




Article

Lab and Field Warming Similarly Advance Germination Date and Limit Germination Rate for High and Low Elevation Provenances of Two Widespread Subalpine Conifers

Lara M. Kueppers ^{1,2,3,*} , Akasha Faist ⁴, Scott Ferrenberg ⁵ , Cristina Castanha ², Erin Conlisk ⁶ and Jennifer Wolf ³ 

¹ Energy and Resources Group, University of California, 310 Barrows Hall #3050, Berkeley, CA 94720, USA

² Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Rd., Berkeley, CA 94720, USA; ccastanha@lbl.gov

³ School of Natural Sciences, University of California, 5200 N. Lake Rd., Merced, CA 95343, USA; jenn.r.wolf@gmail.com

⁴ Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003, USA; afaist@nmsu.edu

⁵ Department of Biology, New Mexico State University, Las Cruces, NM 88003, USA; ferrenbe@nmsu.edu

⁶ Point Blue Conservation Science, 3820 Cypress Dr. #11, Petaluma, CA 94954, USA; erin.conlisk@gmail.com

* Correspondence: lmkueppers@berkeley.edu; Tel.: +1-510-486-5813

Received: 30 September 2017; Accepted: 7 November 2017; Published: 11 November 2017

Abstract: Accurately predicting upslope shifts in subalpine tree ranges with warming requires understanding how future forest populations will be affected by climate change, as these are the seed sources for new tree line and alpine populations. Early life history stages are particularly sensitive to climate and are also influenced by genetic variation among populations. We tested the climate sensitivity of germination and initial development for two widely distributed subalpine conifers, using controlled-environment growth chambers with one temperature regime from subalpine forest in the Colorado Rocky Mountains and one 5 °C warmer, and two soil moisture levels. We tracked germination rate and timing, rate of seedling development, and seedling morphology for two seed provenances separated by ~300 m elevation. Warming advanced germination timing and initial seedling development by a total of ~2 weeks, advances comparable to mean differences between provenances. Advances were similar for both provenances and species; however, warming reduced the overall germination rate, as did low soil moisture, only for *Picea engelmannii*. A three-year field warming and watering experiment planted with the same species and provenances yielded responses qualitatively consistent with the lab trials. Together these experiments indicate that in a warmer, drier climate, *P. engelmannii* germination, and thus regeneration, could decline, which could lead to declining subalpine forest populations, while *Pinus flexilis* forest populations could remain robust as a seed source for upslope range shifts.

Keywords: climate change experiment; Niwot Ridge; *Picea engelmannii*; *Pinus flexilis*; seed germination; seedling development

1. Introduction

Tree species persistence and migration in the face of climate change hinge on successful seedling recruitment within and beyond species' present geographic ranges [1]. Climatic tolerances of seedlings or saplings are likely narrower than adult tolerances since seedlings and saplings are more shallowly rooted, are shorter, and have much less leaf area than adults, which results in reduced access to both

above- and belowground resources [2,3]. Recent work has shown that the climatic niche of juveniles is a subset of that of adults for many species [2,4,5], and that few juveniles are found beyond the upper or northward range limits of adults [5,6]. This suggests that while some tree populations may have started “leaning” upslope [7] and northward with climate change, there are still poorly understood constraints on early life history stages within and beyond current range limits, requiring additional research on the regeneration niche [8] for accurate predictions [9–11].

At and above the tree line ecotone (as defined in [12]), recruitment may be a bottleneck to upslope expansion by subalpine trees, and will rely on seed produced at lower elevations, which may or may not be adapted to high-elevation sites with warming. Population level, or ecotypic, variation in growth rates and other quantitative traits is widely observed in forest trees [13–15], even in new seedlings [16,17]. Common garden experiments comparing provenances of forest trees indicate that phenological, growth, stress resistance, and reproductive traits vary such that realized population niches are well matched with their current geographic occurrences (e.g., [17–20]). Similarly, growth sensitivity to interannual climate variation depends on population position in climate space for both *Pinus contorta* (lodgepole pine) and *Pseudotsuga menziesii* (Douglas fir) [21,22]. While a species', or even a population's, fundamental niche can be broad for a particular trait (e.g., relative growth rate, cold hardiness), interactions among genotypes and tradeoffs between stress tolerance and growth create narrow “seed zones” for populations of even broadly distributed species [18]. When such genetic differences are incorporated into individual and population growth models for the widespread *P. contorta*, dramatic differences are seen in projected future growth rates under climate change between local and optimal (seeds sourced from elsewhere in the species range) populations [23,24].

When considering climate impacts on tree line ecotone populations, comparatively little work has focused on low-elevation populations that are potential sources for both seeds and genetic material [25–28]. Declines in recruitment and regeneration at low elevations could reduce the size of these populations, potentially altering the pace of high-elevation population establishment [24]. Loss of low-elevation populations may also disproportionately affect genetic diversity [25]. In the Western United States (U.S.), low-elevation populations experience not only warmer growing seasons, but also substantially longer frost-free seasons and drier midsummer soils relative to elsewhere in species' distributions. As the climate warms, an even longer growing season is expected [29,30], and in the absence of additional growing season precipitation, soils are expected to dry down more quickly following snowmelt [31,32]. With less buffering capacity than adults, seedlings may be particularly vulnerable to changes in snowpack and soil moisture near lower edges of their distributions [33]. Thus, to project sizes of low-elevation populations that are the seed sources for tree line populations, we need to understand the sensitivity of low-elevation recruitment to changing microclimate. Further, understanding how high-elevation seed provenances perform under low-elevation conditions can provide insight into their suitability for future high-elevation climate.

Microclimate variables are known to affect seed germination and seedling development. For many species, growing degree days or similar metrics are used to integrate temperature over developmental time, predicting days until 50% of seedlings have emerged (e.g., [34]). Soil moisture levels affect seed hydration, and thereby germination, with slowed or aborted germination at insufficient moisture levels, leading some to integrate soil moisture and temperature into “hydrothermal” models [35,36]. Climate changes that alter these factors have the potential to alter overall rates of germination, and the timing of germination and full seedling development in current and novel habitats [37]. However, not all species from a common environment have the same climate sensitivity [38,39], nor do all populations of a single species [40]. Germination and early growth have been shown to vary among seed sources for three subalpine conifers, with first year seedling root:shoot ratios, growth rate, and date of growth cessation that corresponded to seed source elevation, but with differences in species' clinal trends, suggesting species-specific responses to temporal climate variation at the highest elevations [41].

Here we focus on two co-occurring, widely distributed subalpine tree species in the Western U.S., *Pinus flexilis* James (limber pine) and *Picea engelmannii* Parry ex Engelm (Engelmann spruce) [42],

both with bioclimatic envelopes that are expected to shift upslope over the 21st century [43,44]. *P. flexilis* is relatively sparse throughout its range, while *P. engelmannii* forms continuous forest cover or open parkland areas up to the tree line. A five-year field warming experiment found that recruitment (combined germination and survival) by these two species declined with +4 °C warming in low-elevation subalpine forest, and did not increase with warming at and above the tree line [45]. Summer water additions alleviated some negative effects of warming. The low seed provenance recruited more successfully than the high provenance across all elevations, reinforcing the likely importance of low-elevation populations to tree line change. Here, we focus just on germination and describe 17-week experiments in controlled environment growth chambers that mimicked natural diurnal and seasonal temperature variation to examine: (1) the sensitivity of seed germination and initial seedling development to a 5 °C temperature increase above ambient conditions in low-elevation subalpine forest; (2) the sensitivity of these processes to soil moisture; and (3) whether high-elevation and low-elevation provenances differ from each other in mean rates or in their microclimate sensitivity. We compare differences in germination rate and timing in the lab experiment to those from three years of germination observations in the above field experiment to determine whether the simpler, less costly growth chamber approach yields results comparable to those in the field or can lend insight into field experiment patterns.

2. Materials and Methods

2.1. Seed Collection and Processing

We collected *P. flexilis* and *P. engelmannii* seed in September and October 2008 at Niwot Ridge, on the eastern slope of the Rocky Mountains in Colorado, USA, from “low” (near the lower elevation limit of subalpine forest) and “high” (in the alpine-tree line ecotone) elevation sites. Specifically, we harvested low-elevation *P. flexilis* seed from trees growing between 3050 m and 3150 m elevation and within a 150 m radius of 40°2'15" N, 105°32'59" W. Because cones are sparsely distributed and often high above the ground, we collected low-elevation *P. engelmannii* seed from same-year squirrel caches within 150 m of these same coordinates. We collected high-elevation *P. flexilis* seed from two ridges located 1 km apart centered on 40°2'55" N, 105°34'15" W, and between 3370 and 3450 m elevation. We harvested high-elevation *P. engelmannii* seed from flag trees growing between 3370 m and 3400 m located within a 100 m radius of 40°3'1" N, 105°34'49" W. We followed methods for seed handling and processing described in Schopmeyer (1974). We dried cones in well-ventilated areas at low to moderate temperatures (<28 °C for *P. flexilis* and <45 °C for *P. engelmannii*) until it was possible to extract the seed. The United States Forest Service (USFS) Coeur d'Alene Nursery de-winged the *P. engelmannii* seed. We stored processed seed at 4 °C until November 2008, when it was transferred to a −20 °C freezer.

2.2. Lab Germination Experiments

We performed two lab-based germination experiments, each lasting 17 weeks, which is the length of a typical growing season at 3050 m at Niwot Ridge. We initiated the first experiment on 19 March 2009, and began the second experiment on 14 October 2009. For the first experiment, prior to planting, we stratified *P. flexilis* seed for 42 days at 4 °C and did not stratify *P. engelmannii* seed. For the second experiment, we stratified *P. flexilis* seed for 37 days and *P. engelmannii* for 30 days at 4 °C. For each experiment, we sowed 40 trays (1 seed/cell; 60 cells/tray) with one of each species × seed source elevation combination. Trays contained a 1:1 mixture of autoclaved sand and native soil collected at 3048 m elevation and roughly 300 m north of 40°02'09" N, 105°32'09" W. Soil was included in the germination medium to inoculate the mixture with a native microbial community. Both *P. flexilis* and *P. engelmannii* occur near to where the soil was collected.

We assigned each tray, considered a treatment replicate, to a temperature (field ambient or field ambient +5 °C) and a soil moisture (field capacity or 50% field capacity) treatment. We used two growth chambers (Model E-36VL, Percival Scientific, Perry, IA, USA) configured with desiccant driers

(IAT-50, Innovative Air Technologies, Covington, GA, USA) to maintain the temperature treatments and lower humidity. The two experiments used the same growth chambers, but we reversed the temperature treatment assignments from experiment 1 to experiment 2 to eliminate any chamber bias. Chamber temperature and relative humidity (RH) were based on average 1999–2005 (7 years) hourly 2-m air temperature and RH data measured at the Niwot Ridge, CO, USA (US-NR1) Ameriflux weather station (40°01'15" N, 105°32'04" W; 3050 m) [46], reflecting diurnal and seasonal cycles, beginning with the mean date of snowmelt (May 29 ± 3 days). The “field ambient” treatment, hereafter denoted AMB, experienced the mean Ameriflux site temperature regime, while the “field ambient +5 °C” treatment, hereafter denoted +5C, experienced the mean plus a 5 °C temperature increase at every time point. Chamber temperature and RH were monitored with multiple Hobo Tidbit and U23 Pro v2 Temperature/Relative Humidity sensors (Onset Computer Corp., Bourne, MA, USA) during the two experiments. We calculated field capacity, hereafter denoted FC, and half field capacity, hereafter denoted 0.5 FC, of the soil mixture gravimetrically for each tray. Throughout the experiments we maintained the total tray mass of soil plus water using a pressurized sprayer to apply water to the trays every 2–3 days. At the end of each experiment we determined the water content of each replicate by measuring the average gravimetric water content of eight cells per tray by drying soil at 105 °C for three days, or until constant mass. A diurnal light cycle with daytime photosynthetically active radiation intensity similar to that in a forest understory ($350 \mu\text{mol m}^{-2} \text{s}^{-1}$) was maintained throughout both experiments.

We examined every cell in every tray on each watering day, and recorded each germinant’s developmental stage as no emergence, soil broken, seed coat visible above the soil, cotyledons visible, seed coat shed, or dead. We harvested all germinants, regardless of developmental stage, when 80% of emergents reached the final stage (seed coat shed), but we continued to maintain the treatments and harvested additional germinants as they reached the seed coat shed stage. Following harvest, we measured several morphological metrics including number of cotyledons, shoot and root length, and shoot and root mass after drying the germinants at 65 °C for three days (until constant mass).

2.3. Field Experiment and Germination Observations

Details of the field experimental design are provided in Kueppers et al. [45], and summarized briefly here. High- and low-elevation seed were sown into common gardens in small gaps in a stand of mature trees near the low elevation edge of subalpine forest (3060 m) at Niwot Ridge, 300–400 m below local tree line and across the road from the Niwot Ridge Ameriflux site. The 20 circular, 3-m-diameter garden plots were assigned to four equal climate treatment groups: control; heated; watered, and; heated and watered. We used six 1000-W infrared heaters arrayed around the perimeters of heated and heated and watered plots with constant heater output to raise the mean daily 5–10 cm soil temperature (ECTM and 5TM sensors; Decagon Devices, Pullman, WA, USA) during the snow-free periods (mean $+3.9 \pm 0.17$ °C 2010–2012). Except in 2009–2010, heater output was reduced between November and March, to avoid potentially intermittent snowpack [47]. Heating extended the snow-free season (45 ± 16 days) and reduced summer soil moisture ($-0.02 \pm 0.002 \text{ m}^3 \text{ m}^{-3}$). We designed the watering treatments (2.5 mm week^{-1}) 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 ($+0.01 \pm 0.002 \text{ m}^3 \text{ m}^{-3}$) at 5–10 cm depth.

Each autumn from 2009 to 2011, seed was collected and processed as described above, and sown prior to snow accumulation. When new seed availability was low, we sowed excess seed from a prior year that had been stored at -20 °C. Plots were subdivided into four quadrats, which were randomly assigned to receive *P. engelmannii* or *P. flexilis*, high- or low-elevation seed. We sowed 280–1400 seeds per quadrat per year, depending on seed viability (as determined by X-ray analysis) and availability. To exclude small mammals, we placed hardware cloth cages (1-cm² openings) over

each plot. We surveyed plots every 7–10 days during the 2010–2012 snow-free seasons, recording the number of new emergents each sampling date.

2.4. Statistical Analyses

In the lab experiments, within each chamber all combinations of soil moisture treatment, species, and source elevation were represented with either two or three replicates. Across both experiments, we had five replicate trays for each combination of species, source elevation, temperature, and soil moisture. Due to the between-experiment differences in *P. engelmannii* seed stratification protocol, the trays were not perfect replicates, and experiment was included as a factor in the statistical analysis. Paired measurements of chamber temperature and relative humidity were compared between chambers for each experiment, and to the programmed values, using the Wilcoxon Signed Rank test. We used the non-parametric Median Test in one-way analyses to examine differences in soil moisture among groups of trays. We compared percent germination, time to germination, seedling development time, and all post-harvest morphological metrics using the germination trays as replicates. Following a Shapiro-Wilk test for normality, we transformed germination and physical metric data when necessary to meet the assumptions of parametric statistics. We analyzed germination, development, mortality, and physical metric data across and/or within species with analysis of covariance (ANCOVA) using soil moisture as a covariate.

We estimated effects of heating, watering, provenance, cohort, and their two-way interactions on field seedling germination rate and the day of year when 50% of seeds had germinated (median germination date) for each species separately using linear mixed effects models (function `lme4::glmer` in the R 3.1.2 Language for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria). We included plot as a random effect and, for germination rate only, an observation-level random effect to accommodate overdispersion. Significance levels were estimated using likelihood ratio tests, determining which estimated effects are not consistent with chance under null hypotheses. To test main effects, we directly manipulated the design matrix (`stats::model.matrix`), choosing effect, or deviation, coding (using “`contr.sum`” in R 3.1.2). Thus, the intercepts for the models represent the average germination rate or day of year of median germination, while coefficient estimates represent the deviation from this average. 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). For lab and field results, we consider significant effects to be $p < 0.05$ and marginally significant effects to be $0.1 > p \geq 0.05$.

3. Results

3.1. Lab Experimental Effects on Microclimate

For both experiments, mean chamber temperatures deviated from programmed temperatures by less than 0.5 °C during the weeks measured. The AMB and +5C treatments differed by 5.3 °C ($p < 0.0001$) during the first experiment and by 4.6 °C ($p < 0.0001$) during the second. Relative humidity was 13–16% (absolute %) greater than programmed values ($p < 0.0001$ for all comparisons). However, relative humidity did not differ significantly between chambers during either experiment, as intended.

The soil gravimetric moisture content varied by experiment, temperature treatment, and soil moisture treatment (Table 1), but not between species or seed source elevations (not shown). Median moisture content tended to be higher in the first experiment vs. the second (median θ_m 0.24 vs. 0.15), higher in the +5C vs. the AMB treatment (median θ_m 0.24 vs. 0.19), and as intended, higher in the FC vs. the 0.5 FC treatment (median θ_m 0.24 vs. 0.12). The water content difference between experiments 1 and 2 was unexpected, and may have been due to differences in watering technique, or to differences in soil compaction when the trays were initially filled with soil. The temperature effect on moisture differed from our expectation that the warmer chamber could have drier soils in spite of efforts to maintain moisture levels, and was consistent across both experiments (Table 1).

Table 1. Median gravimetric soil moisture ($\text{g} \cdot \text{g}^{-1}$) among treatment groups for two seedling germination experiments. Experiment ($p < 0.0001$), temperature treatment ($p < 0.01$), and moisture treatment ($p < 0.0001$) differed in one-way median tests. The crossed field ambient (AMB), field ambient +5 °C (+5C), field capacity (FC) and half field capacity (0.5 FC) treatment medians for experiments 1 and 2 are reported individually.

Temperature Treatment	Moisture Treatment	
	0.5 FC	FC
Experiment 1		
AMB	0.20	0.24
+5C	0.21	0.27
Experiment 2		
AMB	0.09	0.19
+5C	0.10	0.26

3.2. Lab Germination Percent

The +5C treatment reduced germination rates relative to the AMB treatment, but only consistently so for *P. engelmannii* (Figure 1, Table 2). For *P. flexilis*, a marginally significant interaction effect resulted from the tendency for higher temperature to increase high provenance germination but decrease low provenance germination (Figure 1, Table 2). Higher soil water content increased germination rate of *P. engelmannii* and high-elevation *P. flexilis* seed, while germination rate for low elevation *P. flexilis* was insensitive to soil water (Table 2).

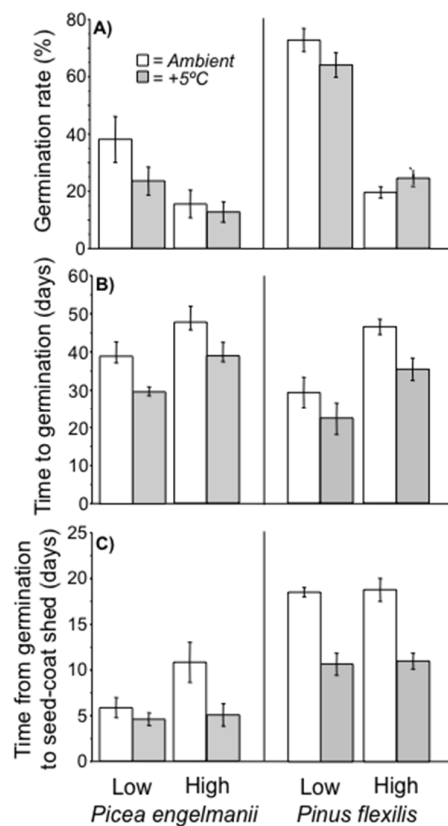


Figure 1. Germination rate (A); time from planting to germination (B); and time from germination to seed-coat shed (C) for low (3100 m) and high-elevation (3400 m) provenances of *Picea engelmannii* and *Pinus flexilis*. Open bars give the ambient treatment means and filled bars give the ambient +5 °C treatment means.

Table 2. Effects of seed provenance (Elevation), temperature treatment, water content, and their interactions, as well as experiment, on seedling germination and development (all models = $p < 0.0001$, degrees of freedom (df) = 7,31, $n = 39$, except df = 7,30 and $n = 38$ for days from germination to seed coat shed models). Variables were transformed prior to analysis. Probabilities < 0.05 are given in bold type.

	Measure	Factor	Coef.	df	SS	F	p
<i>Picea engelmannii</i>	Germination Rate	Elevation (Low)	0.595	1	13.667	32.81	<0.0001
		Experiment	−0.903	1	20.393	48.96	<0.0001
		Temperature Treatment	−0.358	1	4.567	10.97	0.0024
		H ₂ O content	7.326	1	5.512	13.23	0.0010
		Elevation * Temp. Treatment	−0.043	1	0.063	0.15	0.6991
		Elevation * H ₂ O content	2.210	1	0.782	1.88	0.1805
		Temp. Treatment * H ₂ O content	5.724	1	4.859	11.67	0.0018
	Time to Germination	Elevation (Low)	−0.047	1	0.087	21.38	<0.0001
		Experiment	0.088	1	0.191	46.98	<0.0001
		Temperature Treatment	−0.052	1	0.096	23.59	<0.0001
		H ₂ O content	0.231	1	0.005	1.35	0.2548
		Elevation * Temp. Treatment	−0.021	1	0.015	3.67	0.0646
		Elevation * H ₂ O content	−0.120	1	0.002	0.56	0.4576
		Temp. Treatment * H ₂ O content	−0.230	1	0.008	1.92	0.1750
	Time from Germination to Seed Coat Shed	Elevation (Low)	−0.057	1	0.121	5.45	0.0264
		Experiment	0.199	1	0.995	44.83	<0.0001
		Temperature Treatment	−0.115	1	0.456	20.54	<0.0001
		H ₂ O content	0.629	1	0.040	1.79	0.1913
		Elevation * Temp. Treatment	−0.004	1	0.001	0.03	0.8675
		Elevation * H ₂ O content	0.324	1	0.016	0.71	0.4069
		Temp. Treatment * H ₂ O content	−0.464	1	0.030	1.36	0.2520
<i>Pinus flexilis</i>	Germination Rate	Elevation (Low)	1.091	1	45.373	181.39	<0.0001
		Experiment	0.008	1	0.002	0.01	0.9346
		Temperature Treatment	−0.068	1	0.164	0.65	0.4246
		H ₂ O content	0.570	1	0.032	0.13	0.7218
		Elevation * Temp. Treatment	−0.167	1	0.959	3.83	0.0592
		Elevation * H ₂ O content	−2.905	1	1.290	5.16	0.0303
		Temp. Treatment * H ₂ O content	2.794	1	1.127	4.51	0.0419
	Time to Germination	Elevation (Low)	−0.100	1	0.381	118.71	<0.0001
		Experiment	−0.004	1	0.000	0.13	0.7184
		Temperature Treatment	−0.066	1	0.153	47.59	<0.0001
		H ₂ O content	0.472	1	0.022	6.91	0.0132
		Elevation * Temp. Treatment	−0.005	1	0.001	0.26	0.6086
		Elevation * H ₂ O content	0.264	1	0.011	3.32	0.0780
		Temp. Treatment * H ₂ O content	−0.255	1	0.009	2.93	0.0969
	Time from Germination to Seed Coat Shed	Elevation (Low)	0.013	1	0.006	1.09	0.3048
		Experiment	0.012	1	0.003	0.59	0.4486
		Temperature Treatment	−0.109	1	0.382	66.77	<0.0001
		H ₂ O content	0.160	1	0.002	0.42	0.5207
		Elevation * Temp. Treatment	0.013	1	0.005	0.86	0.3610
		Elevation * H ₂ O content	−0.391	1	0.020	3.50	0.0712
		Temp. Treatment * H ₂ O content	−0.056	1	0.000	0.07	0.7942

In general, differences in germination rate due to the temperature and soil moisture treatments were not as large as differences between species and seed source elevations (Figure 1). Germination rates were higher for *P. flexilis* (mean \pm s.e. $45 \pm 4\%$; median 34%) than *P. engelmannii* ($22 \pm 3\%$; 15%), and higher for the low-elevation provenances ($50\% \pm 4\%$; 59%) than the high-elevation provenances ($18 \pm 2\%$; 17%) ($p < 0.0001$ in both cases). The effect of seed source elevation was stronger for *P. flexilis* than *P. engelmannii* (Table 2, Figure 1). Germination rates for *P. engelmannii* also were substantially higher with stratification in experiment 2 than without stratification in experiment 1 (means Experiment 1: $11 \pm 2\%$ vs. Experiment 2: $34 \pm 5\%$) (Table 2).

3.3. Germination Timing and Rate of Seedling Development

Time to germination was significantly reduced in the warmer temperature treatment for both species (by 9 ± 3 days for *P. flexilis*, 9 ± 4 days for *P. engelmannii*; Table 2, Figure 1), but was delayed by increasing soil moisture content for *P. flexilis* (Table 2). The acceleration of germination with 5°C warming was comparable to or less than the difference between low- and high-elevation seed sources (Figure 1). Time to germination was shorter for the low-elevation seed sources than the high-elevation seed sources for both species, but this seed source mean difference was more pronounced for *P. flexilis*

(15 ± 2 days) than for *P. engelmannii* (9 ± 3 days) (Table 2, Figure 1). Low-provenance *P. engelmannii* germination was accelerated slightly more with warming than was high-provenance germination, although this effect was only marginally significant (Table 2). As with germination rate, experiment was important for explaining the germination timing in *P. engelmannii* (Table 2), presumably due to the stratification differences.

The rate of seedling development following germination was estimated as the number of days from emergence to a seedling having fully shed its seed coat. The species differed dramatically, with *P. engelmannii* seedlings taking only 5–10 days to develop to this stage, and *P. flexilis* seedlings taking 10–20 days ($p < 0.0001$, Figure 1). The +5C treatment accelerated seedling development by three days for *P. engelmannii* and eight days for *P. flexilis*, nearly halving development time for both species. Seed source elevation was not a significant factor in rate of development for *P. flexilis*, but low-provenance *P. engelmannii* seedlings developed more quickly than the high-provenance seedlings (Table 2, Figure 1).

3.4. Seedling Size, Mass Allocation, and Mortality

There were comparatively few effects of microclimate and provenance on seedling morphology and mortality. *P. flexilis* seedlings raised in the +5C treatment had slightly less mass (root + shoot) upon harvest than those in the AMB treatment ($p = 0.0435$; 35.5 ± 1.5 mg vs. 41.1 ± 2.5 mg) due to reductions in root mass ($p = 0.0039$), but *P. engelmannii* mass was unrelated to temperature. Unexpectedly, there was greater mass allocation to roots (relative to shoots) in the second experiment compared to the first for both species (*P. engelmannii* means 0.61 ± 0.05 vs. 0.95 ± 0.18 , $p < 0.001$; *P. flexilis* means 0.43 ± 0.02 vs. 0.49 ± 0.02 , $p < 0.001$).

The number of cotyledons reflects the earliest seedling investment in leaf area, while shoot and root lengths reflect allocation for above- vs. belowground resource acquisition. The number of cotyledons produced by the seedlings ranged from 3 to 13, with *P. engelmannii* having 5.4 ± 0.1 and *P. flexilis* having 8.1 ± 0.1 cotyledons on average. For both species, the lower elevation provenances had slightly more cotyledons (*P. engelmannii* difference 0.61, $p < 0.0001$; *P. flexilis* difference 0.54, $p = 0.0109$). Interestingly, for *P. flexilis*, increased moisture led to more cotyledons in the +5C treatment, but fewer in the AMB treatment (interaction $p \leq 0.0001$). *P. engelmannii* root and shoot length did not differ between provenances or microclimate treatments. However, *P. flexilis* seedlings had longer roots in the AMB treatment ($p = 0.0160$), and root length increased with soil moisture ($p = 0.0472$), an effect driven by seedlings in the +5C treatment (interaction $p = 0.0275$). Low-provenance *P. flexilis* also had longer roots than high ($p = 0.0286$). Root length was shorter in the second experiment compared to the first for both species ($p < 0.0001$ for *P. engelmannii* and $p = 0.0258$ for *P. flexilis*), suggesting the above mass difference was not a methodological artifact, but that seedlings in fact had shorter, more massive roots in experiment 2.

Mortality was low in the growth chambers, although up to three individuals per tray (1% of germinants) perished before the end of the experiment. Mortality was lower in experiment 2 (with stratification; $p < 0.0001$), but there were no effects of microclimate or elevation for *P. engelmannii* (Full model adjusted $R^2 = 0.68$, root mean square error (RMSE) = 0.60, $p = 0.0005$). For *P. flexilis*, there were too few observations of mortality to support the full model; a model excluding non-significant interaction effects with elevation revealed that the high-elevation seed source had greater mortality than the low (partial $p = 0.0140$; Full model adj $R^2 = 0.76$, RMSE = 0.39, $p = 0.0447$), and again there were no significant microclimate effects.

3.5. Field Germination Rate and Timing

Field germination rates averaged <15% across treatments for *P. engelmannii*, and <30% for *P. flexilis* (Figure 2). Unlike in the lab germination experiments, heating did not reduce either species' germination rate overall (Table 3). Consistent with the lab germination experiments, *P. engelmannii* germination increased in treatments with added water, but *P. flexilis* did not (Table 3). However, in

the field, we measured germination over three years, finding significant among-cohort (among-year) differences in rates and in heating effects (Figure 3). Germination rates were highest in 2011, a year with late, deep snow and late snowmelt, and lowest in 2012, a year with early snowmelt, little summer rain, and a late onset of monsoon weather patterns. In 2011, heating significantly reduced germination, while it had no effect or increased germination in 2010 and 2012 (Table 3). The year-to-year heating patterns were similar for *P. engelmannii* and *P. flexilis* (Figure 3).

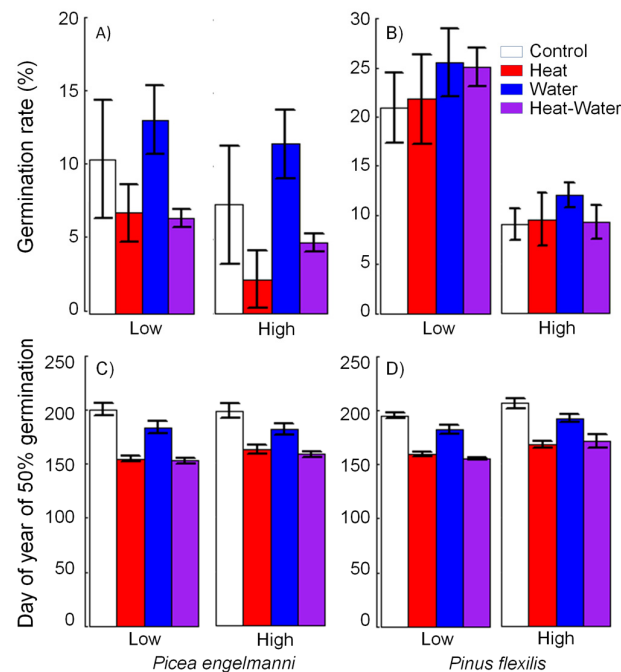


Figure 2. Field germination rate as a fraction of sown seeds for *P. engelmannii* (A) and *P. flexilis* (B) and time to germination expressed as day of year of 50% germination, for the same two species (C,D) for low- and high-elevation provenances, across experimental treatments as observed 2010–2012. Error bars are standard errors of the means across plots and years.

Table 3. Generalized linear mixed effects model coefficients, standard errors (SE), likelihood ratio tests (LRT) and p -values, $P(\chi^2)$, for main effects and all two-way interactions on germination rate of *Picea engelmannii* and *Pinus flexilis*. Random effects variances (for *P. engelmannii* and *P. flexilis*, respectively) are 0.54 and 0.10 for plot and 0.78 and 0.72 for the observation level random effect. Probabilities < 0.05 are given in bold type.

	<i>Picea engelmannii</i>				<i>Pinus flexilis</i>			
	Coef.	SE	LRT (df)	$P(\chi^2)$	Coef.	SE	LRT (df)	$P(\chi^2)$
Intercept	−3.48	0.19			−2.44	0.11		
Heat	−0.08	0.19	0.17 (1)	0.68	0.11	0.11	1.01 (1)	0.31
Water	0.43	0.19	4.82 (1)	0.028	0.07	0.11	0.36 (1)	0.55
Provenance (Low)	0.53	0.09	29.09 (1)	<10^{−5}	0.82	0.08	67.11 (1)	<10^{−5}
Cohort			60.02 (2)	<10^{−5}			102.83 (2)	<10^{−5}
2010	−0.59	0.13			−0.46	0.12		
2011	1.12	0.12			1.50	0.12		
2012	−0.53	0.13			−1.04	0.12		
Heat * Water	−0.12	0.19	0.38 (1)	0.54	−0.13	0.11	1.27 (1)	0.26
Heat * Prov (Low)	0.03	0.09	0.08 (1)	0.78	−0.05	0.08	0.33 (1)	0.57
Water * Prov (Low)	−0.08	0.09	0.82 (1)	0.36	−0.01	0.08	0.02 (1)	0.89
Heat * Cohort			44.11 (2)	<10^{−5}			10.09 (2)	0.0064
2010	0.44	0.13			0.15	0.12		
2011	−0.93	0.12			−0.37	0.11		
2012	0.49	0.13			0.22	0.12		

Table 3. Cont.

	<i>Picea engelmannii</i>				<i>Pinus flexilis</i>			
	Coef.	SE	LRT (df)	P(χ^2)	Coef.	SE	LRT (df)	P(χ^2)
Water * Cohort			0.07 (2)	0.97			5.70 (2)	0.058
2010	0.03	0.13			0.18	0.12		
2011	−0.02	0.12			0.11	0.11		
2012	−0.01	0.13			−0.29	0.12		
Prov (Low) * Cohort			26.52 (2)	<10 ^{−5}			42.25 (2)	<10 ^{−5}
2010	0.66	0.13			0.84	0.12		
2011	−0.56	0.12			−0.50	0.11		
2012	−0.09	0.13			−0.34	0.12		

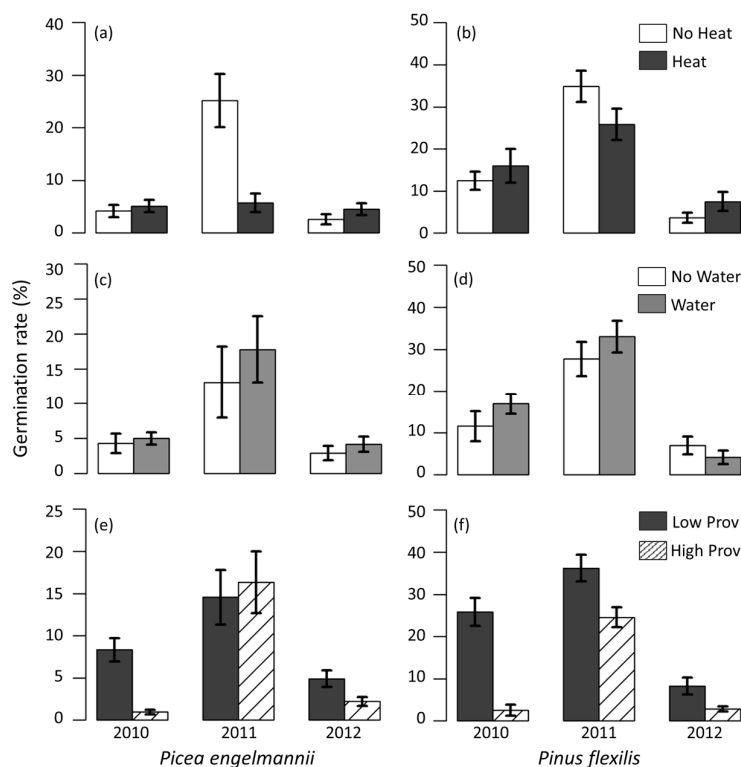


Figure 3. Response of field germination rate as a fraction of sown seeds to heat (a,b), water addition (c,d) and provenance (e,f) for *P. engelmannii* (a,c,e) and *P. flexilis* (b,d,f) in each cohort year. Error bars are standard errors of the means across plots.

As predicted by local adaptation theory, the low-elevation provenances of both species germinated more strongly than the high-elevation provenances, an effect that was larger for *P. flexilis* than *P. engelmannii* in both field and lab experiments (Tables 2 and 3; Figure 2). The provenances did not differ in response (or lack thereof) to warming and water addition, but the low-provenance advantage was strongest in 2010, and weaker or absent in 2011 and 2012 (Figure 3; Table 3).

For both species, the timing of germination in the field was similarly sensitive to microclimate and provenance, with 50% of new germinants emerging by day ~150–200 (~30 May–19 July) on average, depending on treatment (Figure 2). Heating advanced germination by more than two weeks, and summer water addition advanced germination by ~5 days for both species (Table 4). For *P. flexilis*, the heating effect was less pronounced in watered plots (Figure 2, Table 4). There was considerable interannual variability in germination timing, presumably due to different dates of snow disappearance. In particular, germination was later in the late snowmelt year (2011) and earlier in the early snowmelt years (2010, 2012). The heating effect was also smaller in 2011 (by nearly half), likely due to smaller differences in snowmelt date among treatments. Water addition accelerated germination most strongly

in 2012, the earliest melt year, with less pronounced effects in other years (Table 4). Finally, only *P. flexilis* had provenance differences, with the low provenance emerging five days earlier than the high, on average (Table 4).

Table 4. Linear mixed effects model coefficients, standard errors (SE), likelihood ratio tests (LRT) and p -values, $P(\chi^2)$, for main effects and all two-way interactions on day of year when 50% of *Picea engelmannii* and *Pinus flexilis* seeds had germinated. Random effects variances for plot are 64.35 and 9.51 for *P. engelmannii* and *P. flexilis*, respectively. Probabilities < 0.05 are given in bold type.

	<i>Picea engelmannii</i>				<i>Pinus flexilis</i>			
	Coef.	SE	LRT (df)	$P(\chi^2)$	Coef.	SE	LRT (df)	$P(\chi^2)$
Intercept	173.97	2.15			178.45	1.35		
Heat	−17.08	2.15	31.56 (1)	<10^{−5}	−16.38	1.35	46.11 (1)	<10^{−5}
Water	−5.48	2.14	6.80 (1)	0.009	−4.09	1.35	9.12 (1)	0.003
Provenance (Low)	−0.04	1.16	0.00 (1)	0.97	−5.04	1.16	19.64 (1)	<10^{−5}
Cohort			69.79 (2)	<10^{−5}			64.14 (2)	<10^{−5}
2010	−3.66	1.70			−0.47	1.67		
2011	15.00	1.59			12.66	1.61		
2012	−11.34	1.69			−12.19	1.65		
Heat * Water	3.37	2.14	2.88 (1)	0.09	2.69	1.35	4.46 (1)	0.035
Heat * Prov (Low)	−1.59	1.15	2.20 (1)	0.14	0.70	1.16	0.43 (1)	0.51
Water * Prov (Low)	0.10	1.15	0.01 (1)	0.93	−0.27	1.16	0.06 (1)	0.81
Heat * Cohort			44.11 (2)	<10^{−5}			41.51 (2)	<10^{−5}
2010	−4.46	1.68			−1.40	1.66		
2011	10.64	1.59			10.07	1.61		
2012	−6.19	1.67			−8.66	1.65		
Water * Cohort			15.27 (2)	0.0005			18.76 (2)	<10^{−5}
2010	2.98	1.68			3.90	1.67		
2011	3.41	1.59			3.14	1.62		
2012	−6.39	1.67			−7.04	1.65		
Prov (Low) * Cohort			0.11 (2)	0.95			8.17 (2)	0.017
2010	0.12	1.65			1.70	1.67		
2011	0.38	1.57			2.73	1.61		
2012	−0.49	1.57			−4.42	1.65		

4. Discussion

4.1. Climate Sensitivity of Germination and Initial Development

Seeds and seedlings are subject to a great deal of environmental heterogeneity in nature, making it challenging to deduce the dependence of germination and early seedling development on soil microclimate factors with field observations alone [48]. Yet without an understanding of these relationships, it is difficult to predict the effects of climate change on tree recruitment, and thus forest regeneration or range migration [37]. As expected, based on past studies with temperate and boreal conifers (e.g., [34,38,49,50]), both temperature and soil moisture influenced aspects of *P. flexilis* and *P. engelmannii* germination and early development in our lab experiment, which carefully controlled microclimate variables, while still mimicking seasonal variation in temperature. The same effects on germination rate were generally present, but more muted in the field climate manipulation experiment, likely due to interannual variation in both winter and summertime climate. Conversely, due to the overriding importance of date of snowmelt (which was “fixed” in the lab), and strong heating effects on snowmelt in the field, effects of climate manipulations on field germination timing were actually more pronounced than those in the lab (Figures 1 and 2). This highlights that field germination timing is dependent on the combination of melt date and soil temperatures in the weeks following snowmelt.

In the lab, elevated temperatures accelerated the timing of germination and rate of seedling development for both species. A 5 °C temperature increase over the recent historical average near the low elevation edge of subalpine forest at Niwot Ridge, CO, resulted in at least a week’s advance in the timing of germination and up to a week’s advance in the date when a seedling’s cotyledons were expanding. Taken together, this amounts to approximately a two-week head start in the growing season, which typically lasts ~18 weeks. In the field, ~4 °C of warming advanced germination by

more than two weeks, by melting snow and raising soil temperatures earlier in the growing season. Earlier germination and development should result in a longer growing season and thus potentially larger, better-established seedlings. Conversely, earlier germination and development potentially expose seedlings to more environmental stress during a first growing season and may lead to greater mortality risk. In particular, shallow soil moisture can be low even early in the growing season if snowmelt water has pulsed through the soil well before significant summer rainstorms begin. Similarly, if relatively high mid-summer temperatures occur after seedlings have spent seed reserves, seedling carbon balance may be compromised. While our laboratory study was not designed to capture the effects of a lengthened growing season on survival through additional exposure to environmental stress, the *P. engelmannii* mortality rate decreased with earlier germination ($p = 0.032$) in our experiment, which held soil moisture constant over time, indicating that earlier germination could be advantageous for establishment of this species with sufficient soil moisture. In the field, first-year recruitment was reduced with heating for both species, while water addition increased recruitment [45], suggesting that any benefit of earlier germination (also seen in the field) was more than offset by heat and/or water stress during the growing season.

Although germination occurred earlier, higher temperatures depressed overall *P. engelmannii* lab germination rate, even in spite of higher median moisture content in the elevated temperature treatment (Table 1). Knapp and Smith [38] found maximum germination for *P. engelmannii* at a constant 20 °C, with lower rates at higher and lower temperatures. A lab experiment with multiple subalpine species from the Alps also found earlier and stronger germination at ~22 °C for most species as compared to lower temperatures, although for *Picea abies*, there was no significant difference between 22, 16 and 12 °C treatments [50]. Mean growing season temperatures in our experiment, which matched diurnal and seasonal temperature variation in the field, were 9.5 and 14.5 °C in the AMB and +5C treatments, respectively, well below the previously identified optimum. The highest temperatures occurred in week 6, yet still were just 13.6 and 18.6 °C on average. Differences between studies in optimal temperature for germination may be due to differences in methodology including stratification procedure, or to differences in seed provenances, although we found no significant interaction between seed source elevation and temperature treatment (Table 2). Our lab result showing a 38% reduction in low-elevation *P. engelmannii* germination rate at temperatures 5 °C higher than present (Figure 1) was larger than effects seen in the field experiment (Figure 2), wherein elevated temperature reduced germination rate only in 2011 (Table 3). However, 2011, a late snowmelt year, had high germination rates for both species, whereas 2010 and 2012 (early snowmelt years) were both low overall, suggesting that with climate warming, regeneration of *P. engelmannii* in lower subalpine forest may be diminished even in historically favorable snow years with consequences for population persistence. Lower germination was compounded by high seedling mortality to result in near-zero first-year seedling recruitment (0.001–0.0001 seeds recruit) in heated plots [45]. Indeed, when the effects of field heating on recruitment (combined germination and survival) were included in stochastic, demographic models, *P. engelmannii* forest populations declined sharply, due to recruitment failure [51].

For *P. engelmannii*, the timing of germination and seedling development were accelerated in the second lab experiment, and more seeds germinated overall. This experiment included a 30-day stratification at 4 °C to break seed dormancy while the first experiment did not. The observed sensitivity of *P. engelmannii* germination and development to stratification regime suggests that in microsites or years when seeds do not experience a sufficiently long period of soil temperatures in the window required to break dormancy, germination may be inhibited. Changes in the duration of snow cover, the timing of snowmelt, and frequency of freeze-thaw cycles with climate change thus have the potential to alter the number and vigor of establishing *P. engelmannii* seedlings [37]. Among-year differences in field germination rate for *P. engelmannii* cannot easily be used to attribute microclimate effects on break of dormancy vs. other factors that could influence germination rate (e.g., seed desiccation), but there is typically a long period under the snow when temperatures hover just above 0 °C and soils are saturated, favoring the breaking of dormancy. Thus, transition to a shallow or intermittent snowpack,

which results in frozen surface soils followed by more rapid drying following thaw, may present the highest risk.

In our lab experiment, soil moisture was held constant throughout the growing season, unlike temporal patterns in surface soil moisture in the field. However, we reproduced realistic average soil moisture conditions during the germination period for these species. Under these conditions, germination rate increased with increasing soil moisture for *P. engelmannii* and the high provenance of *P. flexilis*. This is consistent with earlier work by Kaufman and Eckard [49], which reported reductions in emergence with soil water potentials to -4 and -8 bars for *P. engelmannii* and *P. contorta*, with greater sensitivity in *P. contorta*. Unlike the earlier study, the lab germination timing and rate of initial seedling development were only marginally sensitive to soil moisture conditions, with *P. flexilis* more sensitive than *P. engelmannii*, although we did not subject seeds and seedlings to excessively low moisture conditions (Table 1; minimum moisture content 7–8%). In the field, added summer water increased *P. engelmannii* germination, but did not affect germination rate of the larger seeded *P. flexilis*. In another field warming and watering study at the tree line, germination rates of subalpine tree species also were not equally sensitive to soil moisture, with *Larix decidua* and *Pinus uncinata* responding positively to higher soil moisture and *Picea abies* appearing insensitive, while *Pinus cembra* was negatively affected, likely due to its vulnerability to soil fungal pathogens [50]. Perhaps due to the more temporally varying moisture conditions in the field, effects of moisture on germination timing were more pronounced than in the lab, with water addition similarly accelerating germination for both species, particularly in 2012.

4.2. Provenance Differences in Climate Sensitivity

Overall, there were few significant or marginally significant interactions between seed source elevation (provenance) and temperature treatment or water content/treatment (Tables 2–4), suggesting generally similar sensitivity of germination and early development by the two provenances to soil climate change. This was consistent in both the field and the lab. Notable exceptions include a marginally greater reduction in days to germination with warming in the low compared to high-elevation *P. engelmannii* provenance, and later germination for low but earlier germination for high-elevation *P. flexilis* with wetter soils in the lab. Finally, germination rate for the high-elevation *P. flexilis* provenance increased with warming and with increasing moisture, but germination of the low-elevation provenance decreased with warming.

While these differences in the temperature and moisture effects were small, we did find large differences in rate of germination and date of germination between provenances for both species, with lower-elevation seed sources germinating earlier and in greater number than high-elevation seed sources, differences that were mostly consistent between lab and field. These mean differences, which suggest a more competitive germination strategy for low-elevation populations, are consistent with Green [41], which found that lower-elevation seed sources of *Abies lasiocarpa* have more competitive values for growth and allocation traits than those from upper elevations. However, contrary to that study, we found few or very small differences in seedling allocation in mountain provenances of *P. engelmannii* and *P. flexilis* differing by ~300–400 m elevation, with only the average cotyledon number being higher in low provenances of both species.

While this experiment did not fully test for local adaptation of seed between low- and high-elevation seed sources (because we did not run a comparable lab experiment using a high-elevation temperature regime), results do suggest that the high-elevation provenance germinates poorly under low-elevation environmental conditions. This could be due to overall reduction in seed quality at the cold edge range limit [52], or to suboptimal germination climate for both species. Either way, with lower germination rates and greater mortality in the high than low *P. flexilis*, and lower germination rates in high than low *P. engelmannii*, coupled with greater mortality with elevated temperatures and lower moisture seen in the field, high-elevation seed sources are clearly at a disadvantage under low-elevation climate conditions both now and under warmed conditions.

If this is not an environmentally mediated maternal effect, but reflects adaptive genetic variation, our results suggest that warming at high elevations could reduce, rather than increase, germination of high-elevation seeds. Findings from both the field and lab experiments are also consistent with respect to effects of warming on timing of germination and overall germination rates in lower-elevation forest, important source populations of both seed and genetic material for upslope range expansion.

5. Conclusions

The magnitudes of differences in lab germination timing and initial development between relatively high- and low-elevation provenances separated by 300–400 m, which corresponds to annual 2-m air temperature difference of only ~2.5 °C [45], were similar to the effect sizes of warming by 5 °C (Figure 1). Provenance differences and warming effects on germination exhibited the same patterns in the field and lab, lending confidence to the use of controlled environment growth chambers for qualitatively assessing these effects in the lab even though the precise magnitude of an effect might differ in the field. That said, interannual variation and seasonal fluctuations in moisture were not replicated in the lab experiment, resulting in an underestimation of the importance of moisture on germination. Subalpine conifer germination and initial development is sensitive both to temperature and soil moisture, with more pronounced effects of warming in *P. engelmannii* than *P. flexilis*. The temperature sensitivity of germination timing and seedling development rate will aid seedlings in tracking an earlier pulse of snow melt water through shallow soil layers with climate warming. However, even moderately drier soils during the growing season may result in lower *P. engelmannii* germination and greater seedling mortality. Together, these experiments suggest that *P. flexilis* will be better able to germinate, and regenerate, in warmer, drier subalpine forests, remaining an important low-elevation source for seed and genetic material for future tree line and alpine populations. Conversely, *P. engelmannii* germination is likely to decline with warming, limiting regeneration at low elevations and contributing to declines in low-elevation subalpine forest populations. Migration to higher elevations may be required to maintain the genetic diversity currently harbored by low-elevation *P. engelmannii* populations.

Acknowledgments: We thank numerous field and lab assistants; E. Brown, R. Butz, A. Carrell, Y. Lu, K. Lubetkin, A. Moyes, and two anonymous referees for comments on earlier versions of the manuscript; and M. Germino, J. Harte, M. Torn, and J. Mitton for helpful advice. We also thank the Niwot Ridge Ameriflux site for sharing climate data and the University of Colorado Mountain Research Station for logistical support. This research was supported by the U.S. Department of Energy, Office of Science, Biological and Environmental Research.

Author Contributions: L.K., A.F., S.F., and C.C. conceived and designed the experiments; A.F., S.F., C.C. and J.W. performed the experiments; L.K. and E.C. analyzed the data; L.K., A.F., and S.F. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Thuiller, W.; Albert, C.; Araujo, M.B.; Berry, P.M.; Cabeza, M.; Guisan, A.; Hickler, T.; Midgely, G.F.; Paterson, J.; Schurr, F.M.; et al. Predicting global change impacts on plant species' distributions: Future challenges. *Perspect. Plant Ecol.* **2008**, *9*, 137–152. [[CrossRef](#)]
2. McLaughlin, B.C.; Zavaleta, E.S. Predicting species responses to climate change: Demography and climate microrefugia in California valley oak (*Quercus lobata*). *Glob. Chang. Biol.* **2012**, *18*, 2301–2312. [[CrossRef](#)]
3. McDowell, N.; Pockman, W.T.; Allen, C.D.; Breshears, D.D.; Cobb, N.; Kolb, T.; Plaut, J.; Sperry, J.; West, A.; Williams, D.G.; et al. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytol.* **2008**, *178*, 719–739. [[CrossRef](#)] [[PubMed](#)]
4. Dobrowski, S.Z.; Swanson, A.K.; Abatzoglou, J.T.; Holden, Z.A.; Safford, H.D.; Schwartz, M.K.; Gavin, D.G. Forest structure and species traits mediate projected recruitment declines in western US tree species. *Glob. Ecol. Biogeogr.* **2015**, *24*, 917–927. [[CrossRef](#)]

5. Lenoir, J.; Gégout, J.-C.; Pierrat, J.-C.; Bontemps, J.-D.; Dhôte, J.-F. Differences between tree species seedling and adult altitudinal distribution in mountain forests during the recent warm period (1986–2006). *Ecography* **2009**, *32*, 765–777. [[CrossRef](#)]
6. Zhu, K.; Woodall, C.W.; Clark, J.S. Failure to migrate: Lack of tree range expansion in response to climate change. *Glob. Chang. Biol.* **2012**, *18*, 1042–1052. [[CrossRef](#)]
7. Kelly, A.E.; Goulden, M.L. Rapid shifts in plant distribution with recent climate change. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11823–11826. [[CrossRef](#)] [[PubMed](#)]
8. Grubb, P.J. Maintenance of species-richness in plant communities—Importance of regeneration niche. *Biol. Rev. Camb. Philos. Soc.* **1977**, *52*, 107–145. [[CrossRef](#)]
9. Conlisk, E.; Syphard, A.D.; Franklin, J.; Flint, L.; Flint, A.; Regan, H. Uncertainty in assessing the impacts of global change with coupled dynamic species distribution and population models. *Glob. Chang. Biol.* **2013**, *19*, 858–869. [[CrossRef](#)] [[PubMed](#)]
10. Keith, D.A.; Akcakaya, H.R.; Thuiller, W.; Midgley, G.F.; Pearson, R.G.; Phillips, S.J.; Regan, H.M.; Araujo, M.B.; Rebelo, T.G. Predicting extinction risks under climate change: Coupling stochastic population models with dynamic bioclimatic habitat models. *Biol. Lett.* **2008**, *4*, 560–563. [[CrossRef](#)] [[PubMed](#)]
11. Franklin, J.; Serra-Diaz, J.M.; Syphard, A.D.; Regan, H.M. Global change and terrestrial plant community dynamics. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3725–3734. [[CrossRef](#)] [[PubMed](#)]
12. Körner, C.; Paulsen, J. A world-wide study of high altitude treeline temperatures. *J. Biogeogr.* **2004**, *31*, 713–732. [[CrossRef](#)]
13. Mitton, J.B. Genetics and the physiological ecology of conifers. In *Ecophysiology of Coniferous Forests*; Smith, W.K., Hinckley, T.M., Eds.; Academic Press, Inc.: San Diego, CA, USA, 1995; pp. 1–36.
14. Aitken, S.N.; Yeaman, S.; Holliday, J.A.; Wang, T.; Curtis-McLane, S. Adaptation, migration or extirpation: Climate change outcomes for tree populations. *Evolut. Appl.* **2008**, *1*, 95–111. [[CrossRef](#)] [[PubMed](#)]
15. Savolainen, O.; Pyhajarvi, T.; Knurr, T. Gene flow and local adaptation in trees. *Annu. Rev. Ecol. Evol. Syst.* **2007**, *38*, 595–619. [[CrossRef](#)]
16. Reinhardt, K.; Castanha, C.; Germino, M.J.; Kueppers, L.M. Ecophysiological variation in two provenances of *Pinus flexilis* seedlings across an elevation gradient from forest to alpine. *Tree Physiol.* **2011**, *31*, 615–625. [[CrossRef](#)] [[PubMed](#)]
17. Rehfeldt, G.E. Adaptation of *Picea engelmannii* populations to the heterogeneous environments of the intermountain west. *Can. J. Bot.* **1994**, *72*, 1197–1208. [[CrossRef](#)]
18. Rehfeldt, G.E.; Ying, C.C.; Spittlehouse, D.L.; Hamilton, D.A., Jr. Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change, and reforestation. *Ecol. Monogr.* **1999**, *69*, 375–407. [[CrossRef](#)]
19. St. Clair, J.B. Genetic variation in fall cold hardiness in coastal douglas-fir in western Oregon and Washington. *Can. J. Bot.* **2006**, *84*, 1110–1121. [[CrossRef](#)]
20. Wu, H.X.; Ying, C.C. Geographic pattern of local optimality in natural populations of lodgepole pine. *For. Ecol. Manag.* **2004**, *194*, 177–198. [[CrossRef](#)]
21. Griesbauer, H.P.; Green, D.S.; O'Neill, G.A. Using a spatiotemporal climate model to assess population-level douglas-fir growth sensitivity to climate change across large climatic gradients in British Columbia, Canada. *For. Ecol. Manag.* **2011**, *261*, 589–600. [[CrossRef](#)]
22. McLane, S.C.; Daniels, L.D.; Aitken, S.N. Climate impacts on lodgepole pine (*Pinus contorta*) radial growth in a provenance experiment. *For. Ecol. Manag.* **2011**, *262*, 115–123. [[CrossRef](#)]
23. Wang, T.; O'Neill, G.A.; Aitken, S.N. Integrating environmental and genetic effects to predict responses of tree populations to climate. *Ecol. Appl.* **2010**, *20*, 153–163. [[CrossRef](#)] [[PubMed](#)]
24. Conlisk, E.; Castanha, C.; Germino, M.J.; Veblen, T.T.; Smith, J.M.; Moyes, A.B.; Kueppers, L.M. Seed origin and warming constrain lodgepole pine recruitment, slowing the pace of population range shifts. *Glob. Chang. Biol.* **2017**. [[CrossRef](#)] [[PubMed](#)]
25. Hampe, A.; Petit, R.J. Conserving biodiversity under climate change: The rear edge matters. *Ecol. Lett.* **2005**, *8*, 461–467. [[CrossRef](#)] [[PubMed](#)]
26. Jump, A.S.; Hunt, J.M.; Penuelas, J. Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. *Glob. Chang. Biol.* **2006**, *12*, 2163–2174. [[CrossRef](#)]
27. Hamburg, S.P.; Cogbill, C.V. Historical decline of red spruce populations and climatic warming. *Nature* **1988**, *331*, 428–431. [[CrossRef](#)]

28. Murphy, H.T.; VanDerWal, J.; Lovett-Doust, J. Signatures of range expansion and erosion in eastern north american trees. *Ecol. Lett.* **2010**, *13*, 1233–1244. [[CrossRef](#)] [[PubMed](#)]
29. Cayan, D.R.; Kammerdiener, S.A.; Dettinger, M.D.; Caprio, J.M.; Peterson, D.H. Changes in the onset of spring in the western United States. *Bull. Am. Meteorol. Soc.* **2001**, *82*, 399–415. [[CrossRef](#)]
30. Schwartz, M.D.; Ahas, R.; Aasa, A. Onset of spring starting earlier across the northern hemisphere. *Glob. Chang. Biol.* **2006**, *12*, 343–351. [[CrossRef](#)]
31. Sacks, W.J.; Schimel, D.S.; Monson, R.K. Coupling between carbon cycling and climate in a high-elevation, subalpine forest: A model-data fusion analysis. *Oecologia* **2007**, *151*, 54–68. [[CrossRef](#)] [[PubMed](#)]
32. Barnett, T.P.; Adam, J.C.; Lettenmaier, D.P. Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature* **2005**, *438*, 303–309. [[CrossRef](#)] [[PubMed](#)]
33. Moyes, A.B.; Castanha, C.; Germino, M.J.; Kueppers, L.M. Warming and the dependence of limber pine (*Pinus flexilis*) establishment on summer soil moisture within and above its current elevation range. *Oecologia* **2013**, *171*, 271–282. [[CrossRef](#)] [[PubMed](#)]
34. Flannigan, M.; Woodward, F.I. A laboratory study of the effect of temperature on red pine seed germination. *For. Ecol. Manag.* **1993**, *62*, 145–156. [[CrossRef](#)]
35. Meyer, S.E.; Debaene-Gill, S.B.; Allen, P.S. Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in *elymus elymoides*. *Seed Sci. Res.* **2000**, *10*, 213–223. [[CrossRef](#)]
36. Rowse, H.R.; Finch-Savage, W.E. Hydrothermal threshold models can describe the germination response of carrot (*Daucus carota*) and onion (*Allium cepa*) seed populations across both sub- and supra-optimal temperatures. *New Phytol.* **2003**, *158*, 101–108. [[CrossRef](#)]
37. Walck, J.L.; Hidayati, S.N.; Dixon, K.W.; Thompson, K.; Poschlod, P. Climate change and plant regeneration from seed. *Glob. Chang. Biol.* **2011**, *17*, 2145–2161. [[CrossRef](#)]
38. Knapp, A.K.; Smith, W.K. Factors influencing understory seedling establishment of Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) in southeast Wyoming. *Can. J. Bot.* **1982**, *60*, 2753–2761. [[CrossRef](#)]
39. Walder, T.; Erschbamer, B. Temperature and drought drive differences in germination responses between congeneric species along altitudinal gradients. *Plant Ecol.* **2015**, *216*, 1297–1309. [[CrossRef](#)]
40. Allen, P.S.; Meyer, S.E. Ecological aspects of seed dormancy loss. *Seed Sci. Res.* **1998**, *8*, 183–192. [[CrossRef](#)]
41. Green, D.S. Adaptive strategies in seedlings of three co-occurring, ecologically distinct northern coniferous tree species across an elevational gradient. *Can. J. For. Res.* **2005**, *35*, 910–917. [[CrossRef](#)]
42. Flora of North America Editorial Committee. *Flora of North America North of Mexico. Volume 2: Pteridophytes and Gymnosperms*; Oxford University Press: New York, NY, USA, 1993; p. 475.
43. Rehfeldt, G.E.; Crookston, N.L.; Warwell, M.V.; Evans, J.S. Empirical analyses of plant-climate relationships for the western United States. *Int. J. Plant Sci.* **2006**, *167*, 1123–1150. [[CrossRef](#)]
44. Monahan, W.B.; Cook, T.; Melton, F.; Connor, J.; Bobowski, B. Forecasting distributional responses of limber pine to climate change at management-relevant scales in Rocky Mountain National Park. *PLoS ONE* **2013**, *8*, e83163. [[CrossRef](#)] [[PubMed](#)]
45. Kueppers, L.M.; Conlisk, E.; Castanha, C.; Moyes, A.B.; Germino, M.J.; de Valpine, P.; Torn, M.S.; Mitton, J.B. Warming and provenance limit tree recruitment across and beyond the elevation range of subalpine forest. *Glob. Chang. Biol.* **2017**, *23*, 2383–2395. [[CrossRef](#)] [[PubMed](#)]
46. Niwot Ridge (Subalpine Forest) AmeriFlux Site Data. Available online: http://urquell.colorado.edu/data_ameriflux (accessed on 14 February 2009).
47. Meromy, L.; Molotch, N.P.; Williams, M.W.; Musselman, K.N.; Kueppers, L.M. Snowpack-climate manipulation using infrared heaters in subalpine forests of the southern Rocky Mountains, USA. *Agric. For. Meteorol.* **2015**, *203*, 142–157. [[CrossRef](#)]
48. Ibanez, I.; Clark, J.S.; LaDeau, S.; HilleRisLambers, J. Exploiting temporal variability to understand tree recruitment response to climate change. *Ecol. Monogr.* **2007**, *77*, 163–177. [[CrossRef](#)]
49. Kaufmann, M.R.; Eckard, A.N. Water potential and temperature effects on germination of Engelmann spruce and lodgepole pine seeds. *For. Sci.* **1977**, *23*, 27–33.
50. Loranger, H.; Zotz, G.; Bader, M.Y. Early establishment of trees at the alpine treeline: Idiosyncratic species responses to temperature-moisture interactions. *AoB Plants* **2016**, *8*, plw053. [[CrossRef](#)] [[PubMed](#)]

51. Conlisk, E.; Castanha, C.; Germino, M.J.; Veblen, T.T.; Smith, J.M.; Kueppers, L.M. Declines in low-elevation subalpine tree populations outpace growth in high-elevation populations with warming. *J. Ecol.* **2017**, *105*, 1347–1357. [[CrossRef](#)]
52. Bykova, O.; Chuine, I.; Morin, X.; Higgins, S.I. Temperature dependence of the reproduction niche and its relevance for plant species distributions. *J. Biogeogr.* **2012**. [[CrossRef](#)]



© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).