimate one component of *in situ* plant stress.

To determine whether experimental treatments affected the capacity of the tree seedlings and an alpine herb to tolerate exposure to high light, we assessed efficiency of Photosystem II *in situ* (ϕ_{PSII})

using a photosynthesis chamber with a fluorometer attachment (6400–40; Li-Cor Biosciences). We measured the ratio of variable to maximum fluorescence, divided by fluorescence during light saturation $\phi_{\text{PSII}} = (F_v/F_m)/F_m$ (Maxwell and Johnson 2000). Measurements were made with an actinic red-light flux of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, with an additional 10% of blue light to maximise stomatal opening, and $400 \mu\text{mol CO}_2 \text{mol}^{-1}$ in the cuvette. Air temperature and humidity in the chamber were set to match environmental conditions by the flow of air into the chamber. Needles of *Picea engelmannii* seedlings were not high enough above ground (often <1 cm height) to reach the opening of the fluorometer chamber, even when the chamber head was positioned on the soil surface, so no fluorescence measurements were taken for this species. Short segments of *Pinus flexilis* needles and *Chionophila jamesii* leaf material were pressed flat in the chamber for measurement. We collected fluorescence data between 9 a.m. and 3 p.m. between 10 and 17 August 2015. Measurements were intended to be completed over the shortest time frame possible so as to be comparable; however, frequent thunderstorms shortened field days, increasing the number of days to complete measurements. Most measurements were taken during clear, sunny conditions.

To quantify the level of moisture stress the plants experienced under the experimental treatments, we assessed the ratio of carbon gain during photosynthesis to water loss via transpiration as WUE *in situ*. We used the same LICOR photosynthesis chamber as for fluorometer measurements. During all gas exchange measurements, CO_2 concentration was set to $400 \mu\text{mol CO}_2 \text{mol}^{-1}$ in the cuvette, and relative humidity and temperature were matched to ambient conditions as above. Instantaneous WUE was calculated as photosynthetic rate A ($\mu\text{mol C m}^{-2} \text{s}^{-1}$)/transpiration E ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$).

To quantify the influence of treatments on the microclimate directly adjacent to target plants, we collected soil temperature at the soil surface and percent volumetric soil water content using a probe (ECTM or 5TM; Decagon Devices, Pullman, WA, USA) over a 0–5 cm depth within a 5 cm radius of each target plant within 1–2 hours of the gas exchange and fluorescence readings. Physiological and microclimate measurements were collected 3 weeks following removal treatments and were intended to capture instantaneous differences in establishing seedlings to inform potential mechanisms driving longer-term survival

differences. Further, because seedling mortality was high, we needed to characterise seedling physiological sensitivity to treatments as soon as seedlings were of sufficient size to handle and had sufficient exposure to the treatments for differences to emerge.

Statistical analyses

To assess plant survival 3 weeks following removal, we ran separate generalised linear mixed effects models for each seedling species, including the water*removal and heat*removal interaction terms as main fixed effects and plot as a random effect with a logit link and binomial distribution (function `lme4::glmer` in R3.3.2 package Matrix). For *Pinus flexilis*, the model only included the fixed effect of removal (because minimal variation in survival prohibited a more complex model fit). To evaluate survivorship at 1 year, we ran separate models for each of the three species. Models for *Chionophila jamesii* and *Pinus flexilis* included the same terms as above, but the model for *Picea engelmannii* included only a water*removal interaction term (we separately evaluated a water*removal and a heat*removal model because very low survival in unwatered plots precluded more complex model fits). To assess treatment effects on ϕ_{PSII} and instantaneous WUE of each species, we again ran separate models for each species as above with heat*removal and water*removal as main fixed effects and plot as a random effect, but with a gaussian distribution and an identity link. Microclimate readings adjacent to plants were analysed with a similar model as above with data for the two species of seedling combined; the alpine herb readings were analysed in a separate model because it was located in an independent array. Survivorship and physiology models were built using the `lme4` package and all graphics were developed in R (R Core Team 2016; Bates et al. 2015). We summarised the models using restricted maximum likelihood, z-tests via Laplace approximation and t-tests via Satterthwaite approximations for degrees of freedom (binomial and Gaussian distributions, respectively). Significance levels were estimated using Wald χ^2 tests (`car::Anova` in R).

Results

The heating treatment increased mean (\pm SE) soil temperature at a depth of 5–10 cm by 1.4°C ($+/- 0.03$) and reduced volumetric soil water content by $0.016 \text{ m}^3 \text{m}^{-3}$ ($+/- 0.001$) over the snow-free growing

seasons of 2010–2014 in the seeded alpine plots (Kueppers et al. 2017). In the unseeded plots, heating increased mean 5–10 cm mean (\pm SE) soil temperature by 1.2°C ($+/-$ 0.07) and reduced volumetric soil water content by 0.012 m³ m⁻³ ($+/-$ 0.0016) during the period of 2010–2015. The watering treatment increased volumetric water content in seeded plots by 0.008 m³ m⁻³ ($+/-$ 0.001) and in unseeded plots by 0.025 m³ m⁻³ ($+/-$ 0.001). Three weeks following removals, the instantaneous microclimate around seedlings was warmer in removal plots (Table 1a, b). The microclimate under *Chionophila jamesii* was cooler with watering and warmer with neighbour removal (Table 1a). Heating amplified the removal effect on temperature, and removal amplified the cooling effect of watering. Watered plots also had wetter soil microclimates, although less so where neighbours were removed (Table 1b).

In partial agreement with our hypothesis that neighbour removal would negatively affect seedling survival, 3 weeks following alpine plant removal, *Picea engelmannii* survival was lower. *P. engelmannii* survival was 92% with neighbours intact and 70% following removal (Table 2; Figure 2) regardless of climate treatments. Survival of *Pinus flexilis* was essentially unaffected, with 98.5% survival regardless of removal or climate treatment.

Seedling survivorship 1 year following removal of neighbouring plants was different among species. In agreement with predictions that *Picea engelmannii* would be more impacted by treatments, the only *P. engelmannii* survivors in heated plots were also watered; in fact, *P. engelmannii* seedlings only survived in watered plots (Table 3; Figure 3) regardless of removal. For *Pinus flexilis*, the early-seral, drought-tolerant species, survivorship did not respond to any of the neighbourhood or climate

treatments. For the alpine herb, *Chionophila jamesii*, removal marginally ($P < 0.1$) reduced survival (Table 3); a larger sample size may provide more statistical power and greater confidence in this result.

In agreement with our prediction that neighbour removal would reduce the efficiency of photosynthesis in conifer seedlings in the alpine, ϕ_{PSII} was lower in *Pinus flexilis* seedlings with neighbours removed, but was not impacted consistently by heating and/or watering (Table 4, Figure 4). Conversely, for the alpine herb, *Chionophila jamesii*, ϕ_{PSII} was unaffected by neighbour removal and was marginally reduced ($P < 0.1$) with additional heat.

Instantaneous WUE was higher in *Picea engelmannii* ($t = 5.85$, $P < 0.0001$; Figure 5) as compared with *Pinus flexilis* and *Chionophila jamesii*. Contrary to our expectations for any of the species, instantaneous WUE was reduced for *P. flexilis* when plants were warmed and neighbours were removed (Table 5).

Discussion

Facilitation predominant in the lower alpine zone

Two of our three species, a late-seral conifer and an alpine herb, benefitted from intact plant neighbours at opposite edges of their respective ranges: above the high-elevation distribution of tree seedlings and near the low-elevation distributional limits of the alpine herb. Prior studies have found patterns of seedling establishment and success to be unique to species life history and physiology (Loranger et al. 2016). While alpine plant species have been traditionally

Table 1. Linear mixed effects model parameter estimates for main effects and two-way interactions for (a) instantaneous surface soil temperature (°C) and (b) instantaneous volumetric soil moisture (%) around seedlings of *Picea engelmannii* and *Pinus flexilis* and individuals of *Chionophila jamesii*, under climate treatments heating, water watering and 3 weeks following neighbour removal (Removal) taken within an hour of physiology measurements, Niwot Ridge, Colorado. $P < 0.05$ is indicated with bold type.

	<i>Picea engelmannii</i> , <i>Pinus flexilis</i> seedlings					<i>Chionophila jamesii</i>				
	Coef	SE	χ^2	df	$P(\chi^2)$	Coef	SE	χ^2	df	$P(\chi^2)$
Table 1a										
Intercept	16.61	1.42				15.23	1.21			
Heating	-0.02	1.64	0.007	1	0.93	-2.23	1.40	0.65	1	0.42
Watering	-1.65	1.64	0.49	1	0.48	-1.65	1.40	3.81	1	0.05
Removal	0.69	1.08	5.98	1	0.01	1.32	0.80	10.47	1	0.001
H*Removal	0.29	1.22	0.057	1	0.81	2.35	0.91	6.76	1	0.009
W*Removal	1.17	1.22	0.92	1	0.34	-1.87	0.91	4.29	1	0.04
Table 1b										
Intercept	6.21	0.54				6.46	1.00			
Heating	-0.08	0.62	1.42	1	0.23	-1.18	1.14	0.10	1	0.75
Watering	1.03	0.62	11.57	1	0.000	3.86	1.14	7.63	1	0.006
Removal	-0.43	0.71	0.29	1	0.59	0.13	1.23	0.02	1	0.89
H*Removal	-1.00	0.80	1.54	1	0.21	2.92	1.40	4.35	1	0.04
W*Removal	1.22	0.80	2.29	1	0.13	-2.73	1.40	3.81	1	0.05

Table 2. Linear mixed effects model parameter estimates for main effects and two-way interactions for survival 3 weeks following neighbour removal (Removal) within heating and watering treatments of *Picea engelmannii* and *Pinus flexilis*, Niwot Ridge, Colorado. Wald χ^2 tests and P -values, $P(\chi^2)$, were calculated for effects of treatments and their interactions. Effects had two levels, so the coefficient for the level not shown is the same magnitude but opposite sign as the coefficient listed. Plot was included as a random effect. $P < 0.05$ is indicated with bold type.

	<i>Picea engelmannii</i>					<i>Pinus flexilis</i>				
	Coef	SE	χ^2	df	$P(\chi^2)$	Coef	SE	χ^2	df	$P(\chi^2)$
Intercept	2.03	0.83				8.82	3.24			
Heating	0.36	0.86	0.60	1	0.44					
Watering	−1.10	0.91	0.03	1	0.86					
Removal	−2.87	1.02	12.04	1	0.001	7.10E-07	1.49	0	1	1.00
H*Removal	0.06	1.08	0.00	1	0.96					
W*Removal	1.53	1.12	1.86	1	0.17					

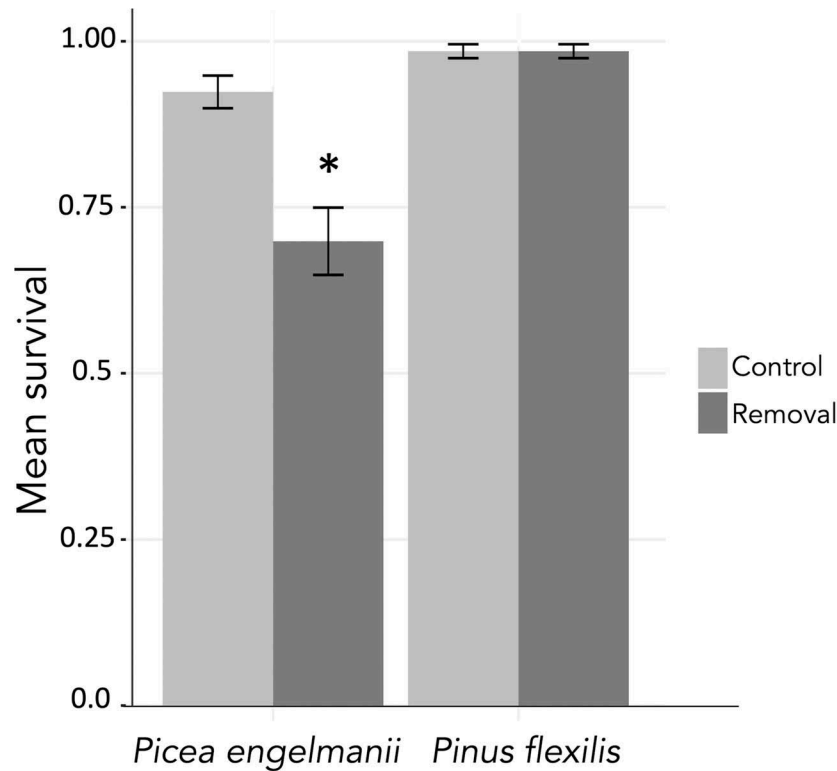


Figure 2. Mean (± 1 SE) survival of *Picea engelmannii* and *Pinus flexilis* seedlings 3 weeks after the removal of all neighbouring plants within a radius of 5 cm, Niwot Ridge, Colorado; (see Table 2). $P < 0.05$ is indicated by an asterisk.

Table 3. Linear mixed effects model parameter estimates for main effects and two-way interactions for one-year survivorship of *Picea engelmannii*, *Pinus flexilis* and the alpine herb *Chionophila jamesii* following neighbour removal (Removal) within heating and watering treatments, Niwot Ridge, Colorado. Wald χ^2 tests and P -values, $P(\chi^2)$, were calculated for effects of treatments and their interactions. Effects had two levels, so the coefficient for the level not shown is the same magnitude but opposite sign as the coefficient listed. Plot was included as a random effect. $P < 0.1$ are indicated with bold type.

	<i>Picea engelmannii</i>					<i>Pinus flexilis</i>					<i>Chionophila jamesii</i>				
	Coef	SE	χ^2	df	$P(\chi^2)$	Coef	SE	χ^2	df	$P(\chi^2)$	Coef	SE	χ^2	df	$P(\chi^2)$
Intercept	−136.60	316.00				−0.05	0.58				−0.24	0.51			
Heating						0.53	0.68	0.99	1	0.32	0.19	0.58	0.13	1	0.71
Watering	124.50	316.00	0.16	1	0.69	−0.11	0.68	1.20	1	0.27	0.22	0.58	1.99	1	0.16
Removal	−1459.90	355.20	0.00	1	0.9901	−1.65	0.91	2.42	1	0.12	−1.15	0.61	2.82	1	0.09
H*Removal						−0.03	0.98	0.00	1	0.97	−0.02	0.66	0.00	1	0.98
W*Removal	1460.00	355.20	16.90	1	3.95E-05	1.56	1.00	2.44	1	0.12	1.04	0.68	2.38	1	0.12

thought to compete with tree seedlings for resources including nutrients, light and water (Billings and Bliss 1959; Tingstad et al. 2015), other evidence has suggested positive associations between plant neighbours – including

shrubs, krumholtz trees, and grasses – and tree seedling density and survival (Weisberg and Baker 1995; Hättenschwiler and Smith 1999; Germino and Smith 1999; Germino et al. 2002; Smith et al. 2003; Maher et al. 2005; Maher and

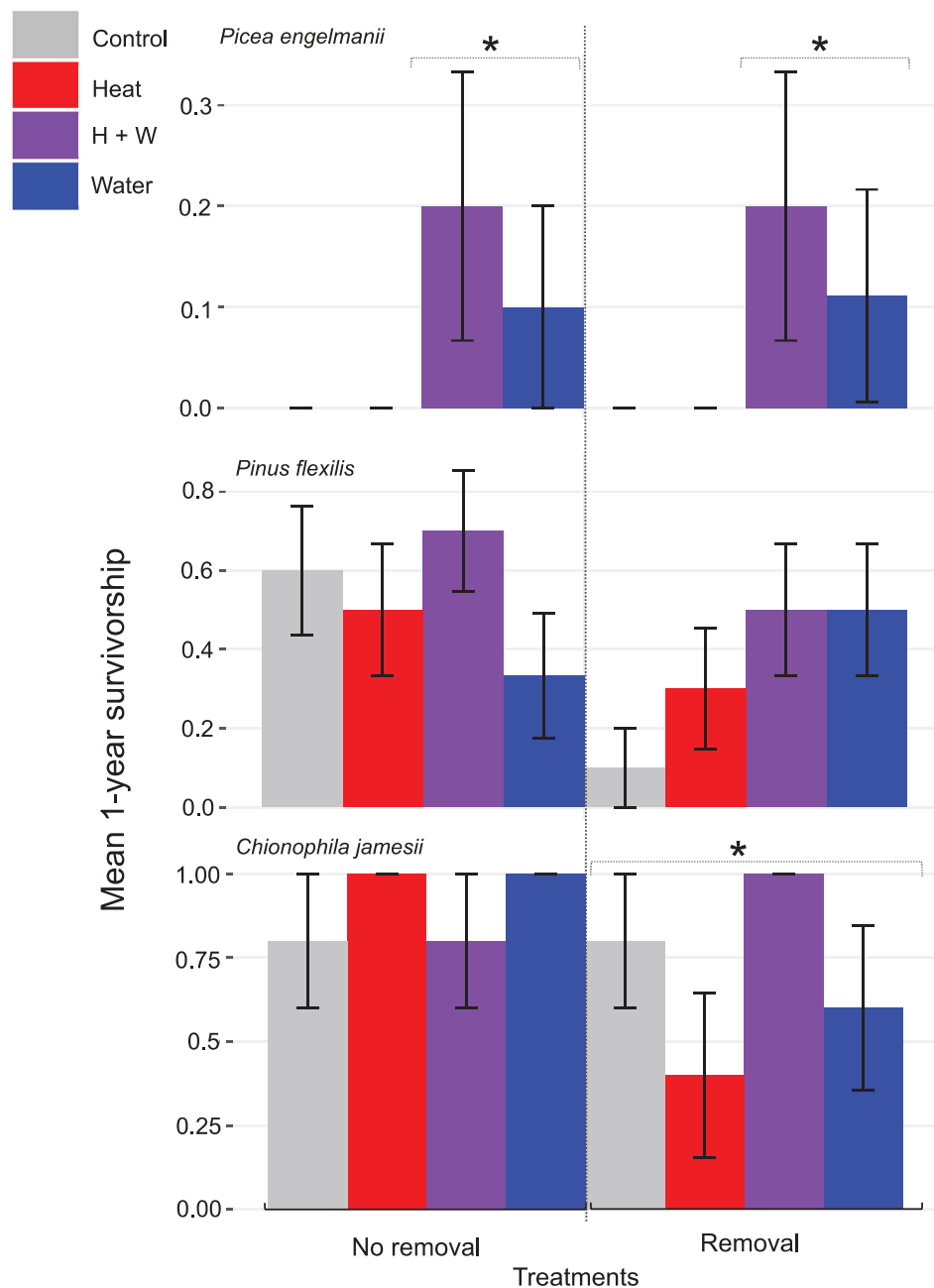


Figure 3. Mean (± 1 SE) survivorship of *Picea engelmannii* and *Pinus flexilis* seedlings and *Chionophila jamesii* individuals 1 year after the removal of all neighbouring plants within a radius of 5 cm, Niwot Ridge, Colorado (see Table 3). $P < 0.1$ are indicated with asterisks.

Table 4. Linear mixed effects model parameter estimates for main effects and two-way interactions for ϕ_{PSII} in *Pinus flexilis* seedlings and *Chionophila jamesii* following neighbour removal (Removal) within heating and watering treatments, Niwot Ridge, Colorado. Wald χ^2 tests and P -values, $P(\chi^2)$, were calculated for effects of treatments and their interactions. Effects had two levels, so the coefficient for the level not shown is the same magnitude but opposite sign as the coefficient listed. Plot was included as a random effect. $P < 0.1$ are indicated with bold type.

	<i>Pinus flexilis</i>					<i>Chionophila jamesii</i>				
	Coef	SE	χ^2	df	$P(\chi^2)$	Coef	SE	χ^2	df	$P(\chi^2)$
Intercept	0.31	0.07				0.20	0.02			
Heating	-0.12	0.08	2.02	1	0.16	-0.02	0.03	2.89	1	0.09
Watering	0.08	0.08	0.26	1	0.61	0.00	0.03	0.15	1	0.69
Removal	-0.09	0.06	10.48	1	0.001	0.03	0.03	1.46	1	0.227
H*Removal	0.02	0.07	0.10	1	0.75	-0.02	0.04	0.14	1	0.71
W*Removal	-0.10	0.07	1.63	1	0.20	0.02	0.04	0.33	1	0.57

Germino 2006; Grau et al. 2013). These previous studies and our findings suggest neighbours can facilitate tree seedling recruitment through several potential mechanisms relevant to seedling

establishment: reducing incoming solar radiation and thermal extremes for seedlings and the soil around them, buffering intense and drying wind, and redistribution of snow.

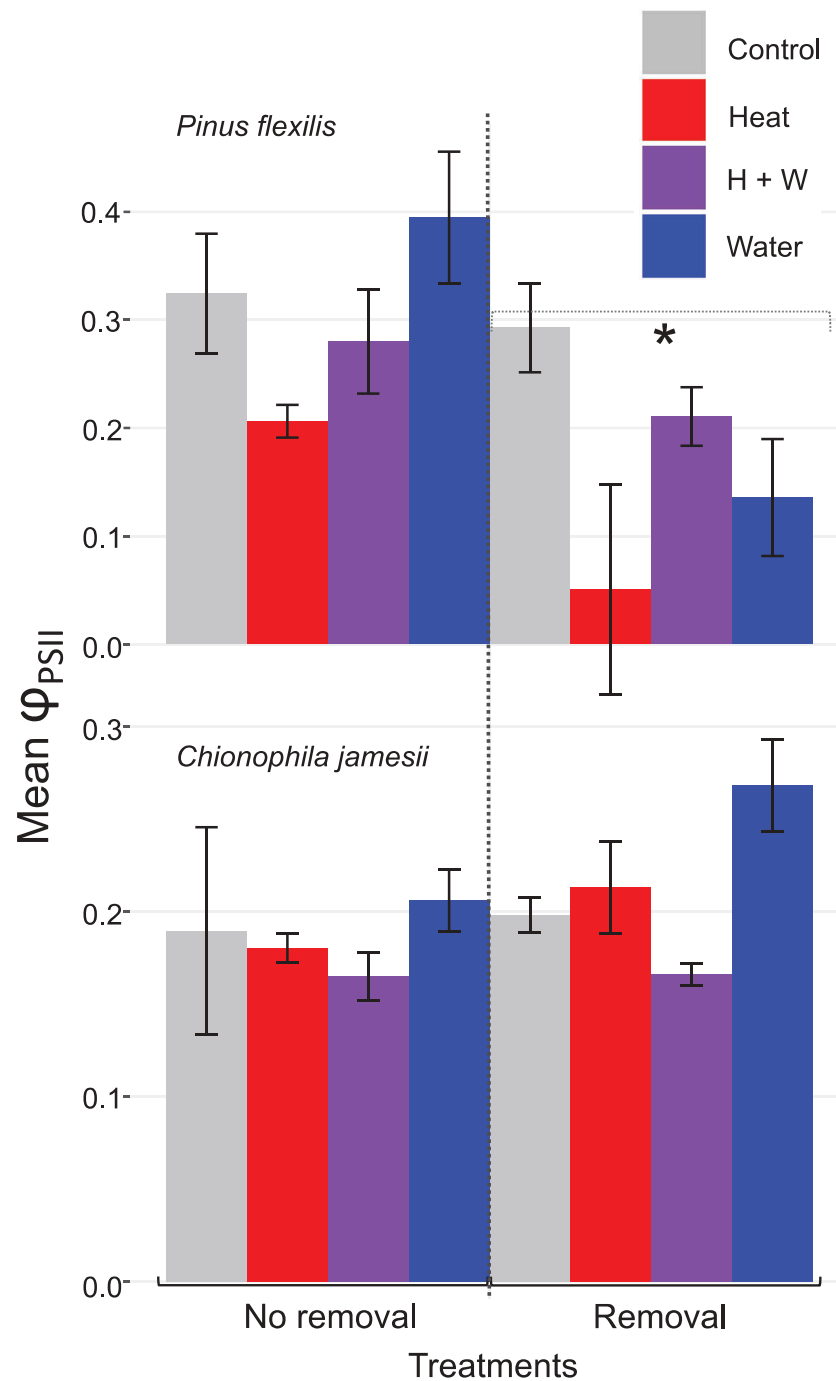


Figure 4. Mean (± 1 SE) photosynthetic efficiency (ϕ_{PSII}) measured in *Pinus flexilis* seedlings and *Chionophila jamesii* individuals 3 weeks after the removal of all neighbouring plants within a radius of 5 cm, Niwot Ridge, Colorado (see Table 4). $P < 0.05$ are indicated with an asterisk.

In the alpine zone, which is above the distributional range of tree seedlings (<3 cm in height), the presence of plant neighbours appears to enhance survival for the mesic and shade tolerant conifer species *Picea engelmannii*. Its seedlings perform better with neighbours intact as they showed greater survival in the first 3 weeks with neighbours. *P. engelmannii* seedlings and saplings in the treeline ecotone of the Medicine Bow Mountains have been reported to occur most frequently near islands of adult trees (Germino et al. 2002), and not to persist

in open exposed microsites (Bansal et al. 2011). Further, cold night-time temperatures have been found to kill *P. engelmannii* seedlings (Helmers et al. 1970). We did not measure night-time minimum temperatures in seedling microsites so are unable to confirm whether neighbouring species provided protection from cold night-time temperatures, but we did record warmer daytime temperatures where neighbours were removed (Table 1a), suggesting a less buffered surface environment. In addition, Maher et al. (2005) observed that tree and

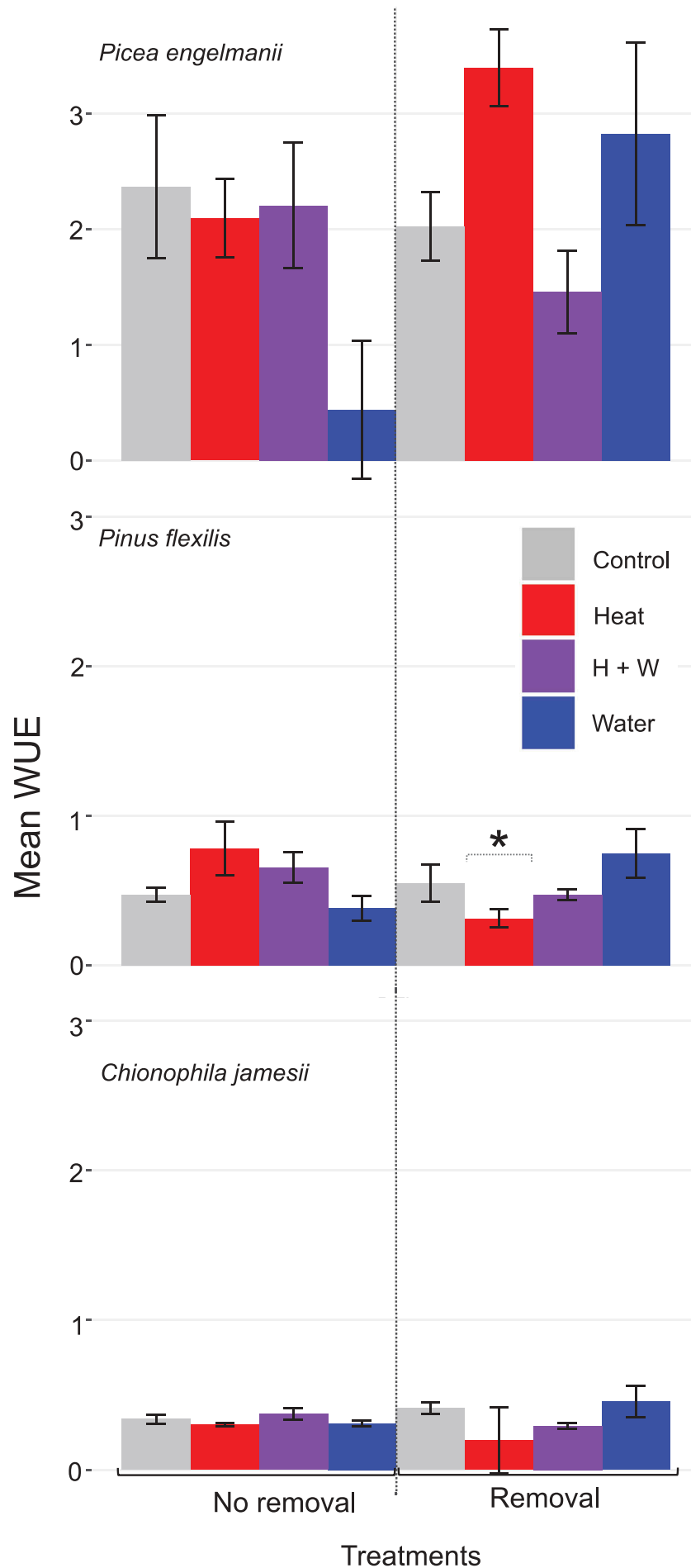


Figure 5. Mean (± 1 SE) water use efficiency (WUE) measured in *Picea engelmannii* and *Pinus flexilis* seedlings and in *Chionophila jamesii* individuals 3 weeks after the removal of all neighbouring plants within a radius of 5 cm, Niwot Ridge, Colorado (see Table 5). $P < 0.05$ are indicated with an asterisk.

Table 5. Linear mixed effects model parameter estimates for main effects and two-way interactions for water use efficiency in *Picea engelmannii*, *Pinus flexilis* and *Chionophila jamesii* following neighbour removal (Removal) within heating and watering treatments, Niwot Ridge, Colorado. Wald χ^2 tests and *P*-values, $P(\chi^2)$, were calculated for effects of treatments and their interactions. Effects have only two levels, so the coefficient for the level not shown is the same magnitude but opposite sign as the coefficient listed. Plot was included as a random effect. *P* < 0.1 are indicated with bold type.

	<i>Picea engelmannii</i>					<i>Pinus flexilis</i>					<i>Chionophila jamesii</i>				
	Coef	SE	χ^2	df	$P(\chi^2)$	Coef	SE	χ^2	df	$P(\chi^2)$	Coef	SE	χ^2	df	$P(\chi^2)$
Intercept	1.51	0.78				0.48	0.15				0.32	0.07			
Heating	1.09	0.90	0.64	1	0.42	0.30	0.17	0.04	1	0.83	0.01	0.08	1.82	1	0.18
Watering	−0.81	0.90	0.62	1	0.43	−0.09	0.17	0.08	1	0.78	0.01	0.08	0.43	1	0.51
Removal	0.52	0.66	0.02	1	0.88	0.05	0.13	0.65	1	0.42	0.08	0.10	0.09	1	0.769
H*Removal	−1.37	0.81	2.89	1	0.09	−0.51	0.16	10.72	1	0.001	−0.20	0.12	0.22	1	0.64
W*Removal	0.46	0.81	0.32	1	0.57	0.25	0.16	2.60	1	0.11	0.05	0.12	2.99	1	0.08

herb cover had additive effects on survivorship and photosynthesis of conifer seedlings (including *P. engelmannii*) except under alleviated water stress, implicating the relevance of soil moisture in seedling establishment as we found in this study.

Inconsistent with our expectations, *Pinus flexilis* survival was not affected by neighbour removal, suggesting neutral interactions with neighbours. Given its ability to tolerate relatively dry conditions on exposed slopes (Rebertus et al. 1991; Letts et al. 2009), this result is reasonable. In the central Rockies of Colorado, solitary pines with no spruce (*Picea engelmannii*) or fir (*Abies lasiocarpa*) neighbours have been reported to have a greater chance of survival than individuals within clumps of six or more trees (Donnegan and Rebertus 1999). Despite its clear tolerance of intense light, this conifer species has not yet extended its distribution and established in the alpine zone. We thus presumed that neighbours would provide some moderation of the large alpine temperature fluctuations and intense solar radiation that would be reflected in physiology even if survival was unaffected. Indeed, neighbour removal reduced photosynthetic efficiency, indicating that seedlings experienced some elevated level of stress but not enough to cause mortality. The relatively high survival rate of *P. flexilis* may be due to its capacity to engage in photo-protective mechanisms. *Pinus albicaulis*, another 5-needled pine that also is able to establish in less protected sites in the alpine treeline ecotone, has less vulnerability to photosystem damage than *P. engelmannii* (Germino and Smith 1999, 2000). Further, when *P. flexilis* seedlings were heated and neighbours were removed, seedling WUE was lower. This could be due to less conservative water use due to changes in the environment, or, more likely, lowered photosynthesis led to the reduced WUE consistent with the *in situ* response by ϕ_{PSII} (Figure 4). In another study at this site, Moyes et al. (2013) found lower rates of growth and maintenance

respiration at high temperature in warmed plots, which suggested respiratory but not photosynthetic acclimation to warmer growing temperatures.

Our site was ideal for testing the stress gradient hypothesis for the native herb *Chionophila jamesii* because the site was situated just above treeline at 3540 m, 200 m above the low-elevation range limit of this species (Ackerfield 2015). Contrary to the SGH, which predicts competitive release following neighbour removal in the lower – compared to upper elevation alpine (Callaway 1995, 1997; Callaway and Walker 1997), we found no benefits of neighbour removal on survival of *C. jamesii* and even observed some negative responses. The presence of neighbours was important for the survival of *C. jamesii* regardless of climate treatment, suggesting facilitation is still the predominant interaction near the low-elevation distributional limit of this species. For the SGH to be further examined for this species, we would need to determine if facilitation is weaker at this low-elevation site relative to a higher site, or if the relevant stress shifts from one factor (e.g., temperature) to another (e.g., soil moisture).

Microclimate and climate change

The success of *Picea engelmannii* in the alpine ecosystem appears to be influenced by the local microclimate. Of all species studied, we predicted that *P. engelmannii* would be most negatively affected by the alpine environment due to its preference for high soil moisture and shade (Kaufmann and Eckard 1977; Gill et al. 2015). In comparison, *Pinus flexilis* displays an adaptive response to drier conditions by maintaining a generally high intrinsic WUE (Lazarus et al. 2017). *P. engelmannii* displayed the highest instantaneous WUE of all three species 3 weeks following neighbour removal, symptomatic of severe water stress. *P. engelmannii* seedlings maintained higher WUE than expected based on prior measurement

over the lifetime of seedlings in the alpine zone and treeline ecotone (Lazarus et al. 2017), which may indicate some amount of stomatal closure to conserve water in the current study. Thus, soil moisture availability is likely to be an important limiting factor for *P. engelmannii* in the alpine. Further, *P. engelmannii* only survived in watered plots, regardless of other treatments, over the 1-year timeframe of the study. Since these plots were also moister (Table 1b), greater survival in moister plots is also consistent with water limitation. Multiple years of data from the same climate change experiment has shown that warming reduced recruitment of *P. engelmannii* above the tree-line primarily due to soil drying (Kueppers et al. 2017). Similarly, photosynthesis and respiration were highest for potted spruce seedlings at moisture deficits less than 10% (Ronco 1970). Indeed, in other subalpine forest studies, *P. engelmannii* was less drought-tolerant than *Pinus flexilis* or *Pinus contorta* and would therefore be expected to require amelioration of the environment before it could establish and eventually colonise (Donnegan and Rebertus 1999; Lazarus et al. 2017).

In a study in the Swiss Alps, seedling success for a number of tree species has not been found to be limited by current climate above treeline (Zurbriggen et al. 2013). In our study, local climatic modification did not significantly alter *Pinus flexilis* seedling survival over 1 year, suggesting that the species may possess adaptive mechanisms that enhance initial seedling growth in the alpine environment compared to other tree species, particularly in the context of climate change and greater water limitation. *P. flexilis* seedlings are generally larger and have deeper roots than those of *Picea engelmannii* (Lazarus et al. 2017), which may allow them to better tolerate both drying associated with warming and the alpine environment without protection from neighbouring species.

For an alpine herb, we predicted that heating would relieve low-temperature constraints to growth, which are expected to some extent even in cold-adapted species, and thus benefits of neighbouring plants were expected to be less under heated conditions (viz. Callaway et al. 2002). Removal of neighbours did lower survivorship, but experimental heating did not change mortality. Although over the full growing season, heating did increase soil temperatures, our instantaneous daytime temperature measurements adjacent to plants following neighbour removals indicate that warmed microsites were only significantly warmer with neighbours removed (Table 1a). Given that

Chionophila jamesii is a relatively long-lived species, it is possible that treatments were not strong enough or that the timeframe of exposure (1 year) was not long enough to elicit a response.

Neighbours, moisture, and range expansion of trees

Understanding the mechanisms that underpin success in the early stages of tree establishment is critically important for projecting the impact of climate change on future tree ranges and potential re-organisation of alpine community composition (Suding et al. 2015). Biotic interactions between tree seedlings and neighbouring species appear to be important for a later-seral conifer, but not so relevant for an early-seral conifer species. In our study, *Picea engelmannii* demonstrates low potential for range expansion – particularly on the south-eastern aspect where we conducted the experiment. All *P. engelmannii* seedlings perished except those that were watered, suggesting a major constraint will be soil drying associated with projected warming. Thus, establishment in the alpine zone may depend on the actual future temperature and summer precipitation. The combination of warmer and wetter conditions following the end of the little ice age in Rocky Mountain National Park have been associated with *P. engelmannii* and *Abies lasiocarpa* colonisation in the treeline ecotone in the last 150 years (Hessl and Baker 1997). *Pinus flexilis* seedlings are larger, have deeper roots, grow more slowly, and exhibit a greater WUE than *P. engelmannii* (Lazarus et al. 2017), which may allow them to better tolerate drying associated with current warming and provide a physiological advantage for establishment within the alpine environment. This species may be able to tolerate competition from alpine vegetation as well as recruit into exposed vegetation gaps.

Conclusions: implications for treeline advances

This study complements niche models and plant demographic studies, which suggest reductions in the current range of *Picea engelmannii* with warming (Dobrowski et al. 2015; Kueppers et al. 2017), by highlighting the limits to facilitation at the leading edge of potential *P. engelmannii* range shifts. The upper edge of its range could be extended with additional summer precipitation and via biotic interactions with neighbours, which may include

shading, which reduces soil drying and needle desiccation, wind buffering, and reduction of incoming solar radiation. Even without enhanced precipitation, the alpine will likely become more open to establishment by *Pinus flexilis* due to its ability to tolerate low moisture conditions. This structural community change will likely have implications for future biodiversity and ecosystem function (Malanson et al. 2007). Tree expansion into the alpine zone could ultimately result in modified local environmental conditions created by a forest canopy such as reduced wind speeds, shading of soils and greater coupling with the free atmosphere (Germino and Smith 1999), which could enhance further seedling recruitment (Dobrowski et al. 2015). Ecosystem consequences of greater tree cover at higher elevations include carbon sequestration and a lower albedo (Jackson et al. 2008) that could, in turn, have feedbacks to the climate system (Bonan 2008).

Acknowledgements

This research was supported by the Office of Science (BER), US Department of Energy Grant No. DE-FG02-07ER64457. We thank the Mountain Research Station and Niwot Ridge LTER at the University of Colorado, Boulder for logistical support. We appreciate the hard work of Scott Ferrenberg, Ethan Brown, and Andrea Campanella for maintaining the experiment and seasonal field assistant Sam Sartwell for assisting with data collection. Thanks to John Harte, Todd Dawson, and David Ackerly for helpful comments. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. government.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Department of Energy, Office of Science (BER) [DE-FG02-07ER64457].

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