



# Drought-induced photosynthetic decline and recruitment losses are mediated by light microenvironment in Rocky Mountain subalpine forest tree seedlings

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## ABSTRACT

Rates of tree mortality continue to rise with climate change, particularly in high elevation subalpine forests. In these systems, which depend on a narrow range of microsite conditions for successful seedling establishment, growing drought frequency and intensity may impose regeneration bottlenecks that further exacerbate population declines. However, it is unclear how regeneration processes tied to species-specific stress tolerances and microsite preferences are affected by varying magnitudes and durations of drought. To simulate moderate (50%) and severe (100% precipitation exclusion) growing-season drought, we installed precipitation exclusion shelters in shade and canopy gap light microenvironments over naturally-regenerating seedlings across a range of size-delimited establishment cohorts spanning first year emergents to large and well-established seedlings of two dominant subalpine forest species – Engelmann spruce (*Picea engelmannii* Parry ex Engelmann) and subalpine fir (*Abies lasiocarpa* (Hook) Nutt.). Seedling survival and gas exchange physiology was assessed to test the interacting effects of drought and light microenvironment on seedling performance, with contrasts to each species' historically preferred microsite under ambient precipitation conditions. Shade partially ameliorated drought effects when precipitation reductions were moderate as illustrated by rates of seedling mortality that were statistically indistinguishable from reference rates for both species, whereas moderate drought in canopy gaps significantly or nearly-significantly increased seedling mortality in several cohorts of both spruce and fir (1.9–2.9 and 2.3–7.5 times greater mortality risk in spruce and fir, respectively). When drought was severe, however, shade induced prolonged net-negative photosynthetic carbon assimilation as low as a  $-1.5 \mu\text{mol CO}_2\text{-m}^{-2}\text{-s}^{-1}$  (growing-season average), which occurred alongside significantly increased mortality in spruce (3.2–9.4 times greater mortality risk). In contrast, mortality was significantly higher in canopy gaps under severe drought for fir (5.5–23.1 times greater mortality risk). Larger seedling size was strongly associated with lower mortality risk and more conservative stomatal behavior (e.g., predominantly  $<0.1 \text{ mol-H}_2\text{O-m}^{-2}\text{-s}^{-1}$  growing-season averages), illustrating the sensitivity of new seedlings to microsite environments. However, persistent declines in photosynthetic carbon uptake observed across all seedling sizes in shaded microsites suggests the potential for lagged mortality and greater susceptibility to recurring drought even for larger and seemingly well-established seedlings. These results demonstrate how acute, intense droughts may alter historical patterns of spruce-fir seedling regeneration and highlight the importance of maintaining suitable microsites when implementing adaptive management strategies to avoid regeneration bottlenecks in forests that are already vulnerable to accelerating drought-induced mortality.

## 1. Introduction

Droughts are increasing in frequency and severity across many forest ecosystems, inducing extensive overstory tree mortality and loss of

ecosystem functions worldwide (Van Mantgem et al., 2009; Allen et al., 2010; Anderegg et al., 2013; Crausbay et al., 2015; Clark et al., 2016; Swann et al., 2023). Increasing droughts are also expected to amplify mortality in seedlings, raising concerns that recruitment will fail to

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offset current and projected rates of overstory loss (Conlisk et al., 2017; Kueppers et al., 2017; Conlisk et al., 2018; Brodersen et al., 2019). Even of short duration or low intensity, droughts can create fundamentally unsuitable conditions for seedling establishment and persistence, especially for species occupying narrow regeneration niches that evolved under cooler and wetter climates, possibly leading to a substantial divergence of future forests from historical compositions and functional structures (Bell et al., 2014; Martínez-Vilalta and Lloret, 2016; Brodersen et al., 2019). Such outcomes are especially probable in high elevation coniferous forests of the western U.S., where climate change effects – especially warming – are accelerated relative to lower elevations (Beniston et al., 1997; Pepin et al., 2015; Dobrowski and Parks, 2016). In these regions, seedling establishment is also strongly controlled by microsite characteristics of soil moisture and canopy cover derived from ecological facilitation (Gill et al., 2015; Buechling et al., 2016; Andrus et al., 2018a,b; Hill et al., 2019; Foster et al., 2020) – conditions which are not mechanistically linked to masting periods of high seed production (Kroiss and HilleRisLambers, 2015; Copenhagen-Parry et al., 2020).

In the Colorado Front Range of the southern Rocky Mountains, the impact of drought remains uncertain as models forecast little systematic changes in precipitation regimes, with total mean annual precipitation projected to decline <5% over the next century (Lukas et al., 2014). However, even in a year with typical precipitation, lower snowpack and earlier snowmelt under higher spring temperatures (Mote et al., 2005) can induce increasingly regular moisture deficits. These seasonal deficits are likely to be further exacerbated by the delayed onset and intermittent failure of summer monsoon rains that are responsible for the majority of growing-season precipitation in the region (Cook and Seager, 2013; Pascale et al., 2017). Indeed, summer precipitation deficits are linked to accelerating rates of adult tree mortality in subalpine forests in the region (Bigler et al., 2007; Smith et al., 2015; Buechling et al., 2017) and recruitment declines in these forests also are anticipated with climate warming (Kueppers et al., 2017; Andrus et al., 2018b). Accelerating rates of mortality in Rocky Mountain subalpine forests threatens not only the productivity and timber economy of these systems, but also critical snowpack dynamics in what is considered the largest water-yielding areas in the region, along with maintenance of wildlife habitat and highly valued recreational opportunities and aesthetic beauty (Alexander, 1987; Mote et al., 2005; Kittel et al., 2015).

Regeneration of the dominant tree species of the subalpine forests in the Rocky Mountains – Engelmann spruce (*Picea engelmannii* Parry ex Engelmann) and subalpine fir (*Abies lasiocarpa* (Hook) Nutt.) – is strongly limited by growing season moisture availability (Gill et al., 2015; Buechling et al., 2016; Andrus et al., 2018b; Hill et al., 2019; Martin and Canham, 2020), which interacts with understory light dynamics and edaphic characteristics (e.g. Bergstrom et al., 2019) in determining regeneration patterns. Spruce and fir have contrasting microsite preferences for germination and seedling establishment; highly shade-tolerant subalpine fir seedlings colonize deeply-shaded microsites often containing thick litter layers in greater abundance – often by a factor of two or three – while moderately shade-tolerant Engelmann spruce seedlings preferentially colonize canopy gaps or disturbed sites without deep litter (Day, 1964; Knapp and Smith, 1982; Hättenschwiler and Smith, 1999; Germino et al., 2002; Redmond and Kelsey, 2018). Combined with the greater longevity of adult spruce trees, these dynamics sustain a roughly co-dominant spruce-fir overstory that can persist for centuries (Shea, 1985; Veblen, 1986; Antos and Parish, 2002; Andrus et al., 2018a).

Increased drought may alter these regeneration dynamics between spruce and fir by degrading microsite conditions that facilitate co-existence (Holmgren et al., 1997; Martínez-Vilalta and Lloret, 2016; Brodersen et al., 2019). While both spruce and fir are considered shade tolerant with some degree of drought tolerance (Wei et al., 2019), even minor shade or drought tolerance advantages in one species could lead to disruption of historical recruitment patterns in future climates. For

spruce, the higher solar irradiance in canopy gaps relative to shaded microsites may exacerbate drought stress via higher temperatures, soil water evaporation, and vapor pressure deficits (Abrams and Mostoller, 1995; Chen et al., 1995); if drought stress increases in gaps, spruce regeneration may be limited to shadier microsites where fir out-competes spruce given its longer taproot capable of reaching through thicker litter layers (Knapp and Smith, 1982). Drought may also be detrimental in deep shade where, despite comparatively higher moisture availability due to lower evaporative demand, it can cause extended reductions in photosynthetic carbon uptake due to lower light availability, promoting depletion of non-structural carbohydrate reserves which may impede water relations and further amplify the risk of mortality (McDowell, 2011; Sala and Mencuccini, 2014; Maguire and Kobe, 2015; Sapes et al., 2021) – particularly for emerging seedlings with inherently narrow carbon budgets (Bansal and Germino, 2008). Indeed, tolerance of drought stress in low light is generally thought to be uncommon in trees, given apparent trade-offs in water stress and shade tolerance traits (Niinemets and Valladares, 2006; Rueda et al., 2017), particularly in seedlings which display a canonization of traits promoting carbon uptake over stress-mitigation strategies (Niinemets, 2010; Augustine and Reinhardt, 2019; Goke and Martin, 2022).

The aim of this study was to evaluate the demographic and physiological responses of naturally establishing Engelmann spruce and subalpine fir seedlings to drought and understory light levels in a subalpine forest in the Colorado Front Range. Patterns of photosynthetic carbon uptake, stomatal conductance, and seedling survival were compared between dense shade and canopy gap microenvironments with two experimental drought treatments, mimicking a weakening (50% precipitation exclusion) and failure (100% exclusion) of the North American monsoon. Given the vulnerability of the smallest seedlings, we expected a single growing-season drought would induce a systematic recruitment failure of new emergents of both species. In addition, we expected that drought effects on gas exchange and survival would be most pronounced in canopy gap microsites, where water stress is elevated due to higher temperatures and evaporative demands, and thus we expected drought will more negatively impact spruce seedlings given the importance of canopy gaps in its regeneration. Physiological trade-offs between drought and shade tolerance suggest that performance of both species will decline when drought occurs in the shade, which again we expected would more strongly impact the less shade-tolerant spruce. Overall, drought could either: (1) further stratify existing microsite specialization between spruce and fir which, if one species displays greater tolerance to drought, has the potential to shift regeneration dynamics towards more asymmetric patterns of overstory dominance, or (2) degrade microsite suitability to an extent that both species become mismatched with current microsites, suggesting forest-wide regeneration failures under prolonged or recurring droughts.

## 2. Methods

### 2.1. Site description

This study was conducted in a mature spruce-fir forest approximately 300 m below timberline at the University of Denver High Altitude Laboratory on Mt. Evans, Colorado, USA (3230 m, 39.66° N, 105.59° W). The site is characteristic of subalpine spruce-fir forests of the southern Rocky Mountains with codominant Engelmann spruce and subalpine fir mixed with infrequent lodgepole (*Pinus contorta* Dougl.), limber pine (*Pinus flexilis* James), and bristlecone pine (*Pinus aristata* Engelm.) amid a sparse understory dominated by *Vaccinium* sp. (L.). Shallow mineral soils at the site are derived from Leighcan family till substratum and Tonahutut-Ohman complex originating from igneous and metamorphic rock (NRCS, 2020). While most of the annual precipitation (mean 780 mm) falls as snow, on average approximately 30% occurs during summer monsoonal rains from July through September (Table S1, NRCS SNOTEL, 2020). Monsoon rains are particularly

important as the study site is far enough from the climatic boundary between the arid west and humid east that even periods of high precipitation in the western Great Plains do not extend sufficiently west to increase moisture in the Front Range mountains of Colorado (Salley et al., 2016). No evidence of any recent disturbances (fire, blow-down, insect outbreak, etc.) was apparent at the site before or during the study.

## 2.2. Experimental design

Twelve seedling monitoring plots were established at the site in July 2019 – six in microsite areas representative of relatively open interior forest gaps (hereafter referred to as “canopy gap” or “gap”) and six in closed canopy, shaded areas (“shade”, Fig. 1). Plots were similar in litter composition, microtopography, and herbaceous cover. Classifications of understory light availability were verified with hemispherical photographs (Nikon COOLPIX 900) taken at seedling height and processed using Gap Light Analyzer (GLA) software v. 2.0. The resulting gap light index (GLI, the combined incident diffuse and direct-beam radiation over a growing season expressed as a percentage of full sun; Frazer et al., 1999) revealed that gap plots averaged 34% GLI ( $\pm 3.7\%$  SE) and shade plots averaged 18% GLI ( $\pm 0.8\%$  SE).

Each plot consisted of three 1 × 1 m quadrats containing naturally regenerating seedlings of spruce and fir defined as stems <5 mm in diameter at the root crown and maximum 20 cm in height encompassing current year emergents to well-established seedlings. Since these species display weak age-size correlations and approximating age from either annual growth rings or terminal bud scars can be unreliable (Schapira et al., 2021), seedlings were classified into approximate developmental and establishment stages based on stem diameter at the root crown as: emergent (mostly cotyledons present, <0.5 mm diameter), small seedling (cotyledons sometimes present, 0.5–1.0 mm diameter), medium seedling (no cotyledons, 1.0–2.0 mm diameter), large seedling (2.0–3.0 mm diameter), and well-established seedling (3.0–5.0 mm diameter, Fig. 2).

Within each plot, one quadrat was randomly designated to receive ambient levels of precipitation while the other two received either 50% or 100% precipitation exclusion treatments from 15 July to 22 September 2019 to mimic reduced or complete failure of monsoonal precipitation. Following widely adopted Drought-Net (2018) designs, 1 × 1 m panels of clear polycarbonate (Suntuf, Palram Americas, Kutztown, PA, USA) were installed 1 m above the soil surface using a PVC frame to achieve 100% reduction, while three pieces of ~15 cm × 1 m roofing were alternated every 15 cm along the frame with two additional ~10 cm × 1 m pieces (5 cm overhang) on opposing sides to create a 50% reduction. Shelters of similar design were found to reduce precipitation as intended, while having insignificant or marginal effects on

soil and air temperatures and light quantity beneath the shelter (Fay et al., 2000; Yahdjian and Sala, 2002; Heisler-White et al., 2008; Cherwin and Knapp, 2012).

## 2.3. Plot throughfall and soil moisture

Effective precipitation (“throughfall”) at the plot level in both ambient and precipitation-exclusion treatments was assessed using rain gauges constructed from 4 to inch diameter PVC pipe capped with funnels to minimize losses from splashing and evaporation. Gauges were placed in a subset of plots for all treatments (5 gap plots and 4 shade plots, 27 gauges total) and emptied on a weekly basis for the duration of the study. Soil moisture (volumetric water content, % VWC) was monitored continuously for the duration of the study in the same subset of plots containing rain gauges using 5 cm electrical conductivity soil moisture probes inserted laterally 5 cm below the soil surface and connected to dataloggers (EC-5 and EM50, Decagon Devices, Pullman, WA, USA). Occasional logger failure created intermittency in soil moisture observations across plots; in these cases, soil VWC was imputed as the average VWC from other plots of similar microsite light classification. Site-level precipitation was retrieved from an on-site SNOTEL monitoring station (NRCS SNOTEL, 2020). The year prior to study (2018) was a drought year in the area, but precipitation deficits had recuperated by early March 2019 after heavy winter snows leading to a typical growing season moisture the year this study was conducted (Table S1), though mild dryness was present in the final weeks of observation (September 2019, NDMG, 2020).

## 2.4. Seedling survival

Seedling survival was assessed on a weekly basis during the period of precipitation reduction. Individuals were considered dead once all needles had browned and seedlings appeared desiccated, at which point they were harvested and measured for stem diameter at the root crown. A final census of all plots was taken to assess survival of remaining seedlings on 22 September 2022. In total, 1,633 seedlings of spruce ( $n = 1,297$ ) and fir ( $n = 336$ ) were monitored for survival throughout the study period (Fig. 2).

## 2.5. Gas exchange

Beginning shortly after the initiation of precipitation exclusion, selections of healthy seedlings ( $n = 5$  to 15 individuals per sampling date of varying sizes across species, precipitation exclusion treatment, and light microenvironments, Fig. 2) were measured for photosynthetic gas exchange using an infrared gas analyzer (LI-6800, LI-COR Biosciences,

### Canopy Gap



### Shade



Fig. 1. Representative images of canopy gap (left) and shade microsites (right), with its associated gap light index (GLI,%) quantified from hemispherical photographs (inset images) at the study sight near Mt. Evans, Colorado, USA (3230 m, 39.66° N, 105.59° W).

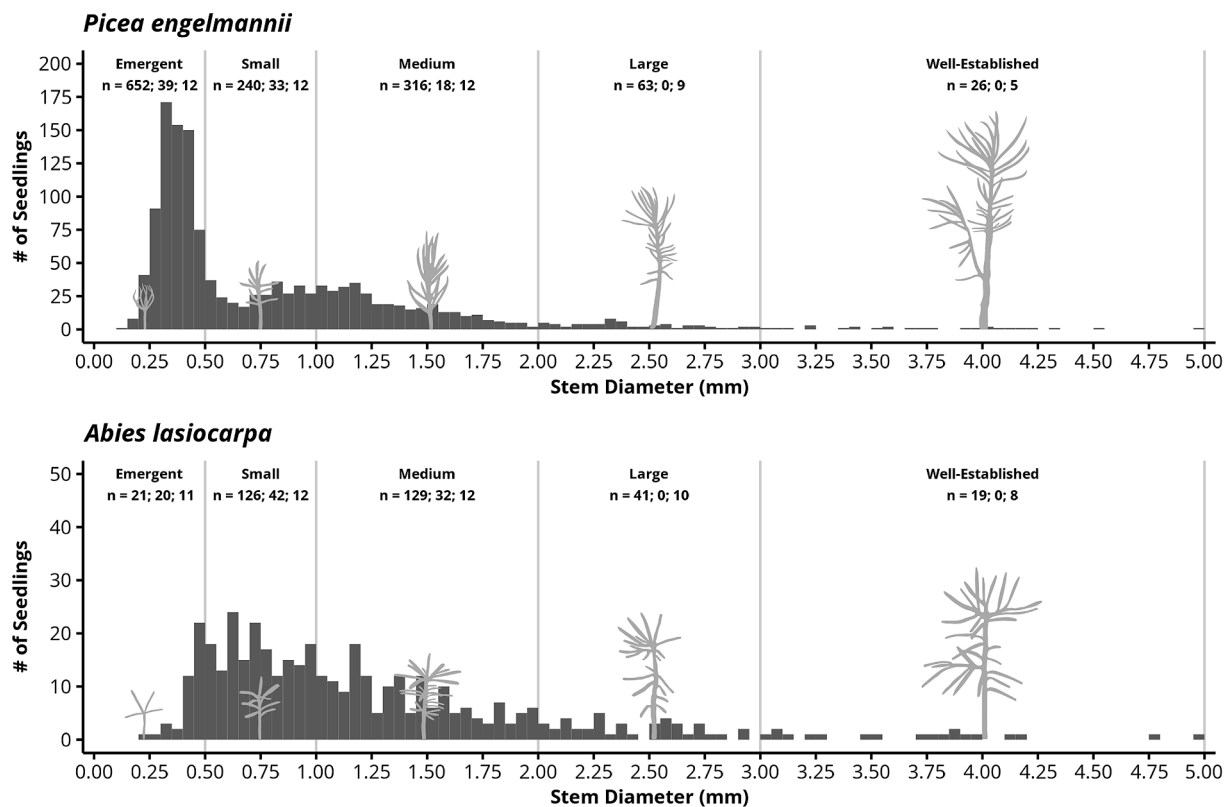


Fig. 2. Distribution of Engelmann spruce (*P. engelmannii*, upper, total  $n = 1387$ , including 90 sampled for gas exchange) and subalpine fir (*A. lasiocarpa*, lower, total  $n = 430$ , including 94 sampled for gas exchange) seedling root-crown stem diameters (mm) and corresponding size classifications across all plots and precipitation exclusion treatments. Inset values specify the number of seedlings (n) monitored for survival, sampled for gas exchange, and number of plots (out of 12) occupied by seedlings within each size classification, respectively.

Lincoln, NE, USA). Since large and well-established seedlings were less abundant among plots and were expected to display greater tolerance to moisture reductions, and responses to precipitation withholding were expected to be greatest at opposing extents of precipitation exclusion treatments, gas exchange measurements were conducted on individuals 2 mm or less in diameter (emergent, small, and medium seedlings) in only the ambient and 100% precipitation exclusion treatments. For seedlings with few leaves, entire crowns were inserted vertically into the chamber maintaining sunwards orientation to the light source. For larger seedlings, sun-oriented branches were inserted horizontally. Chamber temperature was matched to ambient conditions every 10–15 min while humidity was maintained at 1.5 vapor pressure deficit.  $\text{CO}_2$  was supplied at 410 ppm to match typical ambient summertime concentrations (Niwot Ridge Global Monitoring Laboratory, CO, USA, NOAA ESRL, 2020). Net photosynthesis ( $A$ ,  $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and stomatal conductance to water vapor ( $g_{\text{sw}}$ ,  $\text{mol} \cdot \text{H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) was assessed in response to a decreasing gradient of light availability inside the chamber at 0, 50, 100, 200, 400, 600, 800, 1200, and 1600  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  photosynthetically active radiation (PAR). Seedlings were acclimated to chamber conditions a minimum of five minutes prior to measurements. Because seedlings were likely locally acclimated to their microsite light environment (Cater, 2021), maximum photosynthetic flux ( $A_{\text{max}}$ ) and stomatal conductance ( $g_{\text{sw}}$ ) were calculated by averaging the five highest values from each set of measurements (Johnson and Smith, 2005). Measurements were conducted mid-morning two to three times weekly under clear overhead conditions when photosynthetic fluxes and stomatal conductance were expected to be at their peak (Carter and Smith, 1988). Detached leaves from the total leaf sample exposed in the gas analyzer chamber were imaged with a flatbed scanner at 600 DPI (Perfection V850, Epson, Nagano, Japan) and quantified for projected leaf area ( $\text{m}^2$ ) using FIJI v. 1.52 (Schindelin et al., 2012). All

gas exchange measurements are expressed on a leaf-area basis. In total, 90 spruce and 94 fir seedlings were sampled for gas exchange across the period of precipitation exclusion, in addition to the 1,297 spruce and 336 fir seedlings monitored for survival only (total  $n = 1387$  spruce and 430 fir; Fig. 2).

## 2.6. Statistical analyses

### 2.6.1. Seedling survival

The unequal distribution of seedlings across treatments – arising naturally from studying *in situ* seedlings populations (Fig. 2) – required seedling survival to be analyzed non-parametrically. To evaluate the cumulative effect of combinations of precipitation exclusion and light microenvironment on survival, one-tailed Fisher's exact binomial tests were used to determine if mortality was greater than a null expected proportion of gross cumulative survival (pooled survival across all plots). Null proportions were specified separately for each species and each seedling size classification according to well-established microsite recruitment preferences patterns between the two species ("Reference"); rates of seedling gross cumulative survival in ambient canopy gap plots were used as the reference for spruce, and rates of seedling gross cumulative survival in ambient shaded plots were used as the reference for fir, each representing natural levels of "background" mortality for that species in their preferred microsite environment. Thus, the difference in survival ("Net Mortality",  $\Delta\text{Reference}$ ) in the various treatments represents the additional amount of mortality over rates observed in the reference group that is attributable to a given combination of light microenvironment and precipitation exclusion treatment. Pairwise differences in size-specific net mortality among precipitation exclusion and light microenvironment combinations were assessed using the Benjamini-Hochberg procedure to control for overall Type-I error, using

the false discovery rate with a threshold of  $q < 0.05$  (FDR, proportion of incorrectly rejected null hypotheses at  $p < 0.05$ ; Benjamini and Hochberg, 1995). Large and well-established seedlings were excluded as they demonstrated 100% survival during the study.

Additionally, time-to-event probability of mortality was evaluated using Cox proportional hazard regression (R package “survival”, Therneau and Lumley, 2015) with seedling size, precipitation reduction, and light microclimate – and their interactions – as explanatory variables. Mortality risks (hazard ratios, HR) were estimated separately for each species, both overall (“Overall Mortality Risk”) and within each seedling size class (“Size-Specific Mortality Risk”), where hazard ratios represent the multiplicative instantaneous risk of mortality for a given combination of light microenvironment and precipitation exclusion treatment relative to the species-specific reference group across the observation period. Hazard ratios equal to 1 indicate a rate of mortality indistinguishable from the reference group, ratios  $< 1$  a reduced hazard of mortality, and ratios  $> 1$  an increased hazard of mortality (e.g., a ratio of 0.74 indicates a 26% lower risk of mortality relative to the reference group, whereas a ratio of 23 indicates a 23-times greater risk of mortality). As with the null mortality expectations regarding cumulative survival, here we specify seedlings grown in ambient, canopy gap microsites as the reference group for spruce, and seedlings growing in ambient, shaded conditions as the reference group for fir. Hazard ratios were assessed both individually within each seedling size classification (i.e., against the reference group of mortality risk where the hazard ratio = 1; “Size-Specific Mortality Risk”) via a likelihood ratio test to evaluate if the estimated hazard ratio was significantly different than 1, and with pairwise multiple comparison (differences among hazard ratios within each seedling size classification). As before, FDR-correction was applied to control for elevated Type-1 error for multiple comparisons ( $q < 0.05$ ). Although it may be expected that risk of mortality would increase non-proportionally among combinations of precipitation reduction and light microenvironments as the season progressed (thus violating the proportional-hazards assumptions of the Cox model), no evidence of time-dependency was detected (global likelihood ratio  $p > 0.05$  on the linear relationship between time and scaled Schoenfeld residuals, Therneau and Lumley, 2015). Therefore, time-dependent environmental variables such as soil moisture and precipitation were not considered for additional survival analysis. Large and well-established seedlings – along with some combinations of light microenvironment and precipitation exclusion treatment in medium fir seedlings – were excluded from survival analysis because they demonstrated 100% survival during the study which can result in unreliable estimation of hazard for these groups.

### 2.6.2. Gas exchange

Linear models were implemented separately for spruce and fir to test for the effects of seedling size, microsite light environment, precipitation exclusion treatment, and number of days of precipitation exclusion – as well as their interactions – on photosynthetic rate and stomatal conductance. Including plot as a random effect did not meaningfully improve model performance, and so simple multiple regression was used. To test for pairwise differences in gas exchange parameters among precipitation exclusion treatments and light microenvironments, estimated marginal means of both the linear trend (rate of change in gas exchange parameters across the observation period) and the mean response across the period of precipitation exclusion (15 July–22 September 2019) were calculated (R package “emmeans” v. 1.8.1-1, Length 2022). P-values of multiple comparisons were again adjusted via FDR correction ( $q < 0.05$ ). All analyses were conducted in R (v. 4.2.2, R Core team 2022).

## 3. Results

### 3.1. Plot throughfall and soil moisture

Precipitation exclusion shelters created plot-level moisture reductions as intended (Fig. 3). The 100% precipitation exclusion shelters diverted all precipitation in both microsites (i.e., 0 mm throughfall). Relative to ambient levels of precipitation over the entire exclusion period (15 July to 22 September 2019), the 50% precipitation exclusion shelters reduced throughfall by 50.2% ( $\pm 6.78$  SE, for a total reduction of 40.8 mm) in canopy gap microsites and 46.0% ( $\pm 12.10$  SE, for a total reduction of 33.6 mm) in the shaded microsites. Shaded microsites received slightly lower total precipitation – presumably due to interception from the overhead forest canopy.

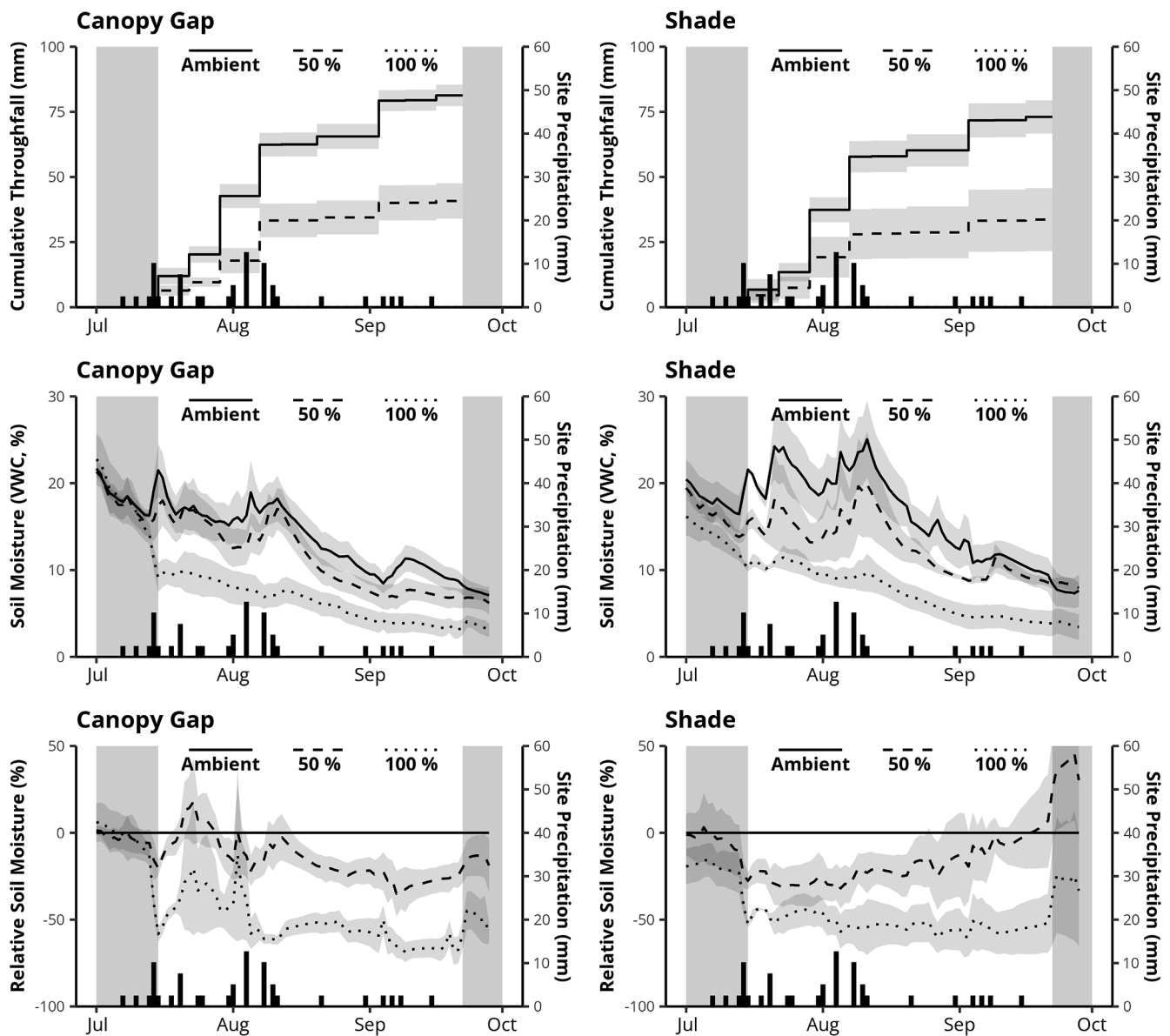
These throughfall reductions corresponded to similar patterns in reduced soil volumetric water content (VWC, %). Relative to ambient soil moisture over the treatment period, daily mean VWC in the 50% precipitation exclusion treatments was reduced on average by 2.2% ( $\pm 0.16$  SE) in canopy gap microsites and 3.9% ( $\pm 0.23$  SE) in shaded microsites, while daily mean VWC in the 100% precipitation exclusions was reduced on average by 7.3% ( $\pm 0.19$  SE) in canopy gap microsites and 9.4% ( $\pm 0.39$  SE) in shaded microsites (Fig. 3). Although the absolute reduction in % VWC was slightly greater in the shaded microsites, relative reductions in percent soil moisture were similar between microsites: daily mean VWC across the treatment period was on average 15.5% ( $\pm 1.30$  SE) and 18% ( $\pm 1.71$  SE) lower relative to ambient soil moisture in 50% precipitation exclusion canopy gap and shaded microsites, respectively, and 52.5% ( $\pm 1.61$  SE) and 52.3% ( $\pm 1.25$  SE) lower in 100% precipitation exclusion canopy gap and shaded microsites, respectively.

### 3.2. Seedling survival

Seedling survival varied strongly with seedling size, precipitation exclusion treatment, and light microenvironment (Fig. 4). Foremost, survival was strongly associated with seedling size; across all precipitation exclusion treatments and light microenvironments, emergents of both species exhibited the lowest survival (ranging from 0.0 to 70.6% gross cumulative survival), followed by small seedlings (57.9–95.5%), and medium seedlings (80.0–100%). There was no mortality during the study in either large or well-established seedlings of either species (data not shown).

The influence of precipitation exclusion and light microenvironment on net mortality compared to the reference group was apparent for both species to varying degrees. For emergent spruce seedlings, 50% precipitation exclusion significantly increased mortality in canopy gaps (+17.9% net mortality;  $p < 0.001$ , Fisher’s exact), but not in shaded microsites (+3.6% net mortality;  $p = 0.210$ , Fisher’s exact) relative to rates mortality in ambient, canopy gap microsites. Mortality further increased under 100% precipitation exclusion, with shaded microsites experiencing the greatest increase in mortality (+45.8% net mortality;  $p < 0.001$ , Fisher’s exact), followed by canopy gaps (+38.9% net mortality;  $p < 0.001$ , Fisher’s exact). For emergent fir seedlings, both levels of precipitation exclusion reduced survival, with seedlings occupying canopy gaps under 100% precipitation exclusion demonstrating 100% mortality (+66.7% net mortality), though this effect was not found to be significantly greater compared to reference rates of survival ( $p = 0.111$ , Fisher’s exact). Net mortality in shaded microsites under 100% precipitation exclusion was however significantly greater than reference rates (+33.3% net mortality;  $p = 0.042$ , Fisher’s exact).

Similar patterns in cumulative survival occurred in small seedlings of both species. In spruce, 50% precipitation exclusion reduced survival significantly only in canopy gaps (+25.5% and +10.4% net mortality,  $p = 0.009$  and  $0.243$  in gap and shade, respectively, Fisher’s exact), while 100% precipitation exclusion significantly increased spruce mortality in both in canopy gaps and shaded microsites – again with shaded



**Fig. 3.** Cumulative plot throughfall (upper, mm), soil volumetric water content (middle, VWC%), and relative soil moisture content (lower,%) among precipitation exclusion treatments (ambient, 50% exclusion, and 100% exclusion) in canopy gap (left) and shaded (right) microsites. Non-shaded regions represent the dates which precipitation exclusion treatments were active (15 July to 22 September 2019). Precipitation throughfall was measured at ground level (e.g., beneath precipitation exclusion shelters) with rain gauges, and soil moisture at 5 cm depth using dielectric moisture probes. Daily precipitation (mm) at the study site retrieved from SNOTEL is also presented as bars. Error ribbons indicate the standard error of the mean.

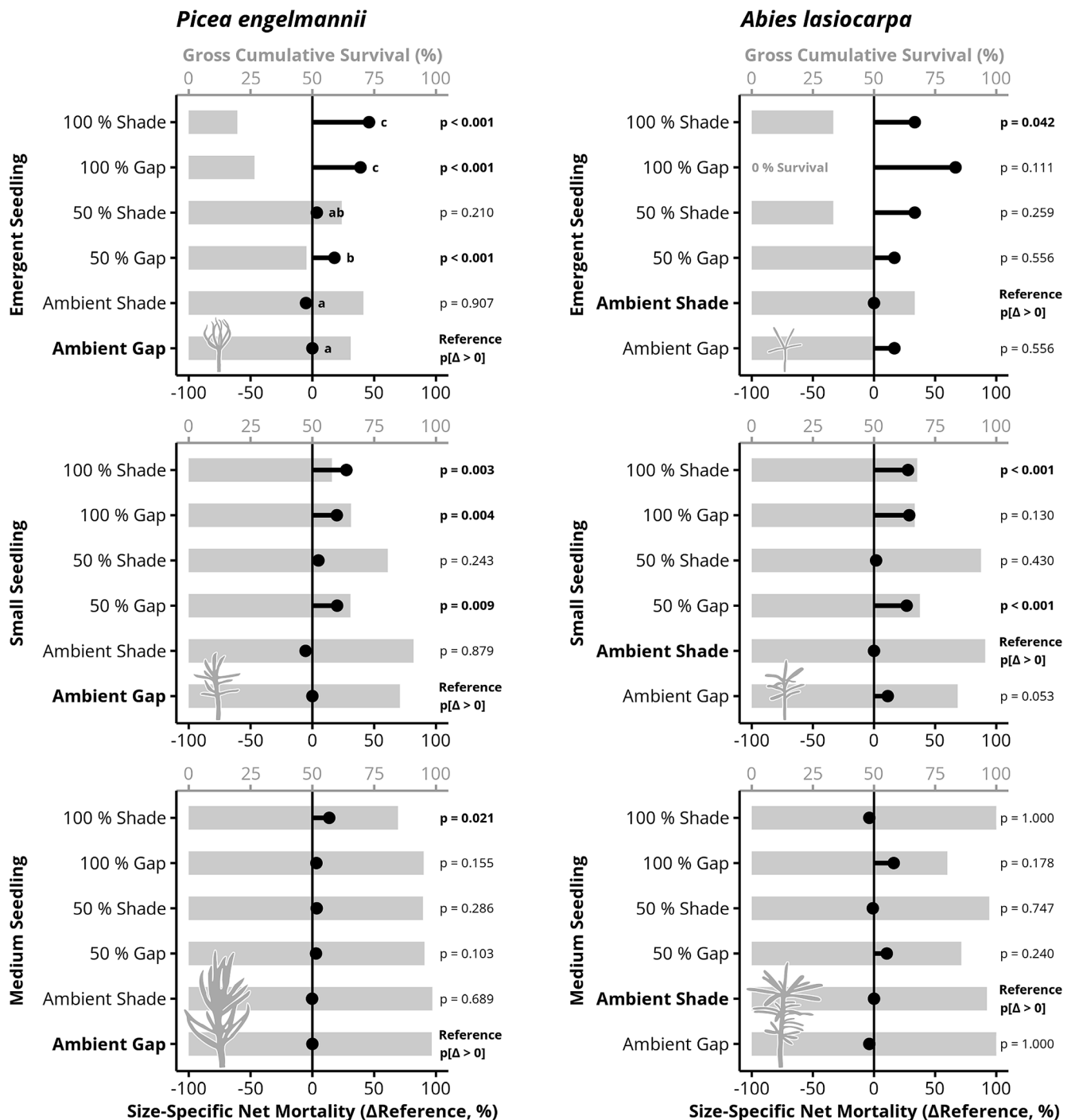
seedlings demonstrating the greatest increase in mortality (+25.3% and +33.0% net mortality in gap and shade, respectively, Fisher's exact). In small fir seedlings, 50% precipitation exclusion corresponded to significantly reduced survival only in canopy gaps (+26.7% and +1.7% net mortality,  $p < 0.001$  and 0.430 in gap and shade, respectively, Fisher's exact). While survival was further lowered under 100% precipitation exclusion (+28.8% and +27.8% net mortality in gap and shade, respectively), this increase in mortality was statistically significant was only in shaded microsites ( $p < 0.001$  and  $p = 0.130$  in shade and gap microsites, respectively, Fisher's exact).

For medium-sized seedlings of both species, precipitation exclusion only slightly reduced survival and only under 100% exclusion. In spruce, a statistically significant increase in net mortality was found only in the shade under 100% precipitation exclusion (+13.9% net mortality,  $p = 0.021$ , Fisher's exact). In medium fir seedlings, the lowest survival was observed in canopy gaps under 100% precipitation exclusion (+16.2% net mortality), though this effect was not significant ( $p = 0.178$ , Fisher's

exact).

Significant pairwise differences among levels of net mortality were found only in emergent spruce seedlings, with seedlings under 100% precipitation exclusion in both shaded and canopy microsites displaying statistically unique levels of net mortality apart from other precipitation exclusion and light microenvironment groupings (FDR  $q < 0.05$ ). No significant multiple-comparison differences among levels of net mortality within any seedling size classifications were found for fir (FDR  $q > 0.05$ ).

Evaluation of the instantaneous mortality risk (hazard ratio, HR) provided further insight on the influence of seedling size and precipitation exclusion, and particularly the influence of light microenvironment on seedling survival between spruce and fir (Fig. 5, Table S2). In emergent spruce seedlings, risk of mortality was significantly elevated relative to the reference group under 50% precipitation exclusion in canopy gaps, and both microsite light environments under 100% precipitation. In these seedlings, the greatest risk of mortality was found in



**Fig. 4.** Gross cumulative seedling survival (%; bars) and net mortality difference from the reference group (ΔReference,%, points) of Engelmann spruce (*P. engelmannii*, left) and subalpine fir (*A. lasiocarpa*, right) seedlings among seedling sizes (emergent, small, and medium), precipitation reduction (ambient, 50% reduction, and 100% reduction), and microsite light conditions (shade, canopy gap). Bolded labels indicate the reference group (net mortality difference = 0, vertical line). P-values specify the one-tailed probability of size-specific net mortality as greater than the reference group (net mortality > 0) within each seedling size classification (Fisher’s exact, bolded where  $p < 0.05$ ). Net mortality percentages not sharing a common letter are significantly different within each seedling size classification (FDR  $q < 0.05$ ).

seedlings occupying shaded microsites under 100% precipitation exclusion where risk of mortality was 3.4 times greater compared to ambient, canopy gap seedlings ( $p < 0.001$ , likelihood ratio). Similarly, the greatest (and significantly so) mortality risk for small spruce seedlings was under shaded, 100% precipitation exclusion conditions (3.2 HR,  $p < 0.001$ , likelihood ratio). Further, the greatest mortality risk for medium seedlings was also found under shaded, 100% precipitation exclusion conditions – the only group with a mortality risk significantly

different than the reference (9.4 HR,  $p = 0.025$ , likelihood ratio). Significant pairwise differences among hazard ratios were found only in emergent spruce seedlings, with seedlings under 100% precipitation exclusion in both shaded and canopy microsites displaying statistically unique hazard ratios apart from other precipitation and light groupings, and seedlings under 50% exclusion in canopy gaps a unique intermediate risk of mortality (FDR  $q < 0.05$ ).

Conversely, mortality risk (though elevated under both levels of

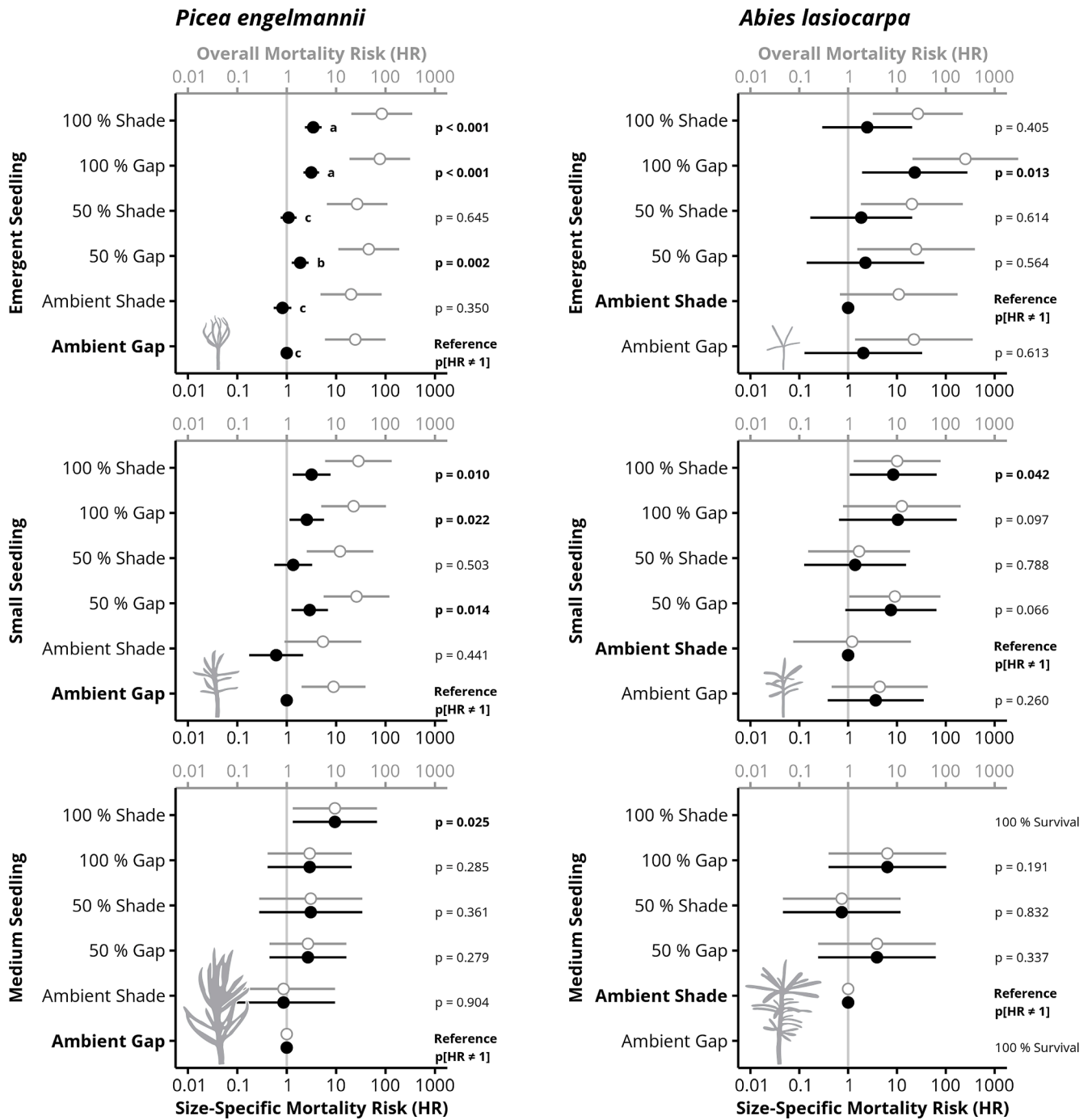


Fig. 5. Cox proportional hazard ratios (HR) of overall seedling mortality (open points) and size-specific mortality (closed points) of Engelmann spruce (*P. engelmannii*, left) and subalpine fir (*A. lasiocarpa*, right) seedlings among seedling sizes (emergent, small, and medium seedling), precipitation reduction treatment (ambient, 50% reduction, and 100% reduction), and microsite light environment (shade, canopy gap). Bolded labels indicate the reference group (HR = 1, vertical line). Error bars indicate the 95% confidence interval of the hazard ratio. P-values specify the likelihood ratio probability of the hazard ratio as different than the reference group (HR ≠ 1) within each seedling size classification. Size-specific mortality risk hazard ratios not sharing a common letter are significantly different within each seedling size classification (FDR q < 0.05).

precipitation exclusion) in emergent fir seedlings was greatest in seedlings occupying canopy gaps under 100% precipitation exclusion – the only group demonstrating significantly different mortality risk relative to the ambient, shaded reference (23.1 HR, p = 0.013, likelihood ratio). In small fir seedlings, mortality risk was again higher with increasing levels of precipitation exclusion, with seedlings occupying canopy gaps under 100% precipitation exclusion demonstrating the greatest mortality risk, though this effect was not significant (HR 14.8, p = 0.097). Shaded seedlings under 100% precipitation exclusion demonstrated the

second highest mortality risk and this effect was significant (HR 8.4, p = 0.042, likelihood ratio). Though mortality risk was also greater in canopy gaps under both levels of precipitation exclusion in medium fir seedlings (9.0 HR and 5.5 HR in 50% and 100% precipitation exclusion, respectively), these effects were not significant. As 100% survival was observed in medium fir seedlings occupying ambient canopy gap sites and 100% precipitation exclusion shade sites, hazard ratios are not estimated for these seedlings. No significant pairwise differences among hazard ratios within any seedling size classifications were found for fir



(FDR  $q > 0.05$ ).

### 3.3. Gas exchange

Linear models of gas exchange patterns revealed that, overall, maximum net photosynthetic rate and stomatal conductance to water vapor declined gradually over the period of precipitation exclusion and were in many cases significantly affected by seedling size, level of precipitation exclusion, and light microenvironment, along with their interactions (Tables S3 & S4, Figs. 6 & 7). Both 100% precipitation exclusion and shaded microsites significantly reduced rates of net photosynthesis and stomatal conductance in both species, though the effect of shade on stomatal conductance was not significant as a main effect in either spruce or fir. Photosynthesis and stomatal conductance were also significantly associated with seedling size; stomatal

conductance in particular showing a strong relationship with seedling size with successively lower rates of stomatal conductance with increasing seedling size.

The rate of decline in photosynthesis ( $A_{max}$ ,  $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and stomatal conductance ( $g_{sw}$ ,  $\text{mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) across the period of precipitation exclusion (15 July–22 September 2019), as well as mean rates of gas exchange over the same period, revealed some significant effects of the combination of light microenvironment and precipitation exclusion treatment, most commonly in emergent seedlings (Figs. 6 & 7). In emergent spruce seedlings, photosynthesis declined most rapidly in seedlings occupying shaded microsites under 100% precipitation reduction. On average, this decline resulted in a net-negative rate of photosynthesis in shaded, 100% precipitation excluded spruce seedlings ( $A_{max} = -1.5 \pm 0.50 \text{ SE}$ ), which was significantly different from all other emergent spruce seedlings (FDR  $q < 0.05$ ). Despite net-negative

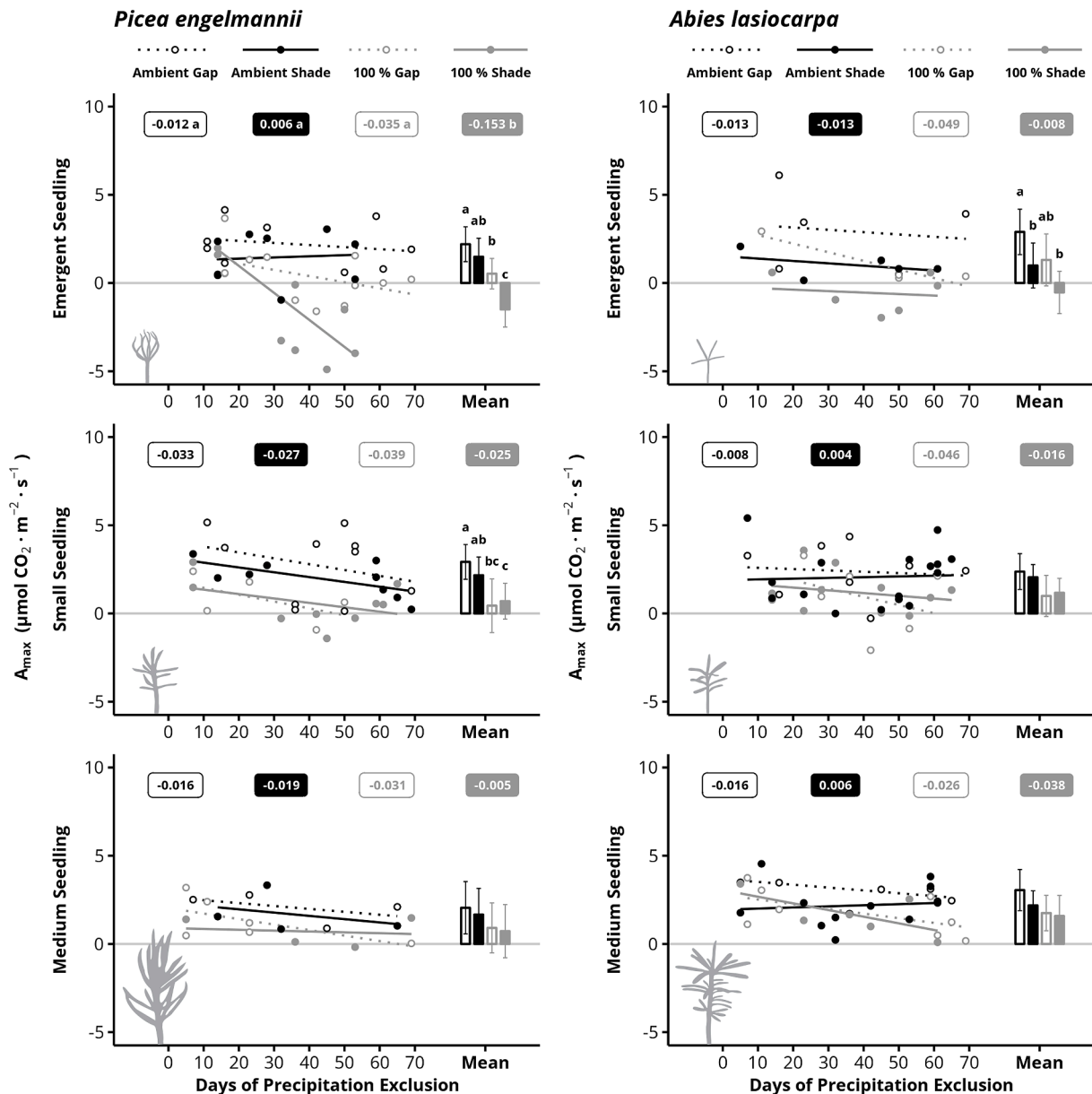


Fig. 6. Observed (points) and linear predictions (lines, Table S3) of maximum net photosynthesis ( $A_{max}$ ,  $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) of Engelmann spruce (*P. engelmannii*, left) and subalpine fir (*A. lasiocarpa*, right) seedlings among seedling sizes (emergent, small, and medium seedling), precipitation reduction treatment (ambient, 100% reduction), and microsite light environment (shade, canopy gap). Inset values specify the slope of the linear prediction. Bars represent the estimated marginal means across the precipitation exclusion period (15 July to 22 September 2019) with error bars indicating the 95% confidence interval. Horizontal gray line indicates net zero photosynthesis. Means and slopes not sharing a common letter are significantly different within each seedling size classification (FDR  $q < 0.05$ ).

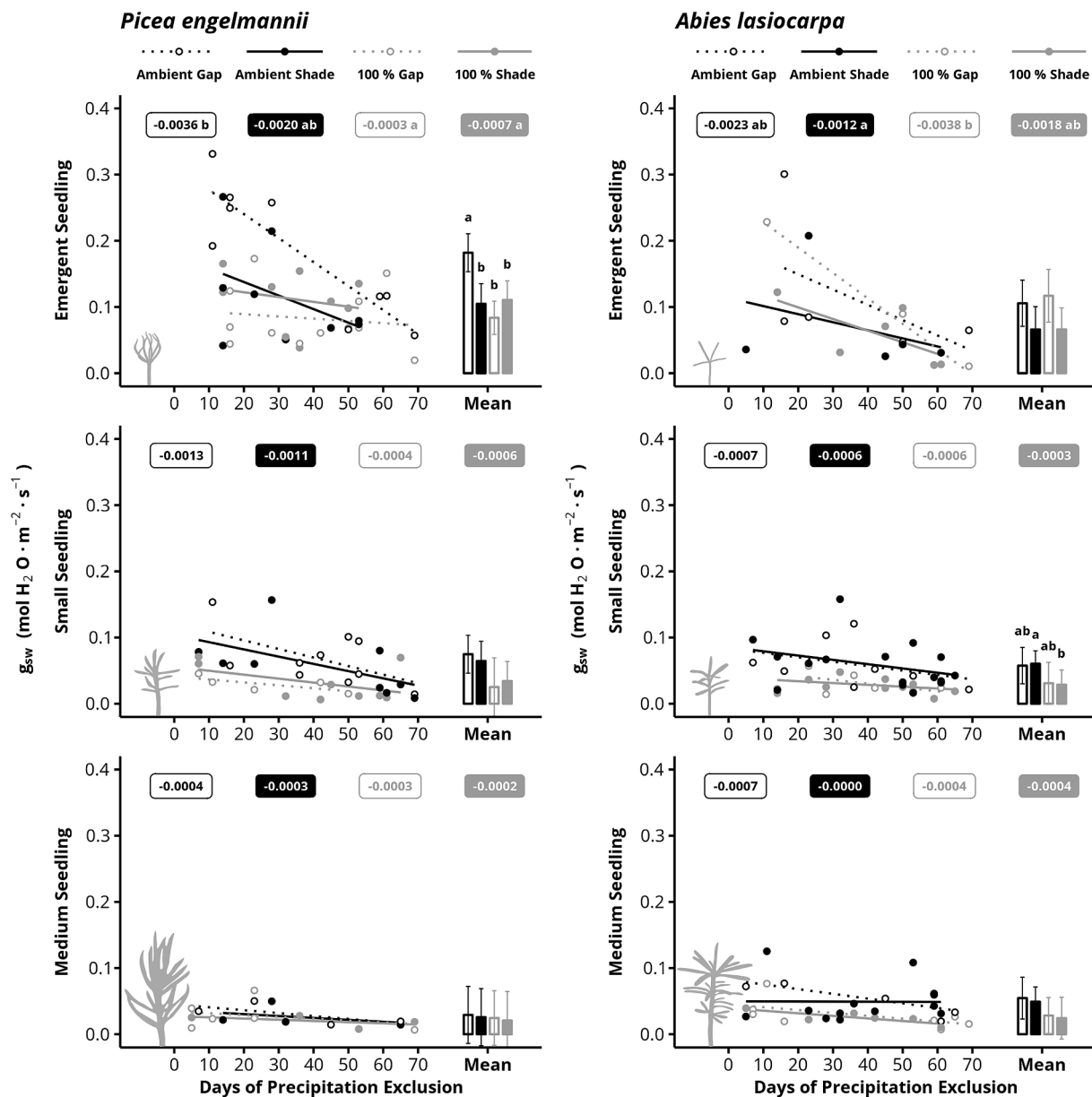


Fig. 7. Observed (points) and linear predictions (lines, Table S4) of maximum stomatal conductance to water vapor ( $g_{sw}$ ,  $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) of Engelmann spruce (*P. engelmannii*, left) and subalpine fir (*A. lasiocarpa*, right) seedlings among seedling sizes (emergent, small, and medium seedling), precipitation reduction treatment (ambient, 100% reduction), and microsite light environment (shade, canopy gap). Inset values specify the slope of the linear prediction. Bars represent the estimated marginal means across the precipitation exclusion period (15 July to 22 September 2019) with error bars indicating the 95% confidence interval. Means and slopes not sharing a common letter are significantly different within each seedling size classification (FDR  $q < 0.05$ ).

carbon assimilation in shaded, precipitation-excluded emergent spruce seedlings, stomatal conductance remained statistically indistinguishable from spruce emergents occupying canopy gaps with excluded precipitation and those receiving ambient precipitation (FDR  $q > 0.05$ ). In small spruce seedlings, photosynthesis was also lower on average under 100% precipitation exclusion ( $A_{\max} = 0.4 \pm 0.76$  SE and  $0.7 \pm 0.51$  SE in canopy gap and shade microsites, respectively), with shaded seedlings under 100% precipitation exclusion showing significantly different rates of photosynthesis (FDR  $q < 0.05$ ). In medium seedlings, though photosynthesis was again lower on average with 100% precipitation reduction, and even more so in the shade ( $A_{\max} = 0.9 \pm 0.71$  SE and  $0.7 \pm 0.76$  SE in canopy gap and shade microsites, respectively), no significant differences among medium spruce seedlings were found (FDR  $q > 0.05$ ).

In emergent fir seedlings, though no significant differences in average rates of photosynthesis were observed, shaded seedlings under

100% precipitation exclusion similarly displayed the lowest (and net-negative) rate of photosynthesis ( $A_{\max} = -0.5 \pm 0.60$  SE). Among these emergent fir seedlings, ambient canopy-gap seedlings displayed the highest rate of photosynthesis ( $A_{\max} = 2.9 \pm 0.66$  SE), which was statistically unique from seedlings occupying shaded microsites under both ambient and 100% precipitation exclusion (FDR  $q < 0.05$ ). Nevertheless, average stomatal conductance in these seedlings was not significantly different from other combinations of precipitation and light microenvironments (FDR  $q > 0.05$ ). Though similar patterns were found in small and medium fir seedlings, these effects were of a smaller magnitude, and none were significant except in small fir seedlings where conductance was significantly lower under 100% precipitation exclusion compared to ambient precipitation in shaded microsites (FDR  $q < 0.05$ ).

## 4. Discussion

### 4.1. Plot throughfall and soil moisture

Precipitation exclusion shelters were effective at creating plot-level precipitation and soil moisture deficits as intended (ca. 50 and 100% reductions in throughfall) and overall created conditions reflective of projected decreases in summer precipitation in areas under ongoing monsoonal weakening and possible outright failure (Cook and Seager, 2013; Pascale et al., 2017). Though shaded microsites received lesser precipitation and proportional exclusion via rainout shelters relative to canopy gap microsites (presumably due to overhead canopy interception; e.g., Gray et al., 2002), soil moisture content relative to ambient conditions was largely similar between shaded and gap microsites under both 50% and 100% precipitation exclusion (<3% and 1% relative difference between shade and gap under 50% and 100% precipitation exclusion, respectively). This convergence in relative soil moisture content despite some differences in throughfall and absolute levels of soil moisture between microsites is likely due to greater surface moisture evaporation from solar radiation in gap plots. These patterns of soil moisture suggest that, while overall water stress may have been somewhat higher in the gap microsites due to slightly lower absolute soil water content, the relative effects of precipitation exclusion on soil moisture were largely uniform. Though we are unable to precisely characterize the physiological degree of moisture stress in the absence of seedling water potential measurements, based on other investigations (e.g., Reinhardt et al., 2015; Kannenberg et al., 2019), the magnitude of reductions in soil moisture content in our study were likely sufficient to induce substantial water stress.

### 4.2. Seedling survival

The effects of precipitation exclusion were evident in the patterns of seedling survival, as notably higher levels of net mortality and elevated risks of mortality (hazard ratio) were observed in seedlings of both species under precipitation exclusion. While mortality rates in spruce and fir emergents can be high under typical precipitation levels, often exceeding 50% in their first year depending on environmental conditions and microsite suitability (Cui and Smith, 1991; Germino et al., 2002; Maher and Germino, 2006), the introduction of moderate (50%) and severe (100%) precipitation exclusion markedly increased mortality compared to ambient rates in emergent seedlings; up to ca. 45% and 67% additional (net) mortality relative to reference survival rates in spruce and fir, respectively. With increasing size, seedlings displayed successively greater survival under precipitation reductions; small and medium seedlings showed  $\geq 50\%$  and  $\geq 75\%$  survival rates, respectively, and notably, there was no mortality observed in either large or well-established seedlings of either species, having presumably developed a greater capacity to withstand substantial moisture deficits for at least one growing season with increasing tree size (Niinemets, 2010). Nevertheless, small and medium sized spruce and fir seedlings can be over a decade in age, and so elevated drought mortality in seedlings of these sizes in just a single growing season is notable since, in the absence of drought, mortality rates are often negligible beyond the third year for these species (Cui and Smith, 1991; Germino et al., 2002; Maher and Germino, 2006). Overall, these results considering growing frequency and intensity of summer droughts makes a systematic recruitment failure of spruce and fir increasingly likely, particularly given the intermittent and highly variable nature of seed crop production and reliance on spring moisture for emergence in these species.

Under ambient conditions, shade did not significantly alter rates of seedling survival of either spruce or fir, despite our expectation that spruce survival would be lower in the shade given its preference for canopy gap colonization (Day, 1964; Knapp and Smith, 1982). Seedling mortality and hazard risk in the shade under the 50% precipitation exclusion, though slightly elevated, was statistically indistinguishable

from seedlings receiving ambient precipitation in both species, suggesting shade has a strong ameliorating effect on moderate drought stress – presumably due to reduced evaporative demand (Abrams and Mostoller, 1995; Chen et al., 1995). In contrast, under 100% precipitation exclusion, net mortality and hazard risk of spruce seedlings were significantly greatest in the shade (though not uniquely higher compared to canopy gaps) even for small and medium seedlings, suggesting the ameliorating effects of shade were outweighed by the negative consequences of low light for the less shade-tolerance spruce (Holmgren et al., 1997). For the highly shade-tolerant fir seedlings, mortality risk was minimized in the shade relative to gap microsites regardless of the level of precipitation, which aligns with historical patterns of microsite colonization in this species (Cui and Smith, 1991; Germino et al., 2002; Maher and Germino, 2006; Andrus et al., 2018a). However, the overall lower abundance of fir seedlings – particularly emergents – at the time of study somewhat limits the generalizability of these results given the higher likelihood of Type-II error associated with smaller sample sizes.

### 4.3. Gas exchange

Measurements of photosynthesis and stomatal conductance also provided evidence for the role of light microenvironment in either ameliorating or exacerbating drought effects in these species. While maximum rates of net photosynthesis and stomatal conductance of both species generally declined over the length of observation in all precipitation exclusion and light environments reflective of typical seasonal physiological patterns (Johnson et al., 2004; Brodersen et al., 2006; Carroll et al., 2019), substantial reductions in carbon gain occurred in emergent spruce and fir seedlings in the 100% precipitation reduction. Notably, net photosynthetic carbon gain became largely negative – and to a striking degree in spruce emergents – after just 4 weeks of precipitation exclusion in shaded microsites. Despite these carbon losses, however, stomatal conductance remained minimally adjusted in emergent seedlings, which likely exacerbated water-loss stress through “leaky” stomata (Hammond and Adams, 2019). Since prolonged negative carbon balances can deplete carbon reserves, leading to impairment of water relations and elevated mortality risk (McDowell, 2011; Sala and Mencuccini, 2014; Maguire and Kobe, 2015; Sapes et al., 2021), these reductions in net photosynthesis in spruce may have contributed to the lack of survival benefit in the shade versus gaps under 100% precipitation reduction. Conversely, prior acclimation to shaded conditions (e.g., down-regulation of metabolic activity, greater allocation of photosynthate to storage; Kobe, 1997) may confer greater tolerance to the combined stresses of drought and shade in shade-tolerant fir as suggested by greater survival and less substantial photosynthetic changes in fir in the shade under precipitation exclusion.

Though precipitation exclusion diminished photosynthesis across all size classes of both species, comparatively higher stomatal conductance in emergent seedlings suggests a systematic lack of physiological regulation under declining moisture availability consistent with a strategy prioritizing carbon gain during emergence and establishment at the expense of stress tolerance and resistance (Augustine and Reinhardt, 2019). Indeed, stomatal constraints on photosynthesis increase with seedling age, reducing transpiration losses and conferring greater water-use efficiency as seedlings grow larger (Cui and Smith, 1991; Goke and Martin, 2022). Though drought was largely non-lethal in larger seedlings, reductions to photosynthesis with increasing water deficit – particularly in the shade – portend that reduced carbon reserves may predispose these individuals to lagged mortality if drought were to recur or persist beyond one growing season (Bigler et al., 2007; Sala and Mencuccini, 2014). Indeed, individual trees displaying poor tolerance to the effects of prior droughts are often those most prone to die under successive periods of drought (Berdanier and Clark, 2016; Caillieret et al., 2017; DeSoto et al., 2020).

Much adaptive management effort in supporting forest resiliency to

drought has centered on the practice of overstory thinning to minimize competition for water resources among adults. Indeed, lower stand densities have been shown to avert the growth and mortality consequences of drought and aridity in many systems (D'Amato et al., 2013; Bottero et al., 2017; Buechling et al., 2017; Zamora-Pereira et al., 2021). However, the impact these practices have on understory dynamics and microsite conditions is unclear, particularly features that feedback on seedling physiology. Our results highlight the strong influence microsite characteristics have on seedling performance and how species-specific microsite controls are altered with drought. Given the critical importance of understory recruitment in determining forest trajectory (Kroiss and HilleRisLambers, 2015; Clark et al., 2016; Carroll et al., 2017; Brodersen et al., 2019; Carroll et al., 2021), evaluating the influence of overstory treatments on understory microenvironments (e.g., Lieffers et al., 1999) in conjunction with a mechanistic understanding of seedling abiotic tolerances and microsite preferences will contribute to our capacity to evaluate and manage forest resilience in current and future climates.

## 5. Conclusions

Forest tree seedling establishment and persistence in a changing climate will have fundamental consequences for future forest structure and function, particularly for species facing increasing seasonal dryness whose regeneration is closely tied to moisture conditions. In this study we demonstrated the potential for a single growing-season drought to alter microsite-facilitated regeneration dynamics in Engelmann spruce and subalpine fir – the two co-dominant species of the subalpine forests of the southern Rocky Mountains. A moderate reduction in precipitation (50%) reduced survival in young seedlings of both species, though shaded microsites ameliorated water stress leading to higher survival. Under complete precipitation exclusion (100%), however, spruce mortality increased markedly in both gaps and shade, as reduced precipitation outweighed the ameliorating effects of shade. Mortality in both species was associated with declining photosynthetic carbon uptake, particularly for emergent seedlings which displayed net negative carbon assimilation in the shade along with poor stomatal regulation despite growing water deficits. Though larger seedlings showed robust tolerance and survival against a complete growing season drought, longer drought studies are needed to characterize post-drought recovery to determine the overall resiliency of seedling regeneration to drought. Effort to support forest drought resiliency has largely focused on management of adult trees – often by reducing overstory density to reduce competition for resources – which inescapably impacts the understory light environment. As these results demonstrate, microsite light environment strongly influenced drought-induced mortality patterns in forest tree seedlings, reflective of contrasting canopy gap colonization patterns and shade tolerances. Therefore, adaptive management planning involving overstory manipulations should consider potential impacts on microsite characteristics to ensure sufficient light microsite quality and availability for seedlings colonization in accordance with species-specific colonization preferences and physiological tolerances.

## CRedit authorship contribution statement

**Alex Goke:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Funding acquisition, Data curation, Conceptualization. **Patrick H. Martin:** Resources, Supervision, Funding acquisition, Methodology, Conceptualization, Visualization, Project administration.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2023.121295>.

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