



Dominant tree species of the Colorado Rockies have divergent physiological and morphological responses to warming



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ARTICLE INFO

Keywords:

Climate change
Phenology
Photosynthesis
Temperature
Forests

ABSTRACT

Increasing temperatures worldwide, as a primary manifestation of climate change, may cause substantial alterations in forests, presenting a major challenge to predicting responses in forest composition and function. Yet, recent empirical research on climate and forests has found patterns at odds with theoretical and modeling expectations. Indeed, the need for an improved mechanistic understanding of climate's effects on forests is clear but controlled field studies that address this issue are lacking. Montane and subalpine systems may be particularly sensitive to changes in temperature yet quantifying temperature effects in real-world conditions can be challenging. We used three common gardens arrayed over a 1200 m elevation and 6 °C mean annual temperature gradient in the Front Range of Colorado to evaluate the temperature responses of seedlings of three widespread and dominant tree species in Colorado: lodgepole pine (*Pinus contorta* var. *latifolia*), ponderosa pine (*Pinus ponderosa*) and aspen (*Populus tremuloides*). Seedlings for the gardens were sourced from populations naturally occurring at the intermediate elevation on this gradient and were planted into identical soils in all three gardens. We focused on *in situ* photosynthetic performance of seedlings, plasticity in spring phenology, and adjustments in leaf morphology. We found no evidence for clear temperature sensitivities in photosynthetic rates of the two coniferous species, neither across a 6 °C range in growing season temperatures between sites nor across manipulated leaf temperatures of 15–30 °C within sites. Likewise, lodgepole pine exhibited uniform leaf size across the temperature gradient; however, ponderosa pine leaf size did increase significantly at the warmest site. In contrast, aspen displayed pronounced temperature sensitivity in photosynthesis and leaf morphology, with maximum observed values at intermediate temperatures which both declined at the colder or warmer temperatures. Relative to the conifers, aspen also showed reduced phenological responses to warming with ~12% fewer growing days at the intermediate site, and 6% at the warmest site. These divergent responses suggest that warming temperatures can alter seedling success in a number of different ways, and taken together, are likely to alter forest composition of Colorado in favor of greater dominance by montane conifer species.

1. Introduction

There is growing evidence that many forests worldwide are undergoing rapid and substantial change as temperatures increase and drought frequency and duration intensify, leading to disruptions such as warming-induced mass mortality events (Allen et al., 2010; Peng et al., 2011; McDowell et al., 2016) and incipient shifts in tree species' distributions (Woodall et al., 2009; Zhu et al., 2012; Buma and Barrett, 2015; Miller et al., 2017). Yet, our mechanistic understanding of how global change drivers are impacting forests is lacking, as empirically based experimental studies of climate impacts in forests are uncommon and have lagged behind other biomes (e.g. grasslands, Knapp et al., 2002; alpine ecosystems, Walker et al., 2006). A host of models have been developed to address the impacts of climate change on forests,

mainly correlative “climate envelope” models (e.g. Box et al., 1999; Iverson et al., 2004) and ecophysiological process models (e.g. VEMAP, 1995; Bugmann, 1996), and both predict large and rapid shifts in tree species ranges. Yet, there is little consensus on how systems such as forests will respond given the assumptions in these models (Pearson and Dawson, 2003; Guisan and Thuiller, 2005; Canham and Murphy, 2016), especially that species distributions remain in equilibrium with climate.

Assessing warming impacts on the ecophysiology of tree seedlings in controlled environmental conditions is a promising area to address this gap, as ecophysiological responses develop quickly and seedlings are small enough for manipulations, but also because the responses and resilience of forests will depend strongly on the regeneration ecology of tree species under current and future climates. Given rapid rates of environmental change and inherent limits on dispersal – most studies

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Table 1

Climate and soil data for the experimental sites. Annual and growing season values for the year 2016 were calculated directly from a weather station and soil probes at each site. Growing season values were calculated May–September. Long term means (calculated for the period 1986–2011) were from 800-m resolution PRISM climate data (PRISM Climate Group, 2004). Soil data are only available for the growing season.

Site	High elevation	Intermediate elevation	Low elevation
Elevation (m)	2750	2470	1567
Long term mean annual air temperature (°C)	3.7	6.6	9.6
Long term mean annual precipitation (mm)	447	567	409
Mean annual air temperature for 2016 (°C)	4.3	8.0	9.5
Mean annual precipitation for 2016 (mm)	484.8	452.6	426.8
Growing season – mean daily air temperature (°C)	10.1	13.3	16.7
Growing season – precipitation (mm)	183	219	171
Growing season – mean soil moisture (%)	16	14	13
Growing season – mean soil temperature (°C)	13.8	16.5	24.5

report mean tree seed dispersal of less than 20 m for all but small-seeded species (e.g. Ribbens et al., 1994; Clark et al., 1998; LePage et al., 2000; Svenning and Skov, 2007; Martin and Canham, 2010; Siefert et al., 2015) – we argue that tolerance and acclimation to changing conditions *in situ* via a combination of fixed and plastic traits will be as important as range shifts in determining how tree species are impacted by climate change. How a plant species acclimates to future conditions will depend on traits such as species-specific predispositions for maintaining minimal hydraulic safety buffers (Choat et al., 2012), limitations in leaf plasticity to acclimate to carbon seeding (Tjoelker et al., 1998), and inherent constraints on shifts in phenology (Roberts et al., 2015).

The effects of warming temperatures on plant carbon relations may be ameliorated foremost by the acclimation of photosynthetic rates to altered conditions (Smith and Dukes, 2013). Photosynthetic acclimation can occur via shifts in the instantaneous response of net photosynthesis to temperature, as well as through changes in the shape and/or base rate of the response, often resulting in a shift in the temperature optimum (Smith and Dukes, 2013). Indeed, evidence suggests that temperate deciduous tree species have an ability to rapidly acclimate photosynthesis to local conditions (Gunderson et al., 2000). However, more studies conducted *in situ* over longer periods are needed to assess how established seedlings respond to novel growing conditions. A key issue in studying warming effects *in situ* on tree ecophysiology is controlling environmental factors that usually co-vary with temperature (e.g. moisture). These co-varying factors can alter observed rates of photosynthesis and the acclimation response in particular. Empirical efforts to study the direct effects of climate on tree species *in situ* have helped address some of these concerns (e.g. Canham and Thomas, 2010; Buechling et al., 2017), but such gradient approaches on extant trees lack controls and compare across individual trees over the species' range. Given the prevalence of adaptation to local climate conditions (e.g. Leimu and Fischer, 2008; Hereford, 2009), treating a species as ecologically equal across its range is likely to bias predictions of growth under future climates (O'Neill et al., 2008; Angert et al., 2011). Thus, while it is clear that forests are in flux, uncertainty remains with respect to how these dynamics will unfold, as replicated controlled experimental studies of the effects of climate on tree performance have lagged behind the models (Petrie et al., 2016).

In this study, we established a series of experimental common gardens along an elevation gradient to investigate the effects of temperature on tree seedling performance while controlling differences in precipitation, light, soil fertility, soil moisture, topography, exposure, and local adaptation. This approach enables the examination of how markedly different temperature regimes alter the physiological, phenological, and morphological performance of tree seedlings of three Rocky Mountain dominant species – two conifers and a deciduous angiosperm. We focused on the response of three key processes expected to determine the main response and acclimation of tree seedlings to warmer temperatures: photosynthesis, the phenology of spring bud break (Saxe et al., 2001), and leaf morphology (Mahan et al., 1997).

These three traits exhibit comparatively strong plasticity to changes in the environment (Jurik et al., 1988; Körner, 2003; Roberts et al., 2015), and identifying trade-offs in these traits is important for predicting whole-plant performance. Warmer temperatures have been shown to result in increased chlorophyll content and thus photosynthetic output (Ormrod et al., 1999). Alternatively, warming can restrict photosynthesis via stomatal closure due to water stress, yet this relationship is highly species-specific (Saxe et al., 2001). Overall, acclimation of photosynthetic optima has been shown to be plastic and can shift dramatically depending on conditions and species (Battaglia et al., 1996). We hypothesized that the ecophysiology of tree species adapted to warm temperatures would be less impacted by higher temperatures than species adapted to cooler environments, but that each species will have some capacity to shift its optimal temperature of photosynthesis based on local conditions. We predicted increases in growing days with higher temperatures will result in the greatest shifts in phenology for the deciduous species, as light capture is maximized by early leaf production rather than retention in the fall (Cannell, 1989). Lastly, global analyses have suggested that gymnosperms increase foliar mass to total mass ratios with warming temperatures at a higher rate than angiosperms (Reich et al., 2014) and that leaf size increases with warming temperatures for both groups (Way and Oren, 2010). Thus, we predicted the conifers would increase leaf size the most with increasing temperature.

2. Materials and methods

2.1. Common gardens

In the summer of 2014, we established three experimental seedling 'gardens' along a 1200 m elevation gradient along the Front Range of Colorado. The 3 sites range from the lower prairie–treeline ecotone at 1560 m to high elevation forests at 2750 m near the upper alpine–treeline, and encompass 3 major forest types in the region. There is a ~3 °C difference between each site for both mean annual temperature (MAT) and growing season temperature (GST, Table 1). At each site, we selected a flat area with full-sun exposure, removed preexisting vegetation and excavated the top 30 cm of soil from two 18.5 m² plots. To homogenize soils in each garden, 30 cm of topsoil was collected from the intermediate temperature site and transported to both the warmest and coldest sites to fill in the excavated plots and tilled using a front-tine tiller. The soil in the intermediate temperature site was excavated in the same manner, returned to the plots, and tilled. Large rocks were removed but no other treatments were performed on the transplanted soil.

We studied 3 tree species characteristic of the montane forest zone (1650–2750 m) – lodgepole pine (*Pinus contorta* var. *latifolia*), ponderosa pine (*Pinus ponderosa*), and quaking aspen (*Populus tremuloides*). Seeds from these tree species were gathered from a single stand for each species in National Forests by the Colorado State Forest Service Nursery (Fort Collins, CO) and germinated in the spring of 2013. Seeds of

lodgepole pine and aspen were gathered at 2450 m and 2600 m, respectively, and ponderosa pine seed was collected at 2250 m. By selecting populations from a single source at mid-elevation, we controlled for the effects of local adaptation allowing our study to mimic how climate change actually operates – with an altered climate for individual trees – rather than studying climate's effect across individuals along environmental gradients as is done in observational studies where local adaptation to climate might confound the results.

The planting pattern at each garden was identical. One year-old seedlings within a species-specific height range were randomly selected from the nursery stock. Each 18.5 m² plot was planted in a 9 × 9 grid with ~23 cm separating each seedling to reduce effects of initial root competition and shading amongst seedlings. The shortest species at time of planting were placed in the southern-most row of each plot followed in order of increasing height to minimize shading. Each plot repeats this pattern twice and staggers one of those sets to allow a total of 81 seedlings per plot (162 seedlings per site). Seedlings were planted in the last week of June 2014, mulched 5 cm deep with untreated chipped wood, and watered to field capacity. Seedlings were watered bi-weekly for the rest of the first growing season to isolate temperature effects and to reduce transplant shock, and plots were weeded bi-annually. The same watering regimen was repeated in 2015, and then it was reduced to approximately once per month in the summer of 2016. Mulch was reapplied once in 2015 after snowmelt to reduce weeds. Beginning in June 2015, data-logging meteorological stations were established at each site to measure air temperature, rainfall, and soil temperature and moisture. The stations had 4 soil volumetric water content and temperature probes per site placed 5cm deep in the soil (Decagon 5TM Soil Moisture and Temperature Sensor), as well as one tipping bucket and air temperature probe per site (Onset HOBO RG3-M).

2.2. Photosynthetic performance

Photosynthetic responses to temperature was assessed from July to early September 2016. From sunrise to midday, we manipulated temperature and assessed photosynthetic rate using a LI-COR 6400 Portable Photosynthesis System on a minimum of 10 individuals per species per site. Under saturating light conditions (1000 μmol m⁻² s⁻¹, LI-COR 6400-02B LED Light Source) and a relative humidity of 40–60%, leaf temperature was increased in 5 °C increments from 15 to 30 °C and photosynthesis was allowed to stabilize before repeated measurements were taken for 2 min and then averaged. The most recent fully expanded leaves were used – 2016 leaves from aspen and a cluster (typically 6) of 2015 needles from the conifers. Leaves were marked between sampling temperatures to ensure the same sets of leaves were used in each estimate of photosynthesis (Ps) and removed from the plant at the end of analysis. The aspen leaves filled the leaf chamber while the projected leaf area of the excised conifer needles was measured using ImageJ software to determine leaf area-corrected Ps estimates. A site-level mean was calculated for each species from every measurement of a species taken at a site, providing a typical photosynthetic response across reasonable daytime temperatures. Additionally, differences in photosynthetic output at each temperature were evaluated within species across the sites. As well as absolute photosynthetic responses, we report a percent of maximum observed photosynthesis where all values are scaled to the highest value for that species for that site (i.e., if the maximum average value for lodgepole at the warmest site was 10 μmol m⁻² s⁻¹ at a leaf temperature of 20 °C and 4 μmol m⁻² s⁻¹ at all other leaf temperatures, we would report 100% at 20 °C and 40% at all other temperatures).

2.3. Spring bud break

Beginning in early April 2016, seedlings were assessed at least twice a week for signs of bud break. We recorded the first date where new leaf

tissue was observed from an opening terminal or axillary bud to assess initiation of annual growth (Shepherd, 1983 – development stage 4), and then estimated growing season days for each species at each site. The start of the growing season was calculated from the date when 50% of the seedlings at a site had broken dormancy while the end of the growing season was arbitrarily set as September 30th. We focused on spring bud break as it displays a dynamic response to climate and is a readily observable phenological response across both deciduous and evergreen species. In contrast, end of season leaf senescence is strongly correlated with photoperiod rather than climate (Lee et al., 2003), though the mechanisms driving dormancy remain unresolved (Richardson et al., 2013).

2.4. Leaf size

Leaf size was estimated via measuring length and width of 10 randomly selected mid-canopy leaves in full sunlight per seedling using microcalipers. The most recent cohort of fully expanded leaves was used – 2016 for aspen, 2015 for conifers. Leaf size per seedling was determined by multiplying length by width for each of 10 leaves/needles then averaged. We did not measure specific leaf area as only non-destructive sampling was appropriate given the sensitivity of seedlings to any defoliation.

2.5. Statistical analyses

Absolute photosynthetic rates and leaf size were both analyzed using one-way ANOVAs using site as the grouping variable. To test for influence of Julian date or air temperature on estimates of photosynthetic rates, we used a multiple linear regression using site, species, air temperature, and Julian date as predictors. These analyses were performed using R software (R Core Team, 2016), the package 'dplyr' (Wickham et al., 2017). Differences between sites within species were further investigated using Tukey's HSD comparisons of means with significance values set at $p < 0.05$.

3. Results

3.1. Photosynthetic performance across sites

Comparing across sites, we observed that average Ps rates differed for aspen and the conifers. The coniferous species showed low variation in mean Ps across the range of elevations (Fig. 1) (lodgepole: $F = 0.733$, p -value < 0.482 ; ponderosa: $F = 0.26$, p -value < 0.772), though lodgepole pine displayed a higher Ps rate than ponderosa pine in each garden. In contrast, Ps in aspen displayed a clear maximum (15.7 μmol m⁻² s⁻¹) at the intermediate temperature site– which is closest to its source location ($F = 61.35$, $p < 0.001$). Non-manipulated ambient factors (soil moisture and time of day) were not significant predictors of photosynthetic rate, while air temperature outside the chamber and calendar date were significant ($p < 0.05$) but not predictive ($R^2 = 0.02$ and 0.009 respectively).

3.2. Photosynthetic performance within sites

Within-site Ps of aspen displayed marked sensitivity to temperature variation only at the coldest site, with the Ps rates being significantly higher at 15 and 20 °C than 25 or 30 °C ($p < 0.001$) (Fig. 2). Similarly, lodgepole pine only displayed within-site sensitivity to temperature variation at the coldest site, with a significant decrease in Ps rate at 30 °C ($p < 0.01$). At the intermediate and warmest sites, none of the species showed significant responses to variation in temperatures. However, there was a non-significant trend for aspen of declining Ps with increasing temperatures at the warmest site. Ponderosa pine did not exhibit significant photosynthetic sensitivity to temperature at any of the sites, but showed a trend of declining Ps with increasing

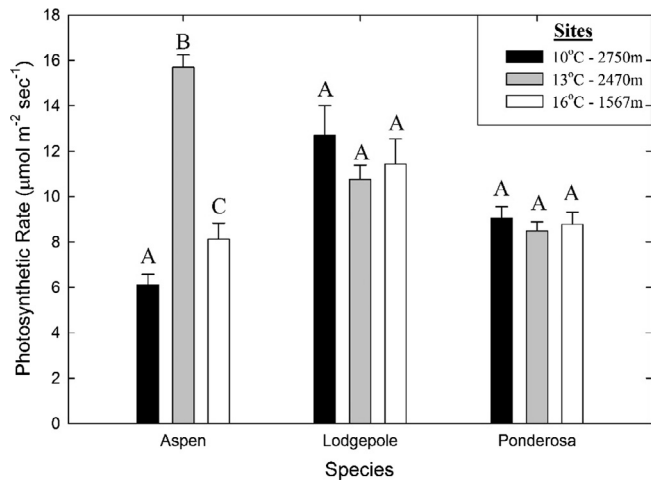


Fig. 1. Mean photosynthetic rates for seedlings of three tree species growing experimentally at three montane sites spanning a range of 6 °C. Photosynthetic rates were measured over a fixed range in experimental temperatures (15–30 °C) in each site. Site temperatures are the mean growing season temperature in 2016. Letters denote significant differences within a species based upon Tukey's HSD and bars indicate 1 standard error.

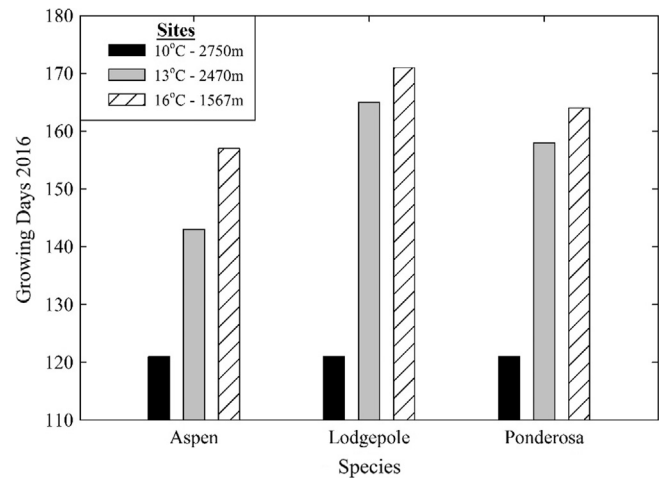


Fig. 3. Growing days by species by site in 2016. Growing days were calculated as the number of days between the date of bud burst (when at least 50% of the individuals of a species at a garden had bud burst) and the last day of September. The estimate of growing days at the warmest site for lodgepole pine may be an under-estimate as nearly all lodgepole had already initiated bud burst on the first day of sampling.

temperatures at the coldest site and a decline in Ps at 30 °C at the warmest site.

3.3. Spring bud break

The number of growing days increased with mean growing season

temperature for all species (Fig. 3). The estimated growing season length was identical for all species at the coldest site. The conifers displayed a strong response in growing season length to the 3 °C of warming between the coldest and intermediate sites (40.5 days increase on average), yet experienced a smaller increase with an additional 3 °C moving between the intermediate and warmest sites (6 additional days for both species). Aspen's phenology increased by 22 days between the

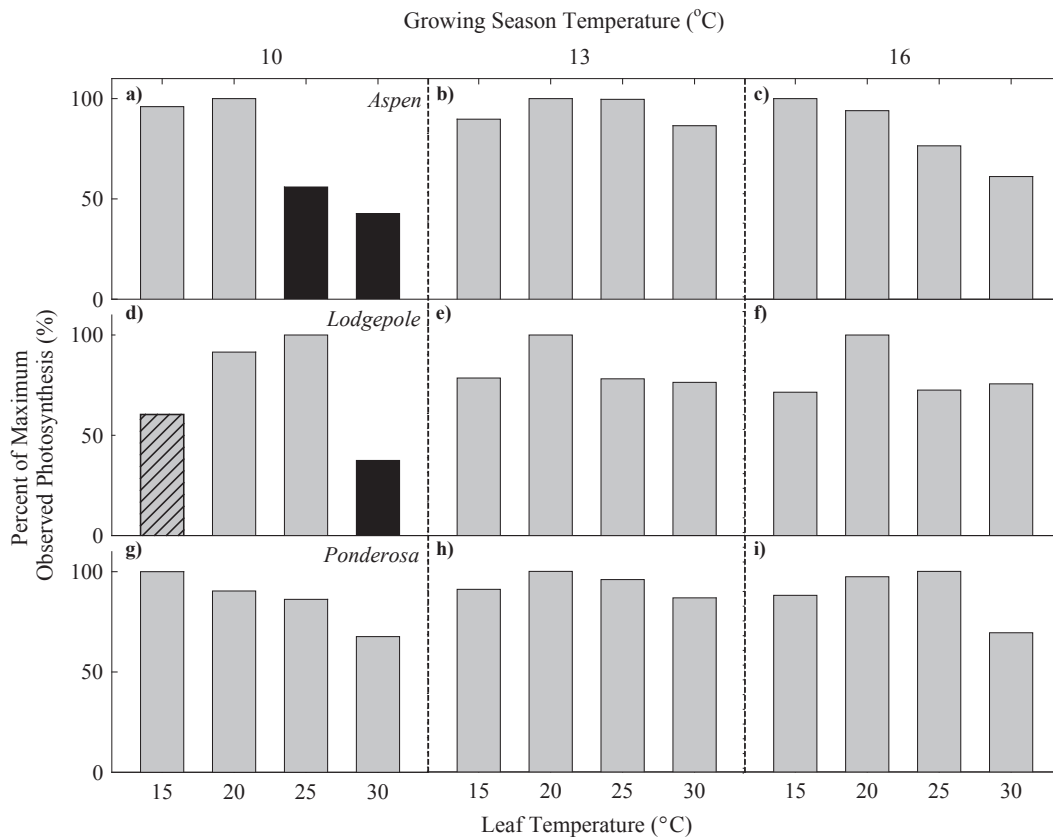


Fig. 2. Mean percentage of maximum photosynthesis measured over a fixed range in experimental temperatures (15–30 °C) at three sites. Different shading of bars denotes statistically significant differences (panels a and d) while cross-hashing (panel d) denotes an intermediate response. Mean absolute photosynthetic responses were calculated per temperature per species per site, and then scaled to be a percentage of the largest average per species per temperature per site combination. Columns denote site moving from coolest to warmest (left to right). Rows indicate species alphabetically.

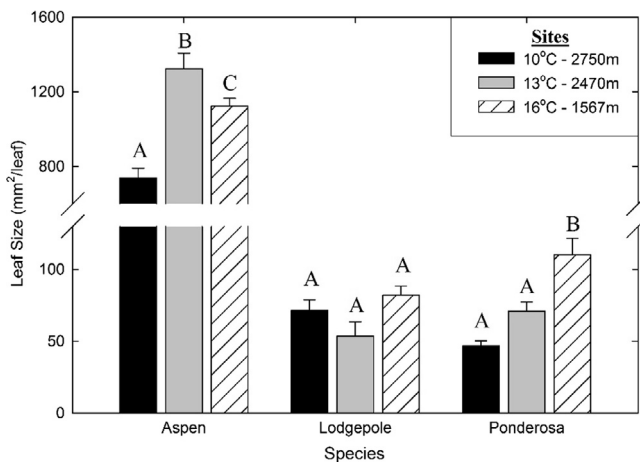


Fig. 4. Mean leaf sizes of fully expanded leaf tissue. Letters denote significant differences within a species based upon Tukey's HSD and bars denote 1 standard error.

coldest and intermediate sites, and 14 days between the intermediate and warmest sites, yet its growing period was shorter than both conifers at the intermediate and warmest sites. These results indicate divergent responses to the effects of warming temperatures on spring bud break per species.

3.4. Leaf size

Needle size varied non-significantly for the coniferous species across the sites, except at the warmest site where ponderosa pine needles were significantly larger (Fig. 4) (lodgepole: $F = 2.084$, $p < 0.135$; ponderosa: $F = 11.81$, $p < 0.001$). In contrast, aspen leaf size was significantly different at each site ($F = 25.74$, $p < 0.001$), with the largest size at the intermediate temperature site and the smallest size at the warm site indicating a clear temperature sensitivity in foliar morphology.

4. Discussion

Facing a rapidly changing environment and shifts in climatic niches in the western US (Lenoir et al., 2009; Bell et al., 2013; Redmond et al., 2015), tree species must migrate with climate, tolerate new conditions, and/or acclimate to novel environments to survive (Aitken et al., 2008). Increasingly, large-scale models predicting species distributions are incorporating within-species variability due to trait plasticity and local adaptation (Valladares et al., 2014; Buechling et al., 2017), and finding that disentangling those two drivers of trait fluidity are both critical for accurate predictions and require controlled replicated *in situ* experiments (Moran et al., 2016). By minimizing differences in local adaptation in our common garden experiment we provide evidence for trait plasticity and the underlying temperature sensitivities of some of the key drivers of seedling growth. We observed three key findings that suggest, as expected, species will not respond uniformly to climate change (Hamann and Wang, 2006). First, aspen displayed a strong degree of photosynthetic plasticity among sites and in leaf size, with both traits achieving their highest values at the intermediate elevation site closest to their seed source location. In contrast, there were no strong Ps patterns for lodgepole pine or ponderosa pine across the sites, though ponderosa pine did increase leaf size at the warmest site. Second, we observed a nearly uniform within-site Ps temperature response for all species between 15 and 30 °C suggesting that fluctuations corresponding to within-day variation in leaf temperature will have minimal effects on plant performance; rather, our data suggest that it is long-term site level temperatures that will impact seedling photosynthetic rates and presumably whole plant performance. Last, the two conifer species demonstrated greater phenological plasticity to

temperature than aspen. A warming of 3 °C from the coldest to the intermediate temperature site increased the growing season for the conifers by nearly three weeks more than for aspen, while the next 3 °C of warming resulted in limited increases for all species.

We originally hypothesized that we would see clear temperature optima that were plastic and responsive to shifts in the growing environment. We found no evidence to suggest this was the case for the species investigated here as we were unable to detect site-specific differences in instantaneous Ps over a range of 15–30 °C (Fig. 2). Interestingly we did observe differences between sites for aspen but not for lodgepole pine or ponderosa pine (Fig. 1), suggesting plasticity is impacting the photosynthetic process at the site level but not to instantaneous or short-term changes in leaf temperature. Turnbull et al. (2002) found a similar pattern in that warming during the day had minimal effects on photosynthetic or respiration rates in *Populus deltoides*, yet warming during both day and night resulted in plastic responses to temperature due primarily to upregulation of the photosynthetic pathway ($V_{C_{max}}$ and J_{max}). Additionally, the weak site-level acclimation of the Ps response we observed in aspen was also found in previous studies of temperature sensitivity and acclimation of Ps in the genus. These studies reported minimal evidence of photosynthetic acclimation – particularly to cooler temperatures – in *P. deltoides* in growth chambers (Ow et al., 2008), or in *P. tremuloides* in common gardens (Dillaway and Kruger, 2010). For acclimation in conifers, Teskey and Will (1999) reported that Ps shifted directionally with temperature in loblolly pine (*Pinus taeda*) grown in growth chambers, with peak Ps occurring at the temperature at which they were established, indicating strong acclimation potential. Likewise, mature Norway spruce (*Picea abies*) exposed to warmer temperatures in whole tree chambers at the Flakaliden research site in Sweden experienced relatively flat temperature photosynthesis response curves (Hall et al., 2013).

As expected, growing days increased with increasingly warmer temperatures. Our results support our hypothesis, although there were some important differences in the effects on warming between the species. A warming of 3 °C from the coldest to the intermediate temperature site started the conifers' growing season nearly three weeks earlier than for aspen – perhaps because an evergreen life form allows for a more rapid end to dormancy as conditions change, at least compared to a deciduous species – while the 3 °C of additional warming resulted in limited increases for all species. This trend suggests marginal increases in temperatures would benefit the montane conifers, rejecting our hypothesis that aspen would benefit the most from increasing temperatures. These findings are similar to the 3 °C warming experiments at Flakaliden which found a two to three week increase in the growing season of Norway spruce due to earlier spring activity (Slaney et al., 2007), and this longer growing season was considered the main reason annual leaf carbon uptake increased 44% in the warmed conifers (Hall et al., 2013). This increased carbon uptake was significant over the growing season despite changes in other costs at higher temperatures including increased respiratory demand and non-photo chemical quenching. Budbreak also has been shown to be strongly regulated by temperature, rather than genotypic differences, in lodgepole pine (Liepe et al., 2016) and *Populus tremula* (Luquez et al., 2008), suggesting that the differential responses of these species are a question of plasticity rather than local adaptation.

While leaf size generally increased with warming as we hypothesized, the response was species-specific with the highest level of leaf plasticity in aspen. Aspen leaf width and length have been shown to be a highly heritable trait between populations (Kagana et al., 2008). As our design controlled for local adaptation by using seedlings from a single stand, our results suggest that not only is this trait heritable, but it is highly plastic within a population as well. This is in contrast to another study in Colorado, however, where aspen displayed minimal plasticity across an elevation/aridity gradient while ponderosa pine decreased in leaf size with decreasing elevation (Anderegg and

HilleRisLambers, 2016). These contradictory results may arise from different study designs, where co-variation in temperature and aridity along gradients in Anderegg and HilleRisLambers (2016) may exert a counteracting influence on leaf traits. That said, Olszyk et al. (2005) found no changes in leaf area or leaf perimeter in Douglas fir (*Pseudotsuga menziesii*) when they isolated the impact of temperature on leaf size in outdoor growth chambers for 4 years. These results mirror our findings for lodgepole pine, reinforcing the conclusion that species responses to environmental change likely will be species-specific and idiosyncratic even in controlled studies.

Taken together, our results suggest warming in the next century will benefit ponderosa pine and lodgepole pine based on the temperature sensitivities and plasticity of the traits investigated herein. Minimal reductions in photosynthetic rates with warming coupled with a higher degree of phenological plasticity suggest that these species will fare better under a warmer climate than aspen. Recent trends in aspen distribution and stand dominance in Colorado support a hypothesis of aspen decline, as numerous studies have found substantial declines in aspen abundance across the state (Worall et al., 2010; Worrall et al., 2013; Coop et al., 2014; Bretfield et al., 2016), with the decline most directly linked to increased aridity, droughts in particular.

Broader extrapolation of these results must be done with some caution, as the long-term implications of this study are unclear. For example, phenological plasticity does not necessarily equate to increases in tolerance or range shifts in all cases (Duputié et al., 2015), nor do mean temperatures explain all phenological shifts as other factors like photoperiod (Marchin et al., 2015) or chilling temperatures (Roberts et al., 2015) can contribute substantially. More fundamentally, this study isolates temperature, but other variables (e.g. moisture) will interact with warming temperatures to determine the net impact of environmental change on these species, and the inability of the gymnosperms to shift photosynthetic rates and needle size could be detrimental under some combinations of environmental change. In this context, plasticity in aspen leaf sizes could be interpreted as a drought avoidance mechanism, where smaller leaves improve water transport efficiencies in drier conditions. Finally, natural disturbances – such as fire and insect outbreaks, which may be increasing in conjunction with climate change – have been shown to increase aspen abundance in Colorado (Buma and Wessman, 2012; Kulakowski et al., 2013), while increases in fire intensity (Harris and Taylor, 2015) are reducing regeneration, particularly at lower elevations, of ponderosa pine (Chambers et al., 2016), Douglas fir and lodgepole pine (Rother and Veblen, 2016).

4.1. Conclusions and future considerations

Our results suggest that of these species, aspen is likely to be the most sensitive to future climate change, as its highest P_s occurred unambiguously in the location and climate of its current seed source and declined markedly at higher temperatures. Despite a longer growing season at higher temperatures, we predict that the strong declines in leaf size and photosynthetic output for aspen at higher temperatures will lower or even restrict growth in this species. In contrast, we predict that conifer performance will benefit from earlier phenology in the warmest site (Fig. 3), as these conifer species appear to be insensitive in leaf size and photosynthetic output across the range of temperatures. This suggests the possibility of divergent trajectories for these species under warming temperatures, favoring the coniferous species while limiting aspen with considerable implications on forest composition and species distributions in Colorado.

Overall, our common garden approach has allowed us to explore the temperature sensitivities of three of the dominant tree species in Colorado in a controlled yet real-world abiotic environment. We observed two contrasting response patterns which mirror the known life-history characteristics of these species. Aspen displayed strong temperature sensitivities in P_s and in leaf size to warming while the

coniferous species benefited substantially via increased growing season length. Accurate predictions of forest distributions and composition will require inputs of whole-plant growth as well as physiological and anatomical characteristics of the primary drivers of seedling performance.

Acknowledgements

We thank Colorado State University Mountain Campus and the Colorado State Forest Service for allowing us to establish our common gardens on their properties. We would also like to thank the Colorado Forest Restoration Institute for aiding in data collection and Dr. Troy Ocheltree for providing equipment necessary to complete this work. This research has been supported by funding from the Colorado Forest Restoration Institute and the Graduate Degree Program in Ecology at Colorado State University.

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