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## **Subalpine Forest Tree Seedling Response to Drought**

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Subalpine Forest Tree Seedling Response to Drought

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A Thesis

Presented to

the Faculty of the College of Natural Sciences and Mathematics

University of Denver

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Alex Goke

December 2020

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Degree Date: December 2020

### **Abstract**

Knowledge of tree species' ability to tolerate drought is necessary to anticipate future forest dynamics with climate change, especially at the seedling stage given their role in shaping forest structure. We used precipitation reduction shelters to mimic drought for subalpine conifer seedlings (*A. lasiocarpa* and *P. engelmannii*) in the Rocky Mountains and compared survivorship and morphological and physiological responses to assess relative degrees of drought tolerance. We detected no significant investment in morphological tolerance traits (e.g. root biomass, leaf:stem area ratio) but substantial reductions in net photosynthesis. While shading partially ameliorated drought effects when precipitation reduction was moderate, complete exclusion caused declines in survivorship in both sun and shade tied to poor carbon balances. We identified a lack of stomatal control as a driver of physiological decline in seedlings suggesting a prioritization of traits that, while useful for early establishment, may portend substantial vulnerability of seedling populations to future drought.

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## **Chapter 1: No Adjustment to Short-Term Drought in Subalpine Forest Tree Seedling**

### **Introduction**

Droughts are expected to increase in both frequency and intensity with ongoing climate change. Droughts linked to climate change have already caused widespread tree mortality across large areas in many forested regions, with deeply adverse impacts on landscape structure and function (Van Mantgem et al. 2009, Allen et al. 2010, Anderegg et al. 2013, Clark et al. 2016). These drought episodes are of particular concern for high elevation forests in mountainous regions where climate change is accelerated (Beniston et al. 1997, IPCC 2014, Pepin et al. 2015, Dobrowski & Parks 2016) and resistance of forests to environmental shifts is strongly affected by competitive interactions (Buechling et al. 2017), divergent species responses (Carroll et al. 2017), and low phenotypic plasticity due to strong local adaptation to narrow bioclimatic envelopes (Valladares et al. 2007, Vitasse et al. 2013, Valladares et al. 2014, Gugger et al. 2015). Recent progress in characterizing the underlying causes of drought-induced tree mortality has highlighted the value of plasticity in mitigating drought stress and reducing likelihood of mortality, especially in traits associated with water and carbon regulation strategies (Richter et al. 2012, Choat et al. 2018). However, there has been little study in how drought affects tree seedlings, despite the critical role seedlings play in forest dynamics, range shifts, and the overall resilience of forests to ongoing climate change (Bell et al. 2014, Martínez-Vilalt & Lloret 2016, Brodersen et al. 2019, Copenhaver-Parry et al. 2020).

Relative to saplings and adults which display strong synchronization to their environment and increased stress tolerance, conifer tree seedlings are thought to be considerably more vulnerable to water stress given their shallow rooting depths and narrow carbon budgets (Grossnickle 2005, Bansal & Germino 2008, Niinemets 2010, Bansal & Germino 2010). These



limitations are most pronounced in newly emerged germinants where trade-offs between investments in leaf development (for photosynthesis and growth) and structural stress-mitigation traits are often observed in conjunction with high mortality rates (Green 2005, Reinhardt et al. 2015, Lazarus et al. 2018, Augustine & Reinhardt, 2019). Instead of satisfying demands for photosynthetic development, seedlings could invest energy in water-stress mitigation strategies such as increased carbon allocation to belowground structures to support water uptake, adjustment of the leaf:sapwood area ratio to promote greater whole-plant water availability, or tighter control of stomatal regulation towards increased photosynthetic water-use efficiency (Choat et al. 2018). However, many studies have demonstrated a striking lack of morphological plasticity in small conifer seedlings – particularly first-year germinants – in response to water deficits (e.g. Aranda et al. 2010, Schall et al. 2012, Augustine & Reinhardt 2019). Indeed, conifer seedlings under water stress may respond with even greater investments in leaf development at the expense of root mass (e.g. Kiorapostolou et al. 2018), suggesting allocation hierarchies prioritizing carbon-gain over drought-mitigation traits are “hardwired” in these seedlings – potentially at the risk of less favorable water status (e.g. greater whole-plant transpiration resulting from increased leaf area). While trees are generally recognized as having increased stress tolerance and morphological acclimation capacities as they grow larger (Niinemets 2010), how and when small seedlings begin to display more conservative physiological controls and alter carbon allocation patterns to develop water-stress mitigation traits more reflective of conspecific adults is largely unknown.

In the present study, we investigated allometry and gas exchange characteristics of naturally-regenerated established seedlings of Engelmann spruce (*Picea engelmannii* Parry ex Engelmann) and subalpine fir (*Abies lasiocarpa* (Hook) Nutt.) over two growing seasons in response to late-season simulated drought in the Front Range of Colorado. Spruce and fir are the dominant tree species in subalpine forests in the Rocky Mountains and have experienced dramatic increases in tree mortality in recent decades suggested as a product of increasing moisture stress (Bigler et al. 2007, Smith et al. 2015). Declining snowpack and earlier snowmelt

has pushed water deficits further into the growing season in across western mountains, simultaneous to declining summer precipitation in the southern Rocky Mountains due to systematic weakening of the North American Monsoon (Mote et al. 2005, Cook & Seager 2013, Hu et al. 2010, Pascale et al. 2017). These changes in the region's moisture regime could impact spruce and fir differently given their contrasting life history strategies. Relative to spruce, fir is characterized by greater seedling rooting depth (Day 1964), slower growth rate (Day 1964, Antos et al. 2000), higher rates of net photosynthesis at lower levels of light saturation (Knapp & Smith 1982), and earlier stomatal closure at the onset of environmental stress, supporting more favorable water use efficiency in stressful environments (Knapp & Smith 1981, Brodersen et al. 2006). Collectively, these factors are hypothesized to lead to the high abundance of fir often observed in subalpine forest understories, while more rapid growth rates and greater longevity in spruce facilitate its overstory co-dominance with seedlings that capitalize on higher light levels when canopy gaps occur (Shea 1985, Veblen 1986, Andrus et al. 2018).

The goal of this study was to evaluate drought response strategies of established high-elevation conifer seedlings and to compare patterns in less-studied small seedlings to adult-sized individuals of these species while also considering implications for forest dynamics. Based on past work in these species on the mechanisms of establishment and adult tree function across natural gradients, species-specific differences in seedling responses to increasing drought frequency and intensity could disrupt the factors that balance the successional dynamics between these co-dominant species, leading to substantial changes in subalpine forest structure, composition and function as ongoing climate change worsens (Conlisk et al. 2017, Lett & Dorrepaal 2018, Brodersen et al. 2019). Though prior studies did not directly evaluate the in situ effects of drought on seedlings of Engelmann spruce and subalpine fir, we predict based on this work that (1) due to lower water demands associated with slower growth rates, fir will display superior drought tolerance than spruce as indicated by greater rates of photosynthesis and water use efficiency under simulated drought, (2) that droughted seedlings will display morphological plasticity (e.g. allocate more growth to roots to alleviate water stress or reduce leaf area to limit

transpiration losses), and that spruce – due to greater water stress from its inherently higher growth rate and delayed stomatal closure in response to stress – will exhibit a greater degree of such plasticity relative to fir, and (3) smaller individuals of both species generally exhibit weaker morphological plasticity and lower photosynthetic water use efficiencies than larger individuals due to narrower carbon budgets and less-regulated stomatal behavior. Taken together, these outcomes may suggest further regeneration limitations imposed by drought on spruce which could disrupt sensitive successional dynamics, potentially favoring complete canopy dominance by fir. Alternatively, since growth rates and available carbon in seedlings of both species are low, neither species may significantly shift its morphology and physiology in response to a late-season drought, leading to drastic reductions in photosynthesis and transpiration which would suggest likely seedling mortality and regeneration limitations in both species under prolonged or recurring drought.

## **Methods**

### *Site Description*

This study was conducted at the University of Denver High Altitude Laboratory near Mt. Evans, Colorado, USA (39.66° N, 105.59° W). At 3,230 m in elevation, the northeastern-facing site is characterized by a dense canopy of mature subalpine fir and Engelmann spruce with a few dispersed individuals of lodgepole pine (*Pinus contorta* Dougl.) and limber pine (*Pinus flexilis* James). The patchy understory is composed primarily of ericaceous species (*Vaccinium* sp. and *Orthillia secunda* (L.) House) along with seedlings and saplings of fir and spruce. Soils consist of mainly Leighcan family till substratum and Tonahutut-Ohman complex derived from igneous and metamorphic rock (NRCS 2020). Mean annual precipitation is 78 cm, most of which falls as snow but with notable rain input from summer monsoons occurring July to September ( $\approx$  30 % of annual precipitation; NRCS SNOTEL 2020).

### *Experimental Design*

Forty 1 x 1 m plots containing naturally regenerated Englemann spruce and subalpine fir seedlings were established throughout the understory of the study site. Plots were selected for

their approximate uniformity in litter composition, consistent microtopography ( $< 15^\circ$  slope), and herbaceous cover ( $< 5\%$  area). Light availability within each plot was quantified using hemispherical camera lens placed at seedling height (COOLPIX 900, Nikon, Tokyo, Japan) and expressed as % of potential direct and diffuse transmitted light based on latitude and topography using gap light analyzing software (GLA v. 2.0, Cary Institute for Ecosystem Studies, Milbrook, NY, USA, Frazer et al. 1999). All plots were located  $> 10$  m from the nearest neighboring plot.

Seedlings within each plot were marked and assessed for height and stem diameter so that a similar number and sizes of seedlings were present in all 40 plots. All seedlings were less than 20 cm in height and between 0.5 and 5 mm in stem diameter, representing a class of post-establishment seedlings residing in the understory prior to canopy in-growth. No more than 20 individual seedlings were contained in any plot to control for possible competitive effects. Half of the plots received a precipitation exclusion treatment (“drought”) and half were used as paired control plots receiving ambient levels precipitation (“ambient”) placed immediately to the side or upslope of the treatment plots while maintaining a 0.5 m buffer to prevent additional precipitation accumulation in control plots from exclusion shelter runoff.

To impose drought treatments via precipitation exclusion, 20 passive rain deflection shelters were constructed above each drought plot. Using validated designs (Yahdjian and Sala 2002, Drought-Net 2018), the shelters were constructed to cover the 1 m<sup>2</sup> plots (1 x 1 m) with roofs angled towards the downhill side of the shelters to drain water away from the target and control plots. The shelters were constructed to reduce precipitation by 100 %. Roofs were made of transparent polycarbonate roofing (Suntuf, Palram Americas, Kutztown, PA, USA) mounted one meter above the soil surface on a frame constructed of PVC pipe. Shelters were in place above the plots from 15 July to 15 September in 2018 and 2019 to mimic summer monsoon failure, thereby creating a summer drought treatment for two consecutive growing seasons. Prior studies have examined the effects of such shelters on the microenvironment underneath and found the effects on light levels and air and soil temperatures were marginal (Fay et al. 2000, Yahdjian & Sala 2002, Heisler-White et al. 2008, Cherwin & Knapp 2012). Soil volumetric water

content (VWC, %) was monitored approximately weekly in treatment and control plots with a handheld electrical conductivity soil moisture probe inserted 5 cm vertically into the soil surface at three random locations within the droughted plots and near the adjacent ambient specimens (HS2, Campbell Scientific, Logan, UT, USA). No seedlings (n = 82 fir and 62 spruce seedlings in drought treatments, 89 fir and 63 spruce seedlings in ambient conditions) died during the study.

#### *Morphological and Physiological Measurements*

At the conclusion of the second growing season of precipitation exclusion (September 2019), a random subsample (n = 67, 14 – 18 individuals per species per treatment) of individuals of both species and in each precipitation treatment were assessed for gas exchange parameters. Net photosynthesis ( $A$ ,  $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), transpiration ( $E$ ,  $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), instantaneous water use efficiency ( $A/E$ ), and stomatal conductance to water vapor ( $g_{\text{sw}}$ ,  $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) was measured using a portable infrared gas analyzer (LI-6800, LI-COR Biosciences, Lincoln, NE, USA). Sun-oriented sprigs of larger seedlings were inserted laterally in the chamber, while the entire seedling of smaller individuals was inserted vertically through the bottom of the chamber, maintaining its original sunwards orientation. Chamber conditions were set to a saturating light intensity of  $1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  photosynthetically active radiation and 410 ppm  $\text{CO}_2$ , representative of levels reported nearby at the Niwot Ridge Global Monitoring Laboratory (CO, USA; NOAA ESRL 2020). Temperature inside the chamber was matched to ambient conditions every 10-15 minutes. All measurements were conducted 15 and 16 September 2019 under clear skies between 9:00 am and 1:00 pm local time. Measurements are reported based on silhouette leaf area of the total chamber leaf sample.

Tree seedling allometry was assessed by excavating seedlings, measuring stem diameter at the root crown to the nearest 0.01 mm to approximate seedling size, and segregating each individual into component leaves, shoots, and roots. Total leaf area was determined via silhouette method using a flatbed scanner (Perfection V850, Epson, Nagano, Japan). Images captured at 600 DPI were thresholded and quantified for projected leaf area ( $\text{cm}^2$ ) using FIJI v. 1.52 (Schindelin et al., 2012). Leaf area for the purpose of gas exchange parameters were

assessed in this same fashion. Leaf:stem area ratio (LSAR, cm<sup>2</sup>) as an approximation of leaf:sapwood area ratio was obtained by dividing leaf area into stem cross-sectional area calculated from stem diameter. Leaves, shoots, and roots were then dried at 70 °C for 72 hours and weighed. From these values we calculated root, stem, and leaf mass fraction (RMF, SMF, LMF, respectively, g · g<sup>-1</sup>) as a proportion of total biomass (TBM, g).

### *Statistical Analyses*

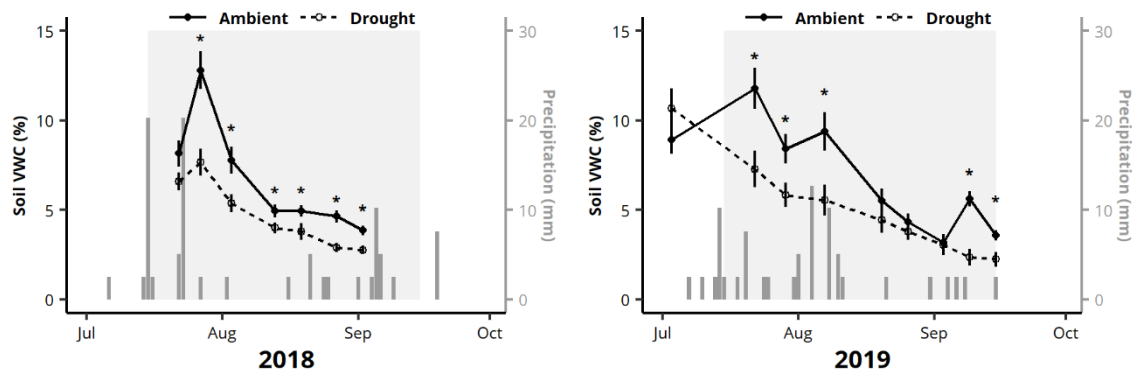
Allometry and gas exchange characteristics were modelled as a function of diameter as a proxy for seedling size, species, and treatment using multiple linear regressions. For the purpose of modelling, root, stem, and leaf mass fractions (RMF, STM, and LMF, respectively) were logit-transformed while total biomass (TBM) and leaf-stem area ratios were log-transformed to improve normality of regression residuals. Plot-level transmitted light was at first also included to account for any light-availability effects on growth and physiology, though no main or interactive effects of light were significant and the term was therefore removed from all models. Predictors were evaluated on the basis of their unstandardized regression coefficients and significance in the model with an alpha critical value of  $\alpha = 0.05$ . Estimated marginal means were then calculated to directly compare the effect of treatment on mean allometry and gas exchange variables while controlling for stem diameter. Tukey post-hoc testing was implemented to evaluate significant differences in these means. Means were back-transformed to their original scale for the purpose of visual comparison. All analyses were conducted in R v. 3.6 (R Core Team, 2020).

## **Results**

### *Soil Moisture*

Relative to ambient conditions, precipitation exclusion shelters were largely successful at reducing soil water content in situ (Fig. 1.1). While no pre-treatment measurements were taken in 2018, average soil water content did not significantly differ between ambient and droughted conditions prior to implementation of the treatment in 2019 (Fig. 1.1). Significant soil dry-down in the drought treatment relative to ambient conditions was then evident within 2 weeks. Average soil moisture remained significantly lower in the drought treatment for the remainder of the

treatment period with the exception of late August and early September 2019 where low levels of precipitation resulted in soil drying within the ambient condition. Slight increases in volumetric water content were observed immediately following larger precipitation events in the drought treatments (e.g. late July 2018), indicating some rain may have blown in laterally during windy precipitation events while subsurface water may have also flowed into the plots from upslope drainage after saturating rains (Fig. 1.1). However, droughted conditions remained on average 2.01 % ( $\pm 0.55$  SE) lower in soil VWC for the duration of precipitation exclusion treatment in 2018, and 2.17 % ( $\pm 0.53$  SE) lower in 2019, accounting for an estimated cumulative reduction of 125 % and 135 % soil VWC deficit-days in 2018 and 2019, respectively.



**Figure 1.1.** Mean soil volumetric water content (VWC, %) between ambient and drought treatments measured periodically before and during the treatments in 2018 (left) and 2019 (right, note: no pre-treatment measurements were made in 2018). Daily precipitation (mm) is also presented. Gray area indicates dates for which the drought treatment was active (15 July to 15 September). Error bars indicate standard error of the mean. Asterisks indicate significant difference between mean VWC for the given date (Tukey HSD,  $p < 0.05$ ).

### *Morphological Plasticity*

Stem diameter was strongly predictive of all measures tree allometry. Notably, root mass fraction was negatively correlated with diameter in both species whereby an increase in stem diameter lead to greater proportion of biomass allocation to above-ground stem and leaf tissues at the expense of root mass (Table 1.1). Species was also highly predictive of root, stem, and leaf mass fractions – with spruce consistently having lower proportional root and leaf masses and higher leaf mass fractions than fir – though not predictive of total biomass, or leaf-stem area ratio.

The effect of treatment was not significantly predictive of any measure of tree allometry or total biomass, nor was the interactions of diameter by treatment, species by treatment, or diameter by species by treatment for most measures. Exceptions included the significant association of the interaction of diameter by species for leaf mass fraction, and diameter by treatment and species by treatment by diameter for stem mass fraction.



**Table 1.1.** Multiple regression estimates (B, unstandardized coefficients, SE, standard errors, df, degrees of freedom, R<sup>2</sup>, coefficient of determination) of diameter, species, and drought treatment predictors on root mass fraction (RMF, g · g<sup>-1</sup>), stem mass fraction (SMF, g · g<sup>-1</sup>), leaf mass fraction (LMF, g · g<sup>-1</sup>), total biomass (TBM, g), and leaf-stem area ratio (LSAR, cm<sup>2</sup>) of Engelmann spruce (*P. engelmannii*) and subalpine fir (*A. lasiocarpa*) tree seedlings.

Predictor	RMF		SMF		LMF		TBM		LSAR	
	B	SE	B	SE	B	SE	B	SE	B	SE
<b>Diameter</b>	-0.28***	0.04	0.15***	0.04	0.14**	.04	1.38***	0.07	0.37***	0.07
<b>Species</b>	0.44**	0.14	0.13	0.13	0.40**	.14	-0.01	0.22	0.24	0.22
<b>Treatment</b>	-0.01	0.13	-0.21	0.12	0.21	.13	-0.05	0.20	0.16	0.20
<b>Diameter x Species</b>	0.08	0.07	0.04	0.06	-0.15*	.07	-0.03	0.10	-0.12	0.10
<b>Diameter x Treatment</b>	-0.03	0.06	0.11*	0.05	-0.08	.05	-0.03	0.09	-0.09	0.09
<b>Species x Treatment</b>	-0.18	0.19	0.34	0.17	-0.21	.19	-0.34	0.29	-0.39	0.30
<b>Diameter x Species x Treatment</b>	0.10	0.09	-0.20*	0.08	0.12	.09	0.14	0.14	0.21	0.14
<b>df</b>	7, 292		7, 292		7, 292		7, 292		7, 292	
<b>R<sup>2</sup></b>	0.34***		0.24***		0.04**		0.85***		0.21***	

\* = p < 0.05, \*\* = p < 0.01, \*\*\* = p < 0.001

When controlling for stem diameter, estimated marginal means of root mass fraction and total biomass were lower in the drought treatment than the ambient treatment for both species, while leaf mass fraction averaged higher in the drought treatment for both species (Table 1.2). Stem mass fraction and leaf:stem area ratio remained similar between treatments in both fir and spruce. However, treatment effects were not significant for any measure of allometry for either spruce or fir.

**Table 1.2.** Estimated marginal means (mean) and standard errors (SE) of the effect of precipitation exclusion (drought vs. ambient) on root mass fraction (RMF,  $\text{g} \cdot \text{g}^{-1}$ ), stem mass fraction (SMF,  $\text{g} \cdot \text{g}^{-1}$ ), leaf mass fraction (LMF,  $\text{g} \cdot \text{g}^{-1}$ ), total biomass (TBM, g), and leaf-stem area ratio (LSAR,  $\text{cm}^2$ ) of Engelmann spruce (*P. engelmannii*) and subalpine fir (*A. lasiocarpa*) tree seedlings. No significant differences between treatment means were found for either species ( $p > 0.05$ , Tukey HSD).

Characteristic	<i>P. engelmannii</i>				<i>A. lasiocarpa</i>			
	Ambient		Drought		Ambient		Drought	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>RMF</b>	0.26	0.01	0.24	0.01	0.31	0.01	0.29	0.01
<b>SMF</b>	0.30	0.01	0.29	0.01	0.26	0.01	0.26	0.01
<b>LMF</b>	0.43	0.01	0.45	0.01	0.41	0.01	0.43	0.01
<b>TBM</b>	0.17	0.01	0.14	0.01	0.23	0.01	0.20	0.01
<b>LSAR</b>	339	22.3	337	22.4	350	22.6	344	22.6

### *Gas Exchange Plasticity*

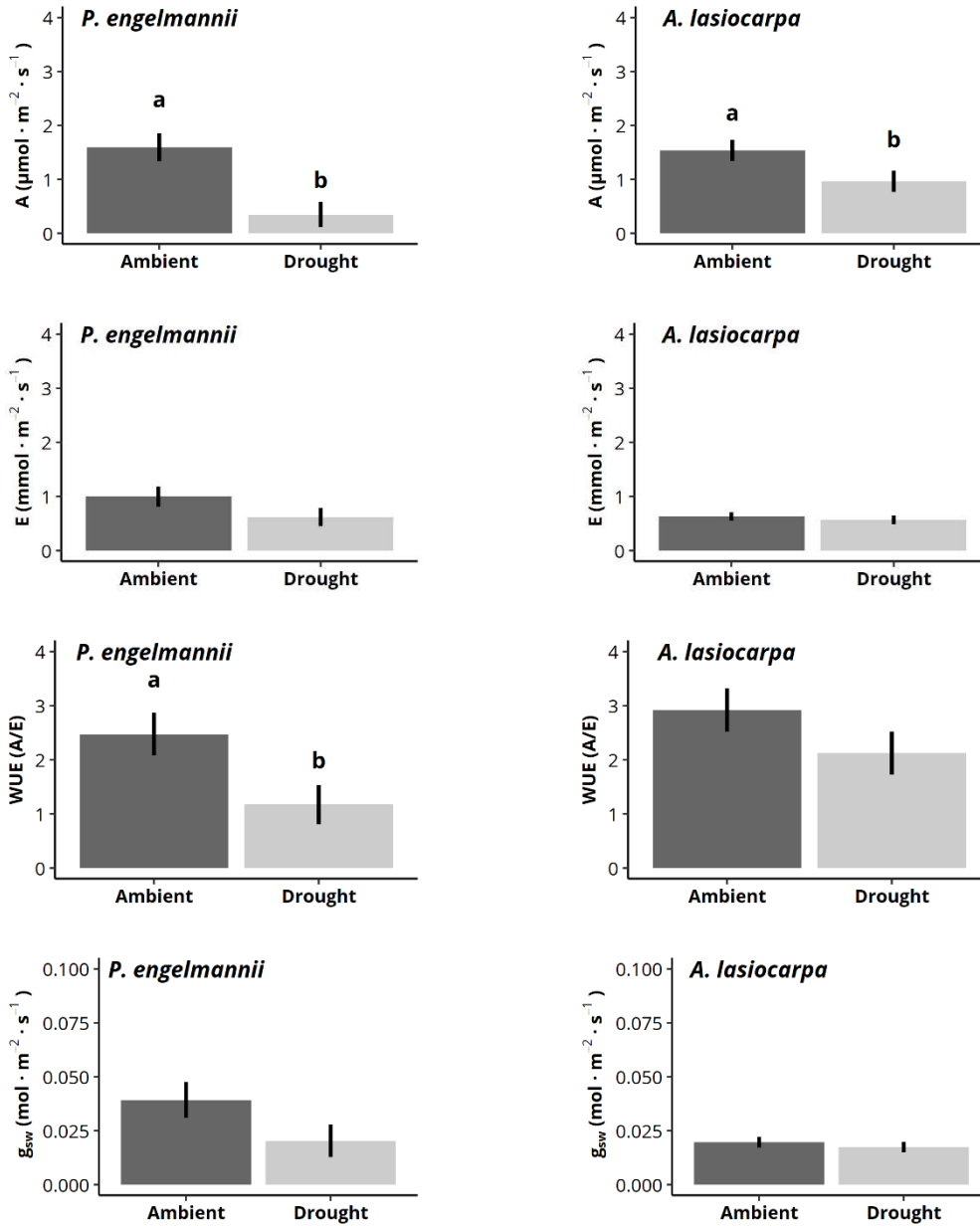
Among gas exchange parameters, only water use efficiency was significantly and positively associated with stem diameter (Table 1.3). Stomatal conductance to water vapor was significantly and positively associated with species, indicating greater overall conductance in spruce. Net photosynthesis appeared negatively associated with treatment, though this effect was not significant. No gas exchange parameter was significantly associated with treatment, nor the interaction of diameter by species, diameter by treatment, species by treatment, or diameter by species by treatment.

**Table 1.3.** Multiple regression estimates (B, unstandardized coefficients, SE, standard errors, df, degrees of freedom, R<sup>2</sup>, coefficient of determination) of diameter, species, and drought treatment predictors on gas exchange parameters of net photosynthesis (A,  $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), transpiration (E,  $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), instantaneous water use efficiency (A/E), and stomatal conductance to water vapor ( $g_{\text{sw}}$ ,  $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) of Engelmann spruce (*P. engelmannii*) and subalpine fir (*A. lasiocarpa*) tree seedlings.

Predictor	A		E		WUE		$g_{\text{sw}}$	
	B	SE	B	SE	B	SE	B	SE
Diameter	0.14	0.20	-0.15	0.11	0.74*	0.35	0.00	0.00
Species	0.18	0.69	0.76	0.40	-0.76	1.24	0.04*	0.02
Treatment	-0.30	0.66	0.00	0.38	0.10	1.18	0.00	0.02
Diameter x Species	-0.04	0.32	0.24	0.8	0.30	0.57	-0.01	0.01
Diameter x Treatment	-0.14	0.78	-0.03	0.16	-0.43	0.50	0.00	0.01
Species x Treatment	-1.66	0.95	-0.77	0.56	-0.84	1.72	-0.04	0.02
Diameter x Species x Treatment	0.53	0.44	0.25	0.26	0.11	0.79	0.01	0.01
df	7, 59		7, 59		7, 59		7, 59	
R <sup>2</sup>	0.22**		0.15*		0.21**		0.14*	

\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$

Adjusted for stem diameter and evaluated individually for each species, estimated marginal means of numerous gas exchange properties were found to be substantially affected by the drought treatment (Fig. 1.2). In both fir and spruce, net photosynthesis was significantly lower for droughted individuals relative to those in ambient conditions, with a greater loss of net carbon gain exhibited by spruce ( $1.249 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  [ $\approx 78 \%$ ] mean reduction in spruce vs.  $0.576 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  [ $\approx 37 \%$ ] mean reduction in fir,  $p < 0.05$ , Tukey HSD). Transpiration was reduced slightly but not significantly for either species. Instantaneous water use efficiency was significantly lower in spruce in response to the drought treatment, and while a loss of efficiency was also found in fir, this effect was not significant. Similarly, stomatal conductance to water vapor was lower, though insignificantly so, for droughted vs. ambient-grown spruce more so than fir.



**Figure 1.2.** Estimated marginal means adjusted for stem diameter of net photosynthesis (A,  $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), transpiration (E,  $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), instantaneous water use efficiency (A/E), and stomatal conductance to water vapor ( $g_{sw}$ ,  $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) for Engelmann spruce (*P. engelmannii*) and subalpine fir (*A. lasiocarpa*) tree seedlings subjected to ambient or drought conditions. Error bars indicate standard error of the mean. Different letters indicate significant difference between means (Tukey HSD,  $p < 0.05$ ).

## Discussion

Relative to ambient conditions, precipitation exclusion shelters were successful at substantially reducing soil water in situ, particularly early in the treatment period when the droughted treatments averaged  $\approx 5 - 8\%$  VWC and the ambient condition averaged  $\approx 8 - 12\%$  VWC (Fig. 1.1). In other tree seedlings, soil moisture below  $10\%$  VWC is often sufficient in meaningfully lowering stem water potentials, indicating our treatments were likely to induce considerable drought stress (Reinhardt et al. 2015, Kannenberg et al. 2019). Indeed, Lazarus et al. (2017) documented meaningful growth declines in conifer seedlings associated with just a mean  $1\%$  reduction in soil VWC imposed from a heating treatment across a single growing season, indicating that even minor reductions to water availability can have considerable consequences in tree seedlings. The seedlings in the ambient condition also likely experienced some degree of water stress in 2018 (15 July – 15 September) when the study region experienced a moderate drought (NDGM 2020) – this likely explains the near-convergence of soil water levels towards the end of the treatment period that season, though water levels remained significantly higher in the ambient condition for most of the 2018 measurements indicating water stress was likely more severe in the drought treatment for the entire duration of study.

Measures of allometry – biomass fraction of roots, stem, and leaves, total biomass, and leaf:stem area ratio – were largely unaffected by two years of summer drought in both spruce and fir seedlings (Tables 1.1 & 1.2). This observation contrasts with our expectation that seedlings would allocate more resources to root development to alleviate water stress or reduce leaf area to limit transpiration losses, and that these adjustments would be more apparent in spruce due to its relatively greater capacity for annual growth (Day 1964, Antos et al 2000). However, we did find a significant negative correlation between stem diameter and proportional root mass, and a significant positive association to proportional stem and leaf biomass and leaf:stem area ratio, demonstrating that seedlings of both species regardless of drought treatment allocate an increasing proportion of resources to leaf development as they grow larger. Though the individuals in our study were already well-established, this behavior could be interpreted as



characteristic of first year germinant seedlings that display little morphological plasticity (e.g. Augustine & Reinhardt 2019) or a 'hardwired' prioritization of leaf area gain over other structural traits, despite accumulating water stress (e.g. Kiorapostolou et al. 2018). In addition, we detected small, non-significant decreases in root mass and increases in leaf mass relative to total biomass in both species in response to drought, trends which could become important over longer periods if, as predicted, summer droughts become more frequent in the region. While mature trees are known to invest more heavily in support structures such as roots and branches as they age (Niinemets 2010), our finding of greater leaf investment across an array of small seedling sizes may reflect a life-history strategy which emphasizes carbon capture and growth rates over survivorship traits to increase the likelihood of the seedling successfully growing out of understory (Andrus et al. 2018). The short growing seasons in high elevation ecosystems like subalpine forests of the Rocky Mountain region can result in a strong selection of traits prioritizing carbon gain (Valladares et al. 2007, Vitasse et al. 2013, Valladares et al. 2014, Gugger et al. 2015), a pattern which could explain the continued investment in leaf development across seedling sizes as observed in our study despite significant reductions in moisture.

Given their extremely slow growth rates associated with these short growing seasons (Antos et al. 2000), it is also possible that significant morphological adjustments could occur in these species at timescales beyond the two years of our study and are likely trait-specific. For instance, McBranch et al. (2018) found no shift in leaf:sapwood area ratios in piñon pine (*Pinus edulis* Engelm.) and one-seed juniper (*Juniperus monosperma* (Engelm.) Sarg.) after 5 years of precipitation reduction and warming, but reductions in leaf and stem growth were found the year following implementation of the treatment in the same population (Adams et al. 2015). Further, size-related shifts in patterns of morphological acclimation in response to water deficit may occur after seedlings break from understory suppression and as saplings or small trees become subjected to changing microclimatic and resource conditions (Niinemets 2010).

Though we found no significant morphological adjustments in response to summer drought, we observed species-specific and size-specific physiological plasticity between

droughted and ambient-grown seedlings. In ambient conditions, spruce and fir displayed similar levels of photosynthesis, but under drought, spruce experienced a dramatic reduction in net photosynthesis (78 % reduction) compared to fir (37 % reduction, Fig. 1.2), supporting our hypothesis that fir maintains comparatively high levels of physiological functioning under water stress (Knapp & Smith 1981, Knapp & Smith 1982, Brodersen et al. 2006). In comparison, Brodersen et al. (2006) noted a  $\approx$  50 % reduction in net photosynthesis in an adult population of both Engelmann spruce and subalpine fir during a summer drought relative to saplings sampled in the same region in prior wet years (Carter & Smith 1988), though differences in life stage and other environmental conditions between the studies make it uncertain if the reduction in photosynthesis was due exclusively to the drought.

Notably, neither species was able to effectively regulate stomatal conductance to increase water use efficiency in the face of persistent water deficits. In fact, we observed the opposite where little adjustment in stomatal conductance paired associated with non-proportional reductions in transpiration relative to photosynthesis resulted in significantly lower average water use efficiencies in the drought treatments relative to ambient conditions for spruce. However, we did find significantly greater water use efficiencies tied to increasing seedling size that might suggest a more active response to water stress with tree age (Table 1.3), similar to the findings of Cui & Smith (1991) in 1- through 4-year-old subalpine fir seedlings. In addition to the lack of a morphological response to reduced water levels by the seedlings in this study, this further suggests carbon gain is strongly prioritized in small seedlings until certain developmental levels are met, such as thresholds in age, size, allometric ratio, or external conditions (e.g. release from low light levels in the understory), at which point individual may display greater morphological and physiological plasticity in response to environment stress as has been observed for saplings and adults of these species (Knapp & Smith 1981, Brodersen et al. 2006).

In the subalpine forests of the southern Rocky Mountains, co-dominance between subalpine fir and Engelmann spruce is maintained by contrasting success between the species under differing conditions. Fir is the superior competitor in the shady understories of mature

forests, substantially outnumbering spruce in the seedling bank. However, spruce is more successful in colonizing canopy gaps due to faster growth and, paired with its greater longevity, allows these species to remain co-dominant in the overstory (Andrus et al. 2018). Our results show that this competitive equilibrium could be disrupted by reductions or failure of summer monsoon precipitation, as fir was far less affected physiologically by drought – with much smaller reductions in net photosynthesis and water use efficiencies at the end of the drought period – suggesting that the competitive advantages of spruce in higher light may be lost by its poorer physiological functioning in water deficits. Spruce may therefore have greater regeneration barriers competing with fir during future periods of increasing drought, potentially leading to short-term understory compositional shifts with substantial consequences for future forest structure in the face of climate change.

## **Conclusion**

Knowledge of species' ability to acclimate to shifts in precipitation regimes at the seedling stage will provide a vital understanding of the underlying traits that convey drought tolerance and how these capacities vary within and among species occupying a critical regeneration bottleneck in high-elevation forest systems. In this study we demonstrate a lack of morphological and physiological plasticity in response to consecutive summer drought in seedlings of the two dominant subalpine climax species of the southern Rocky Mountains – Engelmann spruce and subalpine fir. No morphological adjustments to drought mitigation traits were detected in either species, and both photosynthetic carbon gain and water use efficiency was greatly reduced reflecting poor whole-seedling acclimation to water stress, particularly for spruce. Further, patterns of biomass allocation in response to seedling size did not reflect expected shifts towards greater investment in structural traits over carbon gain structures as seedlings age. However, while no increase in morphological investment was observed, increasing stomatal control with seedling size conferred greater water use efficiency in larger individuals. These results suggest strong conservation of traits that supporting short-term carbon gain at the expense of water stress mitigation well into understory establishment. Increased seedling mortality with climate change-

induced drought is a likely outcome of these responses with potential disruption of successional balances between these two co-dominant subalpine species with long-lasting implications for future forest structure.

## **Chapter 2: Combined Drought and Shade Drive**

### **Photosynthetic Decline and Mortality in Subalpine Forest Tree Seedlings**

#### **Introduction**

Droughts are increasing in frequency and severity across many forest ecosystems worldwide, inducing widespread adult forest tree mortality and loss of ecosystem services (Van Mantgem et al. 2009, Allen et al. 2010, Anderegg et al. 2013, Clark et al. 2016). Droughts are also expected to cause increased mortality in seedlings, raising concerns that recruitment will fail to offset current and projected rates of overstory loss (Conlisk et al. 2017, Kueppers et al. 2017, Conlisk et al. 2018). More frequent droughts may create fundamentally unsuitable conditions for seedling establishment and persistence, especially for species occupying narrow regeneration niches which originally established under cooler and wetter past climates, possibly leading to substantial divergence of future forests from historical structure and extent (Bell et al. 2014, Martinez-Vilalta & Lloret 2016, Brodersen et al. 2019).

Such outcomes may be more likely in high elevation forests such as those in the western U.S. where climate change effects are accelerated relative to lower elevations (Beniston et al. 1997, Pepin et al. 2015, Dobrowski & Parks 2016) and seedling establishment is already inherently limited by moisture availability (Moyes et al. 2013, Gill et al. 2015, Andrus et al. 2018b, Hill et al. 2019, Foster et al. 2020). Indeed, in the Colorado Front Range of the southern Rocky Mountains, though total annual precipitation is projected to decline < 5 % in the next century (Lukas et al. 2014), lower snowpack and earlier snowmelt as temperatures warm (Mote et al. 2005) paired with delayed onset of the summer monsoon responsible for the bulk of growing-season precipitation (Cook & Seager 2013, Pascale et al. 2017) are causing increasingly frequent and intense summer droughts – changes which are implicated in rising rates of adult tree mortality in subalpine forests in the region (Bigler et al. 2007, Smith et al. 2015). Regeneration of

the two co-dominant tree species of this ecosystem – Engelmann spruce (*Picea engelmannii* Parry ex Engelmann) and subalpine fir (*Abies lasiocarpa* (Hook) Nutt.) – is strongly controlled by moisture availability (Gill et al. 2015, Andrus et al. 2018b, Hill et al. 2019) and their regeneration success depends on contrasting microsite conditions, reflecting dynamic trade-offs of stress tolerance and competitive traits through stages of establishment and ontogeny (Andrus et al. 2018a). Superior shade tolerance and rooting depth enables fir seedlings to colonize shady interior forest microsities in greater numbers than spruce (Day 1964, Knapp & Smith 1982), while greater seedling ingrowth in canopy gaps and superior longevity of spruce trees sustains a co-dominant climax-state overstory that, in the absence of major disturbance, persists for centuries (Veblen 1986, Antos & Parish 2002, Andrus et al. 2018a).

Increasing drought may alter the delicate regeneration dynamics between spruce and fir by degrading microsite conditions that currently facilitate co-existence (Holmgren et al. 1997, Martinez-Vilalta & Lloret 2016). For spruce, which occupies canopy gaps and exposed microsities more successfully than fir (Day 1964, Shea 1985, Hättenschwiler & Smith 1999, Germino et al. 2002, Redmond & Kelsey 2018), higher solar irradiance in gaps may exacerbate drought stress through higher temperatures and greater vapor pressure deficits (Abrams & Mostoller 1995, Chen et al. 1995) which in turn may limit spruce to more protected microsities where competition with fir is greater. At the same time, drought in fir-dominated low-irradiance microsities may reduce photosynthetic carbon uptake and increase risk of mortality through depletion of non-structural carbohydrate reserves leading to carbon starvation and death (McDowell et al. 2008, Maguire & Kobe 2015), a particular risk for tree seedlings with inherently limited carbon budgets (Bansal & Germino 2008).

Tolerance to the combined stresses of drought and low light is generally thought to be uncommon in trees given strong negative correlations or trade-offs in water stress and shade tolerance traits (Niinemets & Valladares 2016, Rueda et al. 2017), and particularly for seedlings which display reduced resistance to abiotic stress relative to adults (Niinemets 2010). While both spruce and fir are considered shade tolerant with limited drought resistance (Wei et al. 2019),

even minor advantages in water stress tolerance by one species could lead to displacement of the other as drought exacerbates microsite limitations to regeneration. High elevation spruce-fir forests are extremely slow growing with seedlings that often persist in the understory in excess of 100 years (Antos et al. 2000). Since recruitment declines in these systems are broadly anticipated with climate warming (Kueppers et al. 2017, Andrus et al. 2018b), changes in the performance of existing populations of established seedlings driven by drought-induced alterations to microsite suitability will likely substantially alter forest trajectory with long-term consequences for forest structure and function (Martinez-Vilalta & Lloret 2016).

The aim of this study was to evaluate established seedling demographic and physiological responses to combined drought and shade in naturally regenerating patches of Engelmann spruce and subalpine fir in a subalpine forest in the Colorado Front Range. Patterns of photosynthetic carbon uptake and survivorship were compared between dense shade and interior forest gap microenvironments in combination with complete (100 %) or partial (50 %) precipitation reductions. As both species are considered shade tolerant with comparatively high moisture demands, we expect that drought effects on gas exchange physiology and mortality will be most pronounced in gap sites due increased water stress in higher light. Given the importance of gap colonization in spruce regeneration, this outcome would suggest increased regeneration limitations for spruce with drought. Alternatively, since drought and shade tolerance are generally thought to be non-compatible, physiological performance of both species may be expected to degrade more substantially in the shade than in the forest gap, perhaps most consequentially for fir which may have already limited carbon resources from sustained growth under low irradiances. Regardless, combined drought and shade effects will likely be more pronounced in smaller individuals which display lesser capacitance in tolerating abiotic stress (Niinemets 2010). These outcomes could either (1) reinforce existing microsite differentiation between species, though ultimately favoring fir persistence as gap sites become unsuitable for spruce with drought, or (2) degrade microsite suitability to an extent in which both species become mismatched to their

existing placements, suggesting under prolonged or recurring drought broad regeneration limitations portending substantial compositional shifts towards more pioneering or seral species.

## **Methods**

### *Site Description*

This study was conducted in a mature spruce-fir forest  $\approx$  300 m below timberline at the University of Denver High Altitude Laboratory on Mt. Evans, Colorado, USA (3,230 m, 39.66° N, 105.59° W). The site is characteristic of the subalpine spruce-fir forests of the southern Rocky Mountains composed of co-dominant Engelmann spruce and subalpine fir with scattered 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 78 cm) falls as snow, approximately 30 % occurs during summer monsoonal rains from July through September (NRCS SNOTEL 2020). No evidence of any recent disturbances (fire, blow-down, insect outbreak, etc.) was apparent at the site during the period of study.

### *Experimental Design*

Twelve seedling monitoring plots were established at the site in June 2019 – six in areas representative of relatively open interior forest gaps (“gap”) and six in closed canopy, heavily shaded areas (“shade”). Plots were roughly homogenous in soil and litter composition, microtopography, and herbaceous cover. Classifications of light availability were verified with a hemispherical camera lens placed at seedling height (COOLPIX 900, Nikon, Tokyo, Japan) and processed using Gap Light Analyzer software to calculate the gap light index (GLI) of the combined incident diffuse and direct-beam radiation over a growing season expressed as a percentage of full sun (GLA v. 2.0, Cary Institute for Ecosystem Studies, Millbrook, NY, USA, Frazer et al. 1999). Interior gap plots averaged 34 % GLI ( $\pm$  3.7 % SE) while shade plots averaged 18 % GLI ( $\pm$  0.8 % SE).



Each plot consisted of three 1 x 1 m quadrats containing naturally regenerating seedlings of spruce and fir less than 5 mm in stem diameter and 20 cm in height spanning current year emergents to juvenile individuals. One quadrant was designated to receive ambient levels of precipitation while the other two received either 50 % or 100 % precipitation reduction treatments (50 % PR and 100 % PR, respectively) to mimic reduced or complete failure of July-September monsoonal precipitation, respectively. 1 x 1 m panels of clear polycarbonate were installed 1 m above the soil surface using a PVC frame to achieve 100 % reduction, while slatted panels of the same dimension but half the coverage were used for 50 % reduction (Suntuf, Palram Americas, Kutztown, PA, USA). Shelters of similar design (Yahdjian and Sala 2002, Drought-Net 2018) previously have been found to reduce precipitation as intended while having only marginal effects on soil and air temperatures and light levels beneath the shelter (Fay et al. 2000, Yahdjian & Sala 2002, Heisler-White et al. 2008, Cherwin & Knapp 2012).

To measure the degree to which the shelters affected precipitation in the reduced precipitation quadrats relative to those in ambient conditions, rain gauges were constructed using 4 in diameter PVC pipe capped with funnels to minimize losses from splashing and evaporation. Gauges were placed in a subset of plots (n = 5 canopy gap and 4 shade plots, 27 gauges total) in all three treatments (100 % PR, 50 % PR, and ambient) and emptied on a weekly basis for the duration of the treatment. Soil moisture 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 replication of soil moisture observations across plots, and in these few (< 15 % of days monitored) cases standard error of soil VWC was imputed from the average of neighboring date values. Site-level precipitation was retrieved from an on-site SNOTEL monitoring station (NRCS SNOTEL, 2020). Though the year prior to study (2018) was a substantial drought year, precipitation deficits were recuperated with heavy snowfall in early March 2019 leading to a

largely typical growing season moisture for the year in which this study was conducted, though mild dryness was present in the final weeks of observation (September 2019, NDGM 2020).

Seedling mortality was assessed on a weekly basis during the period of precipitation reduction. Individuals were considered expired once most needles had browned and seedlings appeared fully desiccated, at which point they were harvested and measured for stem diameter at the root crown. At the end of the study period (22 September 2019), seedlings from all plots were assessed for survivorship and stem diameter. Over the course of study, 1,632 seedlings of spruce and fir were monitored for survivorship in total, and 184 for gas exchange characteristics.

### *Gas Exchange*

Beginning shortly after the initiation of precipitation reduction, representative selections of seedlings ( $n = 5$  to 15 different individuals per sampling date of varying size across species and light microenvironments) were assessed for a suite of gas exchange parameters using an infrared gas analyzer (LI-6800, LI-COR Biosciences, Lincoln, NE, USA). Since larger individuals were expected to be relatively more drought tolerant, and drought effects would be more pronounced with greater precipitation reduction, we restricted gas exchange measurements to individuals 2 mm or less in diameter in the ambient and 100 % precipitation reduction treatments. For smaller seedlings, entire crowns were inserted vertically into the chamber maintaining sunwards orientation to the light source. For larger seedlings, sun-oriented springs were inserted laterally. Chamber temperature was matched to ambient conditions every 10-15 minutes while humidity was set to 1.5 vapor pressure deficit.  $\text{CO}_2$  was maintained at 410 ppm reflective of regional levels (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 ( $g_{\text{sw}}$ ,  $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) were assessed in response to a 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). Maximum photosynthetic flux ( $A_{\text{max}}$ ) and stomatal conductance ( $g_{\text{max}}$ ) were calculated by averaging the five highest values from each set of measurements (e.g. Johnson et al. 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 & Smith 1988). All gas exchanges measurements are expressed based on projected area. Detached leaves from the total leaf sample contained in the gas analyzer chamber were imaged with a flatbed scanner at 600 DPI (Perfection V850, Epson, Nagano, Japan). Captured images were thresholded and quantified for projected leaf area (cm<sup>2</sup>) using FIJI v. 1.52 (Schindelin et al. 2012). Since canopies were harvested to determine leaf area for each sample at the time of measurement, different seedlings were assessed at each date for gas exchange parameters.

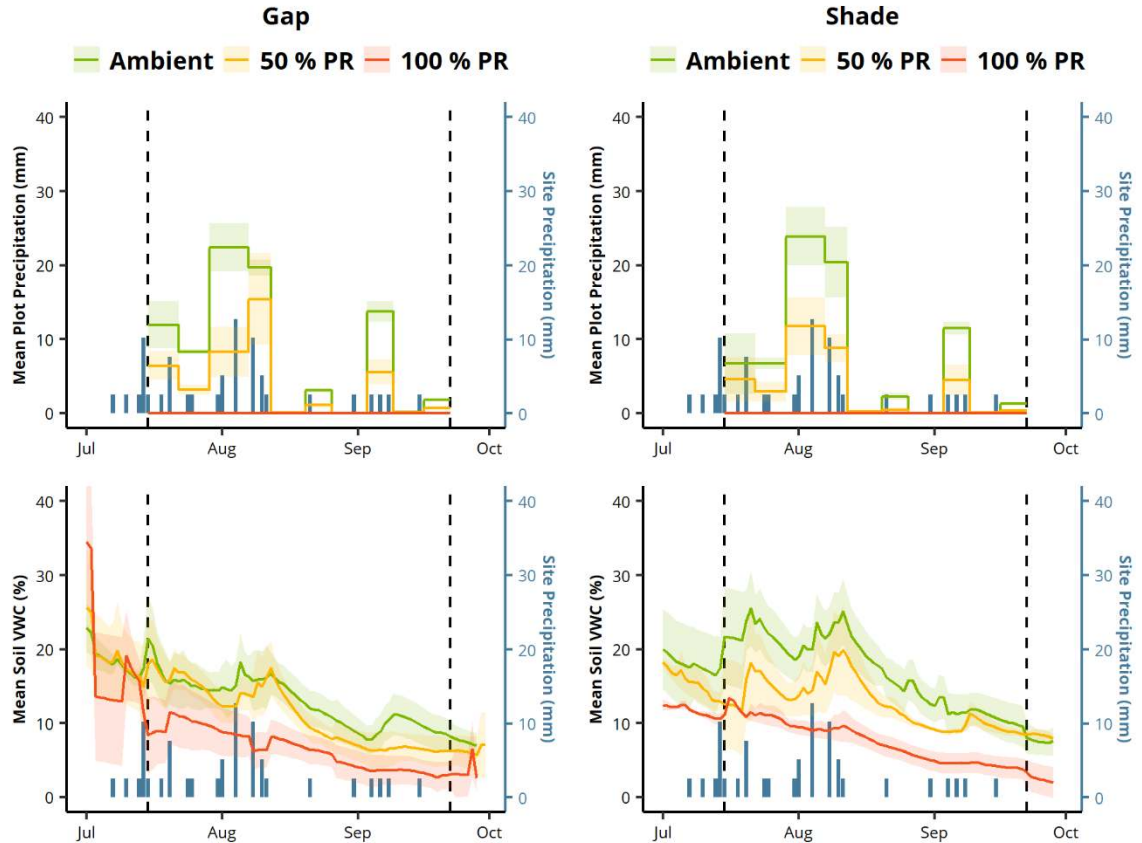
### *Statistical Analyses*

To evaluate the influence of size on seedling responses, seedlings were classified as current-year emergents (only cotyledons present, < 0.5 mm in stem diameter), established small seedlings (cotyledons sometimes present, 0.5–1.0 mm in stem diameter), and large seedlings (no cotyledons, 1.0–2.0 mm in stem diameter) for analysis of gas exchange, with additional small juvenile (2.0–3.0) and large juvenile (3.0–5.0 mm) size classes for observations of survivorship. Survivorship was calculated as the proportion of living seedlings during each week of treatment to gross abundance across all plots within each treatment and microsite environment. Due to overall low abundance of seedlings, some combinations of species, size, and treatment had few individuals (< 10), and in these cases are highlighted in the results. Type III sum of squares ANCOVA was implemented to test for differences in maximum photosynthetic rate and stomatal conductance as an outcome of light environment and precipitation reduction treatment with the number of days of precipitation reduction as a covariate, as drought effects were expected to increase over time as water deficits accumulated. Interaction terms were insignificant for most models, therefore main effects are presented. Simple linear regression was then implemented to visualize relationships among variables. All analyses were conducted in the R programming environment (v. 3.6., R Core team 2020).

## Results

### *Plot Precipitation and Soil Moisture*

Precipitation reduction shelters created plot-level moisture reductions as intended (Fig. 2.1). Relative to ambient precipitation, the 100 % precipitation reduction shelters reduced mean precipitation 100 % (i.e. 0 mm) and the 50 % reduction treatment reduced precipitation to 43.5 % ( $\pm 9.1$  % SE) of ambient leading to proportional reductions in average soil volumetric water content (% VWC) in the plots. Across the treatment period (15 July to 22 September 2019), mean soil moisture was on average  $\approx 3.0$  % VWC lower in the 50 % precipitation reduction plots and  $\approx 7.7$  % VWC lower in the 100 % precipitation reduction plots relative to ambient. Reductions in VWC were more pronounced in the shade plots – mean reductions in VWC were on average  $\approx 4.0$  and  $\approx 9.2$  % lower in the 50 and 100 % precipitation reduction treatments relative to ambient, respectively; gap plots were on average  $\approx 1.9$  and  $\approx 6.2$  % lower VWC in the 50 and 100 % precipitation reduction treatments, respectively.



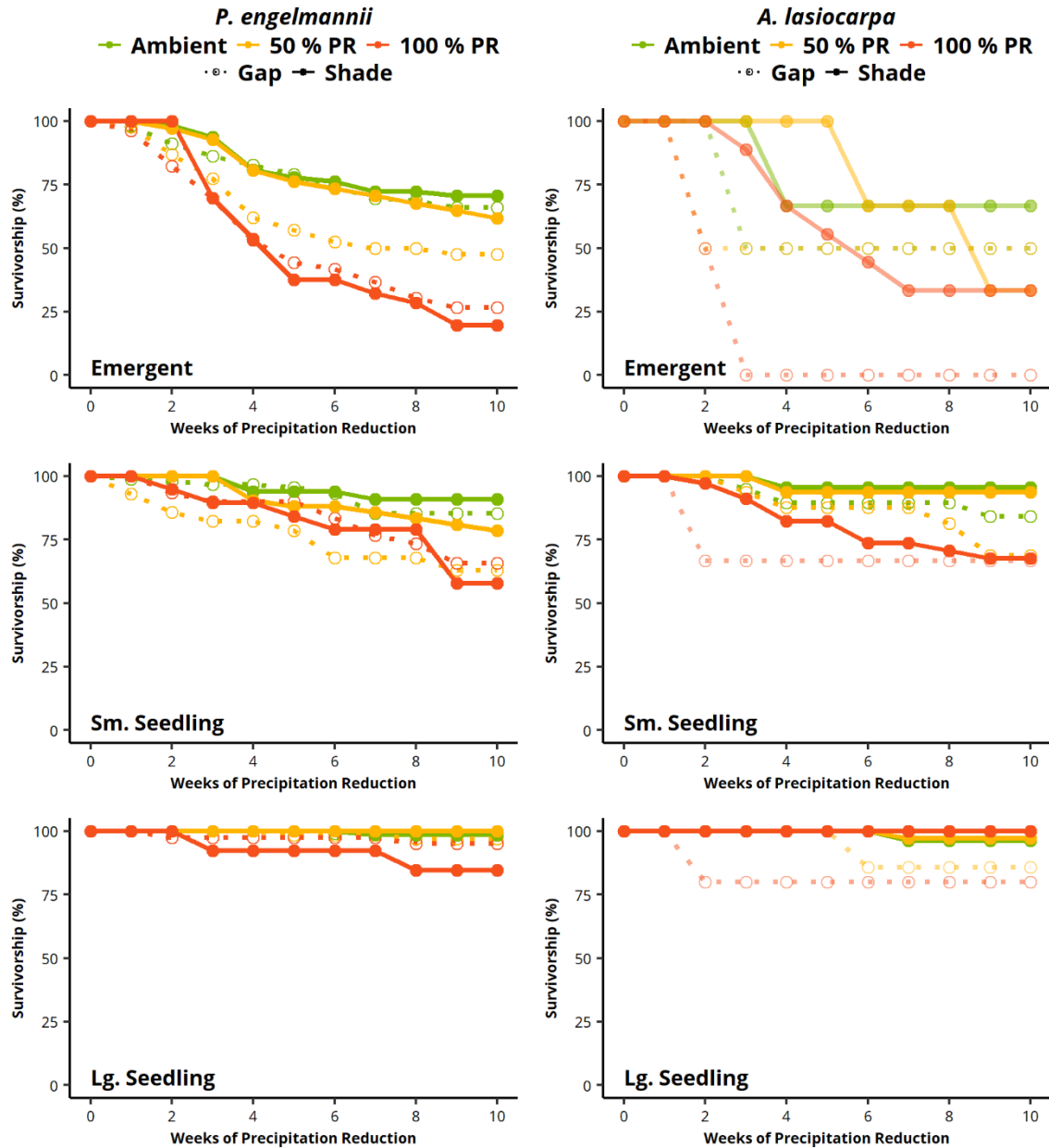
**Figure 2.1.** Mean weekly plot precipitation (top) and mean daily soil volumetric water content (bottom) among precipitation reduction (PR) treatments in canopy gap (left) and shaded (right) canopy microsites. Plot precipitation was measured at ground level (e.g. beneath precipitation exclusion shelters) with rain gauges. Daily precipitation at the study site retrieved from SNOTEL is also presented. Vertical dashed lines show the dates the precipitation reduction treatments were active (15 July to 22 September 2019). Shaded error ribbons indicate the standard error of the mean.

### *Seedling Survivorship*

Survivorship among species, seedling sizes, and treatments is presented in Figure 2.2. Rates of survivorship appeared relatively consistent throughout the monitoring period, with mortality rates increasing under reduced precipitation from rates of mortality in the ambient treatments as soon as two weeks after the initiation of precipitation reduction. Emergents (< 0.5 mm in diameter) of both species exhibited the lowest survivorship (0-65 %) across all precipitation treatments and light microenvironments. Survivorship increased markedly with seedling size – in established small seedlings (0.5–1.0 mm in diameter) there were no instances of mortality

exceeding 50 % of the initial population for either species in any light environment or precipitation reduction treatment, only marginal mortality was observed for large seedlings (1.0–2.0 mm in diameter), and no mortality was observed for either small or large juveniles of both species.

The influence of a drought-light interaction on survivorship was apparent for Engelmann spruce, where emergent seedlings (0–0.5 mm in diameter) in the shade had higher survivorship – nearly equivalent to the survivorship of seedlings in ambient conditions – to those in gaps in the 50 % precipitation reduction. At 100 % precipitation reduction, however, survivorship was similar in gaps and shade and quite low ( $\approx$  25 % or lower). Survivorship was somewhat higher in the shade as well for small established seedlings (0.5–1.0 mm in diameter) at 50 % precipitation reduction, but then lower in the shade at 100 % precipitation reduction. For fir, survivorship was greater in shaded microsites regardless of precipitation reduction treatment or seedling size. However, low abundances of emergent fir seedlings across plots ( $n < 10$  in each combination of microsite and precipitation reduction treatment) poses a degree of uncertainty in these observations. While mortality was overall negligible for large established seedlings (1.0–2.0 mm in diameter) for both species, slightly lower survivorship occurred in the 100 % precipitation reduction in the shade for spruce and in the gap sites for fir.



**Figure 2.2.** Survivorship (%) of Engelmann spruce (*P. engelmannii*, left) and subalpine fir (*A. lasiocarpa*, right) seedlings by size (emergent, < 0.5 mm, small, 0.5-1.0 mm, and large seedlings, 1.0-2.0 mm in stem diameter) during 10 weeks of growing-season precipitation reduction (PR) in shaded and canopy gap microsites. Solid lines and points indicate survivorship of seedlings growing in shaded microsites while dotted lines and open points indicate survivorship of seedlings growing in canopy gap microsites. Lines of lower opacity represent populations of less than 10 individuals. Juveniles (2.0–3.0 and 3.0–5.0 mm in stem diameter) had 100 % survivorship across treatments and microsites during the study and are therefore not shown.

### *Photosynthesis and Gas Exchange*

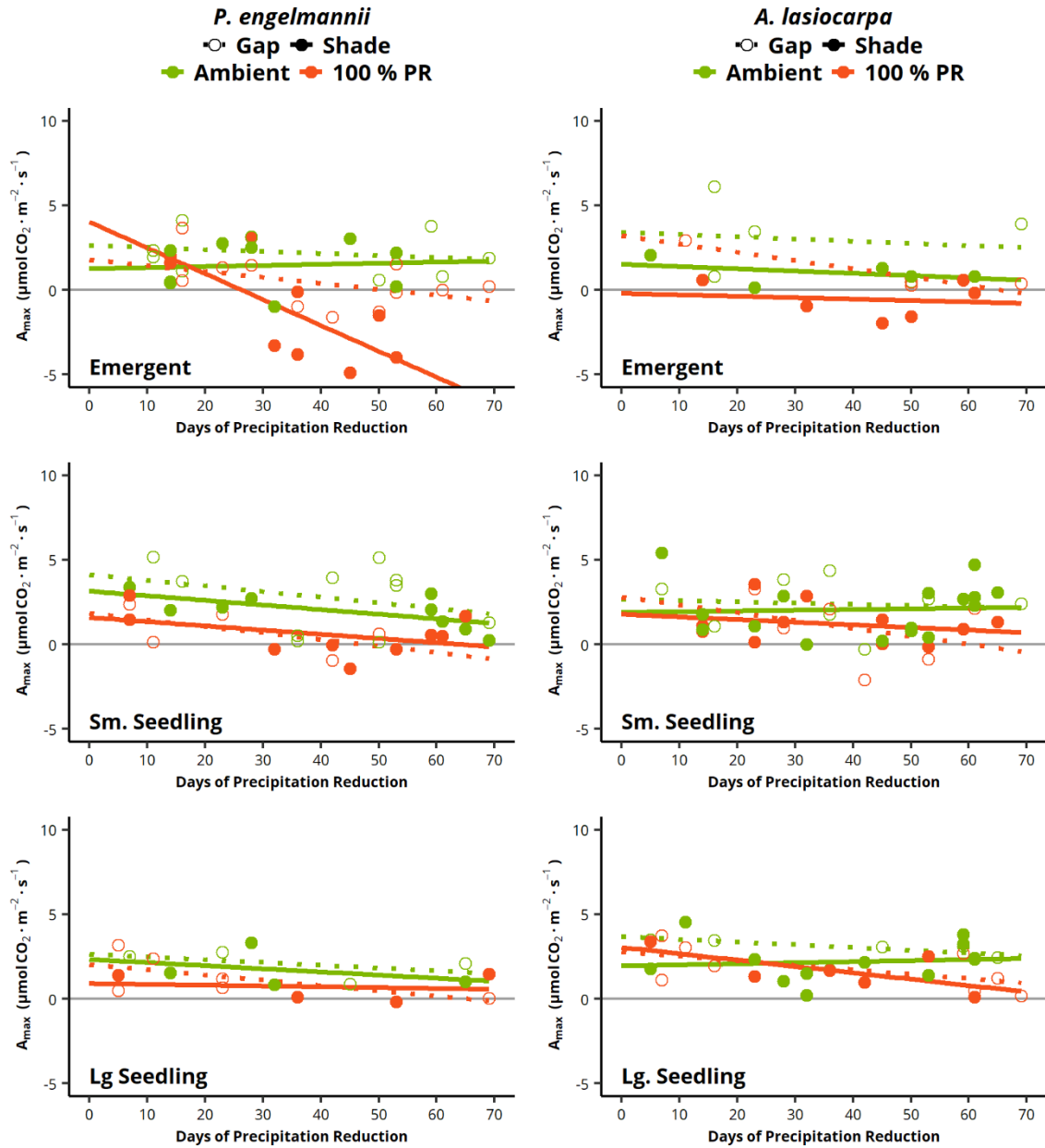
Maximum net photosynthetic rate was strongly and significantly reduced by summer drought for spruce and fir across most seedling sizes (Table 2.1, Fig. 2.3). Emergent seedlings of both species (< 0.5 mm in diameter) experienced a striking loss of photosynthetic carbon gain after  $\approx$  4 weeks of precipitation reduction, when net CO<sub>2</sub> fluxes become largely negative. Shading significantly lowered net photosynthesis as well, leading to pronounced carbon deficits in these seedlings – particularly in emergent spruce seedlings where the interaction between the length and amount of perception withholding, as well as the interaction between these terms and light microenvironment were significant ( $F = 8.14$  and  $4.54$ ,  $df = 1$  and  $1$ ,  $p < 0.01$  and  $0.05$ , respectively). Small established seedlings (0.5–1.0 mm in stem diameter) displayed significant declines in maximum net photosynthetic rate in response to precipitation reduction, but they were less sensitive to the effects of light microenvironment. The largest seedlings (1.0–2.0 mm) showed modest declines in photosynthetic activity over the course of reduced precipitation, though not to a significant degree in spruce.



**Table 2.1.** Main effects ANCOVA table for maximum net photosynthesis ( $A_{max}$ ) as a response to microsite light environment (gap, shade) and precipitation reduction treatment (ambient, 50 % PR, 100 % PR) with the number of days of precipitation reduction as covariate for Engelmann spruce (*P. engelmannii*) and subalpine fir (*A. lasiocarpa*) seedlings of varying sizes (emergent, < 0.5 mm, small, 0.5-1.0 mm, and large seedlings, 1.0-2.0 mm in stem diameter). Type III sum of squares (SS), degrees of freedom (df), and F-values (F) are presented.

Species	Size Class	Parameter	SS	df	F
<i>P. engelmannii</i>	Emergent	Days of Precipitation Reduction	14.51	1	4.61*
		Microsite Light Environment	18.36	1	5.83*
		Precipitation Reduction Treatment	10.90	1	12.99***
	Sm. Seedling	Days of Precipitation Reduction	9.88	1	5.21*
		Microsite Light Environment	4.03	1	0.54
		Precipitation Reduction Treatment	25.60	1	13.48***
	Lg. Seedling	Days of Precipitation Reduction	3.04	1	3.37
		Microsite Light Environment	0.47	1	0.52
		Precipitation Reduction Treatment	3.75	1	4.15
<i>A. lasiocarpa</i>	Emergent	Days of Precipitation Reduction	3.28	1	7.66
		Microsite Light Environment	15.08	1	7.64*
		Precipitation Reduction Treatment	11.81	1	5.98*
	Sm. Seedling	Days of Precipitation Reduction	0.95	1	0.41
		Microsite Light Environment	0.03	1	0.01
		Precipitation Reduction Treatment	12.12	1	5.28*
	Lg. Seedling	Days of Precipitation Reduction	4.28	1	3.75
		Microsite Light Environment	2.31	1	2.04
		Precipitation Reduction Treatment	5.51	1	4.83*

\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$



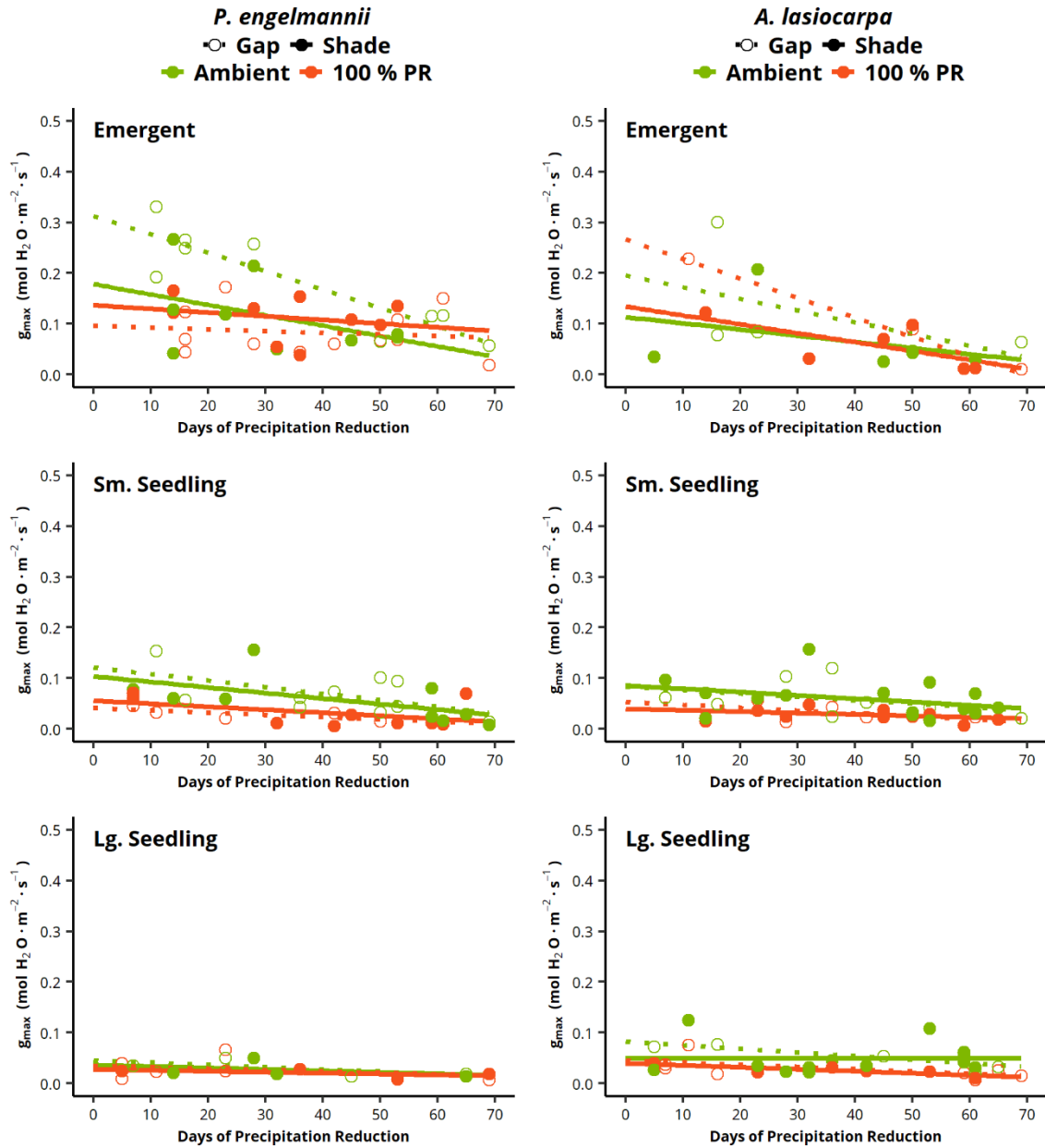
**Figure 2.3.** Maximum net photosynthesis ( $A_{max}$ ,  $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) of Engelmann spruce (*P. engelmannii*, left) and subalpine fir (*A. lasiocarpa*, right) seedlings by size (emergent, < 0.5 mm, small, 0.5-1.0 mm, and large seedlings, 1.0-2.0 mm in stem diameter) during 10 weeks of growing-season precipitation reduction (PR). Lines represent best-fit linear regression models. Solid lines and points indicate max net photosynthesis of seedlings growing in shaded microsites while dotted lines and open points indicate max net photosynthesis of seedlings growing in canopy gap microsites. Horizontal gray line indicates net zero photosynthesis.

Maximum stomatal conductance varied with light availability over the course of precipitation reduction, and seemingly across seedling size as well (Table 2.2, Fig. 2.4). Conductance declined significantly over the period of perception withholding in emergents and small seedlings (0–0.5 and 0.5–1.0 mm in diameter), and this decline was most pronounced in emergents as initial conductance was much higher at the beginning of the observation period, and particularly for spruce where the interaction between the length and amount of precipitation withholding, as well as between precipitation withholding and light microenvironment was significant ( $F = 4.46$  and  $4.5$ , respectively,  $df = 1$ ,  $p < 0.05$ ). Additionally, lower conductance was apparent in response to 100 % precipitation reduction in these smaller seedlings of both species. Overall, conductance appeared much lower in larger seedlings than smaller seedlings. Stomatal conductance did not seemingly vary in response to microsite light environment.

**Table 2.2.** Main effects ANCOVA table for maximum stomatal conductance ( $g_{\max}$ ) as a response to microsite light environment (gap, shade) and precipitation reduction treatment (ambient, 50 % PR, 100 % PR) with the number of days of precipitation reduction as covariate for Engelmann spruce (*P. engelmannii*) and subalpine fir (*A. lasiocarpa*) seedlings of varying sizes (emergent, < 0.5 mm, small, 0.5-1.0 mm, and large seedlings, 1.0-2.0 mm in stem diameter). Type III sum of squares (SS), degrees of freedom (df), and F-values (F) are presented.

Species	Size Class	Parameter	SS	df	F
<i>P. engelmannii</i>	Emergent	Days of Precipitation Reduction	0.4	1	10.91**
		Microsite Light Environment	0.01	1	1.46
		Precipitation Reduction Treatment	0.02	1	5.78*
	Sm. Seedling	Days of Precipitation Reduction	0.01	1	10.1**
		Microsite Light Environment	0.00	1	0.00
		Precipitation Reduction Treatment	0.01	1	10.92**
	Lg. Seedling	Days of Precipitation Reduction	0.00	1	2.95
		Microsite Light Environment	0.00	1	0.19
		Precipitation Reduction Treatment	0.00	1	0.40
<i>A. lasiocarpa</i>	Emergent	Days of Precipitation Reduction	0.04	1	9.36**
		Microsite Light Environment	0.01	1	1.92
		Precipitation Reduction Treatment	0.00	1	0.01
	Sm. Seedling	Days of Precipitation Reduction	0.00	1	5.15*
		Microsite Light Environment	0.00	1	0.00
		Precipitation Reduction Treatment	0.01	1	12.52**
	Lg. Seedling	Days of Precipitation Reduction	0.00	1	3.72
		Microsite Light Environment	0.00	1	0.29
		Precipitation Reduction Treatment	0.01	1	7.67**

\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$



**Figure 2.4.** Maximum stomatal conductance ( $g_{max}$ ,  $\mu\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) of Engelmann spruce (*P. engelmannii*, left) and subalpine fir (*A. lasiocarpa*, right) seedlings by size (emergent, < 0.5 mm, small, 0.5-1.0 mm, and large seedlings, 1.0-2.0 mm in stem diameter) during 10 weeks of growing-season precipitation reduction (PR). Lines represent best-fit linear regression models. Solid lines and points indicate max conductance of seedlings growing in shaded microsites while dotted lines and open points indicate max conductance of seedlings growing in canopy gap microsites.

## Discussion

Precipitation reduction shelters were largely effective at creating plot-level precipitation and soil moisture conditions in line with the intended 50 and 100 % precipitation reduction treatments for the period of study (15 July to 22 September 2019). Precipitation in shade plots was occasionally lower on average than precipitation in gap plots (e.g. 7–12 and 20–26 August), indicating some degree of interception by the overstory in shaded plots. Despite this, seedling water stress was likely somewhat higher in the gap plots across all treatments probably due to greater surface moisture evaporation, and perhaps more so in the final weeks of study as the area experienced the onset of mild seasonal dryness (NDGM 2020). Indeed, average soil moisture was  $\approx 0.8$  % VWC higher in shade plots than gap plots under 100 % reduction, and  $\approx 1.8$  % VWC higher in shade plots than gap plots under 50 % reduction. However, relative differences in water stress may be greater in the shade due to greater stratification in mean soil water content among precipitation reduction treatments. For instance, 100 % precipitation reduction lowered soil moisture relative to ambient conditions by 6.2 % VWC in canopy gaps, and by a greater 9.2 % VWC in the shade. These patterns of soil moisture response to precipitation withholding would suggest that, while water stress may have been slightly higher in the gap microsites, greater relative effects of precipitation reduction may have been manifested in the shade. Overall, precipitation deficits aligned with projected decreases in July-September precipitation brought about by monsoonal weakening (Cook & Seager 2013, Pascale et al. 2017) and were most likely sufficient in inducing substantial water stress given the large reduction of soil moisture content (e.g. Reinhardt et al. 2015, Kannenberg et al. 2019).

As expected, substantially greater mortality was observed in the smaller size classes compared to larger size classes, both in ambient and reduced precipitation conditions. Even under ambient precipitation, typical mortality rates in Engelmann spruce and subalpine fir seedlings is high, often exceeding 50 % in their first year depending on microsite suitability (Cui and Smith 1991, Germino et al. 2002, Maher & Germino 2006). The introduction of moderate (50 %) and severe (100 %) reductions in summer precipitation markedly increased these rates in

current year emergent seedlings by an additional 10–50 % depending on the light microenvironment. Seedlings displayed successively greater survivorship with increasing size even under precipitation reductions as small and large seedlings (0.5–1.0 mm and 1.0–2.0 mm in diameter, respectively) had  $\geq 50$  % and  $\geq 75$  % survivorship rates, respectively, and no mortality at all was observed in juveniles ( $> 2.0$  mm in diameter) of either species, confirming a higher capacity to withstand water stress with increasing tree size (Niinemets 2010). However, somewhat elevated mortality in precipitation reduction treatments of large seedlings classes suggest an overall low tolerance to drought in even well-established seedlings. Indeed, seedlings of such a size are generally over a decade in age, so succumbing to drought in a single growing season is striking, especially 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 & Germino 2006).

Under non-drought conditions, shading facilitates greater survival in first year seedlings of spruce and fir, particularly at upper-elevational range limits where excessive sky exposure can induce low-temperature photoinhibition and death (Cui and Smith 1991, Germino et al. 2002, Johnson et al. 2004, Maher & Germino 2006), but under a 50 % precipitation reduction spruce seedling mortality was greater in the shade than in the gap microsites for emergent and small seedlings. Interestingly, at 100 % precipitation exclusion survivorship was somewhat greater in the gap microsite, suggesting the potential ameliorating of water stress in the shade did not outweigh the negative consequences of low light availability (Holmgren et al. 1997). For emergent fir seedlings, survivorship was greater in the shade than in gap microsites under both 50 and 100 % precipitation reduction which aligns with historical patterns of colonization in this species. However, the low abundance of fir germinants at the site likely bias these observations and should be interpreted with caution.

Physiological measurements of photosynthesis and stomatal conductance also provided evidence for the role of light microenvironment in mediating drought effects in these species, and that smaller seedlings are more susceptible to water stress. While maximum rates of net

photosynthesis generally declined over the length of precipitation reduction for both species in all water and light conditions reflective of typical of seasonal progressions of productivity (Broderson et al. 2006), immediate and substantial reductions to carbon gain were apparent emergent spruce and fir seedlings subjected to 100 % precipitation reduction. Notably, net photosynthetic carbon gain become largely negative – and to a greater degree in spruce – after just 4 weeks of precipitation reduction. Net photosynthesis was also lower in shade versus gap microsites for both species, suggesting greater carbon limitations during drought in the shade, particularly for spruce. Since prolonged negative carbon balances can impose substantial mortality risks as carbon reserves are depleted (McDowell, 2011), these patterns of reduction to photosynthesis in spruce may likely explain the lower survivorship we observed in the shade versus in the gap under 100 % precipitation reduction, and certainly the substantial mortality in response to 100 % precipitation reduction in both species regardless of microsite occupancy. 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 in shade in fir relative to spruce as suggested by lower net changes to photosynthetic accumulation in the shade versus in gap sites we observed here.

Though precipitation reduction significantly lowered photosynthetic uptake across all size classes for both species, larger seedlings appeared more resistant to water stress as demonstrated by lesser degrees of photosynthetic decline. In fact, the largest seedlings measured for gas exchange (1.0–2.0 mm in stem diameter) predominately maintained a net positive carbon balance for the duration of study, likely due to more conservative stomatal behavior as indicated by broadly lower conductance with increasing size. Comparatively, high stomatal conductance in emergent seedlings that persisted throughout the study period indicate lack of physiological regulation under degrading moisture conditions, reflective of a strategy prioritizing carbon gain at the expense of long-term stress resistance (Augustine & Reinhardt, 2019). Indeed, stomatal limitations to photosynthesis have been found to increase with seedling age, conferring greater water-use efficiency as seedlings grow larger (Cui & Smith, 1991).



Though drought was non-lethal in larger seedlings, reductions to photosynthesis with increasing water deficits – particularly in the shade – suggests a possibility for lagging mortality or otherwise predispose these individuals to death if drought were to recur or persist beyond one growing season as implemented here. Indeed, trees displaying low resistance to the effects of prior droughts are often those most prone to die in successive periods of water stress (Cailleret et al. 2017, DeSoto et al. 2020).

## **Conclusion**

Forest regeneration under a changing climate will have deterministic consequences for future forest structure and function, particularly for species whose regenerative ability is inherently tied to moisture conditions. In this study we demonstrate the potential for drought to alter historical microsite-facilitated regeneration dynamics in Engelmann spruce and subalpine fir – the two co-dominant species of the subalpine forests of the southern Rocky Mountains. While moderate precipitation reduction caused reduced survivorship in both species, seedling occupancy in shaded microsites appeared to ameliorate water stress leading to greater survival. However, since fir demonstrates a high capacity to competitively displace spruce from these shaded understory microsites, declines in spruce abundance under moderate precipitation reductions is likely. Complete precipitation exclusion caused considerable mortality as the combined effects of drought and shade led to catastrophic decline in seedling carbon balance, particularly for emergent seedlings which displayed poor stomatal regulation in the face of accumulating water deficits. Broad declines in seedling abundances of both species is therefore likely as microsite suitability declines with drought, further exacerbating regeneration bottlenecks in moisture-sensitive subalpine forests.

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