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Warming and provenance limit tree recruitment across and beyond the elevation range of subalpine forest

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Abstract

Climate niche models project that subalpine forest ranges will extend upslope with climate warming. These projections assume that the climate suitable for adult trees will be adequate for forest regeneration, ignoring climate requirements for seedling recruitment, a potential demographic bottleneck. Moreover, local genetic adaptation is expected to facilitate range expansion, with tree populations at the upper forest edge providing the seed best adapted to the alpine. Here, we test these expectations using a novel combination of common gardens, seeded with two widely distributed subalpine conifers, and climate manipulations replicated at three elevations. Infrared heaters raised temperatures in heated plots, but raised temperatures more in the forest than at or above treeline because strong winds at high elevation reduced heating efficiency. Watering increased season-average soil moisture similarly across sites. Contrary to expectations, warming reduced Engelmann spruce recruitment at and above treeline, as well as in the forest. Warming reduced limber pine first-year recruitment in the forest, but had no net effect on fourth-year recruitment at any site. Watering during the snow-free season alleviated some negative effects of warming, indicating that warming exacerbated water limitations. Contrary to expectations of local adaptation, low-elevation seeds of both species initially recruited more strongly than high-elevation seeds across the elevation gradient, although the low-provenance advantage diminished by the fourth year for Engelmann spruce, likely due to small sample sizes. High- and low-elevation provenances responded similarly to warming across sites for Engelmann spruce, but differently for limber pine. In the context of increasing tree mortality, lower recruitment at all elevations with warming, combined with lower quality, high-provenance seed being most available for colonizing the alpine, portends range contraction for Engelmann spruce. The lower sensitivity of limber pine to warming indicates a potential for this species to become more important in subalpine forest communities in the coming centuries.

Keywords: alpine treeline, climate change experiment, Engelmann spruce, limber pine, Picea engelmannii, Pinus flexilis, seedling demography, species range shift

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Introduction

Shifts in the geographic distributions of species are a widely anticipated consequence of global warming (Chen et al., 2011; Moritz & Agudo, 2013), especially in high-elevation ecosystems where temperature is thought to be a primary constraint (Lenoir et al., 2008; Gottfried et al., 2012; Pauli et al., 2012). In particular, projections using climate niche models suggest rapid upslope shifts in habitat suitable for subalpine trees with warming (e.g., Rehfeldt et al., 2006; Monahan et al., 2013). Low summer temperatures have been observed to limit tree growth above treeline (Rossi et al., 2008; Korner, 2012) and 20th c. warming led to increased tree growth and migration to higher elevations, especially where the magnitude of warming was large (Harsch et al., 2009; Salzer et al., 2009). However, evidence from high-latitude Siberian forests (Frost & Epstein, 2014) to low-latitude cloud forests (Rehm & Feeley, 2015) indicates that moisture can also limit upper treeline position. For example, while Holocene warming generally resulted in tree advances upslope in the western United States (Morgan et al., 2014) and Europe (Schwoerer et al., 2014), fluctuations in temperature, moisture, or both drove regionally synchronous
retreats in treeline elevations (Morgan et al., 2014). At lower elevations, increased exposure to acute and chronic water stress is increasing tree mortality in woodlands and forests (Van Mantgem et al., 2009), including subalpine forests (Smith et al., 2015), although persistent global change effects can be difficult to distinguish from natural modes of variability (Fellows & Goulden, 2012). However, recent studies have also suggested that, with climate change, some populations may shift down slope (Lenoir et al., 2010) due to increased precipitation (Crimmins et al., 2011; but see Stephenson & Das, 2011), net cooling effects from cold-air drainage (Van de Ven et al., 2007), or altered interactions with other species. Thus, upper and lower tree distribution limits can be dynamically sensitive to both temperature and moisture.

Tree recruitment above treeline is required for upward forest range expansion. Similarly, lack of recruitment at the low-elevation edge of a species’ range ultimately leads to range contraction. Seedling germination and establishment are particularly climate sensitive (Walck et al., 2011) and could determine whether and how fast elevation shifts occur (Malanson et al., 2007; Smith et al., 2009). Observational studies that relate periods of tree establishment to particular climatic regimes or compare climate envelopes of adults and juveniles have provided valuable insights (Ibanez et al., 2007; Lenoir et al., 2009; Mclaughlin & Zavaleta, 2012; Zhu et al., 2012; Dobrowski et al., 2015; Millar et al., 2015), but cannot distinguish climate effects on establishment from seed availability or reliably disentangle temperature and moisture effects. To project range shifts with climate change, models require distinguishing among these varied climate and demographic influences on recruitment. Experimental investigations of climate controls on subalpine seedling recruitment have found greater survival and recruitment with greater precipitation or soil moisture, including at and above treeline, albeit with species differences in sensitivity (Gill et al., 2015; Tingstad et al., 2015; Loranger et al., 2016). Experimentally determined effects of temperature on subalpine tree recruitment are somewhat more idiosyncratic, depending on landscape position, neighboring vegetation, and life stage (Grau et al., 2013; Zurbriggen et al., 2013; Loranger et al., 2016). Warming more consistently enhances growth of established seedlings at high elevations (Danby & Hik, 2007; Grau et al., 2013; Zurbriggen et al., 2013). However, most in situ temperature manipulation studies use open-top chambers, which introduce reinforcing and countervailing effects. They reduce wind speed and can decrease nighttime temperatures among other effects, which also alter seedling performance.

Finally, local adaptation to historical climate may leave seedlings poorly adapted to the new climate, requiring dispersal to new, upslope locations to maintain fitness (Rehfeldt et al., 1999; Wang et al., 2010; Valladares et al., 2014). Genetic differentiation has been found across vertical (elevation) distances of just 200–450 m for Rocky Mountain conifers (reviewed in Mitton, 1995). Alternately, gene flow among populations, which is thought to be high for wind-pollinated trees, and phenotypic plasticity may enable populations to track the changing climate and maintain fitness, facilitating both persistence and range shifts (Kremer et al., 2012; Alberto et al., 2013). Quantifying the climatic and genotypic factors affecting recruitment is critical to understanding and projecting how climate change will alter forest distribution and composition.

Here, we report the effects of experimental climate change on seedling recruitment for two subalpine conifers widely distributed in North America, limber pine (Pinus flexilis James) and Engelmann spruce (Picea engelmannii Parry ex. Engelm), at three elevations at Niwot Ridge, Colorado, USA. We used infrared heating and watering during the growing season to separate temperature and moisture effects. Across 5 years, we sowed >750 000 seeds collected locally from high- and low-elevation provenances to assess the importance of genetic variation. Our experiment was designed to answer the following questions: (i) How does warming alter initial tree recruitment and recruitment to 4 years across and beyond the elevation range of subalpine forest? (ii) What effects does supplemental summer water have on recruitment at low and high elevations? (iii) How does seed source affect recruitment and responses to climate treatments? (iv) How do these responses differ between late-seral Engelmann spruce and shade-intolerant limber pine? Our prior work has shown that warming can increase moisture stress in establishing limber pine, reducing photosynthesis and respiration, as well as initial seedling survival (Moyes et al., 2013). At treeline, and with warming in the alpine, temperature and moisture appear to colimit seasonally integrated seedling carbon assimilation (Moyes et al., 2015). We have also found provenance differences in germination and early seedling survival (Castanha et al., 2013), as well as in metabolic rates and seedling size (Reinhardt et al., 2011). This analysis extends our prior work, evaluating the importance of early physiological and demographic responses to later outcomes over a wider range of experimental and interannual climate variability.

Materials and methods

Experimental design

Our study was conducted at Niwot Ridge, on the eastern slope of the Rocky Mountains in Colorado, USA. We established
experimental sites in (i) a stand of mature trees near the low-elevation edge of subalpine forest (3060 m), (ii) an open meadow surrounded by krummholz mats and low tree islands at upper treeline (3430 m), and (iii) an alpine meadow approximately 400 m upslope from local timberline (3540 m; Fig. 1, Table 1). Soils were thin and rocky at all sites, with higher organic matter in the treeline and alpine sites than in the forest, and a ~2-cm organic horizon in the forest site (Castanha et al., 2013). During the period of the experiment, ambient annual air temperature differed by 4.6 °C across the three experimental sites and mean snow-free season length differed by 30 days (Table 1). Snowfall is typically greater at higher elevations at Niwot Ridge due to orographic effects (Kittel et al., 2015), but snow depth is locally variable due to substantial wind redistribution, and was greatest at the treeline site. Aside from an extreme rainfall event in September 2013, which resulted in significant flooding at lower elevations and raised plot soil moisture but did not damage or otherwise directly affect our research sites, climate variability during the years of the experiment (2009–2014) was within the range of historical climate variability documented by the nearest USHCN station in Boulder, CO, from 1931 to 2014.

Each site had 20 circular, 3-m-diameter plots assigned to four groups: control, heated, watered, and heated and watered (Fig. 1). Six 1000-W infrared heaters arrayed around the perimeters of heated plots (following the geometry of Kimball et al., 2008) with constant heater output (following Harte et al., 1995) raised mean daily 5–10 cm soil temperature (ECTM and \( \Delta T \)) to compensate for evaporative losses due to heating, adding water manually with hand-held sprayers once per week, starting 2–3 weeks after snow melt and ending in September. Watering slightly increased soil volumetric water content (+0.01 m³ m⁻³) at 5–10 cm depth at all sites (Table 1).

Seedling recruitment

Our two focal species are widely distributed in Western North America. Engelmann spruce (\( P. \) engelmannii Parry ex. Engelm) occurs at high elevations up to treeline in the Rocky Mountains and in the Cascade Ranges. Limber pine (\( P. \) flexilis James) also occurs up to treeline in the Rocky Mountains, Sierra Nevada, and Great Basin ranges. Unlike the late-seral, shade-tolerant Engelmann spruce, limber pine is often a shade-intolerant pioneer species and can persist on exposed, infertile sites. Both species can live for hundreds or >1000 years. Each autumn, we collected local seeds from high (3370–3570 m) and low (2910–3240 m) elevations (Castanha et al., 2013). Each species and seed provenance was randomly assigned to one of four 1 × 1 m quadrats nested within each 7.1-m² circular plot at the start of the experiment. We sowed 280–1400 seeds per quadrat each autumn (>750 000 seeds total over 5 years), depending on seed viability (as determined by X-ray analysis) and availability. For each provenance and year, we collected 200–1300 limber pine cones from trees and 1500 to 15 000 Engelmann spruce cones from new caches (we estimate an average of 15–70 cones per tree, with some interannual variability). When new seed availability was low, we sowed excess seed from a prior year that had been stored at −20 °C. USPS personnel extracted Engelmann spruce seed from cones and dewinged it in their Coeur d’Alene nursery, and we extracted limber pine seed from cones. Seed was not treated prior to sowing and was carefully blended among source trees to ensure comparable seed went into all plots. Initial trials transplanting glasshouse-started seedlings into the experimental plots were discontinued after we determined that survival rates were elevated for transplants relative to in situ germinants (Castanha et al., 2013). To exclude small mammals, we placed hardware cloth cages (1-cm² openings) over each plot. The exclosures substantially reduced granivory and herbivory (Castanha et al., 2013); remaining noise in the

![Fig. 1 Experimental design for the Alpine Treeline Warming Experiment. Sixty plots were sown annually with high- and low-elevation limber pine and Engelmann spruce seeds in separate quadrants. The alpine site is above treeline, the treeline site is at the upper elevation edge of subalpine forest, and the forest site is near the low-elevation edge of subalpine forest. Soil temperature and moisture sensors recorded microclimate at the center of the four quadrants. Six infrared heaters were suspended at 1.2 m on scaffolding that could be lifted and lowered with the accumulation and melt of snow. Watered plots received 2.5 mm water per week during the growing season.](https://example.com/fig1.png)
### Table 1  Field site climate and treatment effects on microclimate

<table>
<thead>
<tr>
<th></th>
<th>Elev. (m)</th>
<th>Mean air $T$ ($^\circ$C)</th>
<th>PAR (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Wind (m s$^{-1}$)</th>
<th>SL (days)</th>
<th>$\Delta T_H$ ($^\circ$C)</th>
<th>$\Delta SL_H$ (days)</th>
<th>AVWC$_H$ (m$^3$ m$^{-3}$)</th>
<th>AVWC$_W$ (m$^3$ m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>3060</td>
<td>9.8 (0.4)</td>
<td>179 (9)</td>
<td>0.2 (0.0)</td>
<td>158 (9)</td>
<td>3.81 (0.04)</td>
<td>38 (10)</td>
<td>−0.012 (0.001)</td>
<td>0.014 (0.0004)</td>
</tr>
<tr>
<td>Treeline</td>
<td>3430</td>
<td>7.4 (0.5)</td>
<td>448 (13)</td>
<td>3.6 (0.2)</td>
<td>133 (6)</td>
<td>1.72 (0.05)</td>
<td>12 (4)</td>
<td>−0.012 (0.001)</td>
<td>0.008 (0.001)</td>
</tr>
<tr>
<td>Alpine</td>
<td>3540</td>
<td>6.7 (0.5)</td>
<td>446 (12)</td>
<td>5.8 (0.3)</td>
<td>140 (11)</td>
<td>1.38 (0.03)</td>
<td>10 (2)</td>
<td>−0.016 (0.001)</td>
<td>0.008 (0.001)</td>
</tr>
</tbody>
</table>

Daily mean (2010–2014) site climate and treatment effects during the snow-free season, with standard errors in parentheses. Abbreviations are photosynthetically active radiation (PAR); snow-free season length (SL; determined from diel variability in soil temperature in unheated plots); heating effects on 5–10 cm soil temperature ($\Delta T_H$), snow-free season length ($\Delta SL_H$), and 5–10 cm soil volumetric water content (AVWC$_H$); and watering effect on 5–10 cm soil volumetric water content (AVWC$_W$).

Data from damage by animals was likely greatest at the forest site where small mammal pressure was greatest (C. Castanha, personal observation). We tracked the number of seeds sown and number of seedlings surviving to autumn of each year for each cohort. The 2010 and 2011 cohorts were observed for 4 years, the 2012 for 3 years, the 2013 for 2 years and the 2014 for 1 year.

### Statistical analysis

Due to the differences in heating effects on soil and plant temperatures across sites, we estimated effects of heating, watering, provenance and all two-way interactions on seedling recruitment and survival separately for each site using generalized linear mixed effects models (function `lme4::glmer` in R 3.1.2 package mgcv) with a logit link and binomial distribution. We present summary results in figures and tables below, with full results of statistical analyses, including parameter estimates, for first-year and fourth-year recruitment, as well as year-to-year survival of Engelmann spruce and limber pine seedlings in the following tables: first-year recruitment (Tables S1 and S2), fourth-year recruitment (Tables S3 and S4), first- to second-year survival (Tables S5 and S6), second- to third-year survival (Tables S7 and S8), and third- to fourth-year survival (Tables S9 and S10). For models of first-year recruitment, random effects included cohort year, plot within cohort year, and an observation-level random effect to accommodate over-dispersion. For models of fourth-year recruitment and year-to-year survival, cohort year was instead treated as a fixed effect.

Prior to running the statistical models and hypothesis tests, we performed exploratory analyses on first-year recruitment to determine how many interactions to include in hypothesis testing. We used MuMln::dredge to compare all possible models (Burnham & Anderson, 2002). Using a model formulation that includes greater than second-order interactions with a limited data set would have so many terms that no effects would be estimated well. More years of data or more replicates would be necessary to determine full interactions among the four crossed factors employed in our experiment.

Significance levels were estimated using likelihood ratio tests. Using a hypothesis-testing framework, which is consistent with a manipulation experiment, we determined which estimated effects, inclusive of all possible two-way interactions, are not consistent with chance under null hypotheses.

Because GLMM likelihood ratio tests in R do not automatically compute a likelihood ratio for main effects separately from their interactions, to test main effects we directly manipulated the design matrix (stats::model.matrix). In manipulating the design matrix, we chose effect, or deviation, coding (using ‘contr.sum’ in R). Thus, the intercepts for the models represent the average recruitment (or survival) across all factors. The coefficient estimates in the Tables S1–S11 reflect the deviation from this average for a given treatment. Thus, all levels across a factor should sum to zero (where there are two levels, we present just one since the other is the same magnitude but opposite sign). We mention this because this is not the default output in lme4::glmer.

As a measure of the amount of variation explained by each model, we computed both marginal and conditional $R^2$ values following the method of Nakagawa & Schielzeth (2013) using MuMln::r.squaredGLMM (Table S11). Marginal $R^2$ reflects variance explained only by fixed factors, whereas conditional $R^2$ reflects variance explained by fixed and random factors in each model.

### Results

#### Heating effects

Consistent with the hypothesis that warming near the low-elevation edge of subalpine forest would reduce recruitment, we found dramatic decreases in first-year recruitment (survival to end of the first growing season as fraction of sown seeds) (Tables 2, 3, S1 and S2), by 95% and 62% for Engelmann spruce and limber pine, respectively (Fig. 2a, b). Contrary to our expectations that warming at high elevations would increase recruitment, we found, instead, that warming reduced first-year recruitment at treeline (−28.3% and −22.6% for Engelmann spruce and limber pine) and alpine (−16.0% and −11.7%) sites, although these effects were marginal (0.05 < P < 0.11) for limber pine (Fig. 2a, b; Tables 2, 3, S1 and S2).

As seedlings matured, we detected less sensitivity to warming for Engelmann spruce (Tables S3, S5, S7, S9), possibly as a result of reduced sample sizes, culling of more frail individuals, or because of physiological or...
morphological changes. Specifically, first- to second-, second- to third-, and third- to fourth-year Engelmann spruce survival were not significantly affected by warming at any site \((P > 0.06)\). Therefore, the negative effect of warming in the first year carried through to the fourth year at treeline and alpine sites (Table 4, Fig. 3). Heating effects were more pronounced in the 2010 cohort than the 2011 cohort at treeline and in the alpine, although this interaction was marginally significant in the alpine (Tables 4 and S3). At the forest site, no Engelmann spruce seedlings survived to their fourth year in heated plots (lines that stop before year four in Fig. 3e, f), emphasizing the early and acute sensitivity of this species to warming at low elevation. In treeline and alpine sites, 9.3% and 41.3% fewer seedlings survived to their fourth year in heated and unheated plots, respectively (Tables 4 and S3).

Seedling survival actually increased with warming for limber pine seedlings at forest and alpine sites in the second to third year \((P < 0.05; \text{Table S8})\), with a significant heat by cohort year interaction at the treeline site \((P = 0.0072)\). Ultimately, for limber pine, there was no net effect of warming on recruitment to the fourth year; the later benefits of warming reversed, but did not overcome, the negative effect of warming on first-year recruitment in any site (Tables 5 and S4; Fig. 4).

Watering effects

Consistent with our hypothesis that additional summer moisture would enhance recruitment, first-year recruitment increased with watering across sites for Engelmann spruce (Fig. 2c; Tables 2 and S1), but was not significantly different for limber pine at any site (Fig. 2d; Tables 3 and S2). For Engelmann spruce, benefits of watering diminished through time, with effects on year-to-year seedling survival and fourth-year recruitment marginal or not significant \((P > 0.06; \text{Tables S3, S5, S7, S9})\), likely due in part to declining sample sizes. For limber pine, watering increased first-to second-year survival at all sites \((P < 0.05; \text{Table S6})\), and ultimately increased recruitment to the fourth year at the forest site (Fig. 4), where heating significantly diminished the benefits of watering (Tables 5 and S4).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Forest</th>
<th>Treeline</th>
<th>Alpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed to first year</td>
<td>LRT (df)</td>
<td>(P (\chi^2))</td>
<td>LRT (df)</td>
</tr>
<tr>
<td>Heat</td>
<td>46.26 (1)</td>
<td>(&lt; 10^{-5})</td>
<td>11.54 (1)</td>
</tr>
<tr>
<td>Water</td>
<td>4.80 (1)</td>
<td>0.028</td>
<td>10.34 (1)</td>
</tr>
<tr>
<td>Provenance</td>
<td>6.06 (1)</td>
<td>0.014</td>
<td>6.22 (1)</td>
</tr>
<tr>
<td>Heat \times water</td>
<td>2.76 (1)</td>
<td>0.097</td>
<td>0.03 (1)</td>
</tr>
<tr>
<td>Heat \times provenance</td>
<td>0.81 (1)</td>
<td>0.37</td>
<td>1.07 (1)</td>
</tr>
<tr>
<td>Water \times provenance</td>
<td>0.36 (1)</td>
<td>0.55</td>
<td>0.02 (1)</td>
</tr>
</tbody>
</table>

Random effects variances (for forest, treeline, and alpine, respectively) are 1.08, 1.82, and 4.14 for cohort year, 3.92, 0.64, and 0.55 for plot (nested in cohort year), and 2.13, 0.46, and 0.42 for the observation-level random effect. \(P < 0.05\) are given in bold type. All parameter estimates are given in Table S1.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Forest</th>
<th>Treeline</th>
<th>Alpine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed to first year</td>
<td>LRT (df)</td>
<td>(P (\chi^2))</td>
<td>LRT (df)</td>
</tr>
<tr>
<td>Heat</td>
<td>31.91 (1)</td>
<td>(&lt; 10^{-5})</td>
<td>2.60 (1)</td>
</tr>
<tr>
<td>Water</td>
<td>1.38 (1)</td>
<td>0.24</td>
<td>0.05 (1)</td>
</tr>
<tr>
<td>Provenance</td>
<td>17.84 (1)</td>
<td>(2.4 \times 10^{-5})</td>
<td>62.29 (1)</td>
</tr>
<tr>
<td>Heat \times water</td>
<td>0.03 (1)</td>
<td>0.85</td>
<td>0.19 (1)</td>
</tr>
<tr>
<td>Heat \times provenance</td>
<td>0.42 (1)</td>
<td>0.52</td>
<td>0.01 (1)</td>
</tr>
<tr>
<td>Water \times provenance</td>
<td>0.08 (1)</td>
<td>0.78</td>
<td>0.09 (1)</td>
</tr>
</tbody>
</table>

Random effects variances (for forest, treeline, and alpine, respectively) are 1.25, 1.37, and 1.31 for cohort year, 1.00, 0.44, and 0.11 for plot (nested in cohort year), and 1.16, 0.60, and 0.86 for the observation-level random effect. \(P < 0.05\) are given in bold type. All parameter estimates are given in Table S2.
At treeline, any benefits of water addition to fourth-year limber pine recruitment were contingent on heating, with benefits in unheated but not heated plots (Tables 5 and S4).

**Provenance effects**

Contrary to our expectation that seeds would be locally adapted, the low-elevation seed provenance had greater first-year recruitment than the high-elevation provenance for both Engelmann spruce and limber pine, at all sites (Tables 2, 3, S1 and S2). For Engelmann spruce, the biggest differences were at the forest and alpine sites, where low-provenance recruitment was roughly double that of high (Fig. 2e), with a smaller provenance effect at the treeline site (Table S1). For limber pine, the provenance effects were similar across sites (Fig. 2f). The Engelmann spruce high-elevation provenance disadvantage was not significant at any site for older seedling survival ($P > 0.07$; Tables S5, S7, S9), and cumulative fourth-year recruitment was not significantly different between provenances at treeline or alpine sites (Tables 4 and S3). As limber pine seedlings matured, the high-provenance disadvantage persisted, with worse first- to second- and second- to third-year survival at treeline ($P < 0.03$; Tables S6 and S8), and with 70.4%, 57.8%, and 71.3% less fourth-year recruitment in forest, treeline, and alpine sites, respectively (Tables 5 and S4).

Provenances initially responded similarly to warming and watering, with no significant provenance by treatment interactions for first-year recruitment of either species (Tables 2, 3, S1, S2). For limber pine establishing in the forest, heating diminished the
Table 4 Generalized linear mixed effects model likelihood ratio tests (LRT) and P-values, $P(\chi^2)$, for main effects and all two-way interactions on fourth-year recruitment of Engelmann spruce

<table>
<thead>
<tr>
<th>Engelmann spruce Seed to fourth year</th>
<th>Treeline LRT (df)</th>
<th>P(\chi^2)</th>
<th>Alpine LRT (df)</th>
<th>P(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>4.51 (1)</td>
<td>0.034</td>
<td>4.27 (1)</td>
<td>0.039</td>
</tr>
<tr>
<td>Water</td>
<td>3.33 (1)</td>
<td>0.068</td>
<td>0.21 (1)</td>
<td>0.65</td>
</tr>
<tr>
<td>Provenance</td>
<td>1.55 (1)</td>
<td>0.21</td>
<td>2.65 (1)</td>
<td>0.10</td>
</tr>
<tr>
<td>Cohort</td>
<td>39.18 (1)</td>
<td>&lt;10^{-5}</td>
<td>8.07 (1)</td>
<td>0.0045</td>
</tr>
<tr>
<td>Heat × water</td>
<td>0.96 (1)</td>
<td>0.33</td>
<td>1.01 (1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Heat × provenance</td>
<td>0.16 (1)</td>
<td>0.69</td>
<td>0.14 (1)</td>
<td>0.71</td>
</tr>
<tr>
<td>Heat × cohort</td>
<td>7.74 (1)</td>
<td>0.0054</td>
<td>3.34 (1)</td>
<td>0.068</td>
</tr>
<tr>
<td>Water × provenance</td>
<td>0.36 (1)</td>
<td>0.55</td>
<td>0.14 (1)</td>
<td>0.71</td>
</tr>
<tr>
<td>Water × cohort</td>
<td>0.21 (1)</td>
<td>0.65</td>
<td>1.70 (1)</td>
<td>0.19</td>
</tr>
<tr>
<td>Provenance × cohort</td>
<td>2.65 (1)</td>
<td>0.10</td>
<td>0.91 (1)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

There are no values for forest because only five plots had survivors to their fourth year – all in the control and watered groups. Random effects variances (for treeline and alpine, respectively) are 0.48 and 0.17 for plot and 0.36 and 1.93 for the observation-level random effect. $P < 0.05$ are given in bold type. All parameter estimates are given in Table S3.

low-provenance advantage (a negative interaction), while in the alpine, the low-provenance advantage was enhanced by heating (Tables 5 and S4). These effects emerged with first- to second-year survival in the alpine and with second- to third-year survival in the forest (Tables S6 and S8). At treeline, watering diminished the low-provenance advantage in first- to second-year survival of limber pine ($P = 0.020$; Table S6), but this effect did not persist to the fourth year.

Discussion

Our results suggest that high-elevation mountain forests with water-limited growing seasons, such as in Western North America, are likely to experience recruitment declines or, at best, no new advantage as climate warms, growing seasons lengthen, and evaporative demand increases. Recruitment declines with warming are the opposite of what is required to compensate for increasing adult mortality in Western U.S. forests (Van Mantgem et al., 2009; Williams et al., 2013; Smith et al., 2015). While warming decreased recruitment across all sites for Engelmann spruce, dramatic reductions at the forest site (Fig. 2) likely reflect greater realized experimental warming in this site, which had lower wind speeds and therefore greater increases in soil and plant temperatures with infrared heating (Table 1). We did not test whether tree seedlings establishing at treeline and alpine sites would experience similarly dramatic reductions with temperature increases comparable to those in the forest. However, the fact that modest warming (1–2 °C) reduced spruce recruitment at and above treeline highlights the sensitivity of this species. Decreased Engelmann spruce recruitment with warming is consistent with reported reductions in seed germination at high temperatures and at low moisture (Kaufmann & Eckard, 1977) and with a negative correlation between temperature and radial growth for mature Engelmann spruce in dry sites over the period 1899–1991 (Villalba et al., 1994).

Contrary to our expectations, negative effects of warming can even affect recruitment in sites at and above treeline. Previous studies at treeline have found preferential seedling survival in microsites protected from frost (Maher et al., 2005; Maher & Germino, 2006) and that passive nighttime warming can alleviate low temperature stress and increase seedling survival (Germino & Smith, 1999; Batllori et al., 2009). Unlike studies using open-top chambers, which can cool at night (Danby & Hik, 2007) by limiting convective warming of the surface (reducing sensible heat flux from the air to the surface), infrared heaters raise temperatures during the day and night. Therefore, the negative effects of heating that we observed are not due to unexpected nighttime cooling in heated plots at treeline or in the alpine. While Harsch et al. (2009) found that many alpine treelines have been advancing upslope in response to historical warming, they also found that nearly half have not, suggesting other factors may constrain responses to temperature trends in many sites. Winter damage and low temperature photoinhibition during the growing season have been identified as factors limiting tree establishment even in the presence of warming (reviewed in Harsch & Bader, 2011).

Moisture limitation, and an attendant lack of evaporative cooling, should be considered a significant constraint to recruitment even at and beyond upper treeline. In our experiment, heating decreased soil moisture (Table 1), likely exacerbating moisture stress during germination and initial establishment. First-year limber pine recruitment increased with greater surface soil moisture and shorter growing seasons (Moyes et al., 2013), with second-year seedling carbon assimilation roughly equally limited by temperature and moisture at treeline (Moyes et al., 2015). While warming does alleviate low temperature stress early and late in the growing season in our alpine and treeline sites, warming throughout the growing season appears to counteract this benefit by enhancing moisture stress (Moyes et al., 2015). Further, we found that adding summer moisture increases Engelmann spruce first-year recruitment across all sites and limber pine fourth-year recruitment in the forest. These results are corroborated by a precipitation manipulation...
experiment conducted in a subalpine meadow in Utah, which found that over-summer Engelmann spruce seedling survival was best correlated with soil moisture (Gill et al., 2015). Notably, watering had no effect on limber pine recruitment at the treeline site (Tables 3 and 5), where recruitment was strongest overall (Figs 2 and 4), and where snow melt is latest and the growing season is shortest (Table 1). We hypothesize that warming increased seedling mortality risk by lengthening the growing season and reducing near-surface moisture, elevating incidence of hydraulic failure in sensitive new germinants, and by degrading seedling carbon balance via increased respiration and reduced carbon assimilation, even in normal precipitation years.

For the Rocky Mountains, we have experimentally shown that summer moisture may provide an additional constraint to tree establishment at and above treeline and that warming may exacerbate, rather than
ameliorate, this constraint. Our results for seedlings are qualitatively consistent with observations and predictions of increased adult tree mortality in response to water stress (Van Mantgem et al., 2009; Williams et al., 2013; McDowell & Allen, 2015), and with positive correlations between precipitation and radial growth (Villalba et al., 1994) for adult subalpine trees, but reveal quantitatively greater impacts on vital rates for these more sensitive, younger life stages (Dobrowski et al., 2015). While Western North America is known to have water-limited growing seasons, warming will lengthen mountain growing seasons and increase evaporative demand everywhere, potentially altering the recruitment environment beyond recent historical conditions in mountain ranges worldwide.

The greater initial (first year) Engelmann spruce response to watering may help explain species differences in response to warming. Because limber pine seeds and emergent seedlings are much larger and have deeper roots than emergent Engelmann spruce seedlings, limber pine may be better able to withstand the drying that accompanies warming and benefit sooner from warming at high elevations. Limber pine first-year recruitment was much greater than Engelmann spruce recruitment overall (note y-axis labels on Fig. 2), and limber pine seedlings that survived the first year then were unaffected or benefited from heating (and watering) in later years. Conversely, Engelmann spruce seedlings did not see any benefit from heating at any site. Consistent with our findings, niche model projections using forest inventory data across the Western United States point to greater recruitment declines in Engelmann spruce than limber pine with warming (Dobrowski et al., 2015). Differences in species responses to warming and water stress suggest a potential for shifts in high-elevation forest composition, similar to past climate-driven reorganization of plant communities (Le Roux & McGeoch, 2008). In particular, tree species that successfully establish above current treeline may differ from those that are currently abundant in upper elevation forests. While limber pine is currently a minor component of the forest and treeline communities in Colorado, future warming could provide it a competitive advantage within the forest and in colonizing alpine tundra. Ultimately, future forest composition will also depend on the growth, mortality, and reproductive responses of established trees to climate change, and on species interactions that could change as trees and forest stands mature, and as the environment changes (Boyden et al., 2009; Gómez-Aparicio et al., 2011; Ettinger & Hille Ris Lambers, 2013).

Reorganization of forest communities may be accompanied by changes in the genetic structure of populations. Unexpectedly, the low-elevation provenance had greater recruitment across all sites, including the alpine site, suggesting a clear advantage for low-elevation genotypes, at least at this early life stage. It is not clear why the low provenance generally had higher recruitment. High-provenance seeds were not consistently smaller or less viable than low-provenance seeds but may have differed in less apparent ways due to different maternal environments. Low-provenance seedlings seemed to emerge earlier than high-provenance seedlings, enabling a longer first growing season, although longer growing seasons may not favor survival (Moyses et al., 2013). Low-provenance seedlings also have greater photosynthesis and carbon balance (photosynthesis/respiration) than high-provenance seedlings (Reinhardt et al., 2011). These phenological and physiological differences may reflect a more rapid,
competitive growth strategy yielding more robust low-provenance seedlings regardless of site. In this region, which is largely snow melt-driven (Hu et al., 2010), earlier snow melt is often accompanied by earlier water stress, which frequently is not ameliorated until consistent monsoon precipitation in July and August. Early emergence and high assimilation and growth rates may help low-provenance seedlings avoid desiccation. It is also possible that over the 5 years of our study, climatic conditions happened to be favorable for the low-elevation provenance and that a series of years with less favorable conditions (e.g., frequent summer frost) could have favored the high provenance at high elevations. However, recent warming and associated changes in growing season length (Clow, 2010; Oyler et al., 2015) may have already made treeline and alpine sites more climatically similar to lower elevation sites of the past, making adaptations for tolerating low temperature...
(e.g., frost during the growing season) less advantageous now and in the future. For limber pine, heating enhanced the low-provenance advantage in the alpine site, suggesting that future population establishment in the alpine could depend, in part, on the origin of dispersed seed. More work is needed to assess whether the provenance differences we observed in seedlings are maintained in older trees, which may have additional provenance-specific climate sensitivities (McLane et al., 2011).

Greater recruitment of the low-elevation provenance at high elevations has important implications for forest range shifts. If high-elevation seed, which is most readily available for colonizing alpine and treeline sites, has lower recruitment, tree migration into the alpine will be slowed. At the same time, if low-elevation tree mortality continues to increase (Smith et al., 2015), and if this reduces the number of reproductive trees, the degree of gene flow between high- and low-elevation populations may become an important constraint to rates of migration. Alternately, if gene flow from low elevations can increase the performance of high-elevation seed sources over time, keeping up with climate change, then adaptation and upslope range expansion may accelerate over time (Alberto et al., 2013). More work is needed to understand factors limiting gene flow among populations along elevation gradients, including whether climate change can alter historical constraints such as mismatched reproductive phenology (Schuster et al., 1989). While previous studies have highlighted the importance of climatically adapted genotypes to predictions of future tree distributions and forest productivity (Wang et al., 2010; Valladares et al., 2014), they have typically assumed that adult tree traits are predictive of demographic outcomes and neglected traits shaping the regeneration niche (Grubb, 1977). Such regeneration traits may be critical for determining the pace of range shifts with climate change.

Understanding the sensitivity of young life stages to climate is critically important for projecting the impact of climate change on tree populations and distributions. The climate and provenance effects described here emerged across years that varied substantially in snow melt timing and summer rainfall, and occurred within a forest thought to be maintained by continuous replacement rather than episodic recruitment (Knowles & Grant, 1983). Therefore, given (i) observed increases in mature tree mortality in the forest (Smith et al., 2015), (ii) a clear and large negative effect of warming on seedling recruitment in the forest, and (iii) a negative effect of warming on seedling recruitment above treeline, we expect the elevational range of Engelmann spruce to contract in the coming centuries. Limber pine appears better able to persist within its current range and expand upslope due to compensating positive and negative effects of warming on recruitment in the alpine and in the forest. To quantitatively estimate the consequences of climate-limited recruitment for forest tree population sizes and rates of migration requires integration of our experimental results with data on tree growth, mortality, seed production, and dispersal in spatially explicit demographic models. Such models would also enable us to explore whether episodically favorable conditions within the long lifetime of these species could overwhelm the shifts in mean conditions that we describe here and thereby maintain existing populations or enable an abrupt upslope expansion. Further work is also needed to determine whether climate sensitivities and provenance differences are consistent across life stages, to quantify the climate sensitivities of co-occurring species, and to assess how changing forest composition will affect high-elevation biodiversity and ecosystem function.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

- **Table S1.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on first-year recruitment of Engelmann spruce.
- **Table S2.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on first-year recruitment of limber pine.
- **Table S3.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on fourth-year recruitment of Engelmann spruce.
- **Table S4.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on fourth-year recruitment of limber pine.
- **Table S5.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on first to second-year survival of Engelmann spruce.
- **Table S6.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on first to second-year survival of limber pine.
- **Table S7.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on second to third-year survival of Engelmann spruce.
- **Table S8.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on second to third-year survival of limber pine.
- **Table S9.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on third to fourth-year survival of Engelmann spruce.
- **Table S10.** Generalized linear mixed effects model parameter estimates for main effects and all two-way interactions on third to fourth-year survival of limber pine.
- **Table S11.** Marginal (fixed factors only) and conditional (all factors) $R^2$ values for the models presented in Tables S1–S10.