RESEARCH ARTICLE

Growth strategies and threshold responses to water deficit modulate effects of warming on tree seedlings from forest to alpine

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Abstract

1. Predictions of upslope range shifts for tree species with warming are based on assumptions of moisture stress at lower elevation limits and low-temperature stress at high-elevation limits. However, recent studies have shown that warming can reduce tree seedling establishment across the entire gradient from subalpine forest to alpine via moisture limitation. Warming effects also vary with species, potentially resulting in community shifts in high-elevation forests.

2. We examined the growth and physiology underlying effects of warming on seedling demographic patterns. We evaluated dry mass (DM), root length, allocation above- and below-ground, and relative growth rate (RGR) of whole seedlings, and their ability to avoid or endure water stress via water-use efficiency and resisting turgor loss, for *Pinus flexilis*, *Picea engelmannii* and *Pinus contorta* seeded below, at and above tree line in experimentally warmed, watered and control plots in the Rocky Mountains, USA. We expected that growth and allocation responses to warming would relate to moisture status and that variation in drought tolerance traits would explain species differences in survival rates.

3. Across treatments and elevations, seedlings of all species had weak turgor-loss resistance, and growth was marginal with negative RGR in the first growth phase (-0.01 to -0.04 g g^{-1} day^{-1}). Growth was correlated with soil moisture, particularly in the relatively small-seeded *P. contorta* and *P. engelmannii*. *Pinus flexilis*, known to have the highest survivorship, attained the greatest DM and longest root but was also the slowest growing and most water-use efficient. This was likely due to its greater reliance on seed reserves. Seedlings developed 15% less total DM, 25% less root DM and 11% shorter roots in heated compared with unheated plots. Higher temperatures slightly increased DM, root length and RGR where soils were wettest, but more strongly decreased these variables under drier conditions.

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4. *Synthesis.* The surprising heat inhibition of tree seedling establishment at the cold edge of forests appears to have a physiological basis: newly germinated seedlings have poor moisture stress tolerance, which appears related to marginal initial growth and heavy reliance on seed reserves. Variation in these attributes among tree species at tree line helps explain their different climate responses.

KEYWORDS

alpine tree line, climate experiment, ecophysiology, growth, plant–climate interactions, seedling establishment, water stress

1 | INTRODUCTION

Ecologists tend to view climate limitations to plants in a binary fashion, most crudely as either energy or water limited. Low growing season temperatures are thought to inhibit growth and thus presence of trees at high elevations (Körner & Paulsen, 2004). This notion leads to predictions that forest boundaries will move upslope with warming (Grace, Berninger, & Nagy, 2002; Monahan, Cook, Melton, Connor, & Bobowski, 2013). Indeed, in situ warming studies of established vegetation in the transition from forest through tree line to alpine often show increases in mature plant growth with warming (e.g. Danby & Hik, 2007; Michelsen, Jonasson, Sleep, Havstrom, & Callaghan, 1996). Still, only about half of tree lines studied show upward movement with warming since 1900 (Harsch, Hulme, McGlone, & Duncan, 2009), indicating that temperature is likely not the only constraint in all systems. Indeed, recent studies show temperature and precipitation interactively affect range shifts for many plant species (Crimmins, Dobrowski, Greenberg, Abatzoglou, & Mynsberge, 2011; Harsch & HilleRisLambers, 2016). Upslope forest movement cannot occur without successful tree establishment beyond current tree lines, and the factors that limit establishment may differ from those that limit growth of established trees (Daniels & Veblen, 2004). Recent work suggests that moisture stress impacts carbon relations and survival of seedlings at tree line, and thus might slow or prevent tree line advance (Gill, Campbell, & Karlinsey, 2015; Moyes, Castanha, Germino, & Kueppers, 2013; Moyes, Germino, & Kueppers, 2015). However, each of these studies examined a single species, and none took place during relatively wet years, when seedling establishment is most likely to occur according to dendrochronological studies (e.g. Daniels & Veblen, 2004; Millar, Westfall, Delany, Flint, & Flint, 2015). Even more recent work demonstrated that seedlings of different species respond individualistically to experimental warming from forest to alpine (Conlisk et al., 2017b; Kueppers et al., 2017).

Subalpine conifer seedlings have exceptionally low in situ survival rates at high elevation, especially in their first season, thus creating a demographic bottleneck (Germino, Smith, & Resor, 2002). Climate responses of newly germinated seedlings have a strong influence on tree recruitment patterns across elevation gradients from forest to alpine (Maher & Germino, 2006), impacting population structure several years following experimental seeding (Kueppers et al., 2017) and

decades to centuries later (e.g. simulations of Conlisk et al., 2017a), when maturing individuals have greater climate tolerances. Immature and unhardened tissues of seedlings experience a microclimate that is tightly coupled to that of the ground surface, which is subject to temperature extremes (Geiger, 1950). After seedlings emerge following snowmelt, their environment transitions from freezing and wet to warm and dry or even searing for seedlings growing on duff in forests, resulting in exposure to multiple stress interactions (Germino & Smith, 2000; Germino et al., 2002). Despite these challenges, seedlings must achieve a positive carbon balance or rely on seed reserves during their first growing season to become established. Species differences in adaptive strategies for seedling establishment reflect trade-offs between leaf deployment for carbon gain and risks of leaf area exposure to temperature, light and vapour deficit stress, along with the need to develop roots for adequate water uptake as soil–water deficit develops during summer. These trade-offs may be strongly affected by overall plant size. Seed and seedling size can vary many-fold among conifer species and could strongly influence carbohydrate availability and carbon balance.

Traits affecting uptake and efficient use of water may, therefore, be key components of adaptive strategies for seedling establishment at tree line. Water uptake per unit leaf area can be increased by raising the root mass fraction (RMF, ratio of root to whole-seedling dry mass [DM]). Water can be obtained from drier soils via physiological adjustments such as reduction of turgor loss points (Ψ_{TLP}, shifting towards more negative MPa thresholds), the water potential at which wilting occurs (Bartlett, Scoffoni, & Sack, 2012). Photosynthetic water-use efficiency (WUE), the amount of carbon fixed per unit of water lost to transpiration, can be increased by reducing stomatal conductance relative to photosynthesis.

We asked whether and how tree species differences in response to water deficit relates to their establishment along the gradient from forest to alpine. We compared growth and moisture stress responses in first-year seedlings of three species planted in common gardens subject to a factorial of heating and watering treatments below, at and above tree line. This combination of an elevation gradient with warming and watering treatments enabled us to observe species differences in seedling responses to a broad set of climatic conditions. If water deficit indeed limits establishment in the forest to alpine transition, we predicted that: (1) effects of increased temperatures on seedling

growth would be contingent upon water availability; and (2) differences in species sensitivity to water stress would reflect differences in strategies for accessing, physiologically regulating and efficiently using water. More specifically, we predicted that limber pine, which typically had higher initial survival in the common gardens (Castanha, Torn, Germino, Weibel, & Kueppers, 2013; Kueppers et al., 2017), would have the greatest drought tolerance, while Engelmann spruce, with the poorest overall survival, would have the least drought tolerance.

2 | MATERIALS AND METHODS

Entire seedlings were harvested at *c.* 3 and again at 9 weeks of age following germination/emergence in the experimental plots, and we then measured DM of above and- below-ground parts, root length, RMF and relative growth rates (RGRs) from seed to 3 weeks and from 3 to 9 weeks, turgor-loss point, and photosynthetic WUE (as $\delta^{13}C$). We analysed the effects of "site" and "treatment" on the dependent variables with "categorical environment" models. We also analysed the effects of soil temperature and moisture on the dependent variables with "continuous environment" models.

2.1 | Species

Across much of the North American Rocky Mountains, three species dominate the transition from high-elevation forest to tree line: limber pine (*Pinus flexilis* James, PiFl), Engelmann spruce (*Picea engelmannii* Parry ex Engelm., PiEn) and lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex. Loud., PiCo), with subalpine fir (*Abies lasiocarpa* (Hook.) also

common. PiEn is widespread and, along with *A. lasiocarpa*, is dominant at tree line. PiFl is a pioneer species that is widely distributed and is present but not locally abundant at tree line except in patches. PiCo is widespread and dominant in the subalpine forest but typically absent at tree line, except under specific edaphic conditions. In common gardens, first-year survival of PiFl seeded below, at and above tree line at Niwot Ridge, CO, USA, far exceeded that of PiEn and of PiCo, and at least one demographic phase of seedlings of all species were sensitive to water limitation (Castanha et al., 2013; Conlisk et al., 2017b; Kueppers et al., 2017; Moyes et al., 2013).

2.2 | Sites and experimental plot design

We established three research sites along an elevation gradient at the University of Colorado Mountain Research Station in the Front Range of the Colorado Rocky Mountains (Figure 1). Site characteristics are described in detail in Castanha et al. (2013). The Forest site (3,060 m a.s.l.) is dominated by mature PiCo and PiEn, with PiFl and subalpine fir (*A. lasiocarpa* (Hook.) Nutt.) also present. The Tree line site (3,430 m a.s.l.), located at the cool (upper) edge of the local subalpine species range, is an open meadow surrounded by krummholz mats and flag trees of *A. lasiocarpa*, PiEn and occasional PiFl individuals. The Alpine site (3,540 m a.s.l.) is located on Niwot Ridge above the local subalpine tree species current range and is dominated by forbs, grasses and sedges. The snow-free period is longest at the Forest site and shortest at the Tree line site, which receives and traps snow from upwind (Table 1). The abundance and form of trees along the transition from the Forest to Tree line sites has, to our knowledge, not changed appreciably in recent decades, although there have been reports
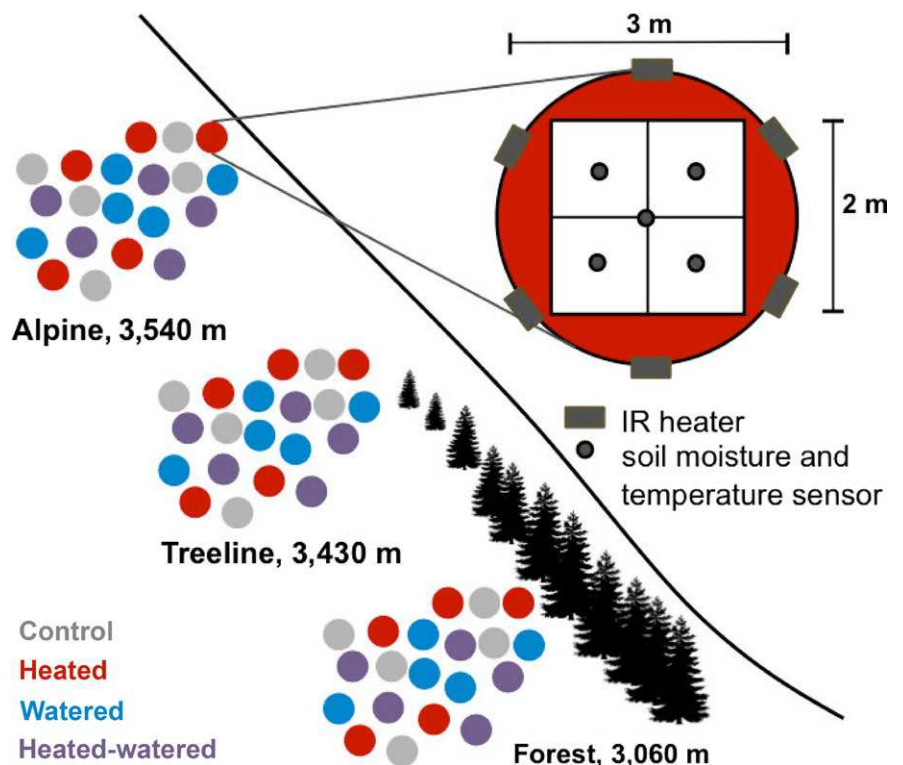

FIGURE 1 Experimental design—common gardens were planted at three elevations (Forest, Tree line and Alpine). At each elevation, there were five replicate 3-m diameter plots of each of four treatments—control, heated, watered and heated + watered. Quadrants within plots were planted with different species-provenance combinations (see text)

TABLE 1 Average daily mean (*SE*) site climate and treatment effects during the snow-free season for 2013. Abbreviations are snow-free season length (SL; determined from diel variability in soil temperature in unheated plots); heating effects on 5- to 10-cm soil temperature (ΔT_H), snow-free season length (ΔSL_H) and 5- to 10-cm soil volumetric water content (ΔVWC_H); and watering effect on 5- to 10-cm soil volumetric water content (ΔVWC_W)

Site	Elev. (m)	Mean air T (°C)	Mean min air T (°C)	Mean max air T (°C)	Mean soil T (control) (°C)	Wind (m/s)	Mean soil VWC (control) (m³/m³)	SL (days)	ΔT_H (°C)	ΔSL_H (days)	ΔVWC_H (m³/m³)	ΔVWC_W (m³/m³)
Alpine	3,540	6.2 (0.55)	3.0 (0.6)	9.7 (0.6)	7.9 (0.1)	5.7 (0.3)	0.25 (0.02)	139	1.51 (0.08)	10	−0.01 (0.002)	0.01 (0.002)
Tree line	3,430	7.0 (0.55)	3.4 (0.5)	11.3 (0.6)	8.7 (0.2)	3.4 (0.2)	0.28 (0.02)	127	1.66 (0.13)	11	−0.01 (0.001)	0.01 (0.002)
Forest	3,060	9.4 (0.44)	4.3 (0.4)	15.9 (0.5)	9.2 (0.4)	0.2 (0.0)	0.20 (0.01)	154	3.54 (0.08)	38	0.00 (0.002)	0.02 (0.001)

suggesting new tree establishment above tree limit at Niwot Ridge (Daly & Shankman, 1985) and height growth of krummholz in nearby tree lines of the Front Range (Weisberg & Baker, 1995).

At each site, twenty 3-m diameter experimental plots were assigned to one of four treatments: heated (H), heated and watered (H+W), watered (W) and control (C). Heated plots were warmed using overhead infrared heaters (Moyes et al., 2013, 2015). Watered plots received 2.5 mm of water once per week. Watering began 2–3 weeks after snowmelt and continued until the end of September. Total precipitation for Niwot Ridge during the 2013 water year was 940 mm, maximum snow depth was 150 cm, and mean temperature was 2.8°C. These represent 116%, 75% and 130%, respectively, of multi-year means (1990–2016, 2005–2017 and 1990–2016, respectively, NWCC, 2017). Soil temperature and volumetric water content (VWC) were measured at 15-min intervals by sensors installed at 5- to 10-cm depth in the centre of each 1-m² quadrant (Figure 1, ECTM or 5TM; Decagon, Pullman, WA, USA).

2.3 | Seed collection and sowing

In fall 2012, we collected, cleaned and sowed seeds from local low- (2,900–3,100 m) and high- (3,300–3,450 m) elevation populations (hereafter referred to as low and high provenances) for each species. Each plot was divided into four permanently marked 1-m² quadrants (Figure 1), which were in turn divided into one hundred 10 × 10 cm cells. We sowed high-elevation limber pine (PiFl High), low-elevation limber pine (PiFl Low), high-elevation Engelmann spruce (PiEn High) or low-elevation Engelmann spruce (PiEn Low) into seventy 10 × 10 cm cells in separate quadrants. In another 24 cells per quadrant, we sowed high-elevation lodgepole pine (PiCo High) in the PiFl quads or low-elevation lodgepole pine (PiCo Low) in the PiEn quads. To avoid edge effects, the six outermost corner cells of each quadrant were not seeded. Hardware cloth exclosures were installed over each quadrant to protect seeds from predation.

2.4 | Seedling surveys and harvest

In spring 2013, starting 3 weeks after snowmelt, we surveyed plots weekly for new emergent seedlings until we observed a peak in emergence. A subset of entire seedlings from the peak cohort was carefully harvested at *c*. 3 weeks (24 ± 2 [*SD*] days, Time 1) and 9 weeks (62 ± 7 [*SD*] days, Time 2) after emergence (Table S1). Snowmelt, and hence peak emergence, occurred at different times in different plots, and harvest timing was staggered accordingly. Prior to harvest, we marked seedlings at ground level to differentiate above- from below-ground tissue. Seedlings were stored on ice and shipped overnight to the US Geological Survey laboratory in Boise, ID, where root length and Ψ_{TLP} were measured (see below). Seedlings were subsequently oven-dried at 65°C and weighed. Seedlings were harvested from all three sites at Time 1, while the Time 2 collection included only the Alpine and Tree line sites, as seedlings were too sparse for comprehensive sampling at the Forest site. At Time 1, we collected both provenances, while at Time 2, we collected only one provenance at the Alpine site due

to insufficient survival of high provenance seedlings. Two seedlings (PiFl) or two groups of 2–5 seedlings (PiCo, PiEn) were collected from each quadrant of each plot where available, with one individual per set subjected to Ψ_{TLP} analyses.

2.5 | Relative growth rate

We calculated RGR between Time 1 and Time 2 (RGR_{1-2}) as follows:

$$\frac{\ln B2 - \ln B1}{A2 - A1}$$

where B1 and B2 are seedling DM at the first and second collections and A1 and A2 are seedling age in days at the first and second collections. We used the same formula with average oven-dry seed weights for each provenance of each species to estimate RGR from seed to Time 1 (RGR_{seed-1}).

2.6 | Turgor loss point

Following harvest, entire (i.e. roots still attached) seedlings were hydrated overnight, and pressure–volume relationships determined by periodically measuring water potential with a pressure chamber (PMS Instruments, Corvallis, OR, USA) followed by mass as seedlings dried on the lab bench (Tyree & Hammel, 1972). Seedlings dried relatively quickly, and so initial measurements were taken in rapid succession (c. every 2 min). Curve fitting and calculation of water potential at Ψ_{TLP} followed Sack and Pasquet-Kok (2011). Where necessary, we corrected for "plateau effects" by discarding initial measurements (Parker & Pallardy, 1987). At Time 1 (c. 3 weeks of age), PiEn and PiCo seedlings were often too fragile for repeated water potential measurements. Therefore, it was possible to complete only a small number of Time 1 pressure–volume curves for these two species (n = 10 PiCo from five plots, n = 14 PiEn from five plots), preventing treatment and site comparisons.

Rehydrating plant tissue prior to pressure–volume curves has been shown to increase Ψ_{TLP} for anisohydric but not for isohydric species (Meinzer, Rundel, Sharifi, & Nilsen, 1986; Meinzer, Woodruff, Marias, McCulloh, & Sevanto, 2014). Comparisons made in our laboratory showed rehydration did not increase Ψ_{TLP} for the species in this manuscript (Figure S1).

2.7 | Tissue $\delta^{13}C$

We measured tissue $\delta^{13}C$ as an indicator of WUE (Ehleringer, White, Johnson, & Brick, 1990) in the Time 2 seedlings relative to the Vienna Pee Dee Belemnite standard, where

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C} \right) \text{sample}}{\left(\frac{^{13}C}{^{12}C} \right) \text{standard}} - 1 \right) \times 1000‰$$

The $\delta^{13}C$ of plant DM reflects the ratio of CO_2 concentration inside the leaf to CO_2 concentration of the atmosphere during photosynthesis (C_i/C_a; Farquhar, Ehleringer, & Hubick, 1989); higher C_i/C_a leads to greater enzymatic discrimination against ^{13}C and therefore to lower (more negative) $\delta^{13}C$. Increases in C_i/C_a and corresponding decreases in WUE can result from stomatal opening or decreased biochemical demand for carbon. We measured tissue $\delta^{13}C$ with a Picarro G2121-i CO_2 analyser coupled to a Costech 4010 Elemental Analyzer and a Picarro Liaison™ to coordinate gas sample delivery. Whole individuals of the same species collected from the same plot and quadrant were combined prior to grinding to provide sufficient material for analysis.

2.8 | Statistical analyses

We conducted two sets of statistical analyses. The "categorical environment" analyses allowed us to determine the significance of climate manipulation and provenance effects at the end of the growing season (Time 2) at the Alpine and Tree line sites only (seedlings were too sparse for comprehensive sampling at the Forest site at Time 2). The "continuous environment" analyses tested effects of continuous variation in soil temperature and moisture on DM, root length and growth rates at both Times 1 and 2 (c. 3 and 9 weeks) and across all sites. Continuous environment analyses used the quadrant-level measures of soil temperature and moisture in lieu of "site" and "treatment" as fixed factors and incorporated data from the Time 1 collection and from the Forest site (where available). Strong collinearity prevented inclusion of site and treatment with soil temperature and moisture in the same models. Table 2 lists the measurements made at each site and time.

2.8.1 | Categorical environment analyses

To determine significance of responses of total DM, root DM, root length, RMF, $\delta^{13}C$ and Ψ_{TLP} at Time 2, we used linear mixed effect models with a full factorial of the fixed effects of species, site (Alpine and Tree line only), heating and watering (JMP 11.2.0; SAS Institute, Cary, NC, USA). We used the same models for RGR_{seed-1} (including all three sites) and RGR_{1-2} (Alpine and Tree line only).

The sampling design did not allow for both site and provenance effects on any response variable to be included in a full factorial model because, due to insufficient survival of high provenance seedlings, only the low provenance was collected at the Alpine site for Time 2 (Table 2 and Table S1). Site effects were of greatest interest, so for those variables for which preliminary analyses detected significant effects of provenance (specifically, on DM, root DM, root length), models were fit to data from the low provenance only to ensure a balanced site comparison. For those variables that did not vary among provenances (RMF, RGR, Ψ_{TLP}, $\delta^{13}C$), data from both provenances were combined for models with individual plant data (RMF, RGR_{seed-1}) and averaged for models with quadrant-level data (RGR_{1-2}, Ψ_{TLP}, $\delta^{13}C$), in order to have one observation per species per plot and to balance sample numbers for site comparisons.

For variables with one observation per plot per species (Ψ_{TLP}, $\delta^{13}C$, RGR_{1-2}), we included "plot" as a random effect. Where data were available at the individual plant level (i.e. for multiple individuals of the same

TABLE 2 Summary of data collection by site and time. RGR$_{seed-1}$ was calculated for all three sites at the individual level. RGR$_{1-2}$ could only be calculated for Tree line and Alpine sites at the quadrant level

Time	Site	Dry mass/RMF	RGR$_{seed-1}$	RGR$_{1-2}$	Ψ_{TLP}	$\delta^{13}C$
1 (early season)	Alpine	Individual samples	Individual samples	NA	1 sample/quad; PiFl only	NA
	Tree line	Individual samples	Individual samples	NA	1 sample/quad; All spp but limited for PiCo and PiEn	NA
	Forest	Individual samples	Individual samples	NA	1 sample/quad; PiFl only	NA
2 (late season)	Alpine	Individual samples (low provenance only)	NA	Quad average (low provenance only)	1 sample/quad; All spp (low provenance only)	Quad average (low provenance only)
	Tree line	Individual samples	NA	Quad average	1 sample/quad; All spp	Quad average
	Forest	NA	NA	NA	NA	NA

species per quadrant: DM, root DM, root length, RMF, RGR$_{seed-1}$), we included both plot and quadrant nested within plot as random effects.

We included age (days since emergence) as a covariate for responses of DM, root length, RGR and RMF, and harvest date as a covariate for $\delta^{13}C$ and Ψ_{TLP}, based on AIC analyses used to determine which covariates had the most explanatory power. Significant species effects were further investigated using Tukey HSD pairwise comparisons. Dry mass data were log transformed and root length data were square root transformed to homogenize variance.

Due to limited data for PiEn and PiCo at Time 1, Ψ_{TLP} for this collection was analysed using a one-way ANOVA with species as the independent variable. A subset of PiFl from the same five plots as the PiCo and PiEn was included in the analysis to make it balanced and to correct for any possible effects of treatment (the five plots were all from the Tree line site and included control, watered, and heated and watered treatments).

2.8.2 | Continuous environment analyses

Significance of responses of DM, root DM, root length, RMF, RGR$_{seed-1}$ and $\delta^{13}C$ to a full factorial of the fixed effects of species, provenance, soil temperature and soil VWC were determined with mixed effects models. If a variable did not vary with provenance, then provenance effects were removed from the model (occurred for RMF, RGR$_{seed-1}$, $\delta^{13}C$). In that case, data from the two provenances were combined for models run with individual plant data (RMF, RGR$_{seed-1}$) and averaged for models run with quadrant-level data ($\delta^{13}C$). As above, where observations were made at the quadrant level ($\delta^{13}C$), we included plot as a random effect. Where data were available at the individual plant level (i.e. for multiple individuals of the same species per quadrant—DM, root DM, root length, RMF, RGR$_{seed-1}$), we included both plot and quadrant nested within plot as random effects. To generate soil temperature and moisture variables, we averaged daily minimum (T_{min}), maximum (T_{max}), and mean (T_{mean}) temperatures, or daily mean soil VWC over the lifetime (LT) of each individual seedling using the sensors in the quadrant for each seedling, resulting in daily minimum temperatures averaged over the lifetime of the seedling (T_{minLT}), daily maximum temperature

averaged over the lifetime of the seedling (T_{maxLT}), daily mean temperature averaged of the lifetime of the seedling (T_{meanLT}) and daily average soil volumetric water content averaged over the lifetime of the seedling (VWC$_{LT}$), respectively. To avoid collinearity among the temperature variables, we compared alternative models of their separate explanatory power and found AIC scores to be 5–10 points lower (better) for T_{minLT} compared with the other variables, and hence, we proceeded with T_{minLT} and VWC$_{LT}$ to represent the actual microclimate of plots as a predictor variable. We included age (days since emergence) as a covariate for DM, root length, RGR, and RMF and harvest date for $\delta^{13}C$, again based on AIC. We did not run continuous environment analyses for RGR$_{1-2}$ or Ψ_{TLP} because no site or treatment effects were evident in the categorical environment analyses, which already included all the available data for these variables.

3 | RESULTS

3.1 | Dry mass, root mass fraction and root length

Dry mass of whole seedlings at Time 2 at Alpine and Tree line sites was 15.3% lower in heated than in unheated plots, 13.0% greater in watered than in unwatered plots (p = .019, .038, Figure 2b,c, Table 3) and many times greater in PiFl than the other species (least in PiEn, Figure 3a). Root DM was 18.0% greater for Alpine than for Tree line seedlings (p = .024, Figure 2d), 25.1% lower for heated than for unheated seedlings (p = .0024, Figure 2e), much greater in PiFl and least in PiEn (Table 3, Figure 4a). Roots were 11.1% shorter for heated than unheated seedlings (p = .034, Figure 2h) and longer in PiFl than in PiCo and PiEn (Figure 4b). Root mass fraction was 11.1% greater in the Alpine than in Tree line sites, 5.9% less in heated compared with unheated plots (p < .0001, .016, Figure 2j,k), and greater for PiEn than for PiCo or PiFl (Table 3, Figure 4c).

Dry mass and root length responses to continuous variation in temperature and moisture differed among the species. Whole-seedling DM was negatively related to T_{minLT} in PiCo and PiEn but not in PiFl, and also more positively related to VWC$_{LT}$ in PiCo and PiEn than in PiFl (Table 4, Figure 5a,b). Greater soil moisture led to longer PiCo and PiEn roots with more DM but shorter PiFl roots with less DM (Figure 5h,e).
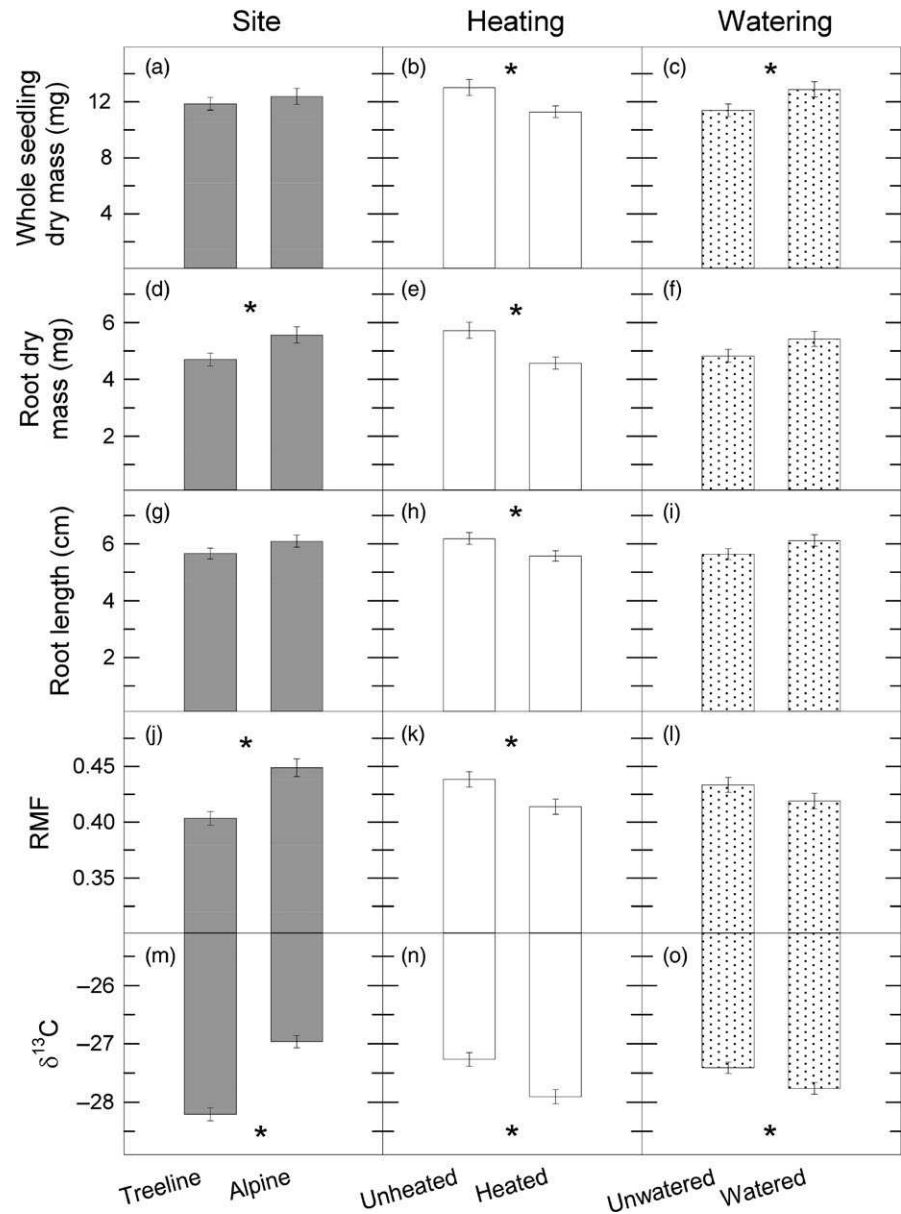
FIGURE 2 Least squares means and standard errors for main effects of site, heating and watering on *c.* 9-week-old (Time 2) seedlings in categorical environment models (Table 3). Asterisks denote significant ($p < .05$) main effects. Data are from Alpine and Tree line sites only

RMF was negatively related to T_{minLT} and VWC_{LT}, and root DM was negatively related to temperature (Table 4). Total DM and root length were inversely related to T_{minLT} where soil moisture was scarce, but positively related in wet soils (Significant $T_{minLT} \times VWC_{LT}$ interaction in Table 4, Figure 5c,i). Dry mass was 7.2% greater, root DM was 7.8% greater, and roots were 7.0% longer in seedlings from the low provenance than in seedlings from the high provenance ($p = .012$, .015, .0004, Table 4). However, these temperature, moisture and provenance effects on DM (for whole-seedling or roots) and root length were small relative to the effects of species and age (Table 4).

3.2 | Relative growth rate

Dry mass tended to decrease during the transition from seed to 3-week-old seedlings (Figure 3a), resulting in negative growth rates for this interval (RGR_{seed-1}, Figures 3a and 6). PiFl had a more negative RGR_{seed-1} across sites and treatments than PiCo and PiEn, with the exception of heated

plots in the Forest site, where PiCo and PiEn had very negative RGR_{seed-1} (see interactions in categorical analysis, Table 3, Figure 6).

In continuous environment analyses, PiFl also had the most negative RGR_{seed-1}, and PiCo had the least negative RGR_{seed-1}. While RGR_{seed-1} increased with soil moisture for all species, it appeared to be somewhat more responsive to soil moisture for PiCo and PiEn than for PiFl (Species × VWC interaction, $p = .06$, Figure 5k, Table 4). Wet soils alleviated growth losses under warming, while dry soils exacerbated them as revealed by RGR_{seed-1} becoming less negative at higher temperatures when soils were wet compared with dry (Table 4, significant $VWC_{LT} \times T_{minLT}$ interaction, Figure 5l).

In the 3- to 9-week interval, RGR was positive for all species, and by Time 2 (*c.* 9 weeks), DM of seedlings had surpassed DM of their seeds in PiCo and PiEn but not PiFl (Figure 3a,b). A significant site × species interaction showed that this species pattern was stronger at the Tree line than at the Alpine site (Table 3). No significant effect of treatment or of age was detected for this growth interval.

TABLE 3 Results (*p*-values) of fixed-effect tests for categorical environment analyses for total dry mass, root dry mass, root length, root mass fraction, $\delta^{13}C$ and Ψ_{TLP} for seedlings at Time 2 (*c.* 9 weeks of age) and RGR for the period from seed to 3-week-old seedling and from 3 to 9 weeks. Models include data for Alpine and Tree line sites only, with the exception of RGR$_{seed-1}$, which also includes the Forest site. Symbols in parentheses represent the direction of the heating, watering, age or DOY harvest effect. Values <.05 are in bold type. Superscript numbers are defined in the first column of the corresponding row. Parameter estimates can be found in Table S2a,b

Source	Log whole-seedling dry mass[a]	Log root dry mass[a]	Root length (square root)[a]	RMF	$\delta^{13}C$	Ψ_{TLP}	RGR$_{seed-1}$	RGR$_{1-2}$
N (individuals[1] or quadrants[2])	223[1]	202[1]	225[1]	335[1]	114[2]	155[2]	544[1]	112[2]
R^2	0.94	0.93	0.74	0.51	0.88	0.36	0.69	0.55
Site	0.4917	**0.0235**	0.1445	**<0.0001**	**<0.0001**	0.6554	**<0.0001**	0.7471
Species	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0009**	**<0.0001**	**0.0001**
Site × Species	0.2562	0.2887	0.362	0.1644	0.7183	0.0551	**<0.0001**	**0.0045**
Heated	**0.0185 (−)**	**0.0024 (−)**	**0.0338 (−)**	**0.0155 (−)**	**0.0021 (−)**	0.8954	**0.0004 (−)**	0.1598
Site × Heated	0.7984	0.2456	0.2655	0.12	0.5919	0.739	**0.0033**	0.5122
Species × Heated	0.1628	0.3434	0.0575	0.7861	0.175	0.5465	**0.0001**	0.6307
Site × Species × Heated	**0.0165**	**0.005**	**0.0035**	0.1053	0.458	0.3719	**0.0001**	0.2862
Watered	**0.0375 (+)**	0.0964	0.0917	0.1414	**0.0179 (−)**	0.6194	0.3347	0.1958
Site × Watered	0.664	0.1632	0.4089	0.4826	0.891	0.1471	0.0954	0.8283
Species × Watered	0.4033	0.1023	0.4028	0.2932	0.7203	0.4318	0.7388	0.7402
Site × Species × Watered	0.2015	0.5901	0.5599	0.8019	0.9596	0.13	0.1097	0.2387
Heated × Watered	0.4387	0.6498	0.4727	0.7985	0.1697	0.9581	0.1258	0.6407
Site × Heated × Watered	0.6385	0.3834	0.1503	**0.0068**	0.3608	0.8263	0.2685	0.8953
Species × Heated × Watered	0.3392	0.8224	0.2336	0.8944	**0.0113**	0.2056	0.7075	0.5674
Site × Species × Heated × Watered	0.7243	0.2827	0.7838	0.6224	0.8856	0.2484	0.6265	0.1583
Mean age (days)[1] or DOY harvest[2]	**0.0307 (+)[1]**	0.0644[1]	0.0808[1]	0.6091[1]	**0.0296 (−)[2]**	**0.0040 (−)[2]**	0.1137[1]	0.9355[1]

[a]Low provenance only.

3.3 | Tissue $\delta^{13}C$

PiFl had the highest average $\delta^{13}C$ (least negative, indicating greater WUE) and PiCo the lowest, with a difference of 2.65‰ (Table 3, Figure 7a). Across species, seedlings in the Alpine site had on average 1.24‰ greater $\delta^{13}C$ than those in the Tree line site (*p* < .0001, Figure 2m). $\delta^{13}C$ was on average 0.64‰ lower in heated than unheated plots, and 0.35 ‰ lower in watered than unwatered plots (*p* = .0021, .018, Figure 2n,o). $\delta^{13}C$ decreased 0.038 ± 0.017‰ per day with collection date (Table 3 and Table S2). $\delta^{13}C$ decreased strongly with increasing T_{minLT} (*p* < .0001) and weakly with increasing VWC$_{LT}$ (*p* = .0216) showed the same strong species effect and did not vary with collection date in continuous environment analyses (Tables 4 and Table S3). At the species level, there was a negative relationship between $\delta^{13}C$ and RGR$_{1-2}$ indicating a trade-off of slower growth for greater WUE in PiFl and the opposite for PiCo (Figure 8).

3.4 | Turgor loss point

At Time 1, Ψ_{TLP} was lowest for PiFl, highest for PiCo, and intermediate for PiEn (Figure 7b), although absolute differences were not large

and values were high overall (i.e. >−1.0 MPa, indicating poor desiccation tolerance). At Time 2, species and harvest date explained most variation in Ψ_{TLP} (Table 3). PiEn had lower Ψ_{TLP} and thus a greater ability to sustain turgor upon desiccation than the other two species (Figure 7c), and Ψ_{TLP} decreased on average 0.025 MPa per day over the course of the collection period over all species, possibly indicating seasonal acclimation (e.g. Colombo & Teng, 1992). Ψ_{TLP} was negatively correlated with DM for all three species over the course of both collections, indicating increased desiccation tolerance with growth (PiCo *r* = −.301, *p* = .0229; PiEn *r* = −.395, *p* = .0022; PiFl *r* = −.254, *p* = .0029, data not shown).

4 | DISCUSSION

In contrast to the traditional emphasis on low-temperature limitations to growth and assumptions of carbon saturation (e.g. Körner, 1998 and subsequent studies) as the dominant factor controlling tree line elevation, our data reveal that tree seedlings across the entire forest to alpine ecotone undergo a phase in which growth is so marginal that RGR becomes negative—a condition only possible with a negative
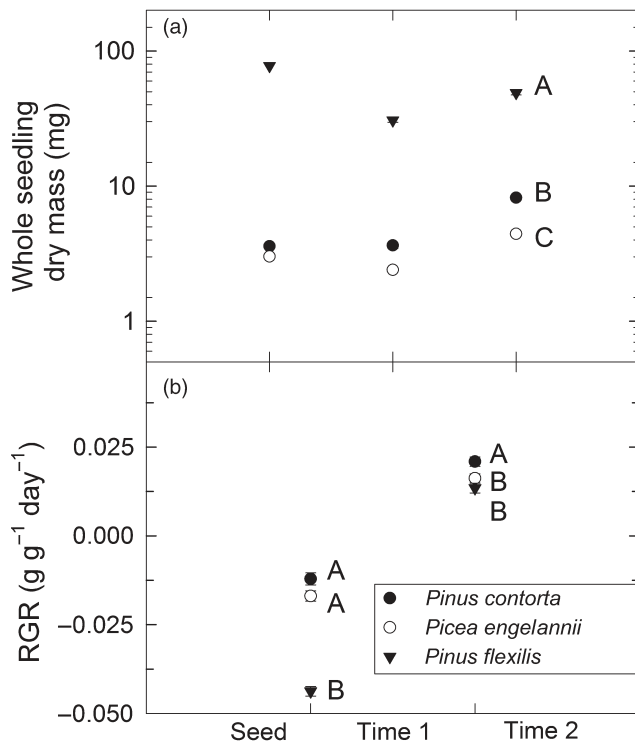
FIGURE 3 Total dry mass per individual for seeds, Time 1 (*c.* 3-week, new germinants, early season) and Time 2 (*c.* 9-week-old, late season) seedlings (a) and RGR_{seed-1} and RGR_{1-2} (b) of *Pinus contorta*, *Picea engelmannii* and *Pinus flexilis*. Letters denote results of Tukey HSD post hoc tests among species within a given time period for categorical environment analyses (species not labelled with same letter are significantly different). Error bars denote standard error and are often smaller than symbols. Seed weights are averages of 50 seeds

carbon balance. Our results also highlight the critical importance of seed carbon reserves for establishment of tree seedlings in the forest to alpine transition by showing that species with greater seed reserves can sustain a more negative RGR and exhibit greater DM, root development and WUE, all of which mitigate water stress impacts. These associations, combined with poor desiccation tolerance of seedlings (high Ψ_{TLP} values), help explain the surprising and repeated observation that warming tends to inhibit tree seedling survival and establishment in the transition from high-elevation forest to the alpine tundra, except under the wettest conditions (Conlisk et al., 2017b; Kueppers et al., 2017).

4.1 | Seed reserves appear to mitigate the impact of water limitation on establishment

Species with larger seeds often have greater establishment rates than smaller-seeded species, particularly under stressful conditions such as moisture limitation (Caddick & Linder, 2002; Hallett, Standish, & Hobbs, 2011; Leishman & Westoby, 1994). Our findings combine with Kueppers et al. (2017) and Conlisk et al.'s (2017b) survival and establishment patterns to suggest that this seed-reserve effect operates at the alpine tree line ecotone and moreover provide generalizable insights on physiological mechanisms underlying the seed-reserve effect.
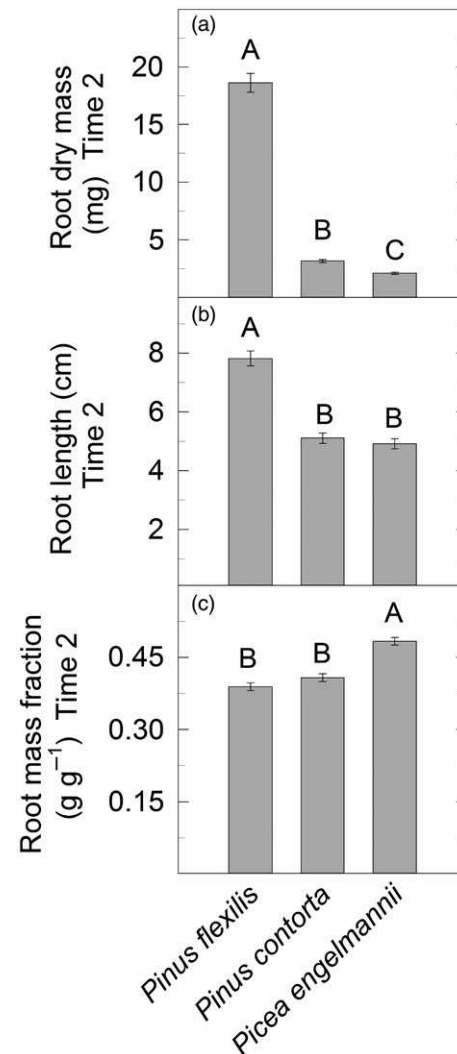

FIGURE 4 Least squares means and standard error for root dry mass (a), root length (b) and root mass fraction (c) at Time 2 (*c.* 9 weeks) by species in categorical environment models. Capital letters denote results of Tukey–Kramer comparisons—species not labelled with same letter are significantly different

Negative RGR and DM reductions indicated that all species used more carbon for growth and maintenance respiration than they fixed during early establishment (Figures 3 and 6). For PiFl, the species with both the greatest seed size and reserves but also the most rapid seed-reserve depletion (Figure 3), early growth (RGR_{seed-1}) and whole-seedling DM (Figure 5k,b) were generally less sensitive to soil moisture than for the smaller-seeded PiCo and PiEn. Inverse relationships between root length and soil moisture in PiFl but not in the other species suggest PiFl seedlings were better able to forage for diminishing water as soils dried, thereby more readily meeting its physiological water needs than the other species (Figure 5e,h). The smaller-seeded PiEn and PiCo appeared less able to increase roots in response to soil drying. Also, Kueppers et al. (2017) found that the watering treatment in our study did not increase survival for first-year PiFL as it did for PiEn, following five annual seedings.

TABLE 4 Results (*p*-values) of continuous environment analyses for whole-seedling dry mass, root dry mass, root length, root mass fraction, RGR for the period from seed to 3 weeks, and δ^{13}C at Time 2 (*c.* 9 weeks of age). Models include data from all three sites with the exception of δ^{13}C, which was not measured at the Forest site. Provenance was removed from models where neither provenance nor any interaction with provenance was significant. Values <.05 are in bold type. Superscript numbers are defined in the first column of the corresponding row. Parameter estimates can be found in Table S3a,b

Source	Log whole-seedling dry mass (Times 1 and 2)	Log root dry mass per (Times 1 and 2)	Square root, root length (Times 1 and 2)	RMF (Times 1 and 2)	RGR$_{seed-1}$	δ^{13}C (Time 2)
N (individuals[1] or quadrants[2])	910[1]	839[1]	912[1]	833[1]	543[1]	114[2]
R^2	0.93	0.92	0.62	0.29	0.65	0.84
Species	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Provenance	**0.0118**	**0.0146**	**0.0004**	–	–	–
Species × provenance	0.0755	0.1195	0.4147	–	–	–
T_{minLT}	0.1167	**0.0017(−)**	0.1486	**0.0145(−)**	0.0947	**<0.0001(−)**
Species × T_{minLT}	**0.0044**	0.0698	0.3809	0.8041	0.2803	0.1608
Provenance × T_{minLT}	0.8197	0.8148	0.4765	–	–	–
Species × Provenance × T_{minLT}	0.6353	0.0801	0.4855	–	–	–
VWC$_{LT}$	**0.0011(+)**	0.3802	0.6229	**0.0027(−)**	**0.0002(+)**	**0.0216(−)**
Species × VWC$_{LT}$	**0.0145**	**0.001**	**<0.0001**	0.1015	0.0608	0.9138
Provenance × VWC$_{LT}$	0.9517	0.8807	0.2613	–	–	–
Species × Provenance × VWC$_{LT}$	0.4157	0.4136	0.1278	–	–	–
T_{minLT} × VWC$_{LT}$	**0.0025**	0.1134	0.0527	0.3327	**0.0019**	0.4268
Species × T_{minLT} × VWC$_{LT}$	0.3465	0.7937	0.8049	0.4484	0.1203	0.9807
Provenance × T_{minLT} × VWC$_{LT}$	0.6618	0.7406	0.3008	–	–	–
Species × Provenance × T_{minLT} × VWC$_{LT}$	0.8465	0.8454	0.923	–	–	–
Mean age (days)[1] or DOY harvest[2]	**<0.0001[1]**	**<0.0001[1]**	**<0.0001[1]**	0.6189[1]	0.2434[1]	0.2391[2]

PiFl seedlings also had the slowest relative growth and the greatest WUE of the three species (Figure 8), linked traits that can combine to help mitigate water limitation effects on survival, provided sufficient carbon reserves are available. Specifically, PiFl's seed-reserve advantage likely allowed its seedlings to initially deploy sufficient roots for adequate water uptake and then endure the reduced RGR (while still having relatively greater absolute DM gain) that accompanies a conservative water-use strategy and its benefits to survival under water limitation.

Although PiFl showed the greatest survivorship in the first growing season (Castanha et al., 2013; Kueppers et al., 2017), it is only a minor component of the forest at high elevations and tree line in Colorado, while PiEn, a species with many fewer reserves, is one of the dominant species at tree line. Moles and Westoby (2004) showed that large-seeded species generally have higher survival rates during early establishment than do small-seeded species, but these rates are not sufficient to compensate for the advantage of greater absolute seed numbers in smaller-seeded species. Our results may substantiate these patterns. While PiFl's seed reserves help it to establish more successfully, PiEn's smaller and therefore much more numerous seed may increase germination opportunities and explain its ultimate dominance in abundance within the tree line community. Alternatively, our results may portend an eventual shift in high-elevation dominance with warming towards species with larger seeds and more stress-tolerant seedlings. Modelling scenarios using seedling demographic data and vital rates from current tree populations show that with warming, PiFl may maintain or increase its populations in high-elevation Rocky Mountain forests, while PiEn may face recruitment limitations (Conlisk et al., 2017a). Indeed, PiFl is more prevalent at drier tree lines in the Great Basin than in the relatively moist southern/central Rockies (Millar et al., 2015).

4.2 | Poor drought tolerance in conifer seedlings indicates need for high soil moisture during establishment

The very high turgor loss points we measured indicate that these conifer seedlings require abundant soil moisture during their first growing season, particularly just after germination at the beginning of the growing season. Stomatal conductance decreased in a threshold-like fashion as midday water potential for PiFL decreased below −1 MPa in our experiment (Moyes et al., 2013). More generally, growing season survival of emergent conifer seedlings was observed to be much higher in a wet year (Germino et al., 2002), and tree line seedling establishment was greater during wet years in dendrochronology studies (Daniels & Veblen, 2004; Lloyd, 1997). Few studies have reported Ψ_{TLP} in first-year conifer seedlings, and the values we

FIGURE 5 Modeled relationships of dry mass, RGR and root length interactions among species with temperature and VWC (Table 4): temperature × species (a, d, g, j), VWC × species (b, e, h, k) and temperature × VWC (c, f, i, l). Models include data from all three sites. For each specified category (species or VWC level), *X*-axis variables were allowed to vary continuously from their minimum to their maximum measured values while all other variables were held constant at their average values. VWC of 13% and 34% (c, f, i, l) represent the lower and upper 97.5% quartiles of measured values of seedling lifetime VWC. Plots with bold lines depict significant interactions (Table 4). Asterisks (*) indicate $p = .0527$ (i), $p = .0608$ (k)
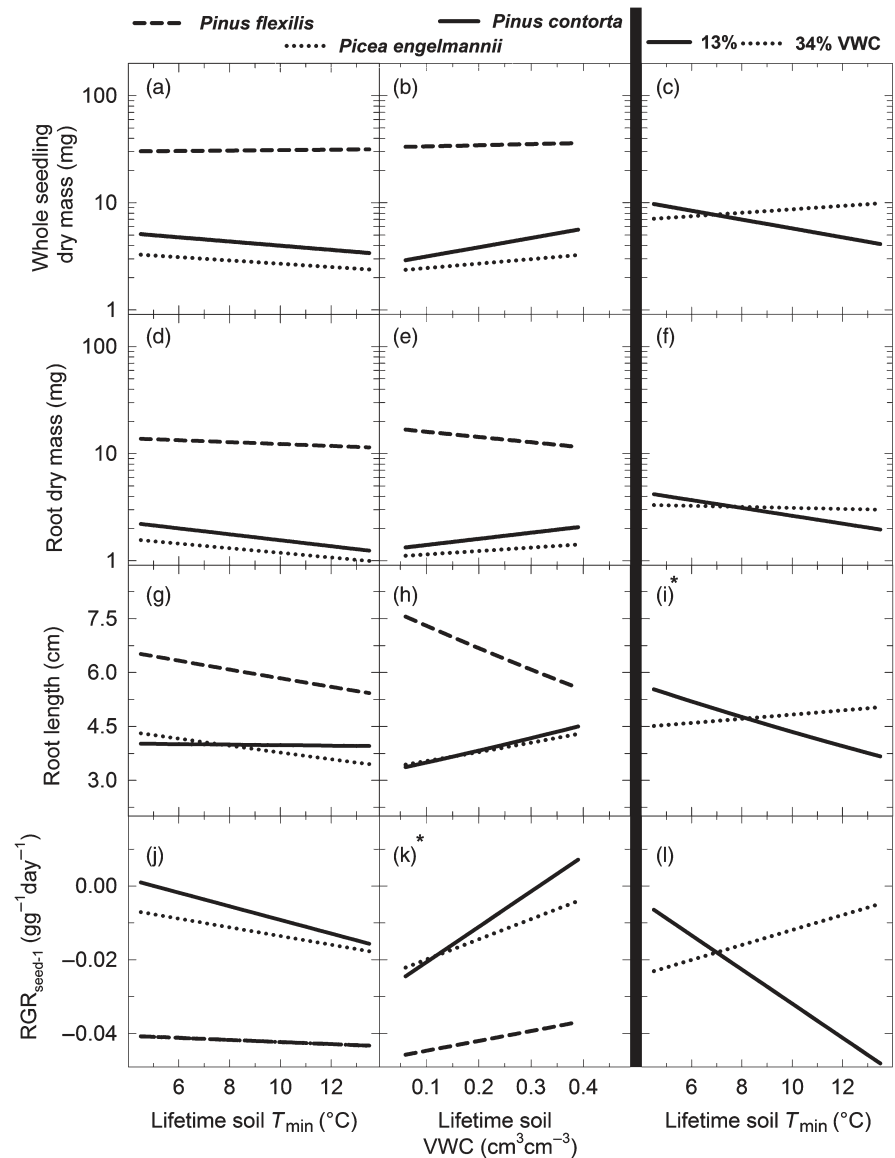
FIGURE 6 Least squares means and standard errors from categorical environment analysis for RGR from seed to *c.* 3 weeks of age for all sites and species
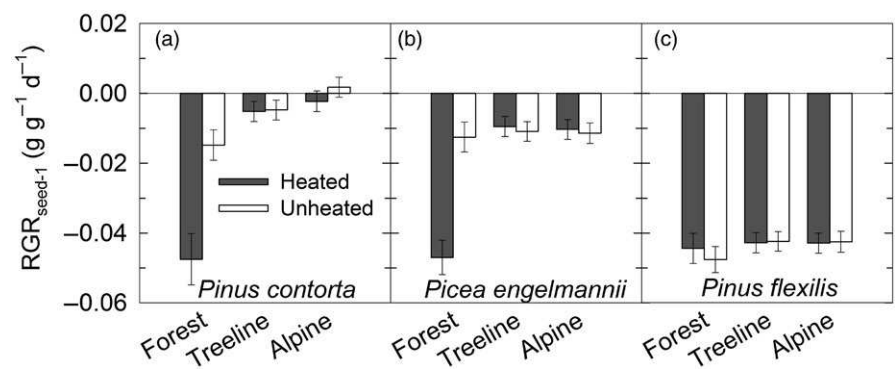
report are higher than those reported for other species, although few have measured plants as immature as ours. Kerr, Meinzer, McCulloh, Woodruff, and Marias (2015) reported Ψ_{TLP} −1.6/−1.9 MPa for first-year *Pinus ponderosa* common garden seedlings from mesic/dry habitat near the end of a relatively wet growing season, comparable to our Time 2. Fan, Blake, and Blumwald (1994) reported Ψ_{TLP}

−1.8/−1.5 MPa for 5-month-old *Pinus banksiana* and *Picea mariana* raised with sufficient water in a growth chamber, *c.* 3 months older than our Time 2 seedlings. Year-old PiFl from the Alpine and Tree line sites measured in late September/early October of 2012 had a Ψ_{TLP} of −2.3 MPa (M. G. Germino, B. E. Lazarus, A. B. Moyes, C. Castanha, & L. M. Kueppers, unpubl. data), substantially lower than what we
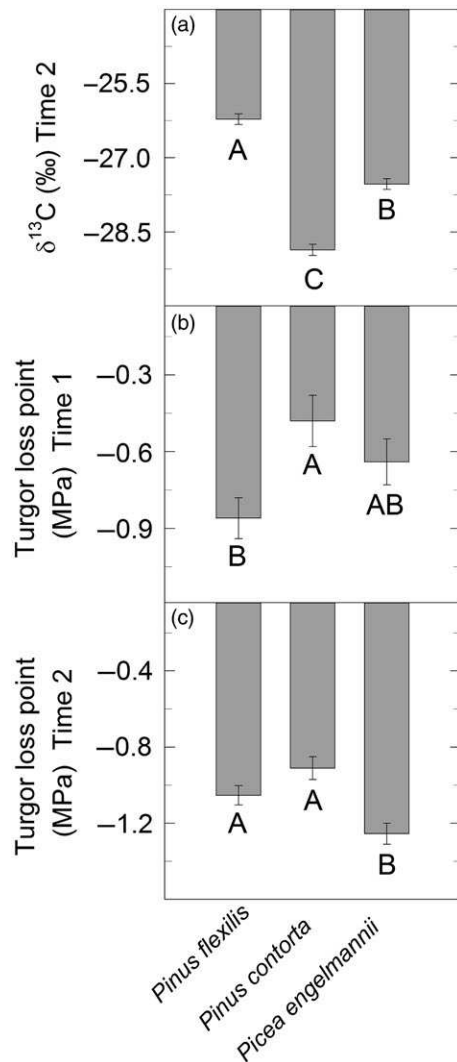
FIGURE 7 Least squared means and standard errors from categorical environment analyses for $\delta^{13}C$ at Time 2 (*c.* 9 weeks) (a), turgor loss point Ψ_{TLP} at Time 1 (*c.* 3 weeks) (b), and Ψ_{TLP} at Time 2 (c). Models include data from Alpine and Tree line sites with the exception of Ψ_{TLP} at Time 1, which includes only Tree line site data. Capital letters denote results of Tukey–Kramer comparisons—species not labelled with the same letter are significantly different

FIGURE 8 Species means (*SE*) for $\delta^{13}C$ plotted against species means (*SE*) for RGR during the period from 3 to 9 weeks (Alpine and Tree line sites only). [Correction added after online publication on 21 September 2017: Figure 8 updated with correct species labels]

measured for first-year PiFl seedlings around the same time of year in 2013 (−1.1 MPa, Figure 7c). This comparison and the time trends in our data suggest seedlings became more drought tolerant as they matured (Figure 7b,c). Hence, moisture stress experienced very early in the first growing season would likely have more negative impacts on growth and survival than moisture stress experienced later.

4.3 | Warmer temperatures negatively affect dry mass and RGR unless soil moisture is abundant

Higher temperatures resulted in slightly greater DM and RGR_{seed-1} when soils were extremely moist, but had a more pronounced negative effect when soils were drier (Table 4, Figure 5c,l). This suggests
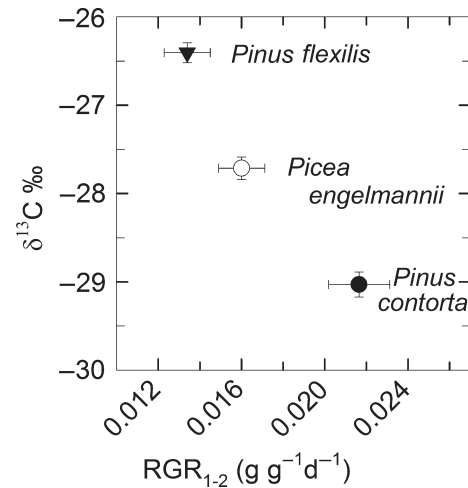
that warming might improve seedling growth and hence establishment only in locations or years with very abundant soil moisture. The positive response of DM and RGR_{seed-1} to increased temperatures only at high soil–moisture content is evidence that temperature and moisture can co-limit establishment at high elevations. After establishment and once plant roots reach a zone of adequate moisture, the factors that limit their continued growth may differ from those that limit establishment (Daniels & Veblen, 2004). Consistent with this, survival for limber pine from the second to the third year of life, when roots had likely reached deeper, more consistent soil moisture, was slightly greater in heated compared with unheated plots (Kueppers et al., 2017).

4.4 | Warming reduced root growth, potentially via increased respiration

Warming (regardless of watering treatment) led to less DM gain by seedlings, which was unexpected (Figure 2b, Table 3). Potential explanations for this include that the responses are (1) part of a growth strategy (i.e. active storage of growth resources such as carbohydrates for use in stress response, at the expense of growth, Wiley & Helliker, 2012) or (2) a reflection of suboptimal growth performance and heat as a stress, perhaps inhibiting carbon balance. In support of the second explanation, Kueppers et al. (2017) found that heating reduced survival for PiFl and PiEn in their first year following germination (which contrasts the above-mentioned greater survival of third year PiFL under heating). Our results further suggest that heating may have reduced growth by limiting root development: heating had a disproportionate effect on root DM compared to whole seedlings (i.e. average reduction with heating of 25.2% vs. 15.3%, respectively, Figure 2e,b). This led to a decrease in RMF with heating (Figure 2k), which was also observed by Way and Sage (2008a, 2008b). We propose that respiration may explain the negative effect of heating on DM gain. Although we did not measure respiration in this study, we

previously observed an increase in respiration with warming for PiFL (Moyes et al., 2013), similar to reports for other species (e.g. Way & Sage, 2008a, 2008b).

As further evidence of the potential importance of respiration for the carbon balance of these seedlings, nearly all growth and physiological variables were better predicted by models including T_{minLT}, which is a nocturnal variable, than by models including T_{meanLT} or T_{maxLT} that would otherwise point to daytime processes such as photosynthesis. Night-time respiration rates increase 1.4- to 4-fold with a 10°C increase in night-time temperatures (Azcon-Bieto, 1992), and carbon utilized for respiration is equal to *c.* 50% of carbon fixed in gross photosynthesis (Amthor, 2000; Farrar, 1985).

Temperature also appears to be the primary factor controlling WUE ($\delta^{13}C$) in this system, a pattern that has been observed in other high-elevation systems. Specifically, $\delta^{13}C$ decreased (and WUE decreased) with increased minimum temperatures resulting from warming or background variation, and $\delta^{13}C$ was more responsive to temperature than to watering or soil moisture (Tables 3 and 4). A decrease in $\delta^{13}C$ with increased temperature has been documented in growth chamber studies (Morecroft & Woodward, 1990, 1996; Smith, Herath, & Chase, 1973) and in subarctic alpine tree line warming studies (Michelsen et al., 1996; Welker et al., 1993). In addition, $\delta^{13}C$ decreased more with increasing temperature than with decreasing soil moisture in subalpine conifers (Hu, Moore, & Monson, 2010) and a variety of subalpine and alpine plant species (Xu et al., 2015). No study points conclusively to a single mechanism by which lower temperatures result in higher plant tissue $\delta^{13}C$, but reduced stomatal conductance at lower temperatures has been implicated (Hu et al., 2010; Morecroft & Woodward, 1996).

4.5 | Overall conclusions—importance of water stress at high elevations

Our results provide new evidence of the importance of moisture stress in tree seedling establishment in the transition from forest to the alpine tundra, showing (1) the species whose growth responses (enabled by large seed reserves) mitigate moisture stress is also the species with the lowest seedling mortality; (2) first-year conifer seedlings have generally high Ψ_{TLP} values and thus poor drought tolerance and are vulnerable to even moderate soil drying early in the growing season; and (3) increased temperatures have negative effects on growth except when soil moisture is very abundant. With warming, initial establishment of tree seedlings in high-elevation forests below, at and above tree line may occur only in sites or years with relatively abundant moisture.

AUTHORS' CONTRIBUTIONS

L.M.K. and M.J.G. conceived and L.M.K., C.C. and A.B.M. conducted the broader Alpine Treeline Warming Experiment. All authors conceived the sampling design. C.C. and B.E.L. conducted the data collection and analyses; B.E.L., C.C., M.J.G. and A.B.M. wrote the manuscript. All authors edited the manuscript and gave final approval for publication.

DATA ACCESSIBILITY

Data available from ScienceBase: https://www.sciencebase.gov/catalog/item/595fc39de4b0d1f9f058665b (Lazarus, Castanha, Germino, Kueppers, & Moyes, 2017).

REFERENCES

Amthor, J. S. (2000). The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. *Annals of Botany*, *86*, 1–20.

Azcon-Bieto, J. (1992). Relationships between photosynthesis and respiration in the dark in plants. In J. Barber, M. G. Guerrero, & H. Medrano (Eds.), *Trends in photosynthesis research* (pp. 241–253). Andover, UK: Intercept Ltd.

Bartlett, M. K., Scoffoni, C., & Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. *Ecology Letters*, *15*, 393–405.

Caddick, L. R., & Linder, H. P. (2002). Evolutionary strategies for reproduction and dispersal in African Restionaceae. *Australian Journal of Botany*, *50*, 339–355.

Castanha, C., Torn, M. S., Germino, M. J., Weibel, B., & Kueppers, L. M. (2013). Conifer seedling recruitment across a gradient from forest to alpine tundra: Effects of species, provenance, and site. *Plant Ecology & Diversity*, *6*, 307–318.

Colombo, S. J., & Teng, Y. (1992). Seasonal variation in the tissue water relations of *Picea glauca*. *Oecologia*, *92*, 410–415.

Conlisk, E., Castanha, C., Germino, M. J., Veblen, T. T., Smith, J. M., & Kueppers, L. M. (2017a). Declines in low-elevation subalpine tree populations outpace growth in high-elevation populations with warming. *Journal of Ecology*, *105*, 1347–1357.

Conlisk, E., Castanha, C., Germino, M. J., Veblen, T. T., Smith, J. M., Moyes, A. B., & Kueppers, L. M. (2017b) Seed origin and warming constrain lodgepole pine recruitment, slowing the pace of population range shifts. *Global Change Biology*. https://doi.org/10.1111/gcb.13840

Crimmins, S. M., Dobrowski, S. Z., Greenberg, J. A., Abatzoglou, J. T., & Mynsberge, A. R. (2011). Changes in climatic water balance drive downhill shifts in plant species' optimum elevations. *Science*, *331*, 324–327.

Daly, C., & Shankman, D. (1985). Seedling establishment by conifers above tree limit on Niwot Ridge, Front Range, Colorado, USA. *Arctic and Alpine Research*, *17*, 389–400.

Danby, R. K., & Hik, D. S. (2007). Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. *Global Change Biology*, *13*, 437–451.

Daniels, L. D., & Veblen, T. T. (2004). Spatiotemporal influences of climate on altitudinal treeline in northern Patagonia. *Ecology*, *85*, 1284–1296.

Ehleringer, J. R., White, J. W., Johnson, D. A., & Brick, M. (1990). Carbon isotope discrimination, photosynthetic gas-exchange, and transpiration

efficiency in beans and range grasses. *Acta Oecologica-International Journal of Ecology*, 11, 611–625.

Fan, S. H., Blake, T. J., & Blumwald, E. (1994). The relative contribution of elastic and osmotic adjustments to turgor maintenance of woody species. *Physiologia Plantarum*, 90, 408–413.

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon isotope discrimination and photsynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 40, 503–537.

Farrar, J. F. (1985). The respiratory source of CO_2. *Plant Cell and Environment*, 8, 427–438.

Geiger, R. (1950). *The climate near the ground*. Cambridge, MA: Harvard University Press.

Germino, M. J., & Smith, W. K. (2000). Differences in microsite, plant form, and low-temperature photoinhibition in alpine plants. *Arctic Antarctic and Alpine Research*, 32, 388–396.

Germino, M. J., Smith, W. K., & Resor, A. C. (2002). Conifer seedling distribution and survival in an alpine-treeline ecotone. *Plant Ecology*, 162, 157–168.

Gill, R. A., Campbell, C. S., & Karlinsey, S. M. (2015). Soil moisture controls Engelmann spruce (*Picea engelmannii*) seedling carbon balance and survivorship at timberline in Utah, USA. *Canadian Journal of Forest Research*, 45, 1845–1852.

Grace, J., Berninger, F., & Nagy, L. (2002). Impacts of climate change on the tree line. *Annals of Botany*, 90, 537–544.

Hallett, L. M., Standish, R. J., & Hobbs, R. J. (2011). Seed mass and summer drought survival in a Mediterranean-climate ecosystem. *Plant Ecology*, 212, 1479–1489.

Harsch, M. A., & HilleRisLambers, J. (2016). Climate warming and seasonal precipitation change interact to limit species distribution shifts across western North America. *PLoS ONE*, 11, e0159184.

Harsch, M. A., Hulme, P. E., McGlone, M. S., & Duncan, R. P. (2009). Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters*, 12, 1040–1049.

Hu, J., Moore, D. J. P., & Monson, R. K. (2010). Weather and climate controls over the seasonal carbon isotope dynamics of sugars from subalpine forest trees. *Plant Cell and Environment*, 33, 35–47.

Kerr, K. L., Meinzer, F. C., McCulloh, K. A., Woodruff, D. R., & Marias, D. E. (2015). Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. *Tree Physiology*, 35, 535–548.

Körner, C. (1998). A re-assessment of high elevation treeline positions and their explanation. *Oecologia*, 115, 445–459.

Körner, C., & Paulsen, J. (2004). A world-wide study of high altitude treeline temperatures. *Journal of Biogeography*, 31, 713–732.

Kueppers, L. M., Conlisk, E., Castanha, C., Moyes, A., Germino, M. J., de Valpine, P., … Mitton, J. (2017). Warming and provenance limit tree recruitment across and beyond the elevation range of subalpine forest. *Global Change Biology*, 23, 2383–2395.

Lazarus, B. E., Castanha, C., Germino, M. J., Kueppers, L. M., & Moyes, A. B. (2017). Data from: Ecophysiological parameters for tree seedlings at alpine treeline (NIWOT). *ScienceBase*, https://www.sciencebase.gov/catalog/item/595fc39de4b0d1f9f058665b

Leishman, M. R., & Westoby, M. (1994). The role of seed size in seedling establishment in dry soil conditions – Experimental evidence from semi-arid species. *Journal of Ecology*, 82, 249–258.

Lloyd, A. H. (1997). Response of tree-line populations of foxtail pine (*Pinus balfouriana*) to climate variation over the last 1000 years. *Canadian Journal of Forest Research*, 27, 936–942.

Maher, E. L., & Germino, M. J. (2006). Microsite differentiation among conifer species during seedling establishment at alpine treeline. *Ecoscience*, 13, 334–341.

Meinzer, F. C., Rundel, P. W., Sharifi, M. R., & Nilsen, E. T. (1986). Turgor and osmotic relations of the desert shrub *Larrea tridentata*. *Plant Cell and Environment*, 9, 467–475.

Meinzer, F. C., Woodruff, D. R., Marias, D. E., McCulloh, K. A., & Sevanto, S. (2014). Dynamics of leaf water relations components in co-occurring iso- and anisohydric conifer species. *Plant Cell and Environment*, 37, 2577–2586.

Michelsen, A., Jonasson, S., Sleep, D., Havstrom, M., & Callaghan, T. V. (1996). Shoot biomass, $\delta^{13}C$, nitrogen and chlorophyll responses of two arctic dwarf shrubs to in situ shading, nutrient application and warming simulating climatic change. *Oecologia*, 105, 1–12.

Millar, C. I., Westfall, R. D., Delany, D. L., Flint, A. L., & Flint, L. E. (2015). Recruitment patterns and growth of high-elevation pines in response to climatic variability (1883–2013), in the western Great Basin, USA. *Canadian Journal of Forest Research*, 45, 1299–1312.

Moles, A. T., & Westoby, M. (2004). Seedling survival and seed size: A synthesis of the literature. *Journal of Ecology*, 92, 372–383.

Monahan, W. B., Cook, T., Melton, F., Connor, J., & Bobowski, B. (2013). Forecasting distributional responses of limber pine to climate change at management-relevant scales in Rocky Mountain National Park. *PLoS ONE*, 8, e83163. https://doi.org/10.1371/journal.pone.0083163

Morecroft, M. D., & Woodward, F. I. (1990). Experimental investigations on the environmental determination of $\delta^{13}C$ at different altitudes. *Journal of Experimental Botany*, 41, 1303–1308.

Morecroft, M. D., & Woodward, F. I. (1996). Experiments on the causes of altitudinal differences in the leaf nutrient contents, size and $\delta^{13}C$ of *Alchemilla alpina*. *New Phytologist*, 134, 471–479.

Moyes, A. B., Castanha, C., Germino, M. J., & Kueppers, L. M. (2013). Warming and the dependence of limber pine (*Pinus flexilis*) establishment on summer soil moisture within and above its current elevation range. *Oecologia*, 171, 271–282.

Moyes, A. B., Germino, M. J., & Kueppers, L. M. (2015). Moisture rivals temperature in limiting photosynthesis by trees establishing beyond their cold-edge range limit under ambient and warmed conditions. *New Phytologist*, 207, 1005–1014.

NWCC. (2017). *Natural resources conservation service, national water and climate center*. Retrieved from https://wcc.sc.egov.usda.gov/nwcc/site?sitenum=663

Parker, W. C., & Pallardy, S. G. (1987). The influence of resaturation method and tissue type on pressure-volume analysis of *Quercus alba* L. seedlings. *Journal of Experimental Botany*, 38, 535–549.

Sack, L., Pasquet-Kok, J., & PrometheusWiki contributors. (2011). Leaf pressure-volume curve parameters. PrometheusWiki. Retrieved from https://prometheuswiki.org/tiki-index.php?page=Leaf+pressure-volume+curve+parameters

Smith, B. N., Herath, H. M. W., & Chase, J. B. (1973). Effect of growth temperature on carbon isotopic ratios in barley, pea and rape. *Plant and Cell Physiology*, 14, 177–182.

Tyree, M. T., & Hammel, H. T. (1972). The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany*, 23, 267–282.

Way, D. A., & Sage, R. F. (2008a). Elevated growth temperatures reduce the carbon gain of black spruce *Picea mariana* (Mill.) BSP. *Global Change Biology*, 14, 624–636.

Way, D. A., & Sage, R. F. (2008b). Thermal acclimation of photosynthesis in black spruce *Picea mariana* (Mill.) BSP. *Plant Cell and Environment*, 31, 1250–1262.

Weisberg, P. J., & Baker, W. L. (1995). Spatial variation in tree seedling and krummholz growth in the forest-tundra ecotone of Rocky Mountain National Park, Colorado, USA. *Arctic and Alpine Research*, 27, 116–129.

Welker, J. M., Wookey, P. A., Parsons, A. N., Press, M. C., Callaghan, T. V., & Lee, J. A. (1993). Leaf carbon-isotope discrimination and vegetative responses of *Dryas octopetala* to temperature and water manipulations in a high Arctic polar semidesert, Svalbard. *Oecologia*, 95, 463–469.

Wiley, E., & Helliker, B. (2012). A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytologist*, *195*, 285–289.

Xu, M., Wang, G., Li, X., Cai, X., Li, X., Christie, P., & Zhang, J. (2015). The key factor limiting plant growth in cold and humid alpine areas also plays a dominant role in plant carbon isotope discrimination. *Frontiers in Plant Science*, *6*, 961. https://doi.org/10.3389/fpls.2015.00961

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