STUDIES

Poor acclimation to experimental field drought in subalpine forest tree seedlings

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Abstract

The ability of tree species to acclimate and tolerate projected increases in drought frequency and intensity has fundamental implications for future forest dynamics with climate change. Inquiries to date on the drought tolerance capacities of tree species, however, have focused almost exclusively on mature trees with scant in situ work on seedlings, despite the central role that regeneration dynamics play in forest responses to changing conditions. We subjected naturally established seedlings of co-dominant subalpine conifer species (Abies lasiocarpa and Picea engelmannii) in the southern Rocky Mountains to 2 years of in situ summer precipitation exclusion, simulating summer drought conditions similar to a failure of the North American monsoon. We compared the morphological and physiological responses of seedlings growing in drought vs. ambient conditions to assess the relative changes in drought tolerance traits as a function of seedling size. Drought treatments had a marked impact on soil moisture: volumetric water content averaged ≈5–8% in drought treatments and ≈8–12% in ambient controls. We detected no significant shifts in morphology (e.g. root biomass, leaf:stem area ratio) in response to drought for either species, but net photosynthesis in drought treatments was 78% lower for spruce and 37% lower for fir. Greater stomatal control associated with increasing stem diameter conferred greater water use efficiencies in larger seedlings in both species but was not significantly different between drought and ambient conditions, suggesting an overall lack of responsivity to water stress and a prioritization of carbon gain over investment in drought mitigation traits. These results indicate a canonization of traits that, while useful for early seedling establishment, may portend substantial vulnerability of subalpine seedling populations to prolonged or recurrent droughts, especially for spruce.

Keywords: Abies lasiocarpa; allometry; drought; gas exchange; monsoon; Picea engelmannii; seedling; subalpine.

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 of 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 relative to lower elevations (Beniston et al. 1997; IPCC 2014; Pepin et al. 2015; Dobrowski and 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, 2021) and low phenotypic plasticity due to strong local adaptation to narrow bioclimatic envelopes (Valladares et al. 2007, 2014; Vitas et al. 2013; Gugger et al. 2015). Recent progress in characterizing the underlying

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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; Chao et al.
2018). However, there are few studies on how drought affects
natural populations of tree seedlings in the field, 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; Martinez-Vilalta and Lloret 2016; Brodersen et al. 2019;
Copenhagen-Parry et al. 2020; Foster et al. 2020).

Relative to saplings and adults, which overall display strong
synchronization to their environment (Martin and Canham
2020) and increased stress tolerance, conifer seedlings are more
vulnerable to water stress given their shallow rooting
depths and narrow carbon budgets (Grossnickle 2005; Bansal
and Germino 2008, 2010; Niinemets 2010; Gill et al. 2015). 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; Reinhart et al. 2015; Lazarus et al. 2018;
Augustine and Reinhart 2019). Instead of prioritizing allocation
to photosynthetic development, seedlings could invest energy
in water-stress mitigation strategies such as increased carbon
allocation to below-ground structures to support water uptake,
adjustment of the leaf sapwood area ratio to promote more
suitable whole-plant water relations or tighter control of
stomatal conductance towards increased photosynthetic water
use efficiency (Chao et al. 2018). However, many studies have
demonstrated a striking lack of morphological changes in small
conifer seedlings—particularly first-year germinants—in
response to water deficits (e.g. Aranda et al. 2010; Schall et al. 2012;
Augustine and Reinhart 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 canalized in these seedlings—
potentially at the risk of less favourable 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
greater (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
Engelm.) and subalpine fir (Abies lasiocarpa (Hooker) Nuttall)
over two growing seasons in response to late-season simulated
drought in the Front Range of Colorado, USA. Spruce and fir
are the dominant tree species across a wide elevational and
mesoclimatic band in subalpine forests in the Rocky Mountains
and have experienced dramatic increases in tree mortality in
recent decades associated with increasing moisture stress
(Bigler et al. 2007; Smith et al. 2015; Andrus et al. 2021). Increasing
temperatures and associated declines in snowpack and earlier
snowmelt have lengthened the period of water deficits across
western mountains, and summer precipitation in the southern
Rocky Mountains is declining due to the systematic weakening
of the North American monsoon (Mote et al. 2005; Hu et al. 2010;
Cook and Seager 2013; 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 has greater seedling rooting depth (Day 1964) and
slower growth rates (Day 1964; Antos et al. 2000), but higher
rates of net photosynthesis at lower levels of light saturation
(Knapp and Smith 1982), and earlier stomatal closure at the
onset of environmental stress, enhancing water use efficiency
in stressful environments (Knapp and 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 in canopy gaps
(Shea 1985; Veblen 1986; Andrus et al. 2018).

The goals of this study were to evaluate monsoon failure-
type drought response strategies of high-elevation conifer
seedlings, and to compare patterns of biomass allocation and
gas exchange between small seedlings and adult-sized
individuals. Though prior studies did not directly evaluate the
in situ effects of drought on co-occurring Engelmann spruce and
subalpine fir seedlings, a body of research suggests species-
specific differences in seedling responses to drought (Conlisk
et al. 2018; Lett and Dorrepaal 2018; Brodersen et al. 2019).
Based on this work, we predict (i) seedlings will acclimate to drought
through morphological and physiological changes (e.g. allocate
more growth to roots to alleviate water stress or reduce leaf
area to limit transpiration losses), and that spruce—due to an
inherently higher growth rate and delayed stomatal closure
which could lead to more rapid desiccation—will exhibit greater
morpho-physiological responses; (ii) due to slower growth and
lower photosynthetic rates, fir will display superior overall
drought tolerance than spruce via greater sustained rates of
photosynthesis and water use efficiency under induced drought;
and (iii) smaller individuals of both species will generally exhibit
weaker morphological changes and lower photosynthetic water
use efficiencies than larger individuals due to narrower carbon
budgets and less-regulated stomatal behaviour. If accurate, these
predictions suggest further recruitment failure for spruce during
growing-season droughts, potentially disrupting successional
dynamics of these two species. Alternatively, since growth rates
and available carbon in seedlings of both species are low, neither
species may sufficiently shift its morphology and physiology,
leading to drastic reductions in photosynthesis and transpiration
that could result in general recruitment failure of these dominant
subalpine species under recurring future drought.

Materials and Methods

Site description

This study was conducted in 2018 and 2019 at the University of
Denver High Altitude Laboratory near Mt. Evans, CO, USA (39.66’N,
105.59’W). At 3230 m in elevation, the northeastern-facing site is
co-dominated by mature subalpine fir and Engelmann spruce
with a few dispersed individuals of lodgepole pine (Pinus contorta
Douglas), limber pine (Pinus flexilis E. James) and bristlecone pine
(Pinus aristata Engelm.). These site conditions are reflective of a
compositional equilibrium characteristic of mature (>200-year-
old) spruce–fir understorey (Andrus et al. 2018). The patchy
understory is composed primarily of ericaceous species (Vaccinium
spp. and Orthezia secunda (L.) House) along with seedlings and
saplings of fir and spruce, with fir occurring in notably greater
proportions than spruce. Soils consist of mainly Leighcan family
ill till substratum and Tonahutut-Ohman complex derived from
igneous and metamorphic rock (NRCS 2020). No evidence of
any recent disturbances (fire, blow-down, insect outbreak,
etc.) was apparent at the site during the period of study. Mean annual temperature is 2.6 °C, and mean annual precipitation is 78 cm, most of which is snow but with notable rain input from summer monsoons occurring July to September (~30% of annual precipitation; NRCS SNOTEL 2020). Monsoon rains are particularly important as the study site is far enough from the climatic boundary between the arid west and the humid east typically located in Great Plains that even periods of high precipitation in the western Great Plains do not extend far enough west to increase moisture in the Front Range mountains of Colorado (Salley et al. 2016). Though total annual precipitation in the study region is projected remain within 5% of historical levels in the next century, reductions in snowpack and monsoon circulation along with rising temperature are likely to exacerbate growing-season moisture deficits (Mote et al. 2005; Lukas et al. 2014). Mean summer temperatures (June–September) at the site during the study periods were similar (12.1% higher in 2018 and 7.5% higher in 2019) to recent averages (1999–2017, NRCS SNOTEL 2020; see Supporting Information—Table S1). Total winter precipitation (October–May) prior to the growing seasons in 2018 and 2019 was 24.7% lower and 4.6% higher than recent averages, respectively.

### Experimental design

Forty 1 × 1 m plots containing naturally regenerated Engelmann 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° slope) and herbaceous cover (<5% area). Light availability within each plot was quantified using a hemispherical camera lens placed at seedling height (COOLPIX 900, Nikon, Tokyo, Japan) and expressed as % of potential direct diffuse transmitted light based on latitude and topography. Light availability within each plot was quantified using a hemispherical camera lens placed at seedling height (COOLPIX 900, Nikon, Tokyo, Japan) and expressed as % of potential direct diffuse transmitted light based on latitude and topography.

#### Supporting Information—Fig. S1

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 of precipitation (‘ambient’) and located 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 run-off.

To impose drought treatments via precipitation exclusion, 20 passive rain deflection shelters were constructed, with one located above each drought plot. Using validated designs (Yahdjian and Sala 2002; Drought-Net 2018), the shelters were constructed to cover the 1-m² plots (1 × 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® Clear, Palram Americas, Kutztown, PA, USA) mounted 1 m 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. Several prior studies have examined the effects of such shelters on the micro-environment and found air and soil temperatures to be minimally altered, with light transmission largely agreeable to manufacturer’s specifications (Fay et al. 2000; Yahdjian and Sala 2002; Heisler-White et al. 2008; Cherwin and Knapp 2012). Therefore, while systematic micro-environmental artefacts may be present, this shelter design has minimized potential light and temperature effects to a degree in which they are likely inconsequential relative to the broader range of conditions the seedlings experience diurnally, seasonally and among plots. Soil volumetric water content (VWC, %) was measured 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 each droughted and ambient plot (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 both species in each precipitation treatment was selected for gas exchange survey measurements. Net photosynthesis (A, μmol CO₂·m⁻²·s⁻¹), transpiration (E, mmol H₂O·m⁻²·s⁻¹), instantaneous use water efficiency (A/E) and stomatal conductance to water vapour (gₛ, mmol·m⁻²·s⁻¹) were measured using a portable infrared gas analyser (LI-6800, LI-COR Biosciences, Lincoln, NE, USA). Sun-oriented sprigs of larger seedlings were inserted laterally in a 3 × 3 cm aperture chamber (LI-6800-12A), while entire leaf areas of smaller seedlings were inserted vertically via a modified gasket in the bottom of the same chamber, maintaining original sunwards seedling orientation. Chamber conditions were set to a saturating light intensity of 1200 μmol·m⁻²·s⁻¹ photosynthetically active radiation using an LED light source (LI 6800-02) and 410 ppm CO₂, representative of atmospheric CO₂ 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 min. Vapour pressure deficit averaged 2.0 (±0.3) for the duration of the sampling period. Measurements, averaged across 4 s, were logged once changes in CO₂ and H₂O concentrations had stabilized (<0.2 μmol·mol⁻¹ and 0.1 mmol·mol⁻¹ standard deviation for CO₂ and H₂O, respectively; ∼5 min per measurement). All measurements were conducted on 15–16 September 2019 under clear skies between 9:00 am and 1:00 pm. Measurements were corrected for silhouette leaf area of the total chamber leaf sample.

Following gas exchange measurements, tree seedling allometry was assessed by excavating seedlings from all plots, 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 (n = 82 fir and 62 spruce seedlings in drought treatments, 89 fir and 63 spruce seedlings in ambient conditions). 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 (cm²) using Fiji v. 1.52 (Schindel et al. 2012). Leaf area for the purpose of gas exchange parameters was assessed in this same fashion. Leaf:stem area ratio (LSAR, cm²) as an approximation of leaf:sapwood area ratio was obtained by dividing leaf area by stem...
cross-sectional area calculated from stem diameter, assuming stems were circular in cross-section. Leaves, shoots and roots were then dried at 70 °C for 72 h and weighed. From these values, we calculated root, stem and leaf mass fraction (RMF, SMF and LMF, respectively, g·g⁻¹) as a proportion of total biomass (TBM, g).

Statistical analyses

Responses of allometry and gas exchange characteristics to treatments were modelled individually for each characteristic with mixed linear effect models. Seedling stem diameter (as a proxy for seedling age), species and treatment, as well as their interactions, were included as fixed effects, and plot was included as a random effect (package ‘nlme’, Pinheiro et al. 2021). For modelling, root, stem and leaf mass fractions were logit-transformed while total biomass and leaf-stem area ratios were log-transformed to improve normality of regression residuals. Plot-level transmitted light assessed with hemispherical photographs was at first also included to account for any light-availability effects on growth and physiology; however, no main or interactive effects of light were significant, and the term was therefore removed from all models. Fixed effects were evaluated on the basis of their unstandardized regression coefficients and significance at α = 0.05. Stem diameter-adjusted estimated marginal means were then calculated to directly compare the effect of treatment on mean allometry and gas exchange variables (package ‘emmeans’, Lenth 2021). Marginal means were estimated using the previous mixed linear effect models, but for each species separately such that treatment and diameter were the only fixed effects. Tukey post hoc testing was implemented to evaluate significant differences in the estimated means of allometry and gas exchange responses between control and drought treatments for each species separately. Means were back-transformed to their original scale for the purpose of comparison. All analyses were conducted in R v. 4.1 (R Core Team 2021).

Results

Soil moisture

Relative to ambient conditions, precipitation exclusion shelters were successful at reducing soil water content in situ (Fig. 1). While no pre-treatment measurements were taken in 2018, significant soil dry-down in the drought treatments was evident within 2 weeks of the start of the precipitation exclusion treatment. Average soil moisture then remained significantly lower in the drought treatment for the remainder of the treatment period. Slight increases in VWC 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 and/or subsurface water may have flowed into the plots from upslope drainage after saturating rains (Fig. 1A). In 2019, average soil water content did not significantly differ between ambient and droughted conditions prior to implementation of the treatment and followed a similar dry-down pattern to 2018. An exception occurred in late August and early September where low levels of precipitation resulted in soil drying within the ambient condition as well, resulting in non-significant treatments differences (Fig. 1B). Overall, droughted conditions remained on average 2.01 % (±0.55 SE) lower in soil VWC for the duration of precipitation exclusion treatment in 2018, and 2.17 % (±0.53 SE) lower in 2019, accounting for an estimated cumulative reduction of 125 % and 135 % soil VWC-days in 2018 and 2019, respectively.

Morphological responses

Stem diameter was strongly predictive of all measures of tree allometry. Notably, root mass fraction was negatively correlated with diameter in both species where larger stem diameters were associated with a greater proportion of biomass allocation to above-ground tissues at the expense of the relative mass of roots [see Supporting Information—Table S2]. Species was also significantly predictive of root and leaf mass fractions—spruce consistently had lower root mass fractions than fir, and higher leaf mass fractions than fir. Species was however not predictive of stem mass fraction, 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 were 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 treatment, species by treatment and diameter by species by treatment for most measures. Exceptions included the significant association of the interaction of diameter by treatment, species by treatment and diameter by species by treatment for most measures.

When controlling for stem diameter, treatment effects were not significant for any measure of allometry for either spruce or fir (Fig. 2). However, estimated marginal means of root mass fraction and total biomass were slightly lower in the drought treatment than the ambient treatment for both species (4.3
and 5.2% lower RMF and 17.5 and 9.8% lower TBM in drought conditions for spruce and fir, respectively, while leaf mass fraction averaged slightly higher in the drought treatment for spruce (4.9%), and lower for fir (4.4%). Leaf:stem area ratio remained similar (<1.5% change) between treatments in both fir and spruce.
Gas exchange responses
Among gas exchange parameters, only water use efficiency was significantly and positively associated with stem diameter [see Supporting Information—Table S3]. Stomatal conductance was greater in spruce. Net photosynthesis was 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.

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. 3). In both fir and spruce, net photosynthesis was significantly lower for individuals in the drought treatment relative to those in ambient conditions, with a greater loss of net carbon gain exhibited by spruce (1.251 µmol CO₂·m⁻²·s⁻¹ [78.2 %, P = 0.046, Tukey HSD] mean reduction in spruce vs. 0.563 µmol CO₂·m⁻²·s⁻¹ [37.3 %, P = 0.001, Tukey HSD] mean reduction in fir). Transpiration was reduced, though not significantly, for both species (38.3 and 11.9 % in spruce and fir, P = 0.135 and 0.476, respectively, Tukey HSD). Instantaneous water use efficiency was significantly lower (53 %, P = 0.029, Tukey HSD) in spruce in response to the drought treatment, and while a loss of efficiency (25.9 %) was also found in fir, this effect was not significant (P = 0.127). Similarly, the reduction in stomatal conductance to water vapour, though insignificant, was greater in spruce than in fir (48.5 and 13.5 % in spruce and fir, P = 0.135 and 0.435, respectively, Tukey HSD).

Discussion
Precipitation exclusion shelters were successful at substantially reducing in situ soil water compared to ambient conditions, particularly early in the treatment period when the droughted treatments averaged ~5–8 % VWC and the ambient condition averaged ~8–12 % VWC (Fig. 1). In the absence of water potential measurements, we are unable to unambiguously confirm induction of plant water stress—reductions in soil VWC may not have amounted to meaningful reductions in physiological water availability, and seedlings may have accessed moisture pools deeper than the 5 cm soil horizon that we assessed for water content. However, in tree seedlings of other species, soil moisture levels <10 % VWC are 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. (2018) documented meaningful growth declines in conifer seedlings associated with just a mean 1 % reduction in soil VWC imposed with a heating treatment in a single growing season, indicating that even minor reductions in water availability can have considerable consequences for tree seedlings. The seedlings in the ambient condition in our study also likely experienced some degree of water stress in 2018 (15 July to 15 September) when the study region experienced a moderate drought (NDMG 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 cumulative 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 2 years of summer drought in both spruce and fir seedlings (see Supporting Information—Table S2; Fig. 2). The lack of significant morphological responses 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). Though the seedlings in our study were established, this behaviour could indicate larger individuals of these species continue to have little morphological responsiveness similar to first-year germinant seedlings which appear to prioritize leaf area gain over other structural traits, irrespective of water stress (e.g. Kiorapostolu et al. 2018; Augustine and Reinhardt 2019). 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. In addition, we detected small, non-significant decreases in root mass and significant 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 a range of small seedling sizes may reflect a life-history strategy which emphasizes photosynthesis and growth rates over stress mitigation 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 the subalpine forests of the Rocky Mountain region can result in a strong selection of traits prioritizing carbon gain (Valladares et al. 2007, 2014; Vitasse et al. 2013; Gugger et al. 2015), a pattern which could explain the continued investment in leaf development across seedling sizes despite significant reductions in moisture, as observed in our study.

Given the extremely slow growth rates in these short growing seasons (Veblen 1986), it is also possible that significant morphological adjustments could occur in these species at timescales beyond the 2 years of our study and are likely trait-specific. For instance, McBranch et al. (2019) 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 changes 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. 3), supporting our hypothesis that fir maintains comparatively high levels of physiological functioning under water stress (Knapp and Smith 1981, 1982; Brodersen et al. 2006). In comparison, Gill et al. (2015) found photosynthetic flux was 42 % lower prior to water addition in droughted 3-year Engelmann
spruce seedlings. Similarly, Broderson et al. (2006) noted a ≈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 and 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 with non-proportional reductions in transpiration relative to photosynthesis resulted in significantly lower average instantaneous water use efficiencies in the drought treatments relative to ambient conditions, especially for spruce. However, we did find significantly greater water use efficiencies tied to increasing seedling size, suggesting greater responsivity to water stress with tree age [see Supporting Information—Table S3], as found for 1- to 4-year-old subalpine fir seedlings (Cui and Smith 1991). The lack of a morphological response to reduced water levels by the seedlings in this study 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). Once such a threshold is reached, individuals may begin to display greater morphological and physiological responsivity to environment stress, as has been observed for saplings and adults of these species (Knapp and Smith 1981; Brodersen et al. 2006). While our study, through the investigation of a broad range of seedling sizes reflective of a naturally regenerating seedling bank (ca. 300 seedlings < 5 mm in basal diameter), has provided preliminary evidence on such canalization of stress insensitivity in seedlings well after establishment, assessments along a finer ontogenetic gradient could help resolve uncertainty in the timing and mechanisms by which seedlings develop stress responses more similar to adult conspecifics.

In the subalpine forests of the southern Rocky Mountains, late-successional co-dominance between subalpine fir and Engelmann spruce is maintained by contrasting life-history traits between species. Fir is more dominant in the shady understories of mature forests, substantially outnumbering spruce in the seedbank. However, spruce is more successful at recruiting from the seedling bank into canopy gaps due to faster growth, and with its greater longevity, this allows spruce to remain co-dominant in the overstory (Andrus et al. 2018). Our results show that at the end of two growing seasons of precipitation reduction fir was far less affected physiologically— with much smaller reductions in net photosynthesis and water use. These results suggest the favourability of spruce in higher light could be reduced by its poorer physiological functioning under water deficits. If periods of increasing drought frequency and severity were to exceed its tolerance, spruce may face greater physiological regeneration barriers than fir, potentially leading to disruption of the delicate regeneration dynamics between the two species, with long-term implications for forest structure and function.

**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. This understanding will further illuminate 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 responses to consecutive summer drought in seedlings of the two dominant subalpine 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 were greatly reduced reflecting poor whole-seedling acclimation to water stress, particularly for spruce. Further, increases in above-ground biomass allocation in response to seedling size did not reflect expected shifts towards greater investment in structural traits over carbon gain structures as seedlings grew in size. 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 support 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, which may in turn affect availability of seedlings for recruitment into larger tree size classes, thus disrupting the regeneration dynamics that maintain species co-dominance.

**Supporting Information**

The following additional information is available in the online version of this article—

**Table S1.** Monthly average temperature (°C), total precipitation (mm) and average snow depth (cm) for years preceding (2017) and during (2018–19) study at the field site near Mt. Evans, CO, USA.

**Table S2.** Linear mixed-effects model estimates (β, unstandardized coefficients, SE, standard errors, df, degrees of freedom, T and P-value) of root mass fraction (RMF, g·g⁻¹), stem mass fraction (SMF, g·g⁻¹), leaf mass fraction (LMF, g·g⁻¹), total biomass (TBM, g) and leaf-stem area ratio (LSAR, cm²) modelled individually as a function of diameter, species and drought treatment as fixed effects and plot as a random effect for Engelmann seedlings.

**Table S3.** Linear mixed-effects model estimates (β, unstandardized coefficients, SE, standard errors, df, degrees of freedom, T and P-value) of net photosynthesis (A, μmol CO₂·m⁻²·s⁻¹), transpiration (E, mmol H₂O·m⁻²·s⁻¹), instantaneous water use efficiency (A/E) and stomatal conductance to water vapour (gₛ, mol·m⁻²·s⁻¹) modelled individually as a function of diameter, species and drought treatment as fixed effects and plot as a random effect for Engelmann spruce (P. engelmannii) and subalpine fir (A. lasiocarpa) tree seedlings.

**Figure S1.** Distribution of Engelmann spruce (P. engelmannii, n = 125) and subalpine fir (A. lasiocarpa, n = 171) tree seedling sizes (stem diameter, mm) subjected to ambient and drought conditions.

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**Contributions by the Authors**

A.G. contributed to conceptualization, methodology, formal analysis, resources, writing, visualization, supervision, and funding acquisition. P.H.M. helped in conceptualization, methodology, resources, writing, supervision, and funding acquisition.

**Conflict of Interest**

None declared.
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Data Availability

The data that support the findings of this study are openly available on GitHub at: https://github.com/atgoke/ManuscriptData.

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