

Warming and the dependence of limber pine (*Pinus flexilis*) establishment on summer soil moisture within and above its current elevation range

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Abstract Continued changes in climate are projected to alter the geographic distributions of plant species, in part by affecting where individuals can establish from seed. We tested the hypothesis that warming promotes uphill redistribution of subalpine tree populations by reducing cold limitation at high elevation and enhancing drought stress at low elevation. We seeded limber pine (*Pinus flexilis*) into plots with combinations of infrared heating and water addition treatments, at sites positioned in lower subalpine forest, the treeline ecotone, and alpine tundra. In 2010, first-year seedlings were assessed for physiological performance and survival over the snow-free growing season. Seedlings emerged in midsummer, about 5–8 weeks after snowmelt. Low temperature was not observed to limit seedling photosynthesis or respiration between emergence and October, and thus experimental warming did not

appear to reduce cold limitation at high elevation. Instead, gas exchange and water potential from all sites indicated a prevailing effect of summer moisture stress on photosynthesis and carbon balance. Infrared heaters raised soil growing degree days (base 5 °C, $p < 0.001$) and August–September mean soil temperature ($p < 0.001$). Despite marked differences in vegetation cover and meteorological conditions across sites, volumetric soil moisture content (θ) at 5–10 cm below 0.16 and 0.08 m³ m⁻³ consistently corresponded with moderate and severe indications of drought stress in midday stem water potential, stomatal conductance, photosynthesis, and respiration. Seedling survival was greater in watered plots than in heated plots ($p = 0.01$), and negatively related to soil growing degree days and duration of exposure to $\theta < 0.08$ m³ m⁻³ in a stepwise linear regression model ($p < 0.0001$). We concluded that seasonal moisture stress and high soil surface temperature imposed a strong limitation to limber pine seedling establishment across a broad elevation gradient, including at treeline, and that these limitations are likely to be enhanced by further climate warming.

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Introduction

Mountain ecosystems occur within elevation-associated climate gradients driven by lapse rate and orographic effects (Lundquist and Cayan 2007; Wieser 2007a, b). Differences in physiological sensitivity of plants to climate are important in the sorting of species with elevation, typically leading to ordered sequences of climax community types arranged in elevation bands (Peet 1978). Climate

models forecast warming of 2–6 °C (median 3.4 °C) across western North America by 2100 (IPCC 2007). This is anticipated to promote uphill redistribution of mountain plant species (Parmesan and Yohe 2003), including displacement of alpine tundra by forest (Moen et al. 2004; Engler et al. 2011) and altered plant communities at low elevation (Weaver 1980).

Local effects of climate on overriding physiological or competitive limitations may produce asymmetric range shifts at upper and lower (i.e., “leading” and “trailing”) distribution edges (Breshears et al. 2008; Kelly and Goulden 2008; Doak and Morris 2010). Carbon imbalances are often cited as physiological mechanisms linking abiotic thresholds to tree species range limits, and explaining observed or predicted responses to climate change. For example, upper elevation limits of subalpine trees occur where growing season shallow soil temperature drops below an average of ~6 °C (Körner and Paulsen 2004). Cold-inhibition of new cell growth, rather than carbon assimilation, has been proposed as the mechanism responsible for alpine treelines (Hoch and Körner 2009; Petit et al. 2010), and forming the rationale for predictions of new tree growth in the alpine. However, the importance of growth limitation by cold during the necessary process of seedling establishment is not yet clear (Reinhardt et al. 2011). At lower elevation, seasonal warm and dry conditions limit seedling establishment by restricting photosynthetic carbon acquisition (Cui and Smith 1991). This limitation is expected to strengthen with warming and may also increase in relevance at the high elevation boundaries of subalpine trees (Johnson et al. 2004; Johnson and Smith 2007).

Field climate manipulations can help to determine how warmer conditions will affect local physiological limitations, but few have been undertaken in high mountain environments due to the associated logistical challenges. High-elevation warming studies have found positive impacts on conifer growth, such as reduced photoinhibition from sun exposure following cold nights (Germino and Smith 1999) and increased stem elongation rates and xylem hydraulic conductivity (Danby and Hik 2007; Petit et al. 2010). However, warming has also been shown to reduce summer soil moisture availability (Harte et al. 1995) and to increase susceptibility to spring frost (Taulavuori et al. 2004; Martin et al. 2010). More studies relating physiological performance (e.g., photosynthesis, respiration, and photoinhibition) and survival of establishing seedlings to climate are needed to predict whether and how tree distributions will shift under future climate conditions.

We investigated the hypotheses that subalpine tree establishment is limited by low temperature at and above treeline and by water stress at lower elevation. We used infrared heaters to warm study plots within three sites spanning 470 m elevation at Niwot Ridge in the Rocky Mountains to evaluate the effects of warming on first-year seedling physiology and

survival. We also included a water addition treatment to alleviate warming-induced moisture reductions and to increase summer water availability above ambient levels. We selected limber pine (*Pinus flexilis*) for its broad geographic distribution, seed mobility by bird dispersal, local presence from mid-elevation to treeline, and ability to colonize relatively dry exposed sites (Letts et al. 2009). We evaluated photosynthesis and respiration rates of field-germinated seedlings over a wide range of conditions in an attempt to identify threshold responses to temperature and soil moisture. We then interpreted these results in terms of the first-year establishment potential of limber pine within and above its current elevation range with warming.

Materials and methods

Sites and experimental design

We conducted all work at Niwot Ridge in the Front Range of the Rocky Mountains in Colorado, USA. We chose three sites on the south-facing slope: near the “warm edge” of current subalpine forest (lower subalpine, LSA, 3,060 m), within the alpine-treeline ecotone (upper subalpine, USA, 3,430 m), and above treeline (alpine, ALP, 3,540 m). Reinhardt et al. (2011) described these sites, and others (e.g., Walker et al. 1994; Monson et al. 2002; Darrrouzet-Nardi 2010) have provided detailed descriptions of the subalpine forest and alpine regions. Tree species at LSA included subalpine fir (*Abies lasiocarpa*), Engelmann spruce (*Picea engelmannii*), lodgepole pine (*Pinus contorta*), and limber pine (*Pinus flexilis*), with a sparse understory of bilberry (*Vaccinium myrtillus*), common juniper (*Juniperus communis* ssp. *alpina*), and herbs. At the USA site, dense, flagged krummholz islands of Engelmann spruce and subalpine fir occurred with a few stunted individuals of limber pine, interspersed within broad patches of alpine tundra vegetation. Alpine vegetation in the USA open spaces and at ALP was similar, primarily comprising short herbaceous perennials and cushion plants (Walker et al. 1994).

At each of the three study locations, we established 20, 3-m diameter, circular treatment areas and assigned them to one of four heating and watering treatments (W, watered; C, control; HW, heated + watered; and H, heated), stratifying by vegetation or canopy cover, aspect, elevation, and distance from krummholz (USA). Study plots were 1 m², positioned within the treatment areas (each area contained three additional plots not used in the current study and a 30-cm buffer area). Heated treatment areas were surrounded by six, 240-V, 1,000-W, IR heaters (Mor Electric Heating, Comstock Park, MI, USA), on circular perimeter scaffolding at 1.2 m height, following the geometry of Kimball et al. (2008). Heaters were run at a constant IR

output continuously from October 2009, supplied by line power extending to each site. Heaters were automatically turned off whenever wind speed exceeded 10 m s^{-1} and heater output was reduced at LSA in June when soil heating effects exceeded $4.5 \text{ }^\circ\text{C}$ above ambient. To offset soil moisture evaporation by the heaters and generate a broader range of summer moisture availability, watering treatments were initiated at each site when average soil moisture in heated plots dropped below that of control plots. Each watered treatment area then received 2.5 mm of water weekly through September. Total water added to plots varied with snowmelt and soil dry-down timing, up to a maximum addition of 30 mm, or about 3 % of mean annual and 20 % of mean July–September precipitation across the transect (Greenland 1989; NWCC 2012).

Limber pine cones were collected in September 2009 from 24 trees growing at 2,900–3,100 m elevation within 1.5 km of LSA. Cones were dried indoors until seed could be extracted. Harvested seeds were pooled, and sowing was conducted in fall of 2009 by placing five seeds in each of 70, $10 \times 10 \text{ cm}$ plot cells at 2.5 cm depth. Plots were visited approximately weekly following snowmelt to record numbers of germinating and dead seedlings. Fraction of seedlings surviving was calculated at the end of the growing season as the number of living seedlings divided by the number observed to germinate (or emerge) on each plot, excluding individuals harvested for destructive measurements. Plots were protected from granivory and herbivory by hardware cloth enclosures.

Soil microclimate and meteorological measurements

Soil moisture and temperature probes (ECTM; Decagon, Pullman, WA, USA) were permanently inserted into the soil vertically in the center of each plot to measure microclimate at 5–10 cm depth, with data recorded every 15 min. Soil probes were calibrated in the laboratory to volumetric water contents ranging from dry to saturated using soil collected adjacent to plots and sieved to remove particles $>2 \text{ mm}$. Meteorological towers erected in the center of each of the three sites measured air temperature and relative humidity (HMP45C; Vaisala, Helsinki, Finland) and wind speed (03101-L; RM Young, Traverse City, MI, USA) at 2 m height, with data logged every 15 min. We determined presence of snow on plots as days with less than $0.5 \text{ }^\circ\text{C}$ diel soil temperature variability, and confirmed that these temperature-based snow cover determinations were consistent with bi-weekly field snow surveys. We then determined snowmelt date (representing the start of the growing season) for each plot as the first snow-free day when all subsequent snow cover events lasted less than four continuous days. Growing degree days (GDD, $^\circ\text{C day}$) were calculated as an integrated measure of exposure to

warm temperature by summing all soil temperature measurements above $5 \text{ }^\circ\text{C}$ for the entire snow-free period in each study plot, and then multiplying by the measurement interval (15 min).

Physiological measurements

Gas exchange and stem water potential measurements were collected from seedlings in three campaigns (10–22 July, 24–31 August, and 29 September–7 October 2010; Fig. 1). All gas exchange measurements were conducted between 0900 and 1600 hours local time (between a few hours after sunrise and several hours before sunset) using a $2 \times 3 \text{ cm}$ leaf chamber with an external LED light source, connected to a photosynthesis system (LI-6400 XT; Li-Cor Biosciences, Lincoln, NE, USA). Seedlings remained small in stature throughout the first year [averaging 3.6 ± 0.5 (SD) cm tall], and in most measurements all cotyledon and leaf material was included inside the chamber during measurements. The chamber was clamped onto each seedling stem in a vertical orientation, positioned to have the light source illuminate seedlings from the side closest to the current natural sun angle. Cotyledons and needles spread in three dimensions inside the chamber volume. Carbon dioxide flowing into the chamber was set to $400 \mu\text{mol mol}^{-1}$ and vapor pressure was kept at ambient levels for all gas exchange measurements. Silhouette areas of sampled material were calculated using image processing software (Image J; Scion, Fredrick, MD, USA) from digital photos taken immediately following gas exchange measurements. Temperature (T) inside the chamber was coupled to ambient air T by the flow of air through the chamber. Leaf temperature inside the chamber during measurements was calculated using energy balance equations in the instrument software. Up to five individuals were sampled for photosynthesis (A_{net}) and dark respiration (R) from each plot (depending on seedling availability), and at least four plots were sampled per elevation and treatment combination in each measurement campaign (LSA $n = 177$, USA $n = 147$, ALP $n = 119$ individuals sampled across all plots, treatments, and campaigns).

We measured light-saturated ($1,200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) A_{net} and then R from each seedling. We determined from preliminary light response measurements that saturation of A_{net} occurred at $400\text{--}800 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Dark respiration rates were recorded after turning off the light source and waiting for stabilization of flux rates, and were regarded as an indication of growth and maintenance respiration, rather than absolute respiration rates in the light during the day (Atkin et al. 2000). Respiration was logged only after remaining stable (no upward or downward trend) for 3–5 min to avoid recording “post-illumination bursts” of light-enhanced dark respiration (Atkin et al. 1998). This

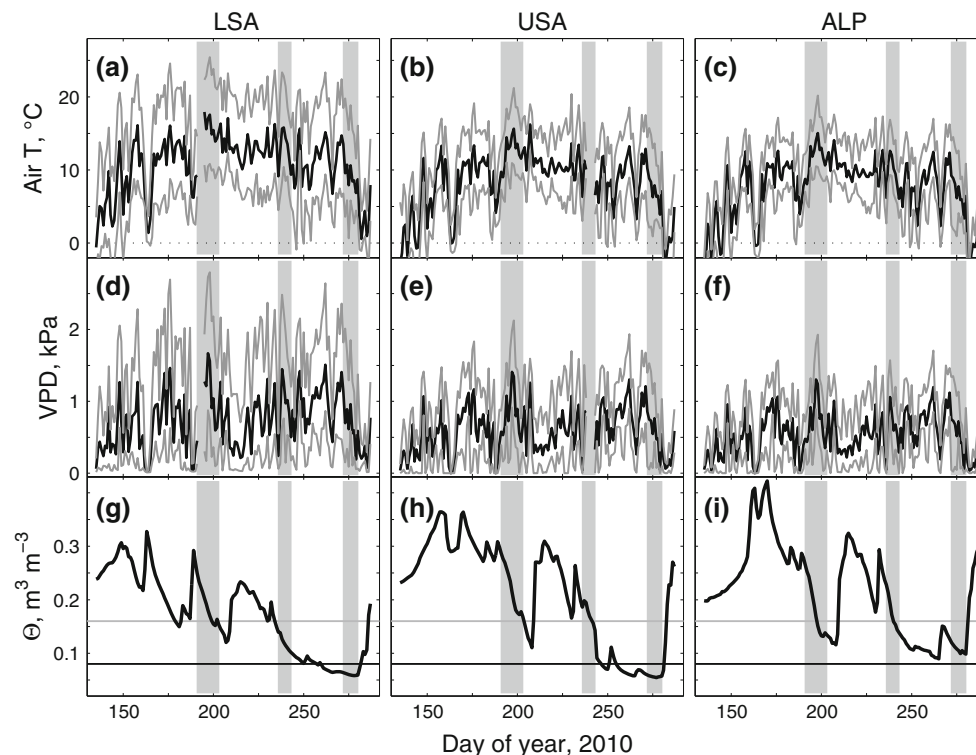


Fig. 1 **a–c** Air temperature and **d–f** vapor pressure deficit (VPD) at 2 m from site meteorological towers, and **g–i** average volumetric soil moisture (θ) from untreated plots at 5–10 cm depth at the three elevation sites for the period of days 135–287 (May 15–October 14). *LSA* lower subalpine forest (3,080 m), *USA* upper subalpine (within

treeline ecotone, 3,400 m), *ALP* alpine (above treeline, 3,550 m). *Black lines* are daily averages and *gray lines* are daily min and max temperature. *Vertical gray bands* show times when gas exchange and water potential data were collected. *Horizontal gray and black lines* show soil moisture values of 0.16 and 0.08 $\text{m}^3 \text{m}^{-3}$

phenomenon was not detected, and 3–5 min of stability was deemed acceptable after initially monitoring seedling respiration for >10 min post-illumination.

Within each measurement campaign, a subset of individuals (88 in total) was excavated immediately after gas exchange measurement, sectioned, and then placed in a pressure chamber (PMS-1000; PMS Instruments, Corvallis, OR, USA) for determination of midday stem water potential. To investigate potential impacts of photoinhibition on seedling gas exchange, five individuals from each elevation and treatment group were sampled for chlorophyll fluorescence (MINI-PAM; Heinz Walz, Effeltrich, Germany) following at least 2 h of dark acclimation (between 2300 and 0330 hours) on the nights of 27–28 August. Photochemical efficiency of Photosystem II (PSII) was calculated as the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) following Maxwell and Johnson (2000).

Analysis approach and statistics

Two-way (site by treatment) analysis of variance (ANOVA) tests were conducted on study plot data ($n = 5$ per site/treatment combination) to assess site and treatment effects on seedling survival, snowmelt date, and soil

moisture and temperature. Data transformations were conducted where necessary to meet model assumptions. All statistically significant results ($p < 0.05$) were evaluated using Tukey honestly significant difference criteria for pairwise comparisons, with Bonferroni corrections for multiple comparisons. Stepwise linear regression was used to determine which environmental variables, if any, were significantly related to seedling survival. Linear and non-linear regression analysis was used to relate seedling physiological measurements to temperature and moisture conditions at the time of measurement.

Results

Seedling physiology

Seedlings sampled for midday water potential (ψ_{midday}) and gas exchange responded strongly and consistently to seasonal reductions in soil moisture across sites and treatments (Fig. 2). Summer reductions of volumetric water content at 5–10 cm depth were associated with low afternoon stem water potential (Fig. 2a), which corresponded with stomatal closure (Fig. 2b) and limited CO_2 assimilation

(Fig. 2c). Based on these observed relationships, θ of 0.16 and $0.08 \text{ m}^3 \text{ m}^{-3}$ were selected as indicating moderate and severe soil moisture stress (e.g., $\psi_{\text{midday}} < -1.5 \text{ MPa}$ and $\psi_{\text{midday}} < -4 \text{ MPa}$, respectively; Fig. 2a). Average values of A_{net} showed an exponential decline with decreasing θ , but there was considerable variation between individual measurements (Fig. 3a). Similarly, dark respiration declined with decreasing θ , but with large variation among individuals (Fig. 3b).

To qualitatively assess responses of A_{net} and R to temperature, we compared A_{net} and R of seedlings to leaf T measured concurrently inside the gas exchange chamber, sampled and pooled across dates and sites (Fig. 4). Leaf T during chamber measurements was coupled to that of air flowing through the chamber, differing by $+0.3 \pm 0.4 \text{ }^\circ\text{C}$ SD, rather than reflecting natural leaf T before insertion into the chamber. However, air T to which seedlings had adjusted during gas exchange measurements encompassed a broad temperature range due to diurnal and seasonal fluctuations. To evaluate effects of water stress on temperature responses, samples were first separated into soil moisture categories intended to reflect minimal ($\theta > 0.16 \text{ m}^3 \text{ m}^{-3}$), moderate ($0.16 > \theta > 0.08 \text{ m}^3 \text{ m}^{-3}$), and severe ($\theta < 0.08 \text{ m}^3 \text{ m}^{-3}$) moisture stress. These categories were largely decoupled from other time-dependent variables, such as seedling age, because midsummer precipitation raised θ above $0.16 \text{ m}^3 \text{ m}^{-3}$ for several weeks at all sites (Fig. 1). Overall responses of A_{net} and R to leaf T were obtained by averaging all data pooled within $5 \text{ }^\circ\text{C}$ temperature categories. Seedlings under minimal drought stress showed highest A_{net} with leaf temperatures of $15\text{--}20 \text{ }^\circ\text{C}$, and A_{net} decreased as leaf temperatures deviated from this range (Fig. 4a). With moderate or severe water stress, A_{net} remained low or negative (net CO_2 efflux under illumination) across the full measured range of leaf T (Fig. 4b, c). Dark respiration generally increased with leaf temperature while θ was above $0.16 \text{ m}^3 \text{ m}^{-3}$ (Fig. 4d), but this trend weakened at moderate soil moisture stress, as many individuals respired at low rates despite high leaf temperatures (Fig. 4e). Under severe soil moisture stress ($\theta < 0.08 \text{ m}^3 \text{ m}^{-3}$), R was relatively low with no apparent influence of leaf temperature (Fig. 4f). Net assimilation in heated and unheated plots was similarly constrained by stomatal conductance (Fig. 5).

Fluorescence measurements revealed generally uniform and high F_v/F_m . Only two individual seedlings showed evidence of substantially reduced photochemical efficiency: $F_v/F_m = 0.41$ (LSA, H + W), and 0.56 (USA, H). These inhibited seedlings also demonstrated net CO_2 loss under saturating light (not shown). Photochemical efficiency (F_v/F_m) from all other seedlings ($n = 58$) ranged from 0.72 to 0.83 , with no relationship between F_v/F_m and A_{net} ($r^2 = 0.008$, $p = 0.54$).

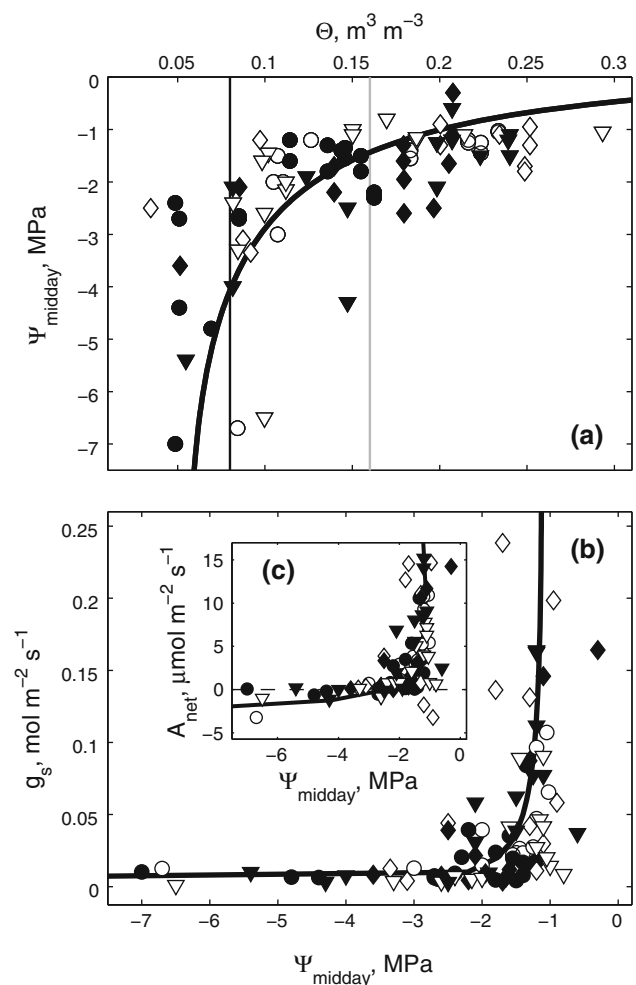


Fig. 2 **a** Afternoon stem water potential (Ψ_{midday}) versus volumetric soil moisture (θ), and **b** stomatal conductance (g_s) and **c** net CO_2 assimilation (A_{net}) versus afternoon stem water potential. *White-filled symbols* are seedlings from unheated (C and W) plots and *black symbols* are from heated (H and H + W) plots; *inverted triangle* LSA, *diamond* USA, and *circle* ALP. *Vertical lines* in **(a)** indicate θ of 0.16 and $0.08 \text{ m}^3 \text{ m}^{-3}$. Lines were fit to transformed data from all sites and treatments, and are **a** $\log(\theta \times 10^6) = 0.30(-\Psi) - 1.83 (-\Psi^{0.5}) + 13.74$, $r^2 = 0.49$; **b** $(-\Psi + 3/8)^{0.5} = 0.03(g_s \times 10^6)^2 - 0.83(g_s \times 10^6) + 6.48$, $r^2 = 0.35$; and **c** $\log(-\Psi) = 0.512 [\log(A_{\text{net}} + 4.23)]^2 - 2.76 [\log(A_{\text{net}} + 4.23)] + 3.86$, $r^2 = 0.46$ (2 lowest values of A_{net} were omitted during curve fitting for **(c)** due to unequal leverage)

Site and treatment effects on microclimate and survival

Throughout the snow-free growing season between average snowmelt date and the first large storm of the following winter (days 135–284), afternoon maximum air temperature was 5.1 ± 1.5 (SD) $^\circ\text{C}$ warmer and afternoon vapor pressure deficit (VPD) was $0.5 \pm 0.3 \text{ kPa}$ higher at the LSA site than the average of the two upper elevation sites, where both measurements were consistently similar (Fig. 1). However, minimum nightly air temperature and

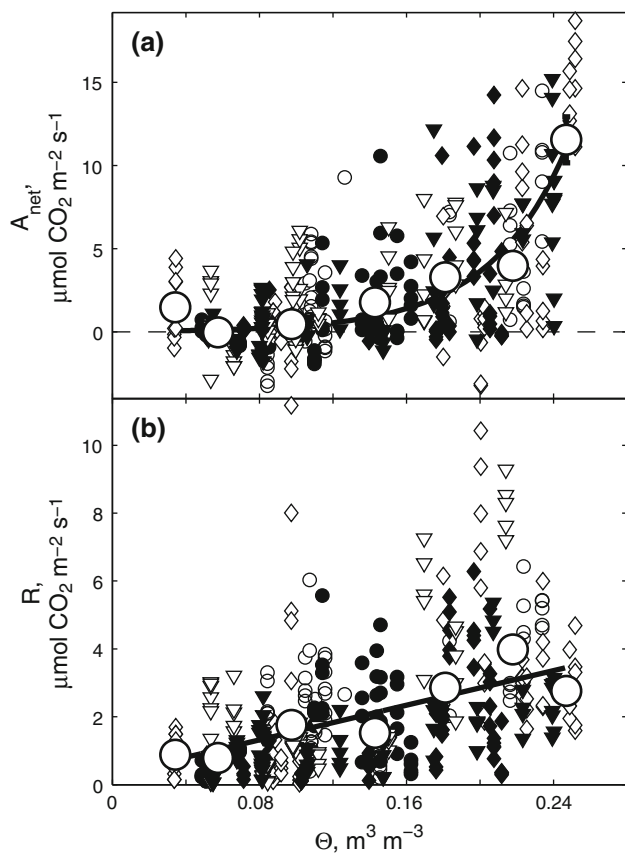


Fig. 3 Net CO₂ assimilation under saturating light (A_{net} , **a**) and dark respiration (R , **b**) versus volumetric soil moisture (θ). Symbols and shapes are as in Fig. 2. Large white circles are averages of θ categories defined by abscissa tick marks, with horizontal and vertical error bars of 1 SEM (smaller than symbols where not seen). Lines were fit to category averages, and are **a** $A_{\text{net}} = 0.032 \times e^{23.64 \times \theta}$, $r^2 = 0.94$, and **b** $R = 13.16 \times \theta + 0.22$, $r^2 = 0.77$

VPD were similar (within 1.0 ± 0.9 °C and 0.04 ± 0.02 kPa) at all three sites throughout the summer. Volumetric water content (θ) was generally lower at the forest site, with an earlier and prolonged period of soil moisture depletion relative to the higher sites (Fig. 1). Total precipitation for the water-year encompassing the 2010 growing season at Niwot Ridge was 85 % of the long-term average (NWCC 2012). The average day of snow melt on study plots varied with elevation and treatment (Table 1; melt timing coincided with peak θ in Fig. 1). Heating caused intermittent snow cover during winter and earlier snowmelt (Table 1), particularly in the LSA forest where wind redistribution of snow was low. This importance of site characteristics on winter heating effects was apparent as a significant site \times treatment interaction affecting snowmelt date (Table 1). Seedling germination occurred primarily within June and July (days 152–212). The growing season abruptly ended at all sites on day 284 (October 11), with the appearance of cold winter weather (Fig. 1).

There was substantial seasonal hysteresis in the relationship between soil T and θ , particularly at LSA, due to cooling in fall before the appearance of winter precipitation (Fig. 6a–c). Seasonal minimum soil moisture occurred weeks later than maximum soil temperature at LSA (Fig. 6a), whereas minimum moisture and maximum temperature occurred at approximately the same time at the high elevation sites (Fig. 6b, c). Although summer air temperature and VPD were higher at the low elevation site (Fig. 1), daily average soil temperature during summer was greater at the two upper elevation sites where soils received direct sun with little shading canopy (Fig. 6a–c).

Histogram plots of 5–10 cm soil temperature provide a relative and somewhat dampened indication of surface temperature variability across sites and treatments (Fig. 6, lower panels). Soil temperature distributions were narrower (had greater kurtosis) at the forest site than at the upper sites, due to larger diel variability in soil temperature in treeline and alpine soil exposed to sun and night sky. The central tendencies of season-long temperature distributions were shifted 2–4 °C warmer by the heating treatments, including periods when soil moisture was limiting (Fig. 6).

Watering did not have a strong effect on 5–10 cm soil temperature distributions within sites (Fig. 6), and had no significant effect on the number of days plots spent below values of θ chosen to indicate moisture stress (Table 1). However, there was a significant treatment effect on survival of seedlings, with greater survival in W than in H plots (Table 1). While heating and watering did not produce significant differences on θ at 5–10 cm depth, significant θ differences were found between sites (Table 1). At the alpine site, plots spent fewer days with $\theta < 0.16$ m³ m⁻³, with almost no occurrence of $\theta < 0.08$ m³ m⁻³ in unheated plots. Survival was also greater in the alpine site than the two lower elevation sites. Heating had a significant effect on soil temperature, including growing degree days and average August/September soil T (Table 1; Fig. 7). August–September temperature was highest at USA, leading to similar growing degree day totals at USA and LSA (Table 1). Stepwise linear regression found that survival was significantly related to both growing degree days (Fig. 7; Table 1) and the number of days plots had $\theta < 0.08$ m³ m⁻³ (Table 1).

Discussion

Physiological sensitivity to temperature and moisture

Contrary to our initial hypothesis, low temperature was not observed to strongly inhibit seedling establishment at any elevation during the snow-free period we evaluated. First-year seedlings emerged 5–8 weeks after snowmelt, and

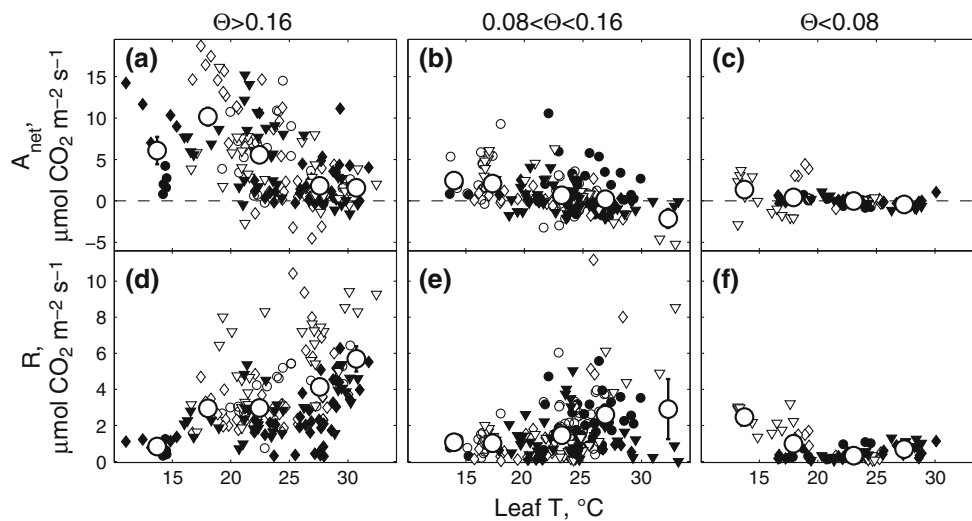


Fig. 4 a–c Net assimilation of CO₂ under saturating light (A_{net}) and d–f dark respiration (R) versus leaf temperature inside the leaf chamber. Data were separated into columns by volumetric soil moisture (θ), according to minimal ($\theta > 0.16$), moderate ($0.08 < \theta < 0.16$), and severe ($\theta < 0.08 \text{ m}^3 \text{ m}^{-3}$) moisture stress.

Symbols and shapes are as in Fig. 2. Large white circles show averages from all sites and treatments within 5 °C categories, corresponding with *abscissa tick marks*, with *horizontal and vertical error bars* of 1 SEM (smaller than symbols where not seen). Category means are not shown for categories with fewer than 3 observations

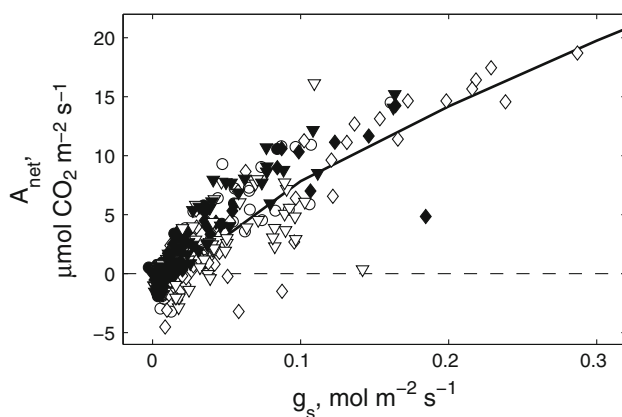


Fig. 5 a Net CO₂ assimilation under saturating light (A_{net}) against stomatal conductance to water vapor (g_s). Symbols and shapes are as in Fig. 2. The *fitted line* is $A_{net} = 52.45 \times g_s^{0.75} - 1.41$, $r^2 = 0.76$

thus largely avoided cold spring conditions. Our composite A_{net} versus leaf T analysis (Fig. 4) suggested that optimal leaf temperature for photosynthesis was 15–20 °C, when soil moisture was not limiting. This optimum temperature range was similar to values reported for seedlings of limber pine from a variety of sites (Lepper 1980), as well as for co-occurring Engelmann spruce (Hadley and Smith 1987). A similar optimum temperature for photosynthesis across our sites with widely-differing conditions (Fig. 1; Table 1) would be consistent with observations indicating that temperate conifer species show relatively low photosynthetic acclimation potential (Atkin et al. 2006; Way and Sage 2008a, b; Wieser et al. 2010). While minimum night air and surface temperatures below 0–2 °C have been

shown to inhibit photosynthesis on the following day for limber pine and co-occurring species (Smith et al. 1984; Johnson et al. 2004), minimum air temperatures during the 2010 growing season (between snowmelt and winter precipitation) and specifically during measurement campaigns, remained above 0 °C (Fig. 1).

A lack of cold night temperatures also contributes to explaining the general absence of photoinhibition observed in chlorophyll fluorescence across sites and treatments in late August. Low incidence of photoinhibition of limber pine at our treeline and alpine sites in summer was also reported for 2009 by Reinhardt et al. (2011). This finding is unique, as photoinhibition is known to impose a strong limitation at other treelines (Ball et al. 1991; Maher and Germino 2006; Bader et al. 2007), where warming of nighttime surface T may improve seedling carbon gain (Germino and Smith 1999). The two individual seedlings that showed inhibited PSII efficiency also showed net carbon loss under saturating light. However, these seedlings were not uniquely exposed to photoinhibitory conditions (radiative cooling to cold night sky followed by high sunlight; Ball et al. 1991), and were more likely in advanced stages of drought stress. Future measurements of chlorophyll fluorescence of overwintering seedlings soon after snowmelt (when seedlings experience coldest night temperature) will be necessary to determine the potential importance of photoinhibition at our sites.

A tendency for lower rates of respiration at high temperature in heated plots relative to unheated plots was apparent (Fig. 4d), and may indicate respiratory, but not photosynthetic, acclimation to warmer growing temperatures to

Table 1 Results of 2-way analysis of variance (ANOVA) of seedling survival (# surviving to end of first year/# germinated), day of snow melt, numbers of days with soil volumetric moisture θ below 0.16 and0.08 m³ m⁻³, average soil temperature at 5–10 cm depth for the months of August and September, and growing season degree days

	ANOVA results			Stepwise regression	
	<i>F</i>	<i>p</i>		<i>b</i> ± SE	<i>p</i>
Survival				–	–
Site	5.63	0.006	LSA ^a USA ^a ALP ^b		
Treatment	4.21	0.010	W ^b C ^{ab} HW ^{ab} H ^a		
Site × Trt	0.54	0.778			
Snowmelt date				0.0003 ± 0.0013	0.818
Site	86.32	<0.001	LSA ^a USA ^b ALP ^b		
Treatment	53.84	<0.001	W ^b C ^b HW ^a H ^a		
Site × Trt	12.17	<0.001			
Days θ < 0.16				–0.0001 ± 0.0018	0.941
Site	5.93	0.005	LSA ^b USA ^a ALP ^a		
Treatment	2.59	0.064	W ^a C ^a HW ^a H ^a		
Site × Trt	2.15	0.065			
Days θ < 0.08				–0.0034 ± 0.0013	0.012
Site	5.18	0.009	LSA ^b USA ^b ALP ^a		
Treatment	2.18	0.103	W ^a C ^a HW ^a H ^a		
Site × Trt	0.93	0.479			
Min θ				0.4996 ± 1.3653	0.716
Site	9.28	<0.001	LSA ^a USA ^a ALP ^b		
Treatment	0.85	0.474	W ^a C ^a HW ^a H ^a		
Site × Trt	1.18	0.333			
<i>T</i> August–September				–0.0036 ± 0.0359	0.921
Site	7.192	<0.001	LSA ^a USA ^b ALP ^a		
Treatment	30.021	<0.001	W ^a C ^a HW ^b H ^b		
Site × Trt	1.710	0.140			
Degree days				–0.0003 ± 0.0001	0.007
Site	22.63	<0.001	LSA ^b USA ^b ALP ^a		
Treatment	65.43	<0.001	W ^a C ^a HW ^b H ^b		
Site × Trt	2.11	0.069			

Superscripted letters denote significant differences, and alphabetic order corresponds with increasing magnitude (i.e. $a < b$). Final model results of stepwise linear regression of environmental variables and survival appear on the right, including coefficients (b), their standard errors (SE) and p values of testing whether $b = 0$ (p)

Results in bold were included in the final model: $p < 0.0001$, $F = 15.555$, $RMSE = 0.176$

improve carbon balance, as observed elsewhere (Ow et al. 2008; Way and Sage 2008a, b; Nedlo et al. 2009). T -response curves from individuals would be needed confirm any acclimation of basal respiration rates or Q_{10} (Atkin and Tjoelker 2003). However, respiration is not diffusion-limited by stomatal restriction in the same way as photosynthesis (Fig. 5), as described by Flexas et al. (2006), a disparity that could lead to carbon limitation under prolonged moisture stress (McDowell et al. 2008; Adams et al. 2009). Way and Sage (2008a, b) showed that respiratory acclimation of black spruce seedlings did not fully compensate for carbon losses under elevated greenhouse temperature. Spruce seedlings altered a number of morphological traits to make up the

balance, including reduced root expansion, which led to enhanced susceptibility to desiccation and greater mortality (Way and Sage 2008a, b). Our field-grown limber pine seedlings showed very low dark respiration rates after soils had dried below 0.08 m³ m⁻³, with little variability in R over a range of leaf T (Fig. 4f). This may indicate a late summer depletion of respiratory substrates (Tjoelker et al. 2009), but further investigation would be needed to determine the extent and importance of this effect.

Seedling photosynthesis remained low under autumn moisture limitation until the growing season ended with the initiation of snow cover. While established trees of the same species have been shown to respond to late season

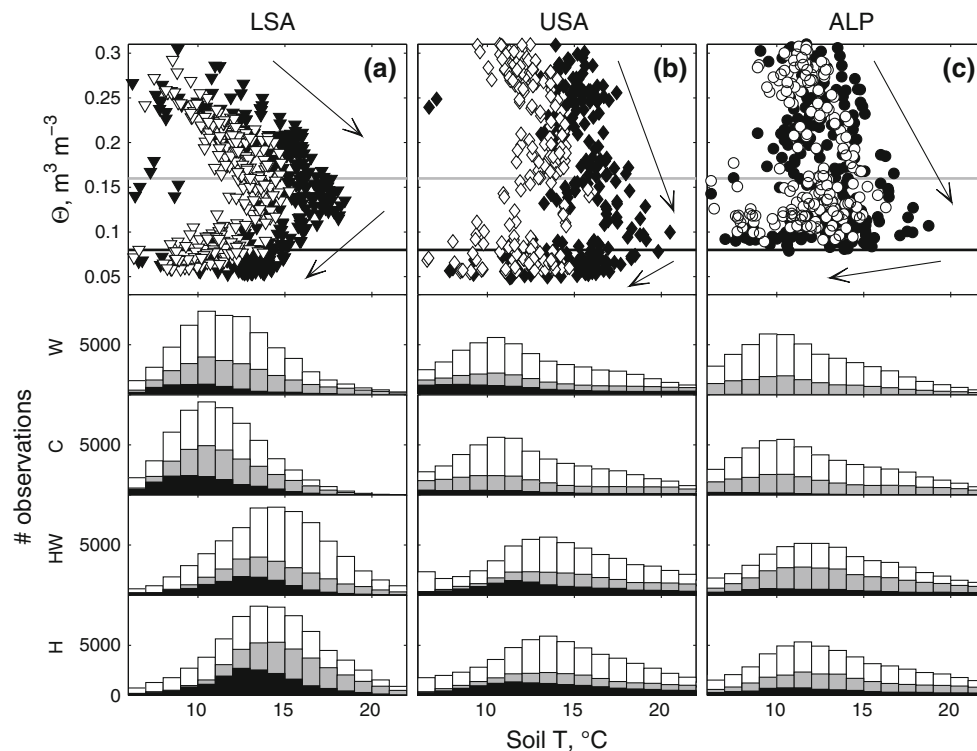


Fig. 6 a–c Daily treatment average ($n = 5$ plots) volumetric soil moisture at 5–10 cm (θ) versus daily average soil temperature at the same depths from days 135–287 (May 15–October 14), emphasizing the summer dry down period ($\theta < 0.3 \text{ m}^3 \text{ m}^{-3}$). Symbols are as in Fig. 2, and arrows show seasonal time course. Lines show θ of 0.16 (gray) and $0.08 \text{ m}^3 \text{ m}^{-3}$ (black). Lower panels histograms of 15-min

soil temperature from all replicate plots at 5–10 cm depth for the same period. White bars are entire dataset, gray bars are periods with $\theta < 0.16 \text{ m}^3 \text{ m}^{-3}$, and black bars include only periods with $\theta < 0.08 \text{ m}^3 \text{ m}^{-3}$. Data below 6 and above 22 °C omitted to emphasize shifts in centers of distribution

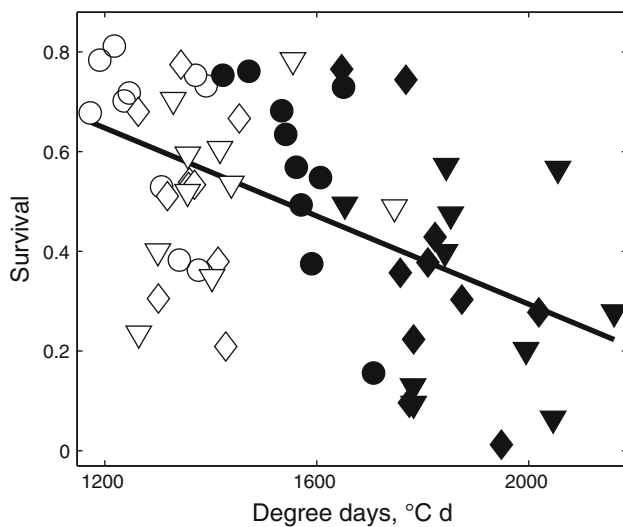


Fig. 7 End of season seedling survival versus growing degree days. Symbols and shapes are as in Fig. 2. The fitted line is $y = -0.0004x + 1.1779$, $r^2 = 0.28$, $p < 0.0001$

moisture availability (Letts et al. 2009), our first-year seedlings (including those in watered plots) remained drought stressed until the abrupt onset of winter, with no

apparent favorable autumn conditions. Most of the seasonal decline in photosynthesis with soil drying in the current study corresponded with low stomatal conductance (Fig. 5a). However, the relative importance of non-stomatal photosynthetic limitations under climate change conditions (Sage and Kubien 2007; Sage et al. 2008), and the potential for first-year seedling carbon uptake following drought-reducing autumn precipitation should be evaluated in further detail.

Soil moisture became limiting to photosynthesis to some degree at all sites and within all treatments over the summer of 2010. Mature limber pine has demonstrated sensitive stomatal responses to soil moisture depletion and high VPD relative to other subalpine trees (Pataki et al. 2000; Letts et al. 2009), but has also been observed to maintain afternoon water potentials near -1.8 MPa despite variable soil and predawn ψ (Fischer et al. 2002). We measured ψ_{midday} below -6 MPa from herbaceous stems of shallow-rooted first-year seedlings (Fig. 2), reflecting heightened vulnerability to desiccation during seedlings' first year. During our water potential measurements, many seedling stems broke apart under increasing pressure before liquid was seen or failed to exude water from cut stems under

maximum chamber pressure (7 MPa). Many stems contained visible lesions in conducting tissues and several may have been embolized or heat girdled (Kolb and Robberecht 1996). These qualitative observations add to the conclusion based on our water potential and gas exchange results (Figs. 2, 3, 4, 5) that increasing temperature may exacerbate physiological challenges to seedling carbon and water balance imposed by seasonal moisture stress at high elevation.

Climate, elevation, and future species distributions

Seedling establishment is a requirement for uphill redistribution of mountain species, and depends largely on sensitivity to microclimate during the first year after emergence (Germino et al. 2002). We found that first-year seedling survival of the drought-tolerant species limber pine was likely in only the coolest plots with the highest water availability, conditions that were most prevalent in unheated plots above treeline. Although water content values of 0.16 and 0.08 m³ m⁻³ were selected as threshold indicators of moderate and severe stress, seedling gas exchange (Figs. 2, 3) and mortality (Fig. 7) responded continuously to seasonal drought stress across all study plots. Notably, the most extreme drought conditions (coincidence of low θ and high soil T) occurred within the treeline site (USA, Fig. 6), where August–September soil temperature was highest (Table 1). Seasonal drought stress in krummholz interspaces may be heightened due to absence of canopy shade relative to the forest and reduction of wind speed relative to the open alpine tundra (Hadley and Smith 1987; Van Mieghroet et al. 2000).

Our results indicated a critical importance of soil moisture to seedling physiology and survival, and our water addition treatment was insufficient to compensate for strong seasonal reductions of soil moisture affecting seedling physiology (watering treatments not separated in Figs. 2, 3, 4, 5 due to similarity). There were no significant treatment effects on indicators of moisture stress calculated from θ (Table 1). This may have been due in part to minimal infiltration to the 5–10 cm sensor depth and/or soil surface evaporation, because our water additions frequently occurred on sunny days. However, a significant treatment effect was found for seedling survival between watered (W) and heated (H) plots (Table 1), the treatments presumably associated with the lowest and highest drought stress, respectively. This result indicates some improvement of seedling survival by watering.

Soil moisture at many alpine forest limits is generally assumed to be continuously adequate for tree growth, often remaining above 0.2 m³ m⁻³ throughout the year (Körner and Paulsen 2004; Mayr 2007; Wieser 2007a, b), but seasonal moisture stress may be important at high elevation in

regions where drier soil conditions occur. Although annual average temperature decreases with increasing elevation, differences in surface radiation balance and snow depth can lead to greater overall climate warming at high elevation (Giorgi et al. 1997), higher summertime extremes of surface temperature (current study; Hadley and Smith 1987; Bansal and Germino 2010), and possibly summer water limitation. A potential for aridity in treeline and alpine environments has been proposed due to biophysical effects of elevation (Smith and Geller 1979; Leuschner 2000), inferred from growth patterns in treeline tree cores (Lloyd and Fastie 2002; Wilmking et al. 2004), and directly observed in physiological measurements collected during unusually dry years (Johnson et al. 2004; Brodersen et al. 2006; Johnson and Smith 2007). We found evidence of an overriding effect of drought stress on seedling CO₂ exchange at high elevation under ambient conditions in a relatively typical year (Figs. 2, 3, 4, 5). Exposure to warm temperature and dry soils across plots was negatively correlated to survival, which was as low as 0 %, indicating the potential importance of first-year survival as an initial filter to seedling establishment and species distribution changes. Our results suggest seasonal moisture limitations to subalpine tree seedlings may be enhanced by climate warming (Table 1; Fig. 7). However, recruitment potential of subalpine trees at high elevation will depend on the frequency of favorable years within longer-term climate variability, and our results were limited to a single species and one growing season. Continued survival of seedlings (including overwintering) and first-year establishment rates during years with greater snow and/or summer precipitation remain to be determined.

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