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ECOPHYSIOLOGY OF PONDEROSA PINE SEEDLINGS AFTER SEVERE FIRE: THE EFFECT OF TREE CANOPY

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by

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THESIS

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ABSTRACT

Ponderosa pine forests evolved with low-severity surface fires to maintain forest structure and process. However, increasingly common high-severity fires often result in complete overstory canopy loss and a shift from forest to shrub- and grass-dominated vegetation that may persist as an alternate stable state due to limited rates of ponderosa seedling regeneration in severely burned areas. Though overstory tree canopy is an important driver of understory plant response, little is known about how the absence of tree canopy following severe fires interacts with changes in year-to-year climate to affect the physiological function of ponderosa pine seedlings. This field study tested whether canopy presence or absence after fire interacts with drought to affect the photosynthetic efficiency, carbon-water balance, and soil nitrogen of ponderosa pine seedlings across three growth years. Photosynthetic efficiency was measured with chlorophyll fluorescence, carbon-water balance was measured with carbon isotopes and stomata density, and soil nitrogen cycling was measured with nitrogen isotopes. Chlorophyll fluorescence results showed no significant difference in photosynthetic efficiency by canopy condition or growth year, though measurements throughout the season are needed to test this question further. Stomata density and carbon isotope results showed a disproportionate effect of overstory canopy absence on the carbon-water balance of seedling foliage in a drought year. Despite increased stomata density, seedlings without overstory canopy had significantly higher carbon isotope ratios. This was most likely due to decreased stomatal conductance as a result of water limitations during the drought. Nitrogen isotopes were also significantly higher for seedlings without overstory canopy, indicating increased rates of soil nitrification. Together these results indicate that canopy loss from high-severity fire has a physiological impact on developing tree seedlings, resulting in disproportionately greater water-stress in a drought year. This finding adds a new perspective on the importance of low-severity fire as a management strategy to increase ponderosa pine forest resiliency; by maintaining overstory canopy, seedlings may be buffered from the physiological stress associated with droughts, which are projected to become more common in the southwest as climate changes.

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INTRODUCTION

Ponderosa pine forests of the semi-arid southwestern U.S. are experiencing a dramatic shift from the historic fire regime of frequent, low-severity surface fires to increasingly common high-severity stand-replacing fires (Swetnam and Baison 1996; Covington and Moore 1994; Allen et al. 2002; Westerling et al. 2006). Low-severity fires maintain the structure of ponderosa pine forests by leaving mature canopy trees intact while thinning understory saplings, shrubs, and grass (Brown and Wu 2005). In contrast, high-severity fires kill mature trees and shift the vegetation from forest to a shrub- and grass-dominated community that may persist indefinitely as an alternate stable ecosystem state (Savage and Mast 2005; Coop et al. 2016; Guiterman et al. 2017). High-severity fire is traditionally defined by the loss of 75% or more of the tree basal area in a forest (Hanson et al. 2009). From 1984 to 2015, 26% of the 3.2 million hectares of southwestern ponderosa pine forests have burned, and 13% of these fires were categorized as high-severity (Singleton et al. 2019). Recolonization of ponderosa pines into severely burned areas is limited by several interacting factors, including distance to mature seed-producing trees (Owen et al. 2017; Chambers et al. 2016), unfavorable climatic conditions for reproduction and germinant survival (Petrie et al. 2016; Stevens-Rumann et al. 2018), and competition with grasses and shrubs (Elliott and White 1987). Efforts to reforest high-severity burn areas by planting greenhouse-grown ponderosa seedlings are also limited in success due to high seedling mortality rates (Ouzts et al. 2015).

A potential cause of low seedling success in severely burned areas is the change in microclimate resulting from the near-complete loss of overstory tree canopy. Broadly, overstory canopy provides physiological costs or benefits to developing seedlings depending on the regional climate (Bertness and Callaway 1994; Holmgren et al. 1997). In environments where water is not limiting and vegetation is abundant, shade from overstory plants can limit photosynthesis and growth, serving as a competitive (negative) interaction (Kitajima and Fenner 2000). In dry environments with high irradiance and hot temperatures, plant canopy can reduce the heat load, transpiration rate, and photoinhibition of seedlings, thus serving as a facilitative (positive) interaction (Holmgren et al. 2012; Valladares and Pearcy 1997). As such, there are several examples of arid-adapted species that rely on the partial shade of 'nurse' plants for successful establishment (e.g. Drezner and Garrity 2003; Valiente-Banuet and Ezcurra 1991; Floyd et al. 2015; Redmond et al. 2018). For example, 66% of piñon seedlings and 71% of juniper seedlings sampled along transects in New Mexico occurred under the canopy of mature trees, suggesting that the facilitation of favorable microsites by overstory trees is a strong driver of successful piñon-juniper regeneration in the southwest (Floyd et al. 2015; Redmond et al. 2018). Similar trends have been found for ponderosa pines. At a site in South Dakota, ponderosa pine seedling density was greatest within the partially shaded canopy edge between unburned and burned forest (Bonnet et al. 2005). Fifteen years after a fire in Arizona, regeneration of ponderosa pines occurred under the unburned tree canopy, but none occurred in areas that burned at high-severity, despite proximity to cone-producing trees (Stoddard, et al. 2018).

Low rates of ponderosa pine establishment in severely burned areas without canopy cover are further exacerbated by drought and hotter temperatures caused by climate change. Drought combined with the canopy-less environment after severe fire results in decreased establishment of ponderosa seedlings due to water stress from lower soil moisture (Savage and Mast 2013). Moreover, drought effects are non-linear with respect to light availability; at highest and lowest light levels, drought effects are the most severe (Holmgren et al. 2012). Thus, intermittent shade provided by overstory tree canopy may be even more important to seedlings during times of drought.

These correlations between ponderosa pine regeneration and canopy cover suggest that the resulting changes in microclimate from canopy loss likely have important physiological consequences for developing tree seedlings (Scheffer et al. 2001). The presence of overstory canopy decreases the soil surface temperature during hot months while increasing soil temperatures during cold months (Breshears et al. 1998). This has the effect of reducing overheating in the summer and reducing frost damage in the winter (Scowcroft et al. 2000). In response to increasing temperature, ponderosa pine seedlings increase transpiration to dissipate heat, resulting in a stem temperature up to 15°C lower than surrounding air (Kolb and Robberect 1996). However, this capacity to dissipate heat is limited by low soil moisture, which significantly reduces the rate of water loss through stomata (i.e. stomatal conductance) (Kolb and Robberect 1996; Novick et al. 2016).

Overstory tree canopy also facilitates improved water relations; the soil beneath tree canopy has a higher water potential, higher daytime volumetric water content, and lower summer potential evaporation (Breshears et al. 1998; Breshears et al. 1997; Vazquez and Bashan 2011). Furthermore, the presence of overstory canopy may facilitate hydraulic redistribution of water from deeper to shallower soil layers, especially benefitting seedlings during dry periods (Brooks et al. 2002). In a study of ponderosa pine seedlings beneath old-growth ponderosa trees in Oregon, 35% of upper soil water appeared to be replaced by hydraulic redistribution during the dry months of July and

August, which allowed stored water to remain in the soil for an additional 21 days (Brooks et al. 2002). The hydrogen isotope values of xylem water in these seedlings indicated utilization of redistributed water during this period (Brooks et al. 2002). Developing tree seedlings are especially sensitive to differences in water availability since they lack the large root networks to access deep soil moisture. More available soil water results in higher xylem water potential, decreased occurrence of cavitation, and ultimately reduced risk for hydraulic failure and mortality (Sperry et al. 2002). More soil water also enables plants to keep stomata open, allowing for higher rates photosynthetic carbon gain (Farquhar and Sharkey 1982) and higher rates of transpiration to maintain optimal leaf temperature (Urban et al. 2017).

The interaction of hotter summer temperatures and reduced water availability experienced by seedlings without overstory canopy affects stomatal conductance, altering the carbon-water balance of plants (Valladares and Pearcy 1997; Novick et al. 2016). One way to quantify stomatal conductance over the lifetime of a leaf is by measuring the ratio of carbon isotopes (^{13}C:^{12}C) in plant tissue. During photosynthesis, Rubisco enzymes discriminate against the heavier ^{13}C, resulting in tissue that is depleted in ^{13}C relative to the ambient air. ^{13}C discrimination is directly proportional to the ratio between the internal (c_i) and the ambient (c_a) CO_2 concentration, which reflects the balance between stomatal conductance and the photosynthetic capacity (Farquhar et al. 1989). This relationship is defined as:

$$\delta^{13}C_{\mathrm{plant}} = \delta^{13}C_{\mathrm{air}} - a - (b - a)c_i/c_a$$

where *a* (4.4‰) is the fractionation occurring during diffusion in the air and *b* (27‰) is the net fractionation resulting from carboxylation in C_3 plants (Farquhar 1989).

The $\delta^{13}C$ values are useful for retrospectively determining water stress (Farquhar et al. 1989). In arid environments like the southwest, drought avoidance via stomatal closure is an important mechanism for survival and growth (Zhang et al. 1997). During drought-induced stomatal closure, C_3-photosynthetic plants including ponderosa pine discriminate less against ^{13}C than ^{12}C, resulting in tissue with a comparatively higher proportion of ^{13}C (Farquhar 1989).

For C_3 plants, stomatal density and $\delta^{13}C$ are closely linked to carbon fixation and water status. In more arid environments, modifications to these traits can help plants avoid drought stress (Sack and Holbrook 2006). An increase in stomata density allows greater rates of CO_2 diffusion into the leaf which decreases the ratio of ^{13}C to ^{12}C.

In addition to experiencing increased water stress, seedlings growing in microclimates following severe fire receive more light than adjacent unburned areas and thus may experience increased photochemical stress. Though usually considered shade-intolerant, ponderosa pine seedlings are known to benefit from intermittent shade produced by nearby mature trees (Oliver and Ryker 1990), and in silviculture, ‘side’ shading of seedlings from nearby tree canopy was deemed essential for producing large, vigorous trees (Pearson 1950). Though light availability is positively correlated with seedling growth, excess light in combination with drought- and heat-stress damages photosynthetic proteins, causing photoinhibition of photosynthesis (Barber and Andersson 1992).

Chlorophyll fluorescence is a useful method to study plant photochemical stress.

Fluorescence measurements of the dark-adapted ratio of variable to maximum fluorescence (F_v/F_m) indicate the maximum potential quantum efficiency of photosystem II. A decrease in F_v/F_m indicates a decrease in the fraction of available photosynthetic reaction centers (Genty, et al. 1989), which indicates overall plant stress. The light-adapted ratio of variable to maximum fluorescence (F_v'/F_m') indicates the proportion of light absorbed by chlorophyll in photosystem II (Genty et al. 1989). A decrease in F_v'/F_m' indicates less light is absorbed by the plant and indicates a decrease in overall photosynthesis (Maxwell and Johnson 2000).

Canopy loss from high severity fire also affects soil nitrogen cycling (Kurth et al. 2014). A change in N-cycling alters the amount of available soil nitrogen, which has important ecological consequences since nitrogen is required for growth and reproduction of ponderosa pines (Heidmann 1984). Nitrogen isotopes of plants, measured as the ratio of ^{15}N to ^{14}N ($\delta^{15}N$), provide insight into N-cycling state because the $\delta^{15}N$ value of foliage generally reflects the $\delta^{15}N$ value of the soil (Craine et al. 2015). Following fire, net nitrification rate by soil microbes is often elevated (Wan et al. 2001), which fractionates against ^{15}N and subsequently enriches the NH_4^+ pool available to plants (Robinson 2001). These elevated nitrification rates usually result in a short-term increase in foliar ^{15}N, reaching a maximum just one year after a burn (Wan et al. 2001). However, after severe stand-replacing fires in southwestern ponderosa forests, nitrification rates may remain elevated for decades (Kurth et al. 2014).

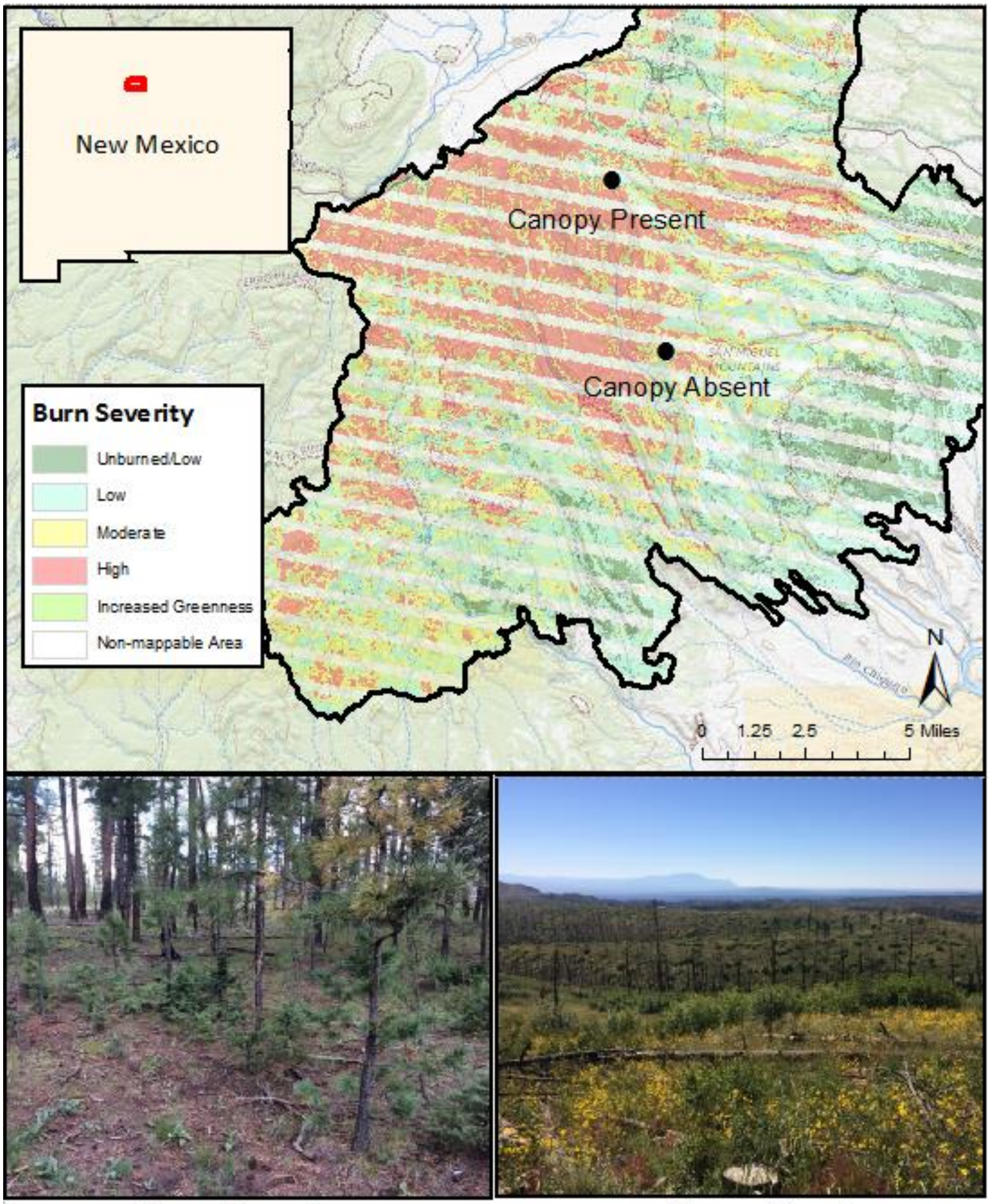
To better understand how canopy loss following severe fire affects ponderosa pine seedling physiology, this study tests whether the absence of overstory tree canopy following severe fire results in greater physiological stress of ponderosa pine seedlings and whether this stress is more pronounced in a drought year. Specifically, this study tests the hypotheses that seedlings in growing in a severely burned area (canopy absent) will exhibit increased water stress indicated by higher $\delta^{13}C$ and increased light stress indicated by lower efficiency of photosystem II compared to seedlings growing in a low-severity burned area (canopy present). These physiological parameters are measured retrospectively across three years of foliage to test whether stress is exacerbated in a drought year. Though previous studies have correlated seedling success with presence of overstory canopy, there is limited research of the specific physiological effects of canopy on ponderosa pine seedlings (but see Bansal et al. 2014). Thus, this study provides a novel field-based test of the potential facilitative ‘nurse plant’ effects of overstory canopy on ponderosa pine seedling physiology.

MATERIALS AND METHODS

Study area

This study was conducted in the Jemez Mountains of northern New Mexico within the perimeter of the 2011 Las Conchas fire (Figure 1). The fire burned more than 156,000 acres, with 30% categorized as high-severity burn (MTBS 2013). Prior to the fire, the study area was dominated by ponderosa pine (*Pinus ponderosa* var. *scopularum*) and mixed-conifer forest (Muldavin et al. 2011). Following the fire, the severely burned areas show minimal conifer regeneration and are dominated by grasses and two resprouting shrub species, Gambel oak (*Quercus gambelii*) and New Mexico locust (*Robinia neomexicana*). The climate is semi-arid and continental, with up to 50% of annual precipitation occurring as monsoonal rains from July through September (Sheppard, et al. 2002). Soils are well-drained sandy loam (NRCS 2018).

Figure 1. Location of sampling sites within the low-severity burned area (canopy present) and high-severity burned area (canopy absent) of the 2011 Las Conchas Fire in the Jemez Mountains of New Mexico, USA.



Canopy Present

Canopy Absent

Sampling design

A total of 20 ponderosa pine seedlings were sampled from two contrasting tree canopy conditions: 10 seedlings naturally regenerating under existing forest canopy (35.8195, -106.4401; elev. 2664m) and 10 seedlings planted by the U.S. Forest Service in 2013 in a severely burned area lacking forest canopy (35.7709, -106.4216; elev. 2401m; USFS 2012). To minimize differences in local precipitation and temperature, the sample sites are less than 10km apart and differ by 263m in elevation. For reference, Ponderosa pines span >1,000m of elevation range in the southwestern U.S., growing from 1,700m to 3,000m (McDowell et al. 2010; Savage and Mast 2005).

Sampling occurred in September 2018 to ensure full needle expansion and incorporation of summer monsoon rains. From each seedling, two fascicles of needles were collected from the current year (2018), one year ago (2017), and two years ago (2016). Needle age was determined by distinguishing annual growth whorls along the main seedling stem (Powell 2009). Needles were consistently collected from the south-facing side of the canopy because δ^{13}C values of foliage in conifer trees differ between south- and north-facing canopy positions (Chevillat et al. 2005).

Microclimate and Climate

For all seedlings sampled, volumetric soil water content in the top 12cm of soil was measured with a HydroSense 620 soil water probe (Campbell Scientific) within 10 cm of the seedling base, averaged across four cardinal directions. Vegetative cover was visually estimated for 1m^2 around each seedling. Seedling height and basal stem diameter were measured. Total canopy cover above each seedling was estimated from photographs taken with an iPhone 5 and analyzed in the Percent Cover app (Public Interest Enterprises, 2017). Seedlings in the high-severity burned area had 0% canopy cover. Seedlings in the low-severity burned area had mean percent canopy cover of 35%.

To test the effect of precipitation on the interannual carbon isotope signatures, mean monthly climate data were downloaded from PRISM (Oregon State University). Winter-spring precipitation was defined as total precipitation from January to May and has been shown to have the strongest effect on total growth of ponderosa pines in the southwest (Szejner et al. 2016). Of the three years tested (2016, 2017, 2018), 2018 was an exceptionally dry year, with 50-60% reductions in winter-spring precipitation from the previous two years.

Chlorophyll fluorescence

To test the hypothesis that absence of overstory canopy results in photochemical stress of ponderosa seedlings, chlorophyll fluorescence was measured with an OS1p Modulated Chlorophyll Fluorometer (OptiSciences). For each seedling, three replicate needles per growth year for the past three years of growth were removed from the plant and stored in zip-top plastic bags. Within one hour of removal, needles were placed in full sun for ~15 minutes prior to measurements of light-adapted chlorophyll fluorescence (F_v'/F_m'). Modulation intensity was set to 50%, saturation intensity was set to 100%, FarRed mode and actinic lighting were off, and Gain was set using the AutoGain feature. The same needles were then placed in a cooler at ambient temperature overnight to acclimate to the dark. Dark-adaptation leaf clips (Hansatech Instruments, Pentney, UK) were placed on the needle to minimize any light exposure during measurement, and maximum quantum yield (F_v/F_m) was measured. Modulation was set to 10%, saturation intensity was set to 85%, FarRed mode was on with intensity of 50% for 10sec, and Gain was set using AutoGain feature.

Stomata Density

In conifers, stomata occur in rows parallel to the long axis of the needles. Total stomata were counted for a precisely measured segment of needle between 1-2mm long using a Nikon SMZ645 dissecting microscope at magnification “2”. One needle was used per growth year for each seedling. Ponderosa pine needles typically have the least variation in stomatal quantity and arrangement toward the center of the needle (Flower-Ellis and Olsson 1993), so all stomata counts were made within 1cm of needle center. Area of the segment was measured with an ocular micrometer. Stomata density was calculated as the number of stomata counted divided by area of segment.

Stable Isotope Analysis

To test the hypothesis that seedlings in severely burned areas experience more water stress than seedlings in unburned areas, isotopic discrimination of ^{13}C to ^{12}C ($\delta^{13}C$) was measured. To remove water prior to stable isotope analysis, two replicate needles from each growth year for each of the 20 ponderosa seedlings were freeze-dried for 24 hours with a FreeZone 4.5Plus (LabConCo, Kansas City, MO). For all needles, the top and bottom thirds of the needle were removed to minimize inter-needle variability (Barszczowska and Jedrysek 2005). The remaining middle portion of the needle was finely chopped with a razor blade. Approximately 4.0mg (±0.3mg) was weighed on a high-precision scale (Sartorius, Germany) and packed into 4x6mm tin capsules (CosTech Analytical, Valencia, CA) for carbon combustion. Whole foliar tissue was used rather than cellulose because for several *Pinus* species, the $\delta^{13}C$ values of foliage and cellulose are similar (Powers et al. 2008). Samples were analyzed for $\delta^{13}C$, $\delta^{15}N$, % carbon, and % nitrogen at the University of New Mexico Center for Stable Isotopes using a Thermo Scientific Delta V mass spectrometer with a dual inlet and Conflo IV interface connected to a Costech 4010 elemental analyzer. Stable carbon ratios were calculated as $\delta^{13}C = R_{\text{sample}} / R_{\text{standard}} - 1*1000$, where R is the molar ratio of heavy to light isotope ($^{13}C/^{12}C$). The carbon isotopic ratio ($\delta^{13}C$) was recorded as deviations per mil (‰) from the Vienna Pee Dee Belemnite international standard (Craig 1957). Nitrogen isotope ratios were calculated similarly to carbon, where R is the molar ratio of ^{15}N to ^{14}N and $\delta^{15}N$ is recorded as deviations per mil in a sample relative to the atmospheric nitrogen standard, N_2 (Coplen 2011; Hoefs 2001).

Statistical analyses

All statistical analyses were conducted in R version 3.5.1 (R Core Team, 2018). Analysis of variance (ANOVA) was used to compare morphology and physiology of ponderosa pine seedlings between two overstory canopy conditions (present, absent) and between needle age (current year, 1-year old, 2-years old needles, which refer to 2018, 2017, and 2016 growth years, respectively). The morphological parameter tested was stomata density. Physiological parameters tested were chlorophyll fluorescence (F_v/F_m, F_v'/F_m'), $\delta^{13}C$, $\delta^{15}N$, %C, and %N. Two-sample *t*-tests were used to compare the following seedling and microsite parameters: seedling height, seedling stem diameter, soil moisture, percent canopy cover, and percent vegetative cover. Normality of data was checked with the Shapiro-Wilk test and homogeneity of variance was checked with the Levene test.

RESULTS

Microclimate and Climate

Soil moisture, estimated soil cover, and seedling stem diameter did not significantly differ between sites (Figure 2). Mean volumetric water content of soil was 15.7% with canopy absent and 18.4% with canopy present (p=0.249). Mean estimated soil vegetative cover was 19% with canopy absent and 11% with canopy present (p=0.124). Mean seedling stem diameter was 3.2cm with canopy absent and 3.8cm with canopy present (p=0.183). Seedling height significantly differed between sites. Mean seedling height was 76.2cm with canopy absent and 103.3 with canopy present (p=0.031).

Mean climate data show that the canopy absent site was slightly warmer and drier than the canopy present site (Table 1). This difference is likely driven by the lower elevation of the canopy absent site. Winter-spring precipitation (January – May) was 50-60% lower for both sites in 2018 compared to 30-year normals, indicating that 2018 was a drought year (Figure 3).

Figure 2. Site microclimate conditions and seedling morphological measurements for 10 seedlings with overstory canopy absent and 10 seedlings with overstory canopy present for the following variables: (A) percent volumetric water content of soil, (B) visually estimated percent soil vegetative cover, (C) seedling stem diameter, and (D) seedling height.

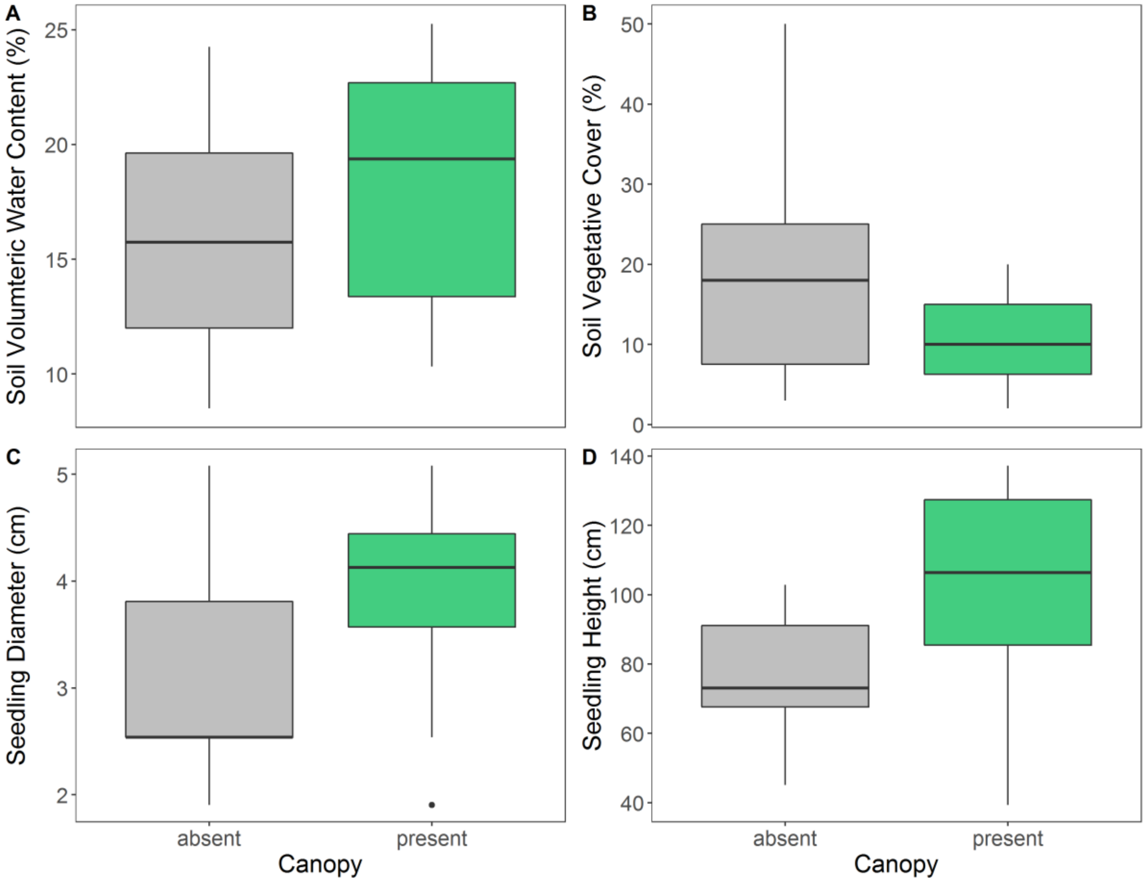
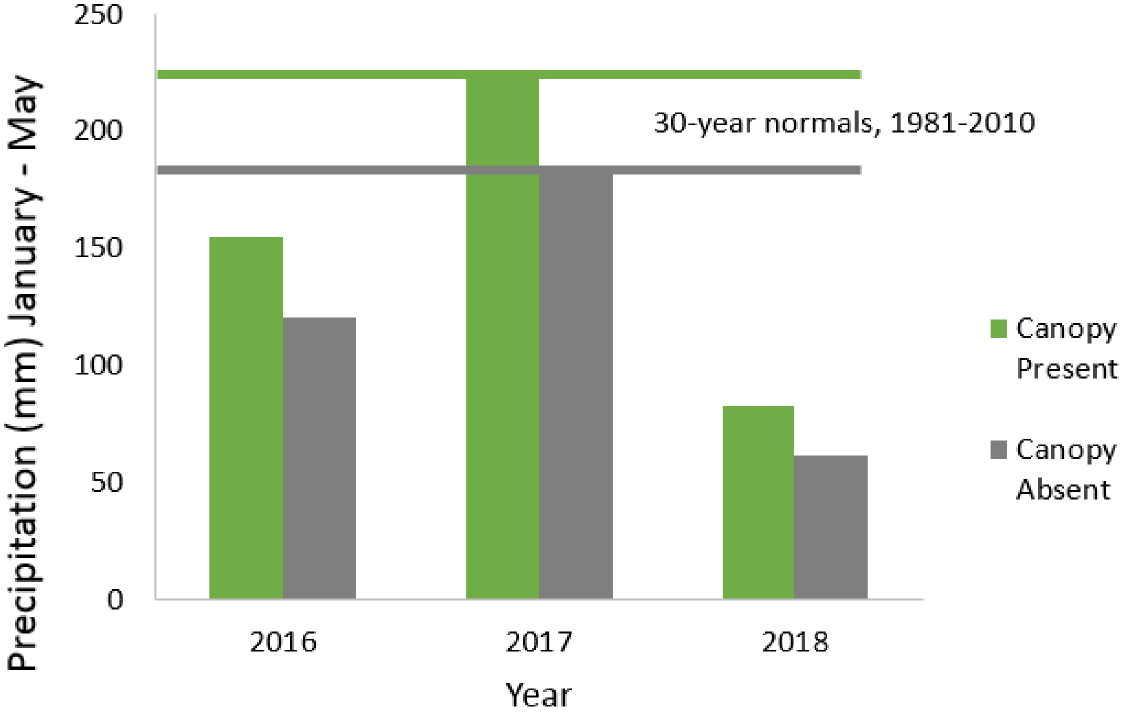


Table 1. Annual climate data for sampling locations in the Jemez Mountains. Data downloaded from PRISM in October 2018. “ppt” is precipitation and “VPD” is vapor pressure deficit.

Year	Canopy	Mean Temp (°C)	Max Temp (°C)	Min Temp (°C)	Mean ppt (mm)	Total ppt (mm)	Max VPD (hPa)
2016	Absent	9.5	29.1	-7.8	33.9	407.3	34.3
2017		10.2	27.2	-5.7	37.1	445.5	31.7
2018		11.6	28.2	-5.9	29.4	235.2	34.8
2016	Present	7.8	26.4	-8.7	39.7	476.5	27.9
2017		8.5	24.7	-6.4	42.8	513.3	25.6
2018		9.6	25.6	-6.7	34.6	276.8	28.8

Figure 3. Total winter-spring (January – May) precipitation for the two sampling locations for 2016, 2017, and 2018 with lines referencing 30-year normals.



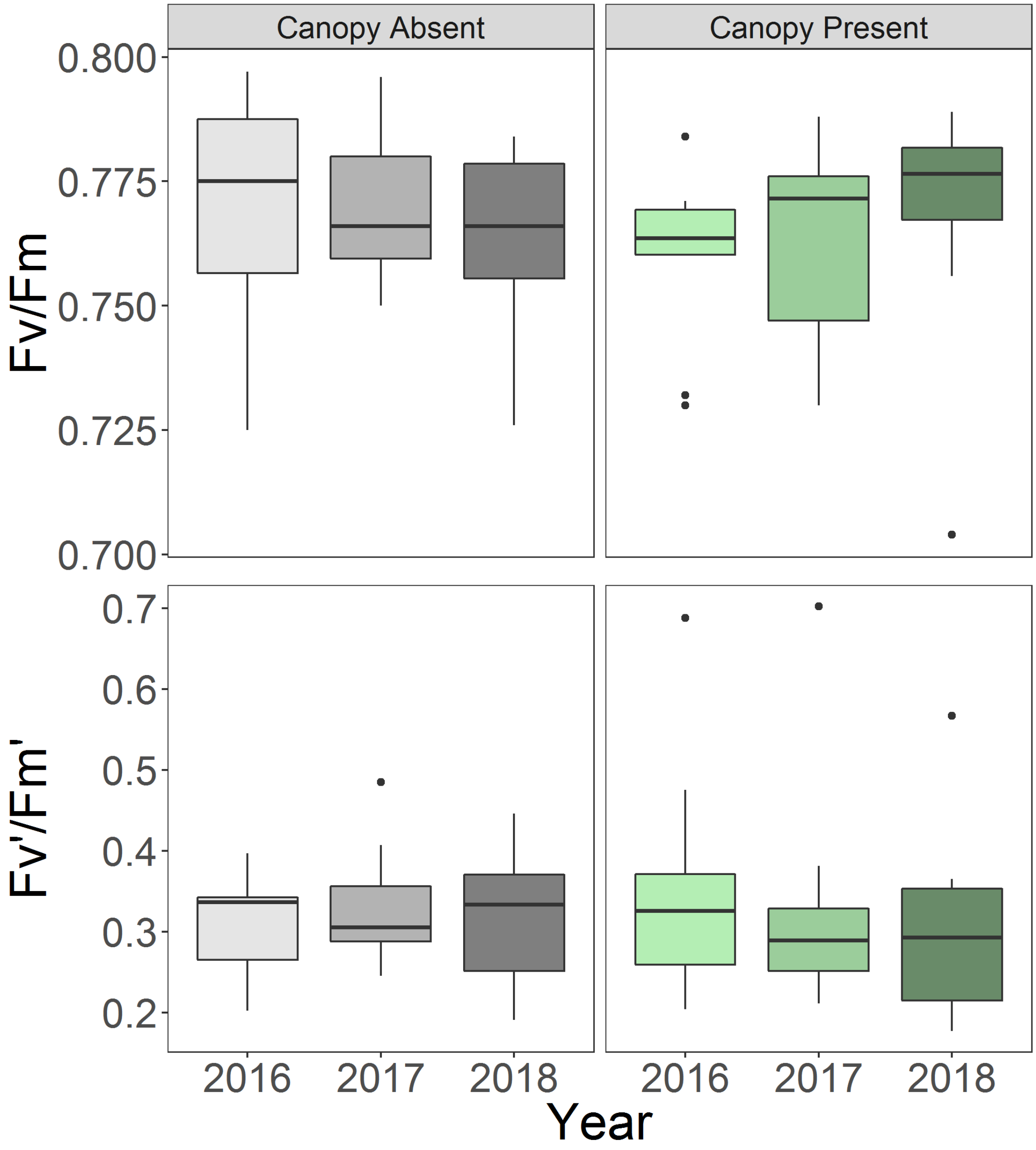
Chlorophyll fluorescence

Mean chlorophyll fluorescence data are presented in Table 2. The dark-adapted chlorophyll fluorescence (F_v/F_m), which measures maximum quantum efficiency of PSII photochemistry, did not significantly differ between seedlings by canopy presence or by needle age (Table 4; Figure 4). Combining all three ages of needles, the mean F_v/F_m for seedlings with canopy present was 0.764 (SD ± 0.019) and with canopy absent was 0.767 (SD ± 0.020). Similarly, the light-adapted chlorophyll fluorescence (F_v'/F_m'), which measures overall quantum efficiency of PSII photochemistry, did not significantly differ between seedlings by canopy presence of by needle age (Table 4; Figure 4). Combining all three ages of needles, the mean F_v'/F_m' for seedlings with canopy present was 0.327 (SD ± 0.071) and with canopy absent was 0.318 (SD ± 0.071).

Table 2. Dark-adapted (F_v/F_m) and light-adapated (F_v'/F_m') chlorophyll fluorescence values [mean ± standard deviation] for current year needles (2018), 1-year old needles (2017), and 2-year old needles (2016) for 10 seedlings growing with canopy absent and 10 seedlings growing with canopy present.

Year	Canopy	F_v/F_m	F_v'/F_m'
2016	Absent	0.77 ± 0.02	0.31 ± 0.06
2017		0.77 ± 0.02	0.33 ± 0.07
2018		0.76 ± 0.02	0.32 ± 0.08
2016	Present	0.76 ± 0.02	0.35 ± 0.14
2017		0.76 ± 0.02	0.33 ± 0.14
2018		0.77 ± 0.02	0.30 ± 0.11

Figure 4. Dark-adapted (Fv/Fm) and light-adapted (Fv'/Fm') chlorophyll fluorescence for current year needles (2018), 1-year old needles (2017), and 2-year old needles (2016) for 10 seedlings growing with canopy absent and 10 seedlings growing with canopy present.



Stomata Density

Stomata density did not significantly differ between canopy conditions or year for 2016 and 2017 needles. For 2018 needles, however, stomata density was significantly higher for seedlings without overstory canopy (p<0.001; Table 4; Figure 5).

Stable Isotope Analysis

Mean foliar $\delta^{13}C$ values differed across needle age (p<0.001), canopy environment (p<0.001), and the interaction of needle age and canopy environment (p<0.001; Table 3; Table 4; Figure 5). Mean foliar $\delta^{13}C$ was significantly higher for seedlings growing without canopy cover across all needles ages, though this difference was especially pronounced during the drought year (2018) when mean $\delta^{13}C$ of seedlings without canopy was 3.22‰ higher.

Seedlings in both canopy environments were depleted in ^{15}N relative to the N_2 air standard. Within each canopy environment, $\delta^{15}N$ did not differ across needle age, so values were pooled for comparison between canopy environments. Mean $\delta^{15}N$ for seedlings with canopy absent was -1.298‰ and with canopy present was -3.919‰. $\delta^{15}N$ of seedlings growing in the severely burned, canopy-absent area was significantly higher (p<0.001; Table 3; Table 4; Figure 6).

Foliar percent carbon (%C) differed across needle age (p<0.001), but not canopy environment (Table 3; Table 4; Figure 7). Mean %C decreased by a difference of 1% per year from 2016 to 2018 needles for seedlings in both canopy environments. Foliar percent nitrogen (%N) significantly differed across needle age (p<0.001), canopy environment (p<0.001), and the interaction of needle age and canopy environment (*p*<0.001; Table 3; Table 4; Figure 7). From 2016 to 2018, foliar %N of seedlings without overstory canopy increased from 1.195% to 1.591%. For seedlings with overstory canopy, %N increased from 1.158% in 2016 to 1.254% in 2018, though this increase of 0.096% is within the ± 0.12% error associated with %N of the standard.

Table 3. Foliar carbon and nitrogen properties [mean ± standard deviation] for current year needles (2018), 1-year old needles (2017), and 2-year old needles (2016) for 10 seedlings growing with canopy absent and 10 seedlings growing with canopy present.

Year	Canopy	$\delta^{13}C$	$\delta^{15}N$	%C	%N
2016	absent	-27.558 ± 0.675	-1.316 ± 1.25	55.222 ± 0.600	1.195 ± 0.165
2017		-26.719 ± 0.855	-1.463 ± 1.14	54.579 ± 0.528	1.372 ± 0.222
2018		-24.467 ± 0.434	-1.115 ± 1.116	53.207 ± 0.566	1.591 ± 0.261
2016	present	-28.843 ± 0.626	-3.903 ± 1.599	54.942 ± 0.665	1.158 ± 0.129
2017		-27.882 ± 0.683	-3.975 ± 1.373	54.645 ± 0.452	1.221 ± 0.113
2018		-27.683 ± 0.755	-3.879 ± 1.362	52.604 ± 0.500	1.254 ± 0.143

Figure 5. Stomata density and foliar δ^{13}C for current year needles (2018), 1-year old needles (2017), and 2-year old needles (2016) for 10 seedlings growing with canopy absent and 10 seedlings growing with canopy present.

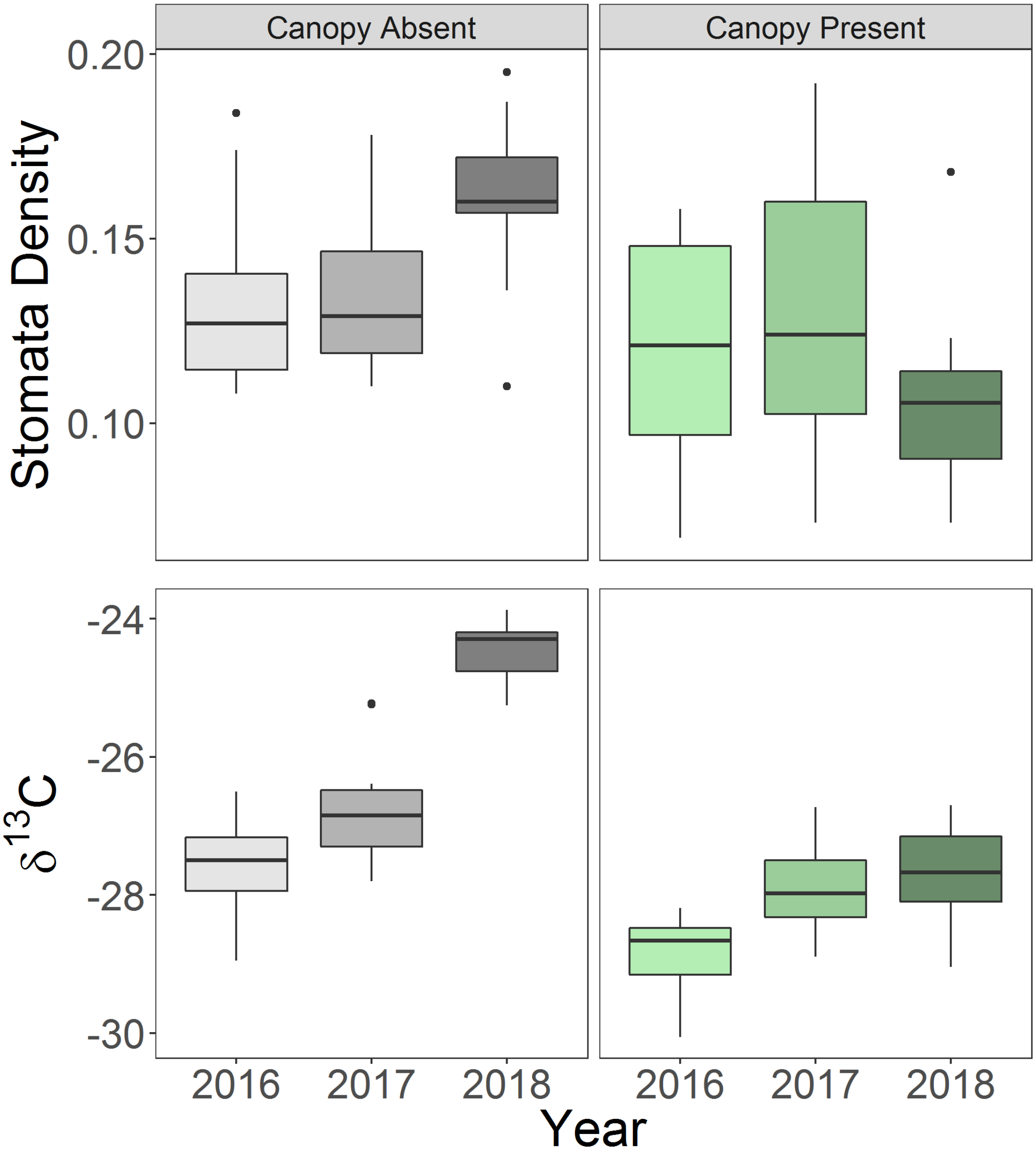


Figure 6. $\delta^{15}N$ for current year needles (2018), 1-year old needles (2017), and 2-year old needles (2016) for 10 seedlings growing with canopy absent and 10 seedlings growing with canopy present.

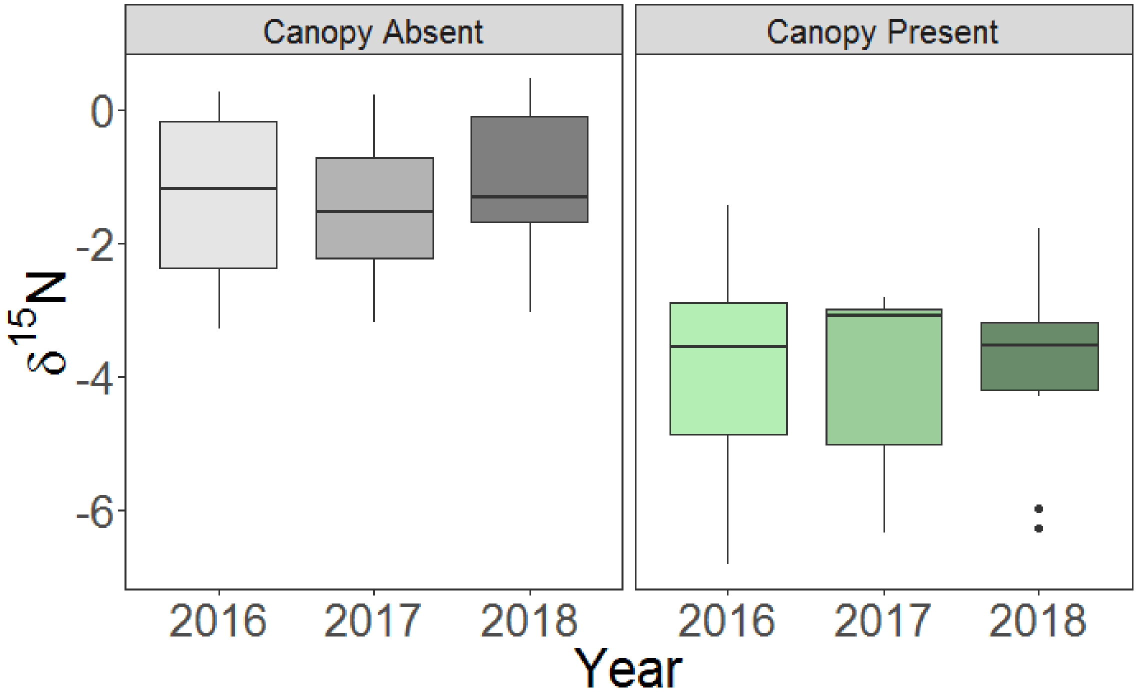


Figure 7. Foliar percent nitrogen and percent carbon for current year needles (2018), 1-year old needles (2017), and 2-year old needles (2016) for 10 seedlings growing with canopy absent and 10 seedlings growing with canopy present.

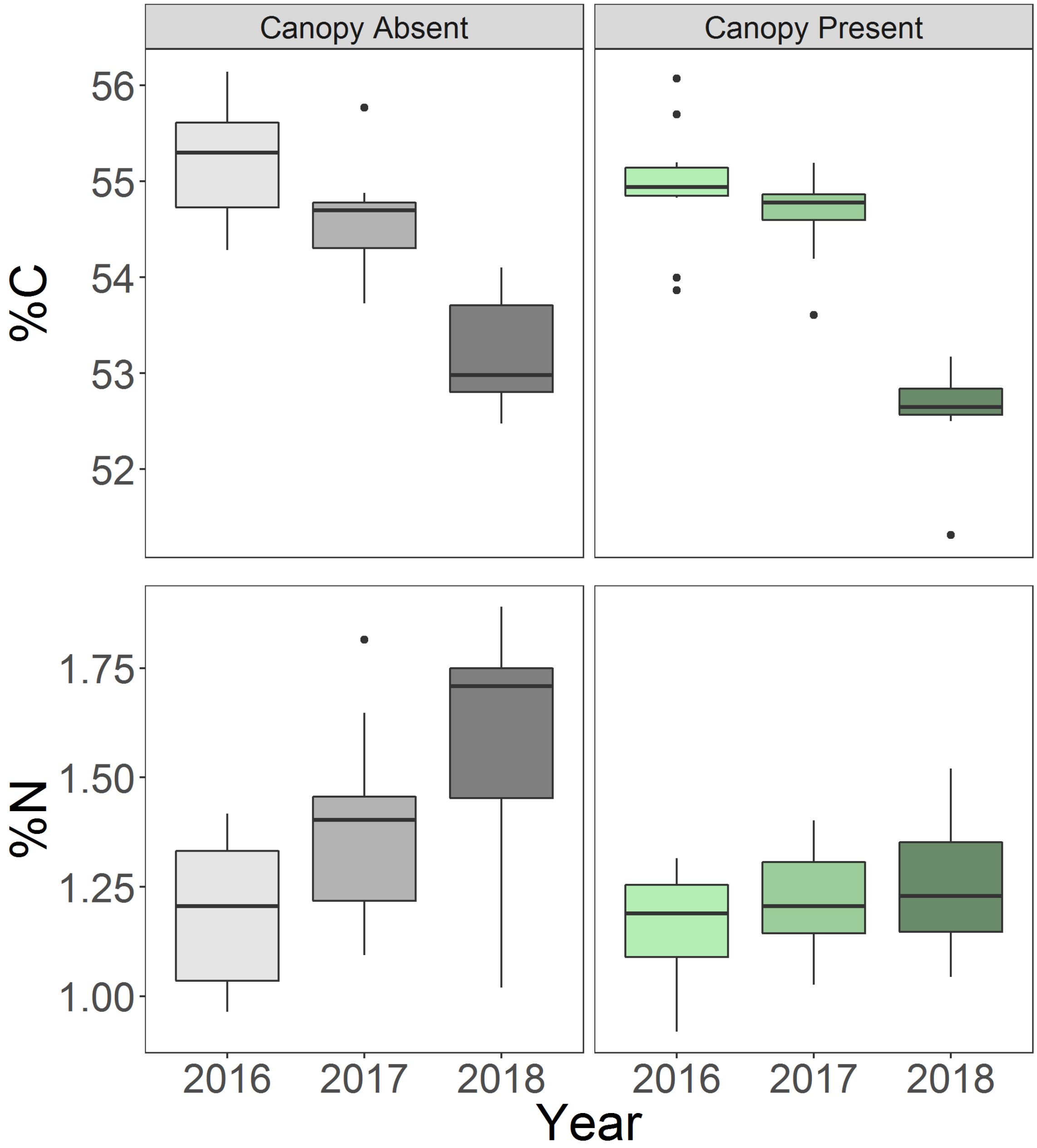


Table 4. Analysis of variance (ANOVA) table for: (A) dark-adapted chlorophyll fluorescence (F_v/F_m), light-adapted chlorophyll fluorescence (F_v'/F_m'), and stomata density (SD); and (B) carbon isotope discrimination ($\delta^{13}C$), nitrogen isotope discrimination ($\delta^{15}N$), percent carbon (%C), and percent nitrogen (%N) for three needle ages (current year needles (2018), 1-year old needles (2017), and 2-year old needles (2016)) for ponderosa pine seedlings growing in two canopy conditions (present, absent). Numbers in bold indicate statistical significance (p<0.01).

A.

		F_v/F_m		F_v'/F_m'		SD	
Source of Variation	df	MS	*F*	MS	*F*	MS	*F*
Needle Age	1	0	0	0.0027	0.25	7E-04	0.796
Canopy Condition	1	0.0002	0.401	0.0014	0.132	0.009	**10.54**
Age x Canopy	1	0.0005	1.198	0.0091	0.847	0.004	**4.916**

B.

		$\delta^{13}C$		$\delta^{15}N$		%C		%N	
Source of Variation	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Needle Age	1	49.52	**93.57**	0.14	0.084	49.4	**123.011**	0.6704	**20.813**
Canopy Condition	1	55.99	**105.8**	107.95	**64.793**	1.17	2.904	0.4807	**14.924**
Age x Canopy	1	9.77	**18.46**	0.08	0.049	0.27	0.682	0.2371	**7.361**

DISCUSSION

Chlorophyll Fluorescence

Foliage from seedlings in both canopy conditions showed no significant differences in chlorophyll fluorescence measurements. This suggests that regardless of needle age or overstory canopy, the relative proportion of open reaction centers in photosystem II and the efficiency of photosynthetic light capture do not differ. This finding disagrees with previous research that showed higher photochemical efficiency of a succulent (Pérez-Sánchez et al. 2015) and deciduous tree seedlings (Yang et al. 2010; Rodríguez-Calcerrada et al. 2008) growing under a nurse plant canopy compared to plants in full sun. However, chlorophyll fluorescence in the present study was measured at a single point in time in late September when hours of sunlight and daytime temperatures are lower compared to annual highs of June and July. Extended periods of high light levels in combination with heat stress can damage photosynthetic proteins, but efficient repair mechanisms can re-assemble damaged light-harvesting complexes (Barber and Andersson 1992). In a greenhouse-simulated heat wave study by Marias et al. (2016), ponderosa pine seedlings exhibited a short-term decrease in F_v/F_m but returned to levels comparable to control seedlings within one week. Therefore, any heat-associated damage to photochemical proteins of seedlings in the present study may have recovered during the monsoon season (mid-July to mid-September), which brings precipitation and cooler temperatures to the southwest. Repeated measurements of chlorophyll fluorescence throughout the growing season are necessary to test whether the absence of overstory canopy results in photochemical stress in seedlings, and how long this stress persists. Any prolonged reductions in photosynthetic efficiency would result in decreased carbon assimilation (Barber and Andersson 1992), and if heat and drought limit repair mechanisms, depletion of carbohydrates and eventual mortality can occur (Adams et al. 2009).

Carbon Isotopes

Ponderosa pine seedlings growing in the severely burned area lacking overstory tree canopy had significantly higher $\delta^{13}C$ compared to seedlings with overstory canopy present. This difference was especially pronounced for needles grown in 2018, which was an exceptionally dry year. At the leaf level, higher $\delta^{13}C$ can be caused by two possible mechanisms relating to carbon-water balance: increased carbon assimilation (A) or decreased stomatal conductance (g_s) (Farquhar et al. 1982). Increased carbon assimilation is directly related to higher rates of photosynthesis. Photosynthesis is generally higher for plants in open habitats with high irradiance (Pothier and Prevost 2002), except when water stress results in stomatal closure, which decreases stomatal conductance (Comstock and Ehleringer 1984). Without overstory canopy, seedlings experience greater irradiance, enabling increased ATP synthesis in the light reactions to power carbon fixation by Rubisco (Björkman 1981). Furthermore, seedlings without overstory canopy had slightly higher stomata density, which would enable higher rates of carbon fixation due to increased CO_2 diffusion into mesophyll tissue (Farquhar and Sharkey 1982). Although %N was significantly higher for seedlings without canopy, which often correlates with greater Rubisco content and increased photosynthesis, the difference is relatively small with high variation; the mean difference for 2018 seedlings is 0.337, but the standard deviation for canopy absent 2018 needles is 0.261. In a study of *Pinus sylvestris*, seedlings growing in areas without overstory canopy due to high-severity fire or clear-cut had elevated foliar nitrogen concentrations but did not differ in photosynthetic rate (Bansal et al. 2014). Without direct measurements of photosynthesis, it is unclear whether seedlings in the burned area have higher $\delta^{13}C$ due to increased photosynthetic carbon assimilation.

Alternately, a decrease in stomatal conductance, g_s, could explain the higher $\delta^{13}C$ values. Water availability to a plant is a major contributor of its carbon isotope composition because of the tight coupling of leaf gas-exchange and stomatal behavior (Diefendorf et al. 2010). In fact, prior research has shown that $\delta^{13}C$ is more closely correlated with stomatal conductance than carbon assimilation (e.g. Ehleringer 1990). Under dry conditions, ponderosa pines typically avoid low xylem water potential by stomatal closure, which results in reduced stomatal conductance (Panek and Goldstein 2001; Kerr et al. 2015; Cregg et al. 2000). This response has the benefit of avoiding cavitation and hydraulic failure associated with water loss (Sperry et al. 2002) but has the cost of limiting photosynthetic carbon gain (McDowell et al. 2008). Although *Pinus sylvestris* seedlings increased stomatal conductance when growing without canopy cover (Bansal et al. 2014), this boreal species may not be as water-limited as ponderosa pines in the semi-arid southwest. Climate data show that 2018 was an especially dry year, with 66% and 63% reductions in winter-spring (January-May) precipitation from 2017 to 2018 at the canopy absent and canopy present sites, respectively. This early-season precipitation comprises the bulk of annual photosynthesis and growth for ponderosa pines (Szejner et al. 2016), and thus should contribute most to the carbon isotope value. If canopy has no effect on seedling water stress, a large decrease in precipitation should affect seedlings in both environments equally. However, this disproportionate difference in $\delta^{13}C$ values during a dry year supports the hypothesis that seedlings growing without overstory canopy experience greater water stress than seedlings with overstory canopy. Thus, this result provides evidence that a mature tree canopy buffers variable climate conditions for ponderosa pine seedlings.

Stomata Density

An interesting result related to the carbon-water balance is the significantly higher stomatal density of 2018 needles growing without overstory canopy. If all other factors are equal, more stomata should result in increased CO_2 diffusion into the leaf, thereby decreasing $\delta^{13}C$. However, for the 2018 foliage, higher stomatal density co-occurred with higher $\delta^{13}C$. Therefore, it is most likely that stomatal conductance decreased.

Percent Carbon

Seedlings in both canopy conditions showed an inverse relationship between foliar %C and needle age; the current-year needles had the lowest foliar %C while the two-year-old needles had the highest foliar %C. The carbohydrate concentration of tissues should only decline when the availability of C from photosynthesis plus storage does not equal C consumption to maintain metabolism (Marshall and Waring 1985). Decreased %C could be due to greater relative storage of carbon in older tissues and/or greater rates of respiration in new tissue.

Nitrogen Isotopes

Following fire, net nitrification rate often increases (Wan et al. 2001), enriching the NH_4^+ pool available to plants (Robinson 2001). The significantly higher foliar $\delta^{15}N$ of ponderosa seedlings growing in the severely burned area is consistent with post-fire studies of *Pinus muricata* in California (Grogan et al. 2000) and *Pinus banksiana* in Michigan (LeDuc et al. 2013). Although elevated nitrification rates usually result in a short-term increase in foliar ^{15}N (Wan et al. 2001), severe stand-replacing fires in southwestern ponderosa forests can result in nitrification rates that remain elevated for decades (Kurth et al. 2014). This could explain why foliar $\delta^{15}N$ is higher for seedlings in the severely burned area compared to seedlings in the low-severity burned area seven years after the Las Conchas fire. Furthermore, N-cycling rates in southwestern ponderosa pine forests are higher in soils dominated by grasses compared to soils dominated by pines, which may be maintained by limited tree regeneration (Kaye and Hart 1998). Overall, these results support the hypothesis that the vegetation community shift from ponderosa to shrub-grassland after severe fire results in an N-cycling state shift from low to high nitrification rates (Kurth et al. 2014).

Implications

This study provides physiological evidence that the absence of overstory canopy following severe fire negatively affects the carbon-water balance of seedlings and suggests that mature ponderosa trees serve as nurse plants for ponderosa seedlings. Microclimate conditions beneath the canopy may ameliorate water stress of tree seedlings during hot and dry periods, decoupling seedling response to regional climate and contributing to overall ecosystem resiliency in an era of changing climate (Bertness and Callaway 1994). The Southwest is projected to be warmer and drier, with increasing drought severity (Cayan et al. 2013). As a result, large severe wildfires are likely to continue occurring at elevated rates (Westerling et al. 2006), resulting in post-fire areas that not only are warmer and drier due to a changing climate but also lack the resiliency buffer provided by overstory canopy (Redmond et al. 2018). The converging ecosystems stresses from climate change and altered fire regime are causing forest changes beyond historical norms (Allen 2014). Thus, it is imperative to understand the physiological mechanisms affecting seedlings in response to these multiple stressors, because seedling success or failure will ultimately determine future forest composition (Bazzaz 1991). These findings also add a new dimension to the importance of restoring the historical fire regime of frequent, low-severity surface fires to ponderosa pine forests of the southwest (Allen et al. 2002). Surface fires not only maintain the overstory canopy and its associated microclimate, but also reduce the risk of future canopy loss from high-severity fire (Walker et al. 2018). Reducing the risk of a high-severity fire and the ecosystem state shift that follows not only preserves the forest in the short-term but may also buffer against heat- and drought-stress of regenerating seedlings, contributing to forest resiliency in the long-term.

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