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Foliar respiration and carbon dynamics of mature piñon and juniper trees in response to experimental drought and heat

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**FOLIAR RESPIRATION AND CARBON DYNAMICS OF
MATURE PIÑON AND JUNIPER TREES IN RESPONSE TO
EXPERIMENTAL DROUGHT AND HEAT**

by

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THESIS

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ABSTRACT

Plant respiration (R) is generally well-coupled with temperature and in the absence of thermal acclimation, respiration is expected to increase as climate change brings higher temperatures. Increased drought is also predicted for future climate, which could drive respiration higher if the carbon (C) cost to maintain tissues (R_m) or grow increases, or lower if substrate or other factors become limiting. We examined the effects of temperature and drought on R as well as photosynthesis, growth, and carbohydrate storage of mature individuals of two co-dominant tree species. Three mature, in-situ piñon (*Pinus edulis*) and juniper (*Juniperus monosperma*) trees were assigned to each of the following treatments: +4.8 °C; 45% reduced precipitation; a combination of both (heat + drought); along with ambient control and treatment controls. R_m measured prior to foliar and twig growth was far more sensitive to drought in piñon, and heat in juniper. Total respiration (R_t , R not partitioned) acclimated to temperature in piñon such that elevated temperature had minimal impacts on R_t ; however, juniper exhibited higher R_t with elevated temperature, thus juniper did not display any thermal acclimation. R_t in both species was weakly associated with temperature, but strongly correlated with pre-dawn water potential, photosynthetic assimilation (A) rates, and in piñon, foliar carbohydrates. For both species, heat caused far more days where $A-R$ was negative than did drought. The consequences of drought alone and heat alone in piñon included higher

R_t per unit growth, indicating that each abiotic stress forces a greater allocation of R_t to maintenance costs, and both drought + heat in combination results in far fewer days that foliar carbohydrates could sustain R in both species. Notably, the much higher A and R of juniper than piñon is consistent with predicted superior carbon budget regulation of juniper than piñon during drought; however, juniper's lack of temperature acclimation in contrast to piñon suggests climate warming may have a greater deleterious impact on juniper carbon balance than piñon.

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Introduction

Global perspective of importance in understanding R response to temperature and drought

The dynamics of plant respiration and photosynthesis, from the cellular to the global scales, play important roles in survival and mortality of forests and are one of the key drivers of CO₂ concentration in the atmosphere. At the plant level, the inability to maintain a positive carbon (C) balance may be implicated in forest mortality during drought (e.g. McDowell et al., 2008). At the global scale, respiration of terrestrial plants consumes up to 50% of gross primary production in temperate forests and 70% of gross primary production in equatorial rainforests, which subsequently impacts annual variations in atmospheric CO₂ (Keeling, 1960; Ruimy, Dedieu, & Saugier, 1996). Increased temperature and drought conditions are thought to drive large-scale forest mortality, and could contribute to a positive feedback loop of C to the atmosphere (Allen et al., 2010; Allison et al., 2009; van Mantgem et al., 2009). Changes in plant C budgets and alterations to the respiration component of photosynthesis may alter global C dynamics and importantly, climate feedbacks (Ryan, 1991a).

C balance of leaves and importance in determining survival

Respiration has evolved as a mechanism to convert lipids and carbohydrates to energy useful in performing work such as maintenance of tissues (R_m), growth, and export of cellular products (Amthor, 2000; Ryan, 1991b). If C expenditures exceed new C supply from photosynthesis then plant survival can only be maintained as long as C is available from storage for use in metabolism and defense against biotic agents (McDowell, 2011;

Sala, Woodruff, & Meinzer, 2012). Among the most critical tissues to this balance is the canopy, where the net flux resulting from photosynthesis and respiration defines if the plant is currently gaining or losing C. Water stress in the presence of elevated temperature has been shown experimentally to drive foliar respiration higher and possibly contribute to earlier plant death (Adams et al., 2009). Thus, a leaf-level C budget can be a useful tool for inferring the survival trajectory of a plant if conditions reduce photosynthesis or increase respiration.

Review of response to drought and temperature

Respiration rates are dynamic and depend on a number of biotic and abiotic factors. Water deficit typically reduces the rate of photosynthesis and growth, and respiration may decline if substrate is limiting or growth ceased (Fatichi, Leuzinger, & Körner, 2014; Körner, 2003; McDowell, 2011). Alternatively, even if growth stops and photosynthesis is reduced or eliminated, R_m may increase during drought if osmoregulation, repair or senescence costs become elevated (Flexas, Bota, Galmés, Medrano, & Ribas-Carbó, 2006; Turnbull et al., 2001). Respiration is known to be coupled with temperature in the short-term when other conditions are not limiting, such as substrate supply, sink demand, enzyme capacity or adenylate availability (Atkin & Tjoelker, 2003; Hartley et al., 2006; Körner, 2003). Respiration can respond exponentially to increases in temperature, which may tip the scale of the C balance toward the negative (respiration exceeds photosynthesis) under elevated temperatures unless photosynthesis concurrently rises or the temperature dependence of respiration declines. Respiration may acclimate to temperature changes by reducing the base respiration rate or lowering the factor by which

respiration increases over a concurrent temperature increase (e.g. Q_{10}), but factors such as drought are important in determining this response (Atkin, Bruhn, Hurrey, & Tjoelker, 2005; Atkin & Tjoelker, 2003; Ryan, 2010). The contrasting responses of respiration to common abiotic stressors such as drought and increased temperature illustrate the need to understand these dynamics in the face of changing climate.

Introduction of species, statement of hypotheses

We measured respiration and the associated physiological processes that may regulate it in the leaves of two co-dominant tree species, one-seed juniper (*Juniperus monosperma*) and piñon pine (*Pinus edulis*) while imposing the following treatments: 45% reduction in soil water (drought, D); +4.8 °C elevated temperature (heat, H); both simultaneously (heat + drought, HD); ambient control (control, C); and chamber control (chamber control, CC). We conducted this study with mature, in-situ trees in the Southwest USA, an area expected to undergo increases in both drought and temperature in the coming decades (IPCC, 2013; Seager et al., 2007). *P. edulis* is known to close stomata and cease photosynthetic activity during drought yet maintains relatively high leaf water potential, while *J. monosperma* sustains some gas exchange while experiencing lower leaf water potential (Limousin et al., 2013). Little is known regarding the response of these species to temperature changes, alone or in combination with drought. Our goals were to investigate the responses of foliar respiration to drought and temperature, explore the potential drivers of their responses, and determine their potential impacts on branch level carbon balance including growth. Our hypotheses thus are:

- 1) heat will increase both nightly and seasonal respiration in the absence of thermal acclimation,
- 2) drought will lower both nightly and seasonal cumulative respiration,
- 3) heat + drought will result in no change in nightly and seasonal respiration, given balancing effects of drought and heat, but will reduce daytime photosynthesis and hence the balance of photosynthesis minus respiration (A-R),
- 4) the daily and seasonal foliar carbon balance and growth will be reduced in drought, heat and heat + drought compared to controls, and
- 5) treatment effects will be greater for *P. edulis* than for *J. monosperma*.

Material and Methods

Field site and experimental design

This study was implemented in June 2012 in a piñon-juniper woodland on a ~one-hectare parcel of land located on Frijoles Mesa, encompassed by Los Alamos National Laboratory, 7.9 km south of Los Alamos, New Mexico, USA. The site is at 2150 m m.s.l. on Hackroy clay-loam soils ranging in depth from 40-80 cm over a layer of meters-thick volcanic-ash-derived tuff. Two micrometeorological stations located 100m apart collected a data record of ambient temperature, humidity, wind speed, wind direction, and precipitation (see Garcia-Forner et al., (In press) for more detail). During periods of data loss or sensor malfunction, a nearby (~1km away) meteorological tower was used to complete the data record, in addition to providing solar radiation data (The Weather Machine, <http://environweb.lanl.gov/weathermachine/default.asp>).

Open-top-chambers (OTCs) and precipitation-diversion (drought) troughs were used to impose heat and drought treatments to test our hypotheses. Fifteen mature juniper and piñon trees (>10 cm diameter at breast-height) were selected and three of each were assigned to the following treatment groups: control (C, un-manipulated control trees), chamber control (CC, trees in ambient-temperature OTCs to account for chamber effects), drought (D, trees in the drought plot), heat (H, trees in OTCs maintained at +4.8 °C) and heat and drought combined (HD, trees in heated OTCs and in the drought plot). Air temperature in the H, HD and CC treatments was controlled using custom-built clear polycarbonate-walled and aluminum-framed OTCs surrounding the canopies, and conditioned air provided by air-exchange package heat pump, heating, ventilation and air-conditioning units (RJPL and RLPL, Ruud Manufacturing, Atlanta GA, USA).

Temperature measurement and control was achieved using CR1000 dataloggers and two CS215 temperature and relative humidity probes (Campbell Scientific, Logan UT, USA) per chamber installed at 1.0 m above ground and at 2/3 the height of the chamber. The drought plot was constructed using parallel polycarbonate troughs installed at ~1.3 m above the ground, similar to those detailed in Pangle et al. (2012). Additional details of infrastructure and temperature control can be found in Garcia-Fornier et al. (In press). Treatments were imposed 11 months prior to the initial data collection.

Gas exchange measurement

All data were collected in 2013 for this study. In order to test hypotheses that drought and heat will differentially impact foliar C dynamics, we periodically measured photosynthesis and respiration. Maximal light-saturated assimilation (A) and nighttime CO₂ efflux (respiration, R) was monitored approximately once per month from April to October using LI6400 and LI800 infra-red gas analyzer-based systems (IRGAs), respectively (Li-COR Inc., Lincoln NE, USA). For A measurements, see Garcia-Fornier (In press) for details. We considered A equal to zero when the CO₂ values of the sample and reference IRGAs of the LI6400 differed by less than one $\mu\text{mol/mol}$. Nightly respiration was measured using custom efflux chambers constructed of polycarbonate boxes shaded from sunlight by surrounding them with aluminum foil, and sealed from ambient air with neoprene strips and titanium dioxide rope-caulk (Weatherstrip and Caulking Cord, Mortite Inc., Kankakee IL, USA). South-facing branches 10 cm – 60 cm in length were sealed within efflux chambers and connected via 3.175mm diameter Synflex tubing (Synflex 1300, Eaton Corporation PLC, Dublin, Ireland) to a custom-

built, valve-, pump- and CR1000 datalogger-operated automated CO₂ measurement system. Ambient air was circulated through the chambers at all times except during the ~five minute measurement cycle of any given chamber, with each chamber sampled ~every 75 minutes. During the measurement cycle, air was circulated through the chamber and the slope of CO₂ buildup in the closed loop between the chamber and IRGA was used to calculate CO₂ efflux ($\mu\text{mol s}^{-1}$). Half (15) of the trees were measured on the night of the date reported herein and the other half were measured the following night. For CO₂ efflux measurements, the same branches were measured repeatedly throughout the growing season with two exceptions when one branch died and another was accidentally damaged, in which cases they were harvested and new branches were selected. Foliar growth was quantified every two to four weeks as described in Adams et al. (In press). To calculate C flux on a leaf area basis, foliage samples were collected for each measurement of A, and in November for nightly CO₂ efflux. Leaves were weighed, measured on a LI3100 Leaf Area Meter, dried at >60 degrees °C for >24 hours and re-weighed. To prevent the loss of leaves due to browning or senescence over the course of the season on the nightly CO₂ efflux branches, leaves were gently agitated one to three times between measurement periods and shed leaves were collected and measured for mass and leaf area, and leaf area applied as appropriate to efflux calculations. Nighttime CO₂ efflux was categorized as supporting maintenance processes (R_m ; cell repair, protein turnover, gradient maintenance, export) during the period prior to observed foliar or twig growth, which was on and before May 22 for piñon and on and before July 25 for juniper. CO₂ efflux that occurred after growth began was not further parsed into components and was deemed ‘total respiration’ (R_t).

Assimilation and CO₂ efflux scaling

On May 15 2012, we conducted a photosynthetic assimilation diurnal, from which we determined the relationship between maximal assimilation and cumulative daily assimilation. For that diurnal, we measured A using LICOR 6400 instruments, on the same foliage of five *J. monosperma* and five *P. edulis* trees approximately every two hours from sunrise to sunset. The instrument and measurement parameters were the same as was detailed in Garcia-Forner et al. (In press) with the following exceptions: 1) ambient light conditions were monitored throughout the day with a quantum sensor (LICOR 9901-013 External Quantum Sensor) and based on these data, light conditions inside the leaf chamber for each two hour measurement cycle were set to approximate ambient conditions of 200, 1100, 1800, 2050, 1900, 1100 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation; 2) air temperature was also monitored throughout the day and the LI6400 block temperature was set to approximate ambient conditions of 15, 22.5, 26, 29, 30, 28 and 27 °C. For piñon, the daily cumulative assimilation value equaled the maximal rate if it were sustained for 6.0 hours, and for juniper the maximal rate if sustained for 7.9 hours.

On the day of measurements in 2013, A was scaled to a daily cumulative value by multiplying the measured maximal values on a given day by the relationship between maximal A and cumulative daily A as determined by the diurnal conducted in May 2012. Respiration was estimated between measurements in the same night (measured approximately every 75 minutes) using linear interpolation. In October, thermocouples were inserted into each sealed CO₂ efflux chamber and placed nearby or in contact with

foliar tissue and temperature was recorded. Foliar temperature prior to October was estimated by using the relationship between ambient or OTC air temperature and the temperature recorded inside the efflux chamber in October. Although the observed hysteresis in the response of CO₂ efflux with temperature was usually minimal, every two CO₂ efflux and foliar temperature data points were averaged in order to reduce any potential effects of hystereses.

Each night, the relationship between estimated foliage temperature and CO₂ efflux (de Wit, Brouwer, de Vries, & Setlik, 1970) was determined by fitting a two-coefficient exponential curve to the data using the following equation:

$$\text{efflux} = B_0 * \exp^{(B_1 * T)} \quad (\text{Eqn 1}),$$

where: T = temperature (degrees C), B₀ is the rate of efflux at T = 0, and B₁ represents the strength of the relationship between temperature and CO₂ efflux. B₁ can be used to determine the Q₁₀, the factor by which efflux increases given a 10-degree temperature increase, following:

$$Q_{10} = \exp^{(B_1 * 10)} \quad (\text{Eqn 2}).$$

When CO₂ efflux was only measured for a portion of the night (usually due to weather delaying equipment setup), efflux was estimated for the rest of the night by using the Q₁₀, B₀ and estimated foliar temperature if the B₁ parameter was significant in the exponential model fit (p<0.05). If a non-significant relationship with temperature was found, Q₁₀ was fixed for that tree on that night at Q₁₀ = 1 and mean efflux during the measurement period was applied to scale outside the measurement period for the rest of the nighttime hours. Total nighttime CO₂ efflux was estimated for the period between sunset and sunrise the next day, as determined by observed solar radiation values less than 15 μmol

$\text{m}^{-2} \text{ s}^{-1}$ of photosynthetically active radiation.

We estimated total daytime A and nighttime R between measurement periods using measured A and Q_{10} respectively, along with our 15-minute ambient and OTC air temperature record. A was interpolated between measurement dates and for a daily cumulative value of A, instantaneous maximal A was multiplied by the appropriate scale for each species as specified. Q_{10} for a given period between measurements was applied backward and forward to the midpoint (in number of days) between measurement periods. For example, if a measurement period was separated by 28 days, the Q_{10} was used from the given measurement period for that night, and 14 days before and after that measurement date. The nighttime foliar temperature between measurement periods was assumed to be coupled with air temperature and thus, ambient or OTC temperature values were used directly in the calculation of CO_2 efflux. Respiration flux was then calculated using the estimated Q_{10} and measured ambient or OTC air temperature, and summed for the duration of the night as given by the solar radiation record.

Growth measurements

To test our hypothesis that growth will be negatively impacted by all imposed treatments, we monitored foliar elongation (both species) and buds (piñon only). Two shoots on each branch, one dominant (following the direction of growth of the main axis), and one nearby subordinate (within 10 cm of the dominant shoot but perpendicular to the main axis) were selected for repeat growth measurements following Girard et al. (2011, 2012) and marks were made near the distal end of each with a permanent marker in early spring. Every two to four weeks, bud growth (in piñon) and foliage length (in both

juniper and piñon) was measured with digital calipers or a ruler. After foliar emergence in piñon, the needle lengths of a subset of four needles per distal tip were measured. Relative bud growth in piñon and foliage growth in juniper was calculated by subtracting the initial measurement from each subsequent measurement. In addition to repeated measurements, at the end of the growing season we counted the number of distal tips that grew and the number of distal tips that did not grow for each piñon branch. Whole-branch bud growth was estimated by multiplying the average end-of-season relative growth of the two distal tips per branch by the fraction of the number distal tips that did grow versus the number of distal tips that did not grow, for an estimate of mean bud growth in millimeters weighted by the fraction of distal tips that grew.

Leaf water potential, relative extractable water and NSC measurements

To quantify the levels of moisture stress in our trees, leaf water potential was measured within two days (and often on the same day) of the A and nightly efflux measurements, with the exception of the first measurement of 2013 when leaf water potential and A was measured 12 days prior to the respiration measurement (no rain fell during this period). Pre-dawn and mid-day leaf water potential was measured following Garcia-Forner et al. (In press). To better understand when and how much water was available for use by the trees in our study, we calculated relative extractable water at a daily timescale using the BIJOU water balance model following Granier et al. (1999). We parameterized the model using data from our meteorological record (precipitation, air temperature, relative humidity, wind speed and solar radiation) along with leaf area index measured at 12 random locations outside the drought plot with a LAI 2000 Plant Canopy analyzer. We

assumed that maximum extractable water in the soil type present at our site was 100mm (websoilsurveys.nrcs.usda.gov) and we estimated that the drought structure intercepted and removed 45% of precipitation.

To investigate the relationships between foliar gas exchange and tissue carbohydrates, foliar and twig tissues were sampled for non-structural carbohydrates (NSC) in June of 2013, and were processed according to Dickman et al. (2014). We used NSC data to correlate R in grams of C with NSC in grams of C. To do this for starch, we assumed that starch was comprised of free glucose molecules, with no glycosidic bonds, which would result in a conservative (underestimate) of the amount of C present, and reduce any observed treatment differences.

Statistical analyses

Linear mixed-effects, random-intercepts models were constructed to investigate the relationships between C fluxes, time and other physiological and abiotic variables. The parameters of interest were input as fixed effects, while the individual trees nested in the individual OTCs were input as random effects. Trees in the ambient control treatment were considered to be nested in the same (fictional, outdoor) OTC. Each model was built starting with a full version including all parameters and interactions, and progressively reduced to simpler models by removing non-significant terms until the simplest meaningful model with the lowest Aikake Information Criterion (AIC) value was derived. AIC values within five units of one another were considered equivalent and the model with the fewest number of explanatory terms was selected. Control and chamber control treatments were found to be statistically indistinguishable from one another in

measures of leaf water potential, soil moisture and vapor pressure deficit (data not shown), thus both control groups were pooled into one group, control + chamber control (C+CC) and contrasts were only made between treatment groups (drought, heat and heat + drought) and the combined control group (C+CC). We used two-way ANOVAs to analyze data that satisfied the assumptions of independence (e.g., R_m , Fig 2). Post-hoc analysis with linear mixed effects models and ANOVA was performed with Tukey's HSD post-hoc test. Prior to each analysis, parameters were log or square root transformed as necessary to satisfy assumptions of normality. We used an alpha critical value of $\alpha=0.05$ in determining statistical significance.

R-squared (r^2) was obtained for linear mixed effects models following Nakagawa and Schielzeth (2013) and adapted by Jon Lefcheck (<http://jonlefccheck.net/2013/03/13/r2-for-linear-mixed-effects-models/>). Non-linear model r^2 was calculated following recommendations given in Kvalseth (1985).

All analyses and figures were completed using R statistical software, version 3.0.2 (R Core Team 2013) and the packages *nlme* for non-linear and linear mixed effects models (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2013), *ggplot2* (Wickham, 2009), *scales* (Wickham, 2012), and *reshape2* (Wickham, 2007) for graphics and *plyr* (Wickham, 2011) for data manipulation.

Results

Environmental conditions

Our experimental site experienced lower than average precipitation for most of the growing season (Fig 1a) and through late July, relative extractable water content in soils remained less than 0.4, the point at which most plants undergo water-mediated reductions in transpiration (Fig 1b, Granier et al., 1999). By September 1st our site had received less than 36% (147 mm) of the 25-year mean (414 mm) in annual precipitation. However, one week in mid-September brought 42% (182 mm) of 2013 precipitation, which served to rapidly recharge soil water to field capacity (Fig 1b). Mean temperature was above average; from May to October, ambient temperature was 19.0 °C, compared to the 25-year mean of 15.7 °C.

Treatment effects on respiration

Cumulative piñon R_m prior to observed growth (before May 22) was reduced in drought and drought + heat compared to controls, while heat was not significantly different from controls (Fig 2a). After twig and needle growth began, increased variability within treatments was observed and no significant differences between treatments in R_t were found (Fig 3a). Juniper exhibited higher R_m prior to growth (before July 25) in the heat treatment compared to drought and controls and no differences were identified between controls and drought, or heat + drought (Fig 2b). The total cumulative estimated CO₂ efflux for the entire growing season was about double for juniper than for piñon, and was consistently higher in juniper heat than controls (Fig 3b, Fig 3a).

Predawn water potential (Fig S1a, Fig S1b) and A (Fig S2a, FigS2b) both exhibited a gradual decrease for both species to a minimum in June and an increase following precipitation to a maximum in October. Water potential values of controls in both species were at times higher than in drought or heat groups and the range for piñon was from approximately -3 MPa to -1 MPa, while the range in juniper was from approximately -6.2 MPa to -1 MPa.

Predawn leaf water potential shared an exponential relationship with R_t in both species, and in piñon, drought interacted with water potential, exhibiting increased R_t at high water potentials and decreased R_t at low water potentials compared to controls (Fig 4a). In juniper, R_t was higher in the heat treatment over the range of predawn leaf water potential than controls, drought or drought + heat (Fig 4b). Piñon assimilation was positively correlated with nighttime CO_2 efflux (Fig 4c) and no differences were found between controls and treatments. Juniper A was also positively correlated with R, but heated trees respired more per unit A than did controls, drought or heat + drought. Q_{10} approached or equaled 1 at least once during the season for each treatment group and each species, and overall Q_{10} was quite variable (Fig S3a, Fig S3b). Piñon Q_{10} did not show differences between treatments at any time points, but generally increased over the season from 1.2 to a maximum of 2.4 in October (Fig S3a). Juniper controls showed the least variability, with a seasonal average of 2.2 and drought, heat, and drought + heat varied between 1 and 2.3 (Fig S3b).

Treatment effects on foliar carbon dynamics

Total June foliar NSC was higher in juniper than piñon and was positively correlated with temperature-corrected CO₂ efflux. When species were combined, a significant relationship between R_t and NSC is observed (Fig 5a). For piñon branches that exhibited foliar growth in 2013, June twig starch was well-correlated with R_t in August (Fig 5b). Assimilation for both species was often higher in the controls than in heat, drought or heat + drought groups (Fig S2a, Fig S2b). Over the growing season, the estimated cumulative CO₂ assimilated minus the cumulative CO₂ respired was reduced in all treatment groups compared to controls in both species (Fig 6a, Fig 6b). Piñon branches spent an average of 5.0, 36.3, 54.7 and 50.7 days in a negative carbon status (i.e. nightly respiration exceeded daytime assimilation) in controls, drought, heat and heat + drought, respectively, with heat dominating this response (Fig 7a, Fig S6a). The mean number of days spent in a negative carbon balance for juniper controls, drought, heat and heat + drought was 1.0, 14.7, 15.0 and 36.7 days respectively, and juniper heat + drought was significantly different than controls (Fig 7b, Fig S6b).

Impacts on growth and NSC

Piñon cumulative R_t was correlated with needle length in branches that grew leaves (Fig 8a). R_t was as high for branches that did not grow needles as those that did exhibit significant growth (Fig 8b). Piñon mean bud and needle lengths throughout the growing season were often higher in controls than in drought, heat and heat + drought, but differences were rarely significant. When scaled to the whole branch, total bud growth in piñon was higher in controls than in heat + drought, though drought and heat did not

differ from either controls or heat + drought (Fig S4). Most of the control piñons grew needles (five of six trees), as did all of the branches in heat and one each of the branches in drought and heat + drought. Using the June rates of R_t and total foliar starch measurements, we calculated that foliar starch in piñon could supply R_t for over twice as long as treatment groups, but this difference was not significant (Fig 9a). In juniper, heat and heat + drought significantly reduced the length of time that foliar starch could supply R_t (Fig 9b).

Discussion

Treatment effects and mechanisms regulating R

In testing hypothesis 1, we found that elevated temperature differentially affected piñon and juniper. At no time in 2013 did we observe heated piñon differing from controls (Fig 3a), which is consistent with thermal acclimation (Atkin & Tjoelker, 2003). Examining the piñon Q_{10} values in October, after water limitations had been removed due to a historically large rainfall event and when A and leaf water potential were at their seasonal high points, provides further support for this interpretation. On this sampling period, Q_{10} values indicated consistently strong temperature- R_t relationships across species and treatments, and the mean piñon Q_{10} in the heat treatment was lower than (though not statistically different from) controls (Fig S3a). In contrast to piñon, heated juniper exhibited consistently higher R than controls during the periods both before and after the initiation of growth (Fig 2b, Fig 3b), and no evidence in October Q_{10} values indicate that juniper experienced thermal acclimation (Fig S3b). Heated juniper displayed higher R_t than control trees for both a given leaf water potential (Fig 4b) and A (Fig 4d), again demonstrating that temperature caused a greater respiratory cost for heated junipers, consistent with a lack of acclimation.

While acclimation differed between species, other treatment effects were similar across species. We found that imposed drought decreased R_m in both piñon pine and juniper, consistent with hypothesis 2 (Fig 2a, Fig 2b). Additionally, for much of the year the relationship between R_t and either water potential or A was much more significant than the relationship between R_t and temperature, suggesting that temperature is not the most important driver of R_t in this system during a relatively dry year (Fig 4a-4d, Fig S3a, Fig

S3b, Fig S5). Both species experienced substantial periods of no growth in stems (S. Sevanto, personal communication) leaves or buds: two months for piñon, and four months for juniper. During this time of zero above-ground growth, lower R_m under drought (Fig 2a, Fig 2b) may be attributed directly to metabolic rates lowered by drought impacts on cellular water potential (Fig 4a, Fig 4b), or indirectly to water-mediated reductions in A (Fig 4c, Fig 4d) and consequent reductions in C availability for maintenance processes such as protein turnover, ion and osmotic gradient maintenance. Reductions in R_t during drought should benefit these species by reducing NSC consumption during periods of low A, however, reducing R_t may also have consequences on reducing the ability to repair damaged tissues, to grow, and to defend (Atkin & Macherel, 2009).

Piñon R_t rates in the drought treatment increased to match those of controls after soil moisture, A, and foliar growth increased in July (Fig 3a). The cumulative R_t in droughted piñon equaled that of controls by the end of the growing season (Fig 3a), which indicates that after growth onset, foliage incurred increases in maintenance costs, higher growth costs, or the combination of both, relative to the control trees. Droughted piñon trees experienced lower R_t at lower leaf water potentials than controls, but also experienced higher rates of R_t when water was available (Fig 4a), thus R during periods of high water availability was higher than for controls. Since the relationship between nighttime R_t and A was the same for drought and controls (Fig 4c), but the relationship between R_t and water potential differed in drought than controls (Fig 4a), it is plausible that water exerts a more direct control of R_t than does A. In fact, the strong correlation between the amount of starch stored in twigs in June and nighttime R_t in August when needle

elongation was occurring (Fig 5b), suggests that piñon foliar growth and associated R may have been fueled from stored C. Consistent with this, bud growth began in June when rates of A were indistinguishable from zero (Fig S2a), indicating that this growth was fueled by C from storage rather than recently assimilated C.

Juniper drought trees frequently exhibited lower cumulative R_t than controls throughout the season (Fig 3b). We observed good relationships between R_t and both leaf water potential and A (Fig 4b, Fig 4d), which strengthens the argument that either or both of these parameters influence R_t in juniper. Because drought did not differ from controls in these relationships or interact with leaf water potential or A, we exercise caution in speculating which parameter may be more important in driving R.

We predicted in hypothesis 3 that the combination of heat + drought would counterbalance the effects of drought alone and heat alone, and we found no evidence of this in piñon but some evidence to support this hypothesis in juniper. Heat + drought, and drought alone, both had lower R_m in piñon than controls (Fig 2a, Fig 3a). By the end of the season, heat + drought and drought alone both rose to meet rates of R_t in controls. Heat + drought also had similar effects to drought on foliar growth, with only one of three trees in each treatment producing needles. Thus, in piñon, heat + drought was often different than controls, but not different than the effect of drought alone. Since our results suggest that piñon trees in the heat treatment alone may have experienced thermal acclimation, this process may have occurred in heat + drought as well and the reductions in R_m we observed relative to the controls may have been dominated by a response to drought. In contrast, our measures of R_t in juniper heat + drought were intermediate between the effects of heat and of controls (Fig 2b, Fig 3b). Though R_m in heat was

different from controls, heat + drought was not different from either (Fig 2b). Over the course of the measurement period, mean heat + drought R_t was consistently between, but not statistically different from R_t in controls and heat (Fig 3b), suggesting that elevated R_t from the higher temperatures experienced by heat + drought was balanced by reductions in R_t from reduced water.

Piñon twig starch in June was correlated with August R_t in trees that grew leaves (Fig 5b), which is consistent with the idea that the accumulation of NSC during drought may benefit growth and R_t later when drought has ceased (e.g. Körner, 2003; McDowell, 2011; Dickman et al., 2014). In contrast, we found that juniper foliar total NSC was at best weakly correlated with nightly R_t in growing trees approximately one month later ($r^2=0.37$, $p=0.08$). This suggests that juniper may rely on recently assimilated C and piñon relies more upon storage to fuel metabolic processes. Juniper generally had higher foliar NSC than piñon, however, and piñon was on the lower end of a continuum between NSC and R (Fig 5a). This result is consistent with previously observed patterns in these species, where juniper operates as a relatively more anisohydric species, continuing A during drought when piñon behaves in a relatively more isohydric manner, (Martínez-Vilalta, Poyatos, Aguadé, Retana, & Mencuccini, 2014) ceasing stomatal conductance and thus eliminating recent C supply from A (McDowell et al., 2008; Limousin et al. 2013). Regardless of the mechanisms that may explain these phenomena, recognizing the patterns of carbohydrates remains an important goal in further developing predictive models of drought impacts on survival and mortality (McDowell et al., 2013).

Impacts on foliar carbon balance

Drought and heat had a significant negative impact on foliar-level C balance (as indexed by A-R) of both species (Fig 6a, Fig 6b). The clear relative differences between control groups and treatment groups are large and consistent over time and across species. We were careful not to underestimate A for our A-R calculations, thus conclusions about the frequency of A-R below zero are conservative (Fig 7a, Fig 7b). Piñon spent an average of 36.3, 54.7 and 50.7 days with negative A-R in drought, heat and heat + drought respectively, compared with 5.0 days for controls (Fig 7a). Other studies have documented the tendency for piñon to reduce transpiration and therefore A to zero during periods of low soil moisture (Limousin et al., 2013; West, Hultine, Sperry, Bush, & Ehleringer, 2008) and thus it is not surprising that all treatments sustained some amount of time when whole-plant R_t was fueled by NSC storage. Though drought, heat, and heat + drought were statistically indistinguishable from one another, the trend is suggestive of exacerbated periods of low A-R for piñon trees experiencing drought, and even more so for those experiencing hotter temperatures. The trend in juniper is similar to that of piñon, with drought, heat, and heat + drought trees spending 14.7, 15.0 and 36.7 days respectively in a negative C balance compared to 1.0 days for controls (Fig 7b). That drought alone or heat alone was not significantly different from controls however, is to be expected; juniper typically maintains some new C inputs via continued A during drought, and in the presence of ambient levels of soil moisture, we may expect the same from trees that are heated. That foliar A-R in juniper is substantially negatively impacted by the combination of heat and drought suggests that these conditions may alter whole-plant C balance over time, consistent with the previously observed periods of low NSC of

droughted and dying juniper (Dickman et al., 2015). Thus, while juniper clearly has superior A-R balance over piñon during drought, it also exhibits large impacts of drought that suggest it is also vulnerable to future, warmer droughts (McDowell et al. *in revision*; Breshears et al., 2005). We suggest that the reader exercise caution in interpreting this index of time spent when A-R is less than zero, as it should not imply absolute time spent, or the absolute amount by which foliar C is impacted, and only that our imposed treatments likely affect whole-plant C dynamics, as measured at the shoot level.

Consequences of lowered foliar carbon balance

One potential consequence of a reduced A-R is a concomitant reduction in growth. Juniper grew very little in 2013 across all treatments (Adams et al., In press). In piñon pine however, of the trees that grew needles in 2013, (five of six controls, all heated trees and one each of drought and heat + drought trees) needle growth was correlated with cumulative R_t (Fig 8a). Although we did not partition the growth components from R_t , this relationship indicates that growth and R_t are linked, an idea that is well established (Ryan, 1991b; Amthor, 2000) and unchanged regardless of whether the demand for growth drives R_t or if R_t drives growth. Piñon trees in the drought and heat groups respired as much cumulative nighttime C as did controls, but foliar and bud growth was reduced or eliminated in trees that were subjected to drought, heat, or both (Fig 8b, Fig S3). Trees that experienced reduced foliar growth may have allocated a larger portion of C than controls to export, storage, or root growth. These consistent reductions in foliar growth should force greater reliance on older, less efficient photosynthetic machinery that

unless replaced with new leaves at some point, would promote net reductions in A and increase their susceptibility to carbon starvation.

Implications of drought and heat on piñon and juniper

All of the trees we studied were exposed to a natural drought in addition to the artificial drought we imposed upon them. Though we did not witness mortality, the stress responses we did observe are likely on a spectrum of responses with survival on one end and mortality on the other. Though difficult to observe, elucidating thresholds along this spectrum is useful in understanding and predicting continued survival versus gradual versus punctuated mortality. Drought has been shown to decrease A and result in the accumulation of NSC concurrent with growth reductions, and in the absence of new C inputs via A, tissues must therefore rely on stored C (Sala et al. 2012; Dickman et al., 2014). If the canopies of our trees were to rely on the stored foliar starch that was present during the driest time of year (June), that starch reserve would become depleted much more rapidly in heated juniper (Fig 9b). The trend appears to be similar for piñon, though this result was not statistically significant (Fig 9a, $p=0.131$). Although we saw that drought reduced R_t , continued R_t is necessary for plant survival and our results suggest that R_t could deplete an important C pool when exposed to heat in the presence of moderate (natural in our experiment) or severe (artificially imposed in our experiment) drought. Had the 2013 drought continued, we speculate that we may have observed mortality and that C starvation may have been an important component in distinguishing survival from mortality.

Overall, our results reveal that co-dominant piñon and juniper respond differently in foliar R_t , A-R, growth, and NSC dynamics to drought and to heat. Piñon, which is historically viewed as a relatively isohydric species, experiences a more negative C balance during seasonal and prolonged droughts than the relatively more anisohydric juniper (Dickman et al., 2015; McDowell et al., 2013). Thus, it is logical that piñon R_t acclimates to temperature while juniper did not, since it may be adaptive for piñon to be more plastic in its regulation of R_t due to its wider swings in A than in juniper. Despite these differences between species, R_t in both appears to be driven by soil moisture or A, singularly or in combination, and is much less regulated by temperature during drought than has been observed in more mesic species under wetter conditions (e.g. Atkin and Tjoelker, 2003). Temperature does, however, play a role in the C balance for both species and elevated temperature alone or in combination with drought may expose both species to risk of mortality in a hotter, drier climate. This is a novel finding for *J. monosperma*, which has persisted on the landscape through drought in many regions where *P. edulis* has not (Breshears et al., 2005). We recognize the need for additional manipulative experiments such as this, perhaps utilizing isotopic tracers to further investigate the drivers and sources of R_t , growth, and NSC fluxes in all plant compartments with the goal of understanding the mechanisms behind stress responses and ultimately to model and predict vegetation patterns and global C fluxes.

Figures

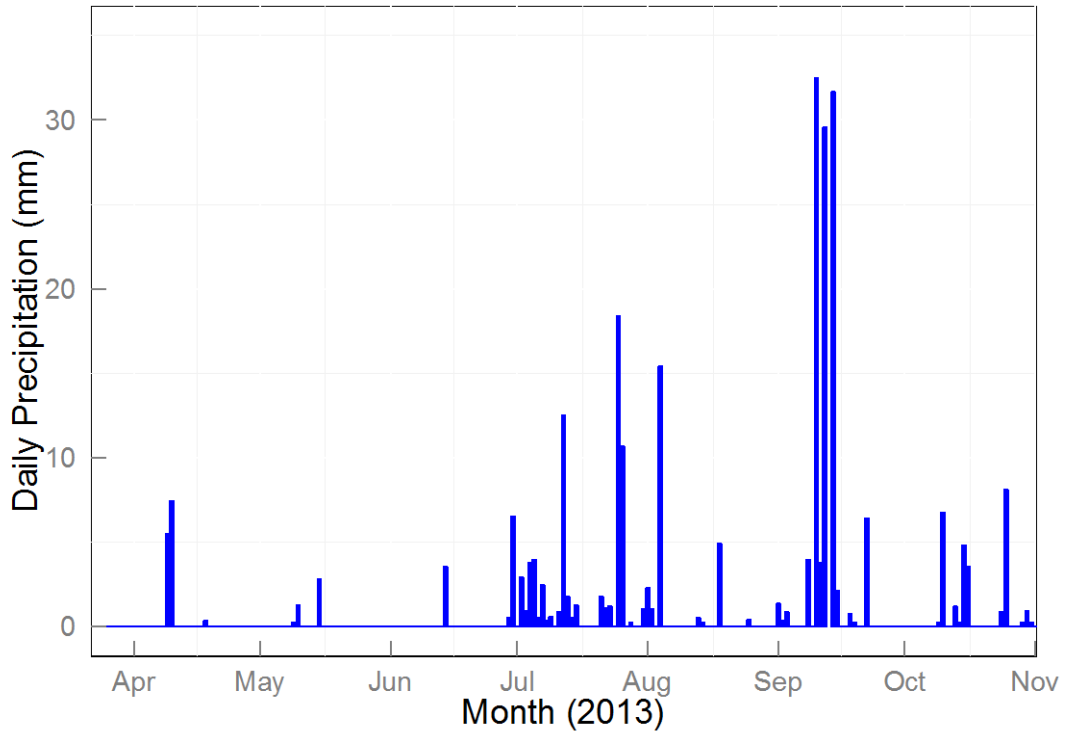


Figure 1a. Daily precipitation during the period of measurement from April through October 2013. Each bar represents a daily mean from three rain gauges. Error bars are omitted for clarity.

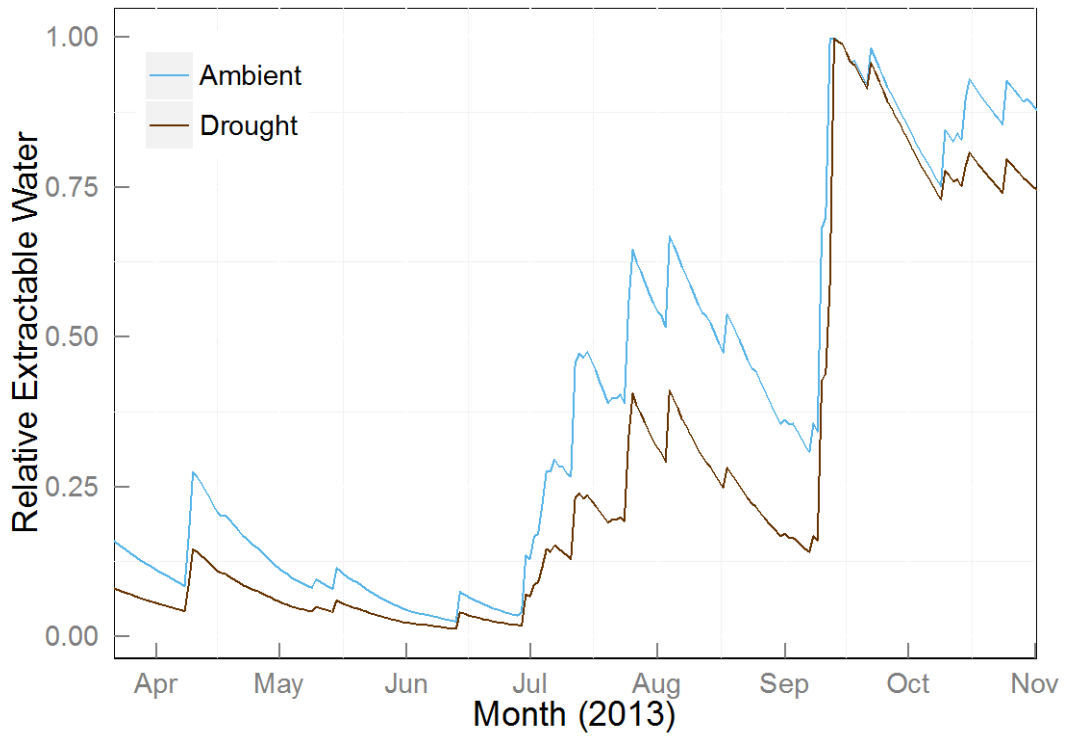


Figure 1b. Relative Extractable Water as estimated from the BIJOU water balance model.

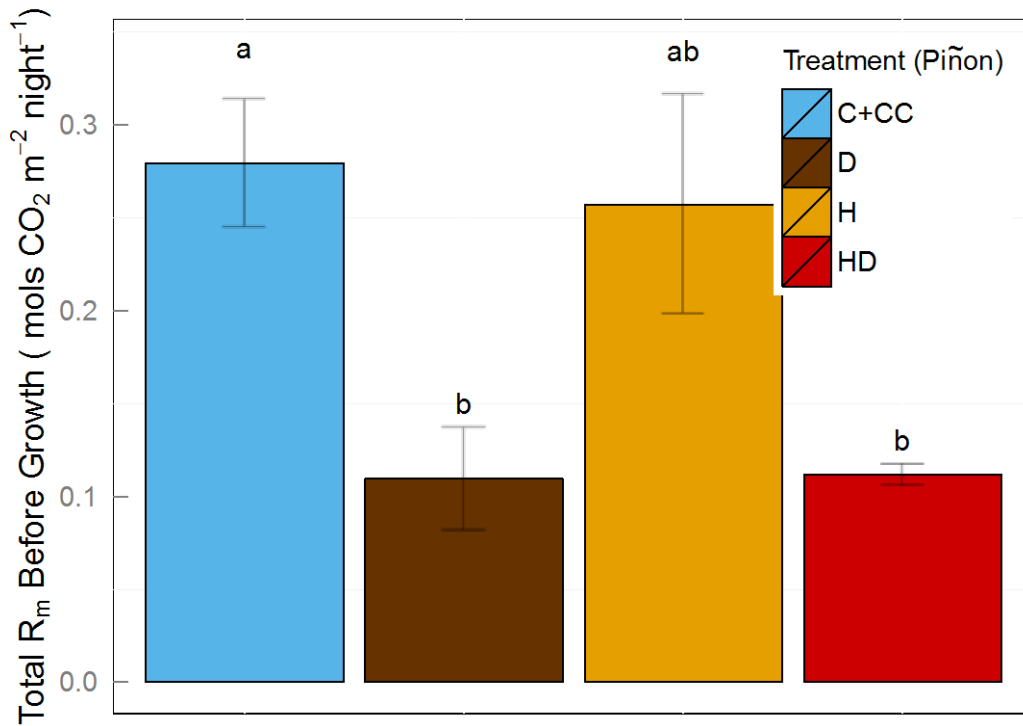


Figure 2a. Maintenance respiration (R_m) prior to initiation of foliar or bud growth in piñon foliage. Error bars represent +/- standard error of the mean. Letters indicate significant difference from controls ($p < .05$).

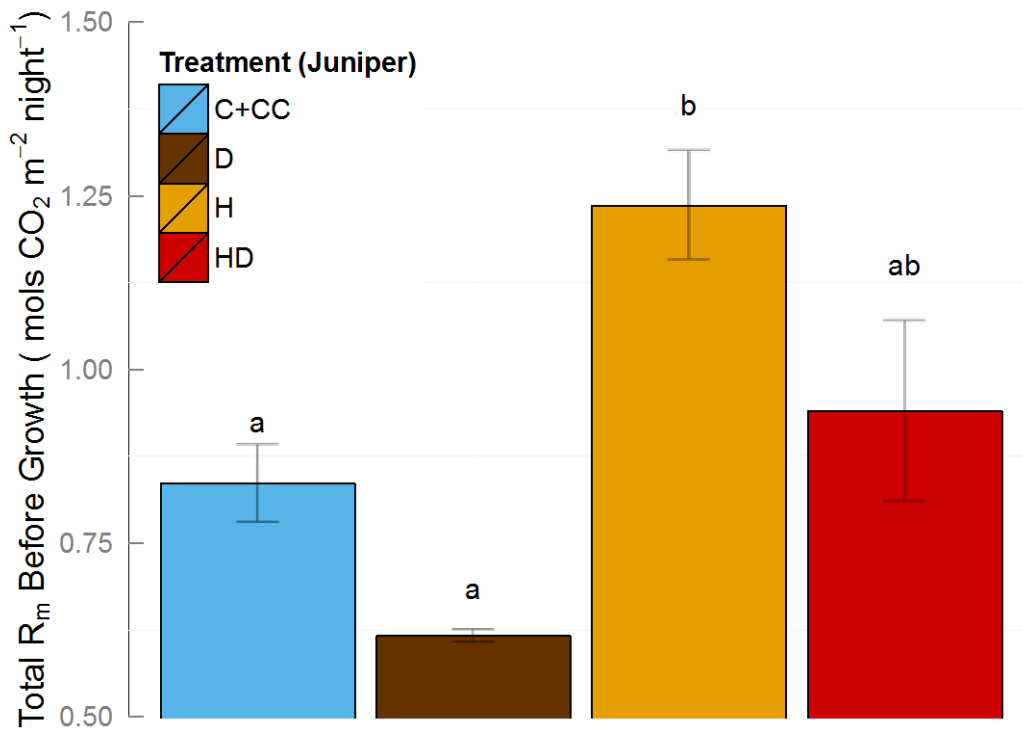


Figure 2b. Maintenance respiration (R_m) prior to initiation of foliar or bud growth in juniper foliage. Error bars represent +/- standard error of the mean. Letters indicate significant difference from controls ($p < .05$).

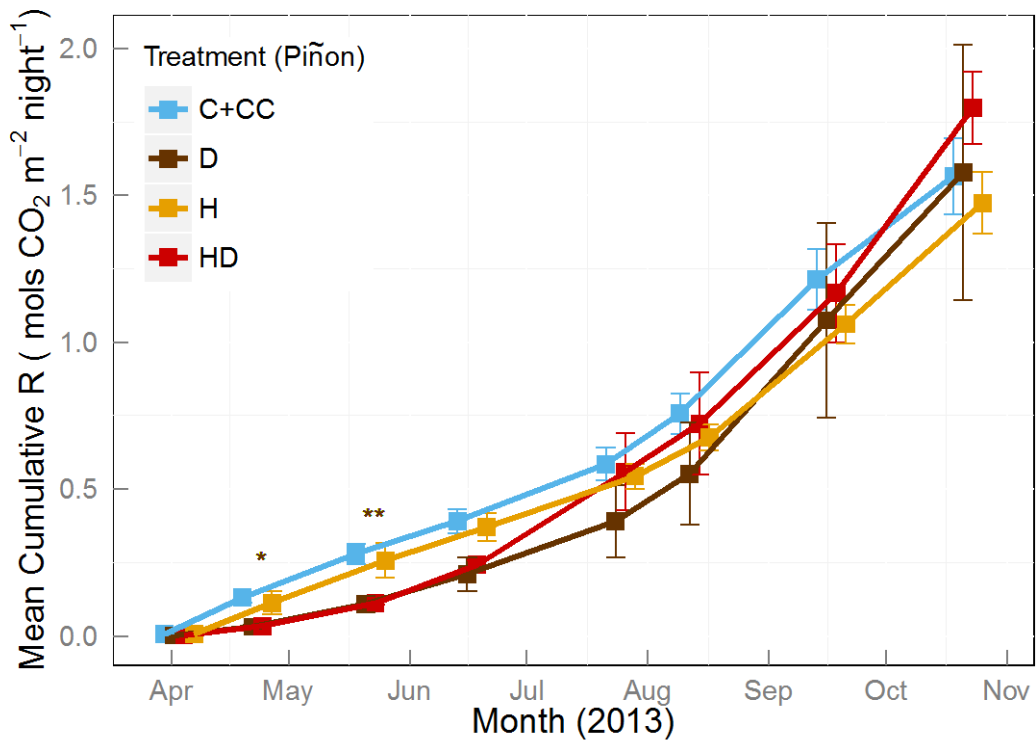


Figure 3a. Piñon cumulative respiration in foliage over the growing season. Error bars represent +/- standard error of the mean. Asterisks indicate significance from controls (C+CC): * p<.05; ** p<.01; *** p<.001. HD significance is only denoted when HD differs from C+CC but H and D do not.

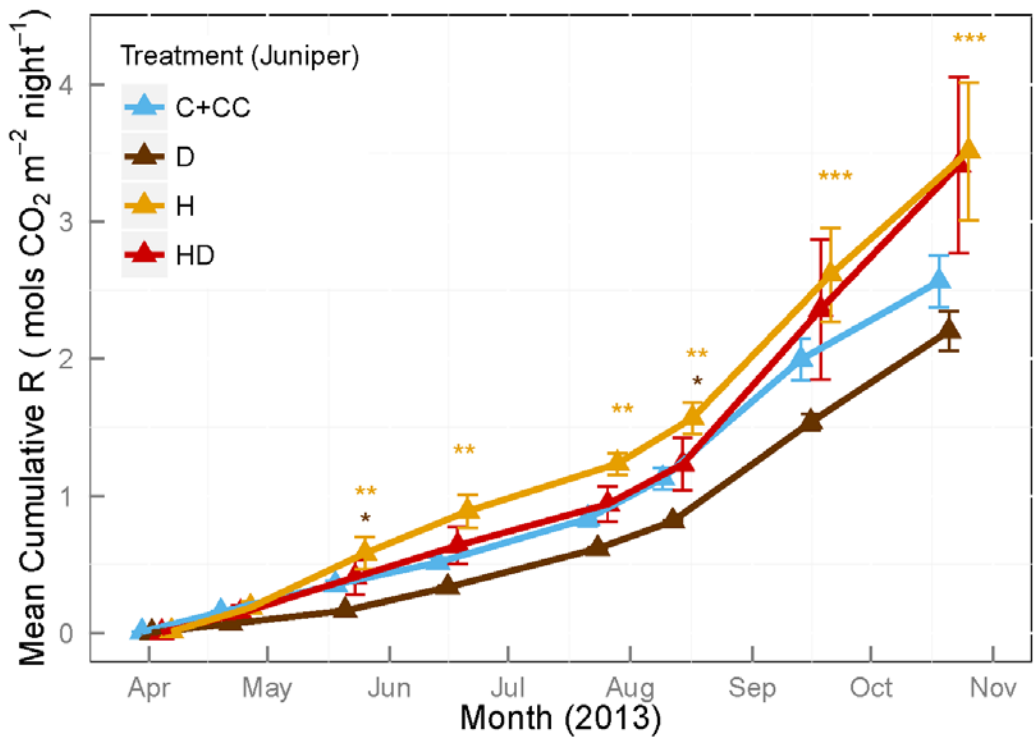


Figure 3b. Piñon cumulative respiration in foliage over the growing season. Error bars represent +/- standard error of the mean. Asterisks indicate significance from controls (C+CC): * p<.05; ** p<.01; *** p<.001. HD significance is only denoted when HD differs from C+CC but H and D do not.

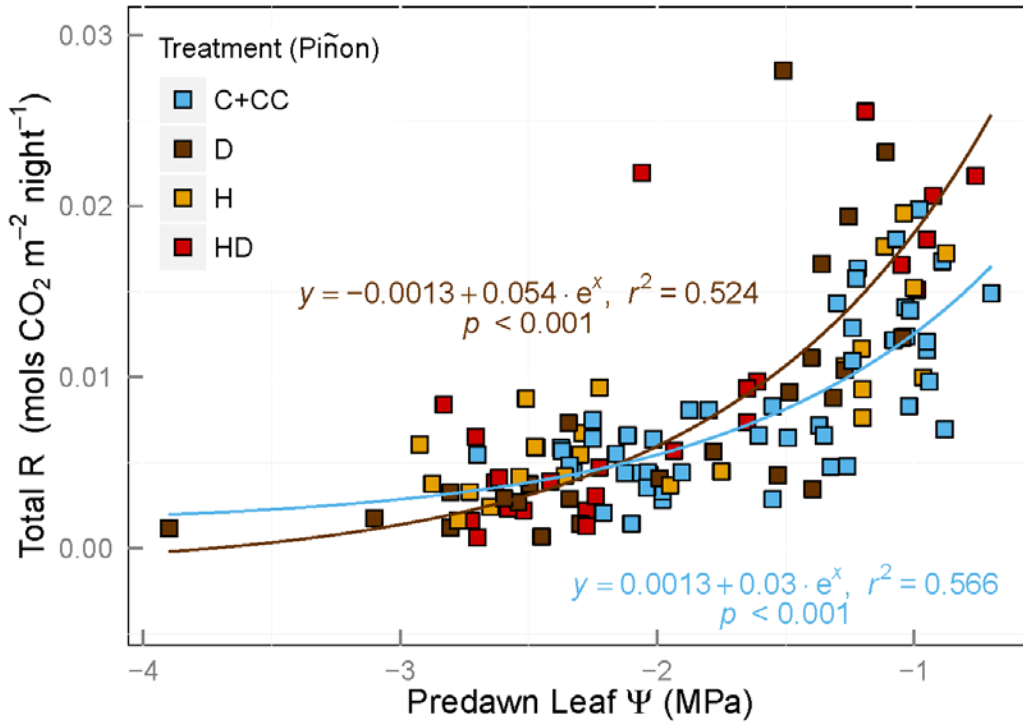


Figure 4a. Piñon nighttime respiration as a function of predawn leaf water potential over the growing season. Solid lines represent exponential curve fits to the data; the drought treatment (brown line) was the only treatment significantly different than controls (blue line).

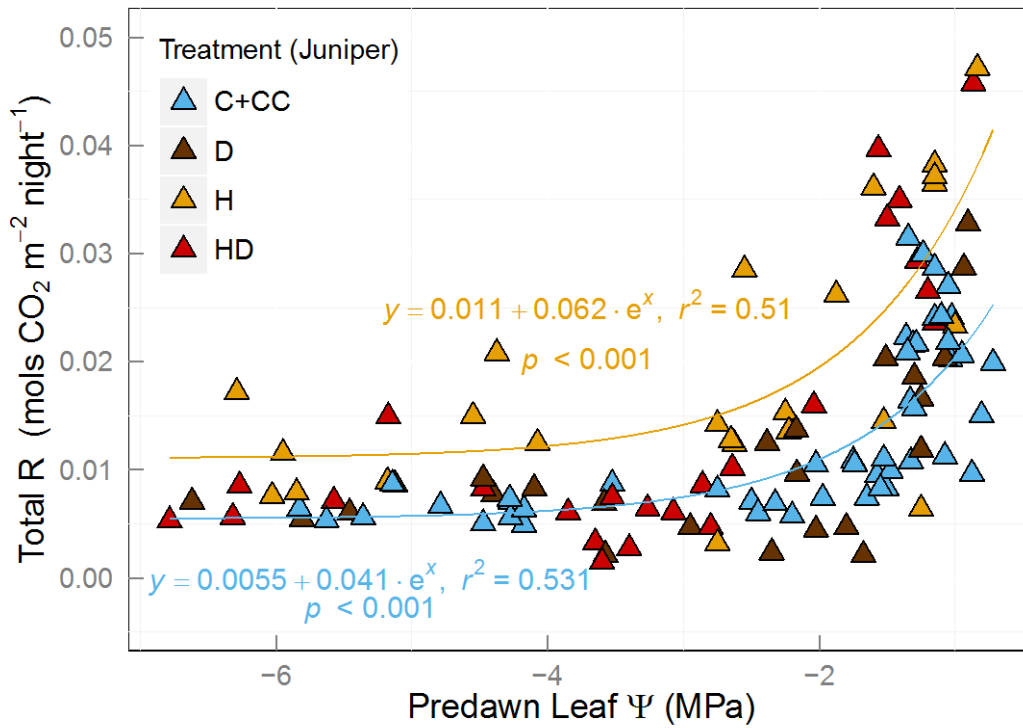


Figure 4b. Juniper nighttime respiration as a function of predawn leaf water potential over the growing season. Solid lines represent exponential curve fits to the data; the heat treatment (orange line) was the only treatment significantly different than controls (blue line).

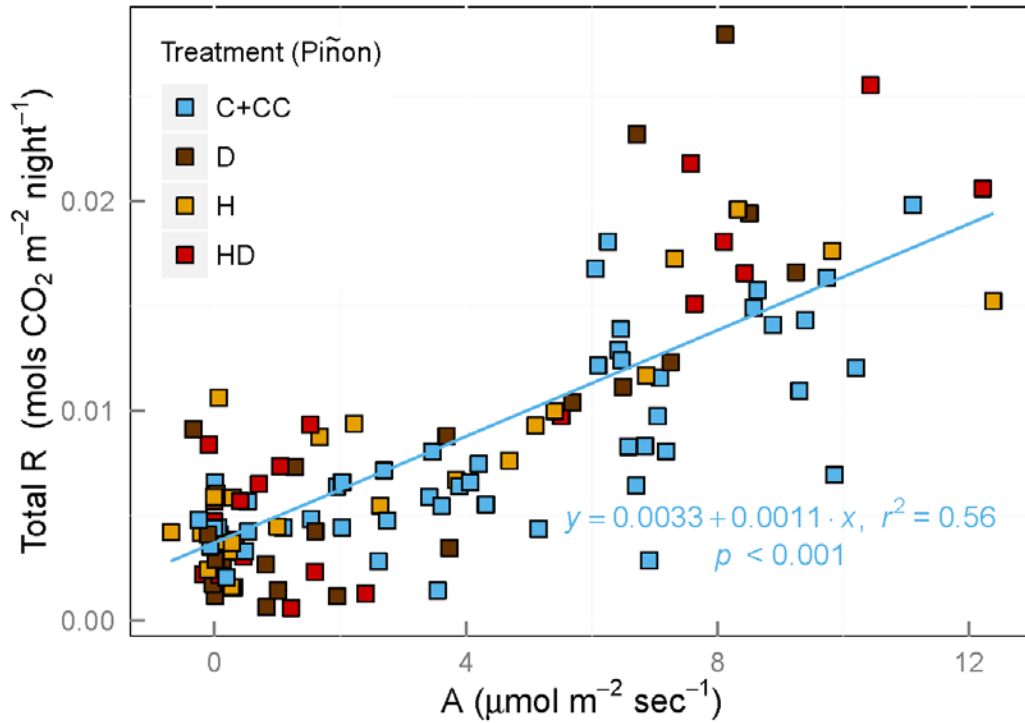


Figure 4c. Piñon nighttime respiration as a function of assimilation over the growing season. The solid blue line represents a linear regression fit to the control data. No differences were observed between treatments and controls.

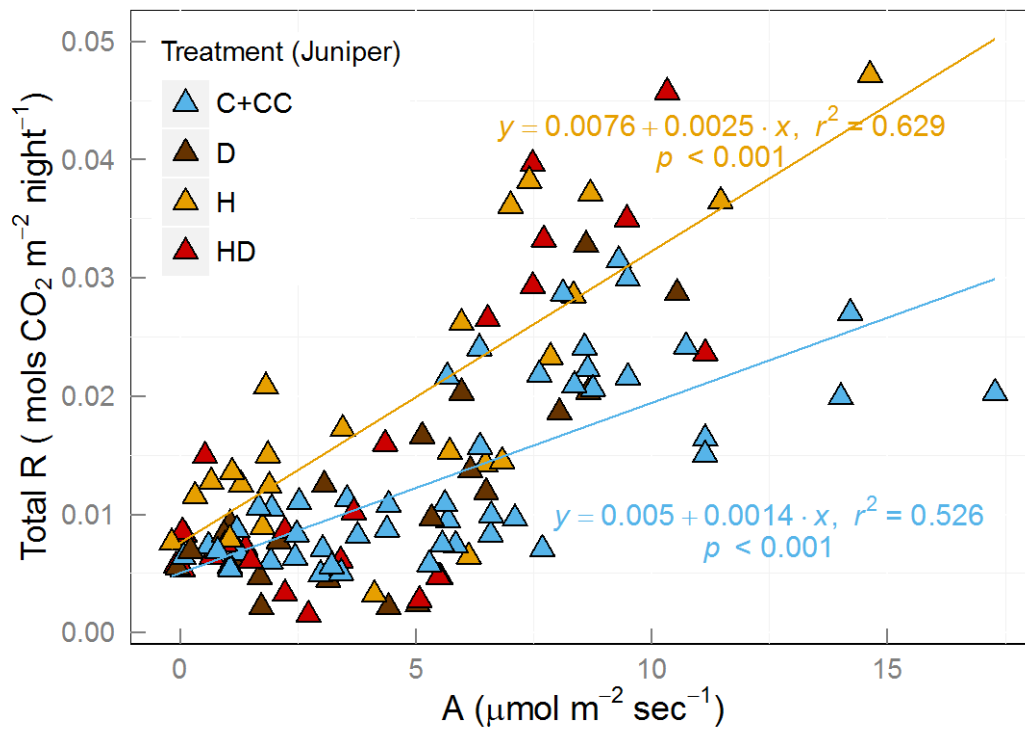


Figure 4d. Juniper nighttime respiration as a function of assimilation over the growing season. Solid lines represent linear regression fits to the data; the heat treatment (orange line) was the only treatment significantly different than controls (blue line).

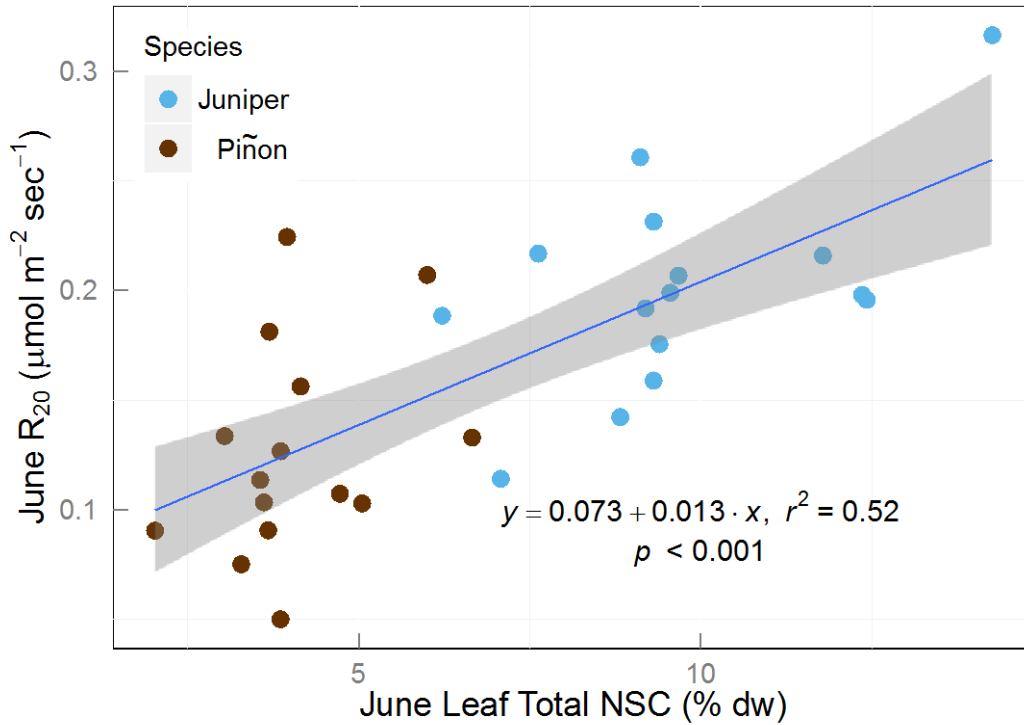


Figure 5a. Respiration temperature corrected to 20 °C as a function of June foliar NSC. The solid blue line represents a linear regression fit to the data and the shaded area represents a 95% confidence interval (CI).

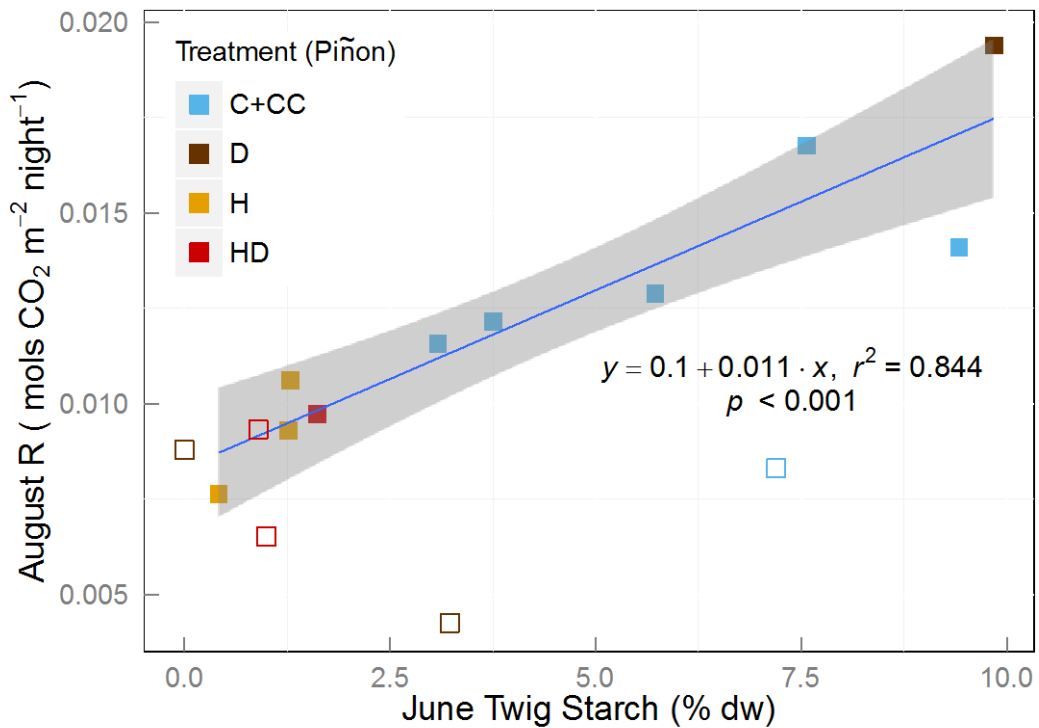


Figure 5b. August respiration as a function of June twig starch. Closed symbols represent trees that grew needles in 2013 and open symbols represent trees that did not grow needles in 2013. The solid blue line represents a linear regression fit to the closed symbols and the shaded area represents a 95% CI.

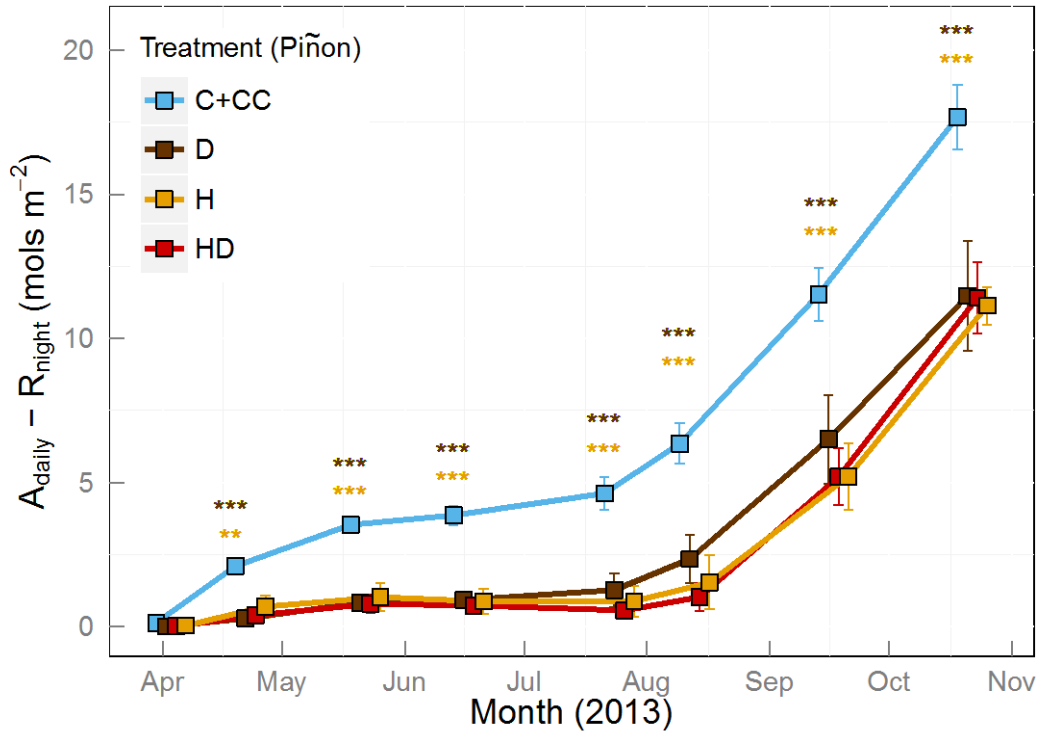


Figure 6a. Piñon cumulative estimated foliar carbon balance over the season. Error bars represent \pm standard error of the mean. Asterisks indicate significance from controls (C+CC): * $p < .05$; ** $p < .01$; *** $p < .001$. HD significance is only denoted when HD differs from C+CC but H and D do not.

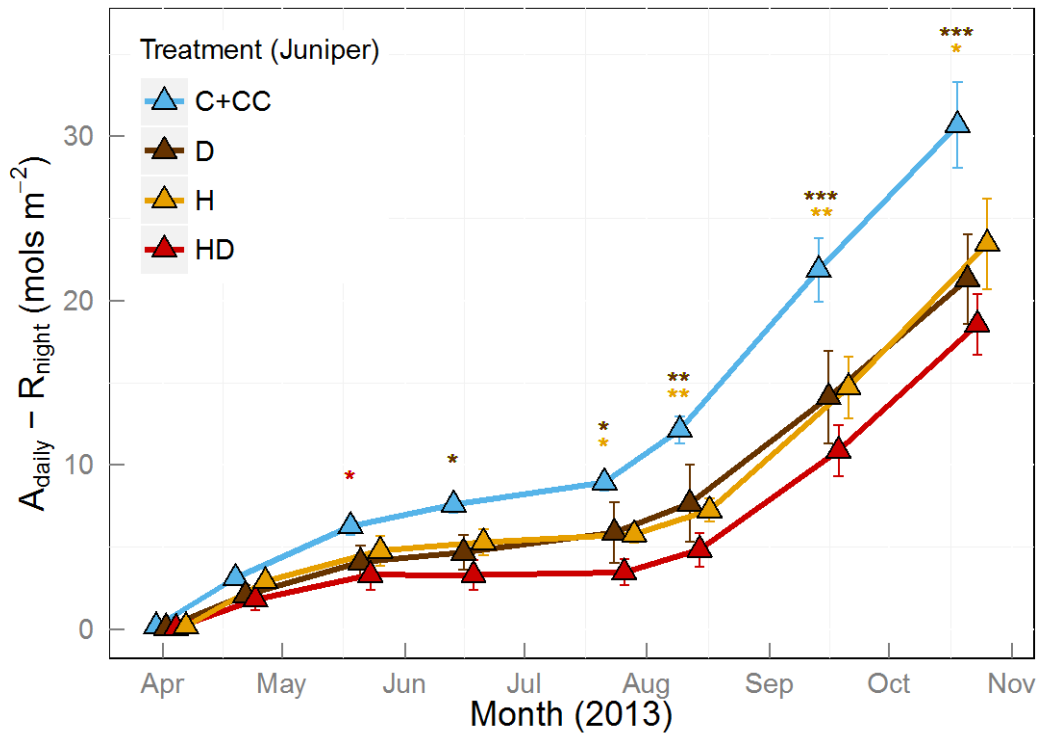


Figure 6b. Juniper cumulative estimated foliar carbon balance over the season. Error bars represent \pm standard error of the mean. Asterisks indicate significance from controls (C+CC): * $p < .05$; ** $p < .01$; *** $p < .001$. HD significance is only denoted when HD differs from C+CC but H and D do not.

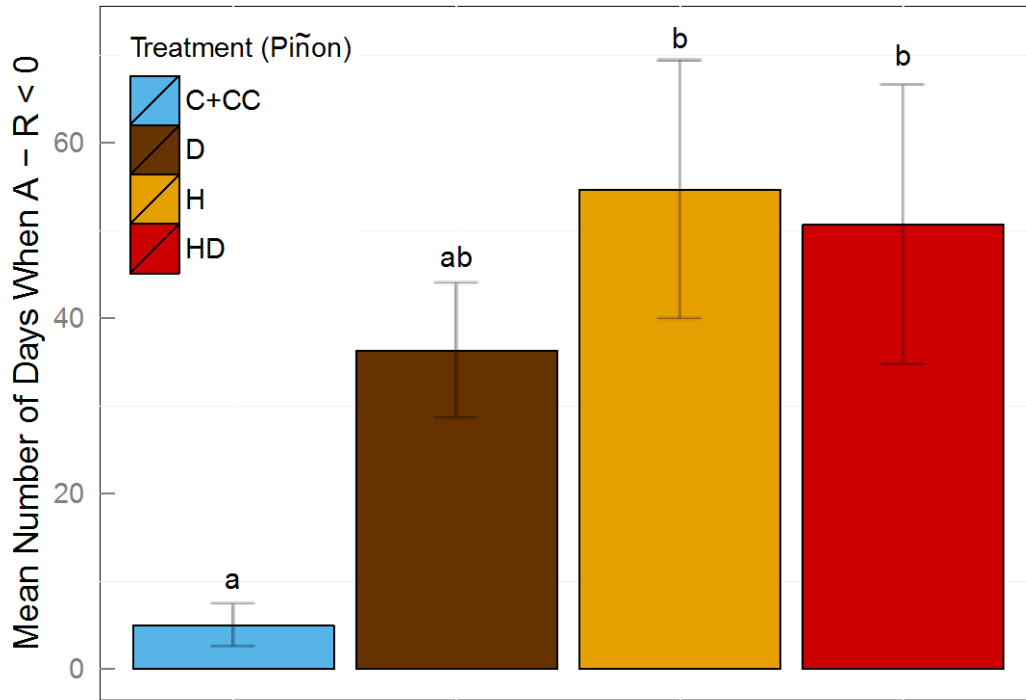


Figure 7a. Piñon number of days when foliar A - R was less than zero. Error bars represent +/- standard error of the mean. Letters indicate significant difference from controls ($p < .05$).

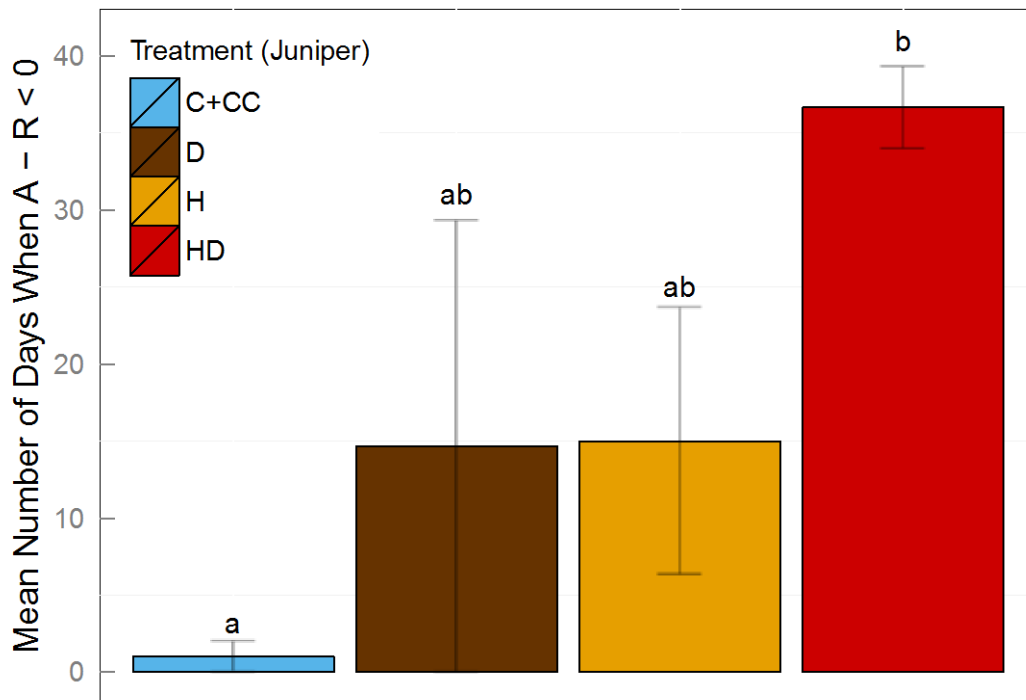


Figure 7b. Juniper number of days when foliar A - R was less than zero. Error bars represent +/- standard error of the mean. Letters indicate significant difference from controls ($p < .05$).

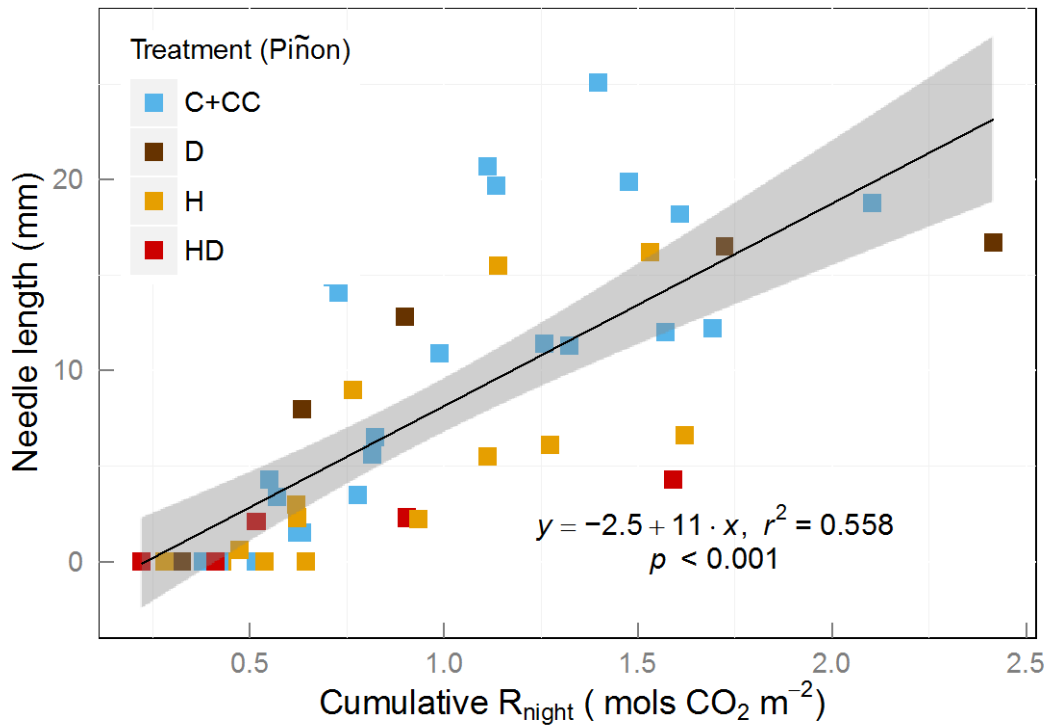


Figure 8a. Piñon needle length as a function of cumulative nighttime respiration in trees that grew needles in 2013. The line represents a linear regression fit to all data and the shaded area indicates a 95% CI.

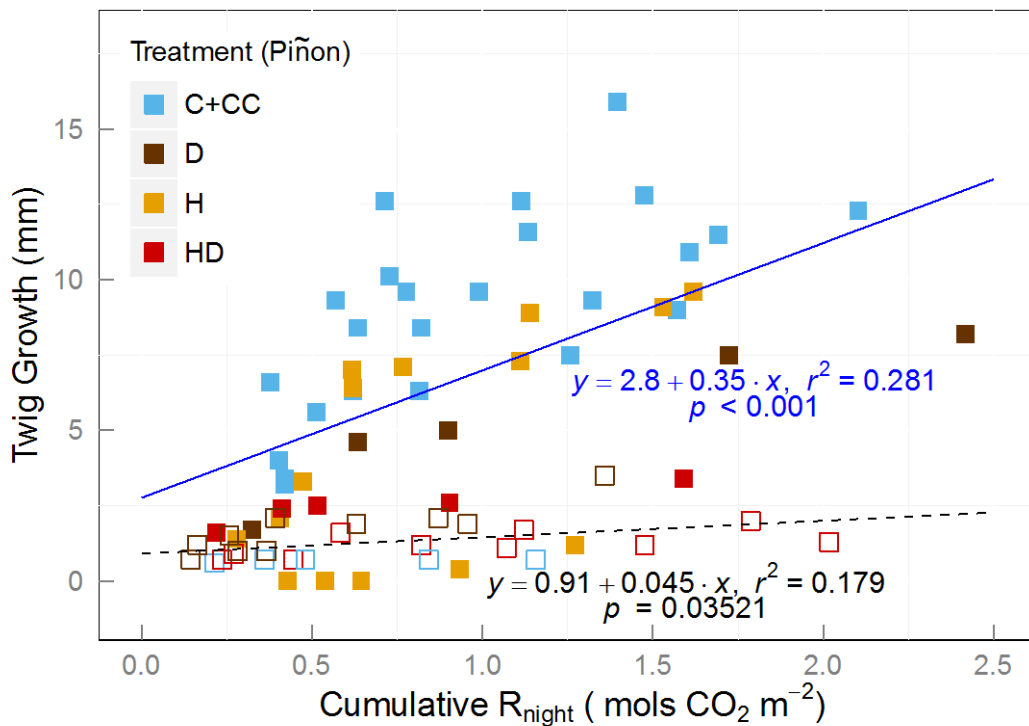


Figure 8b. Piñon twig growth for all branches as a function of cumulative nighttime respiration. Closed symbols indicate branches that grew needles in 2013 and open symbols indicate branches that did not grow needles. The solid blue line represents a linear regression fit to the controls that grew needles and the black dashed line represents a linear regression fit to all treatments that did not grow needles.

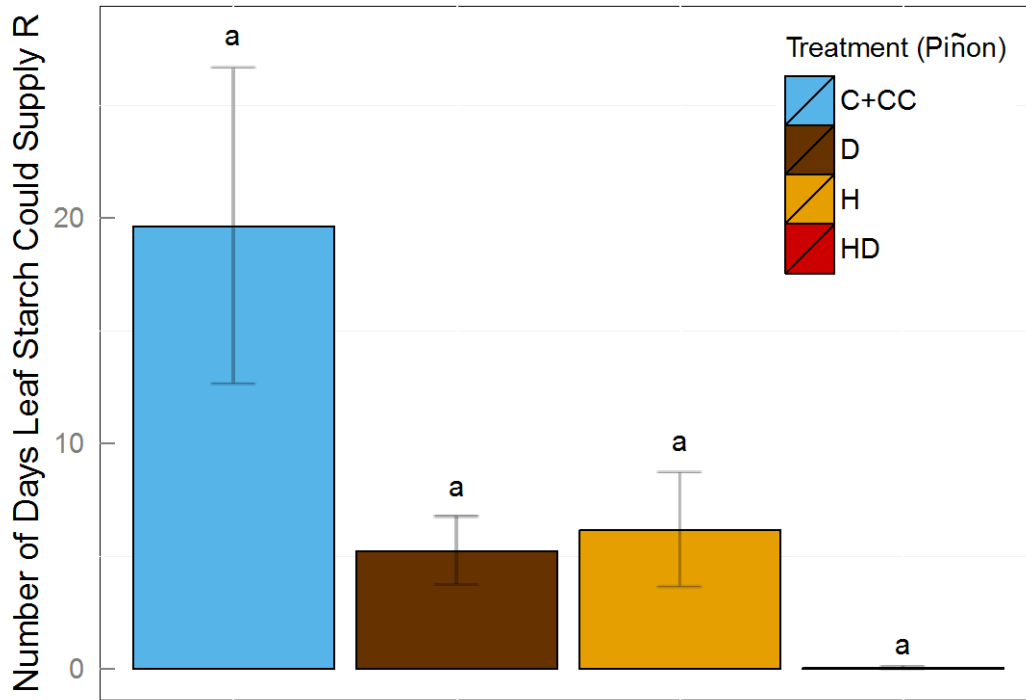


Figure 9a. The number of days in piñon that the foliar starch present in June could supply respiration at the rate of R in June without further C inputs. Error bars represent +/- standard error of the mean. Letters indicate significant difference from controls ($p < .05$).

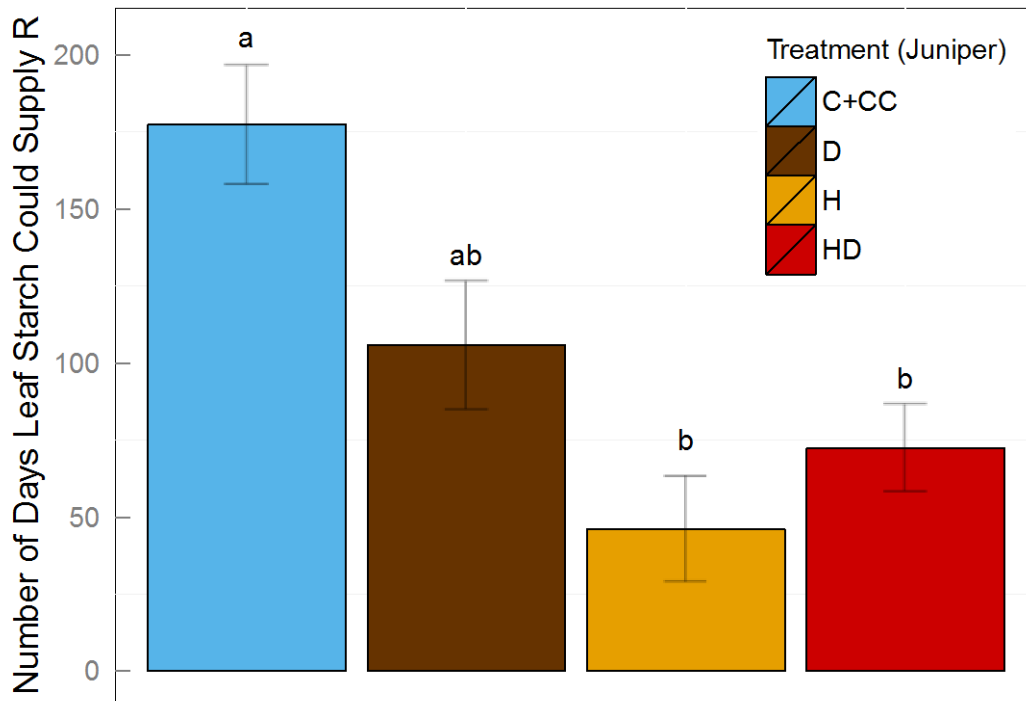


Figure 9b. The number of days in juniper that the foliar starch present in June could supply respiration at the rate of R in June without further C inputs. Error bars represent +/- standard error of the mean. Letters indicate significant difference from controls ($p < .05$).

Appendices

APPENDIX A: Supporting Information

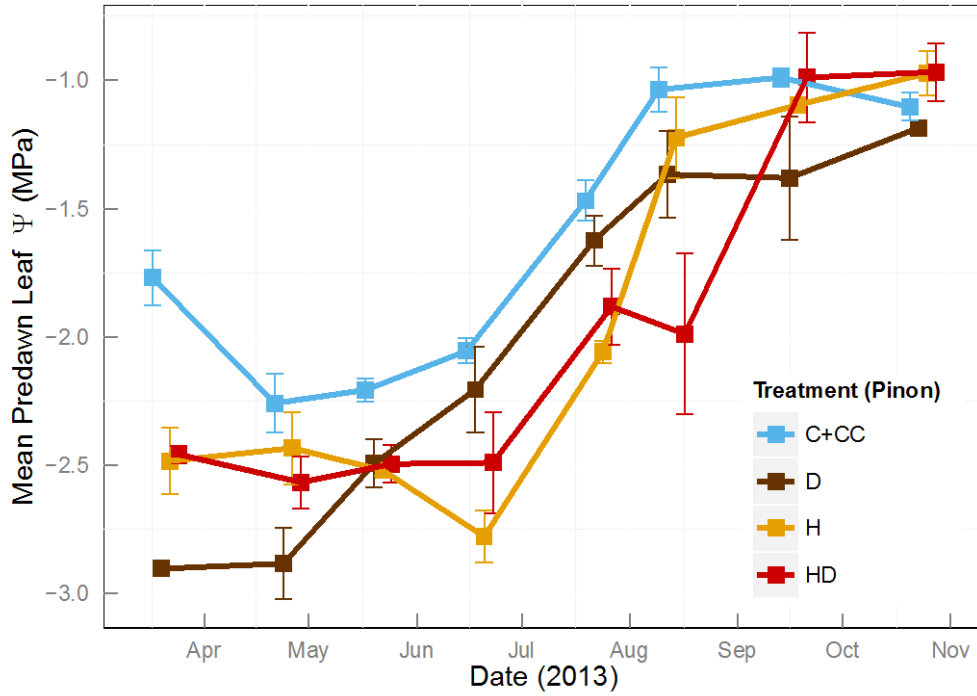


Figure S1a. Piñon mean predawn leaf water potential. Error bars represent +/- standard error of the mean.

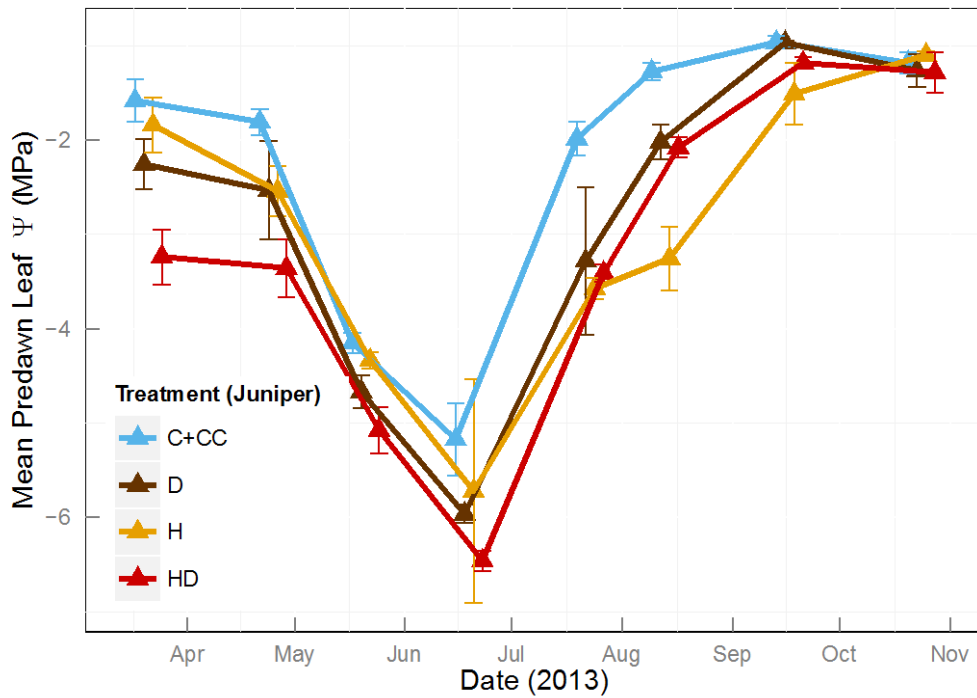


Figure S1b. Juniper mean predawn leaf water potential. Error bars represent +/- standard error of the mean.

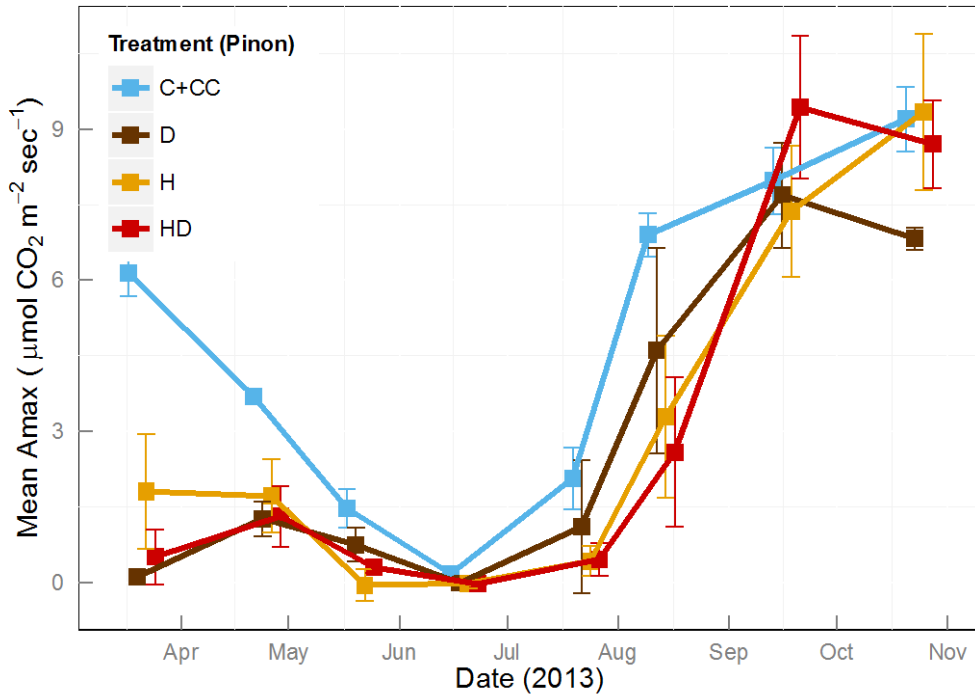


Figure S2a. Piñon mean maximal assimilation over the growing season. Error bars represent +/- standard error of the mean.

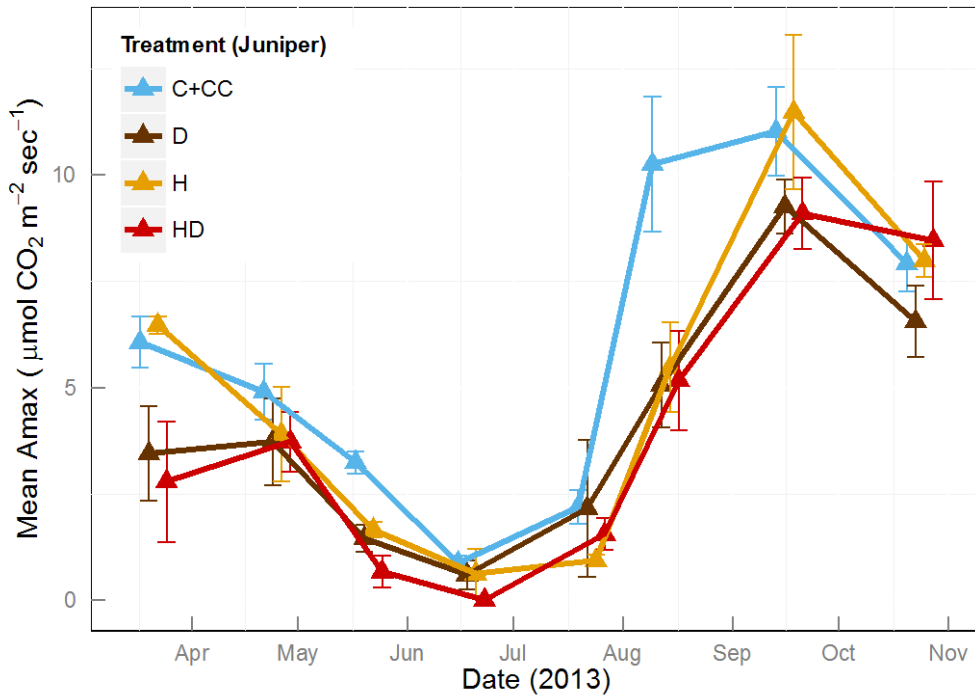


Figure S2b. Juniper mean maximal assimilation over the growing season. Error bars represent +/- standard error of the mean.

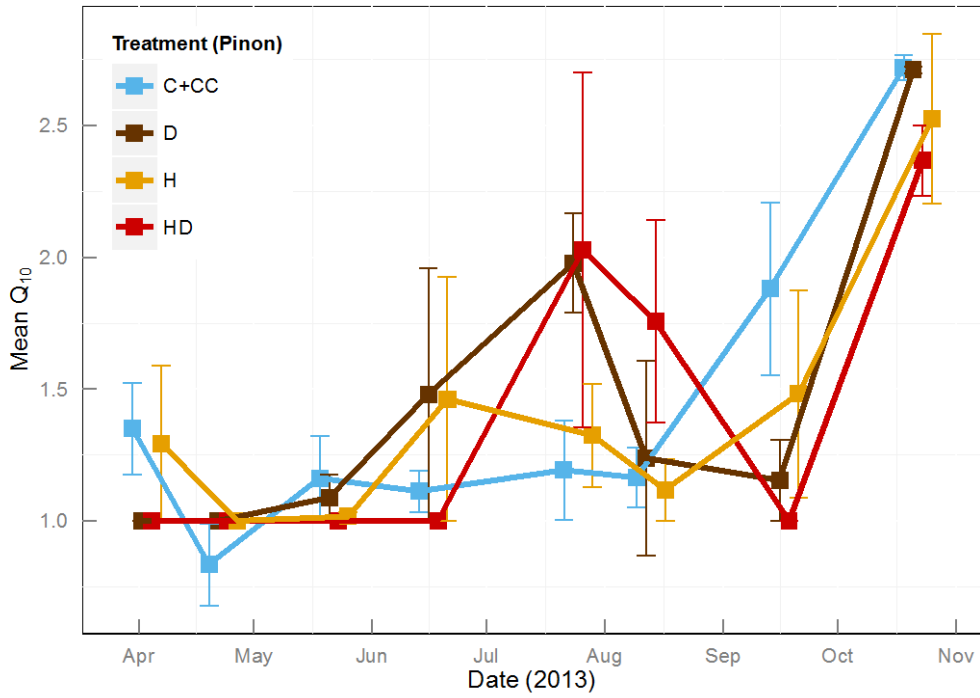


Figure S3a. Piñon mean Q_{10} over the growing season. Error bars represent +/- standard error of the mean.

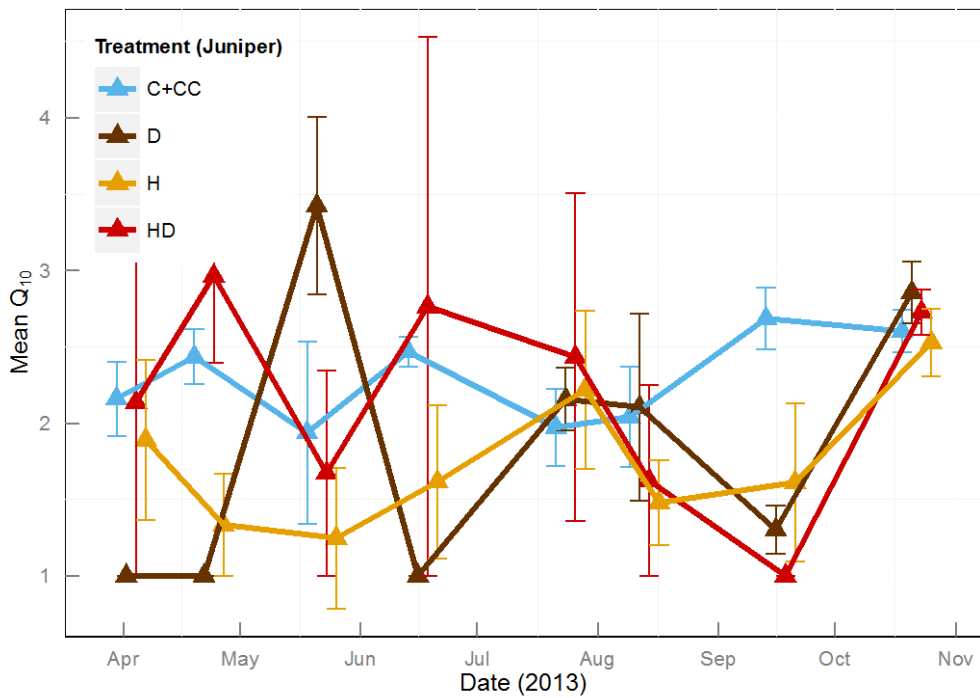


Figure S3b. Juniper mean Q_{10} over the growing season. Error bars represent +/- standard error of the mean.

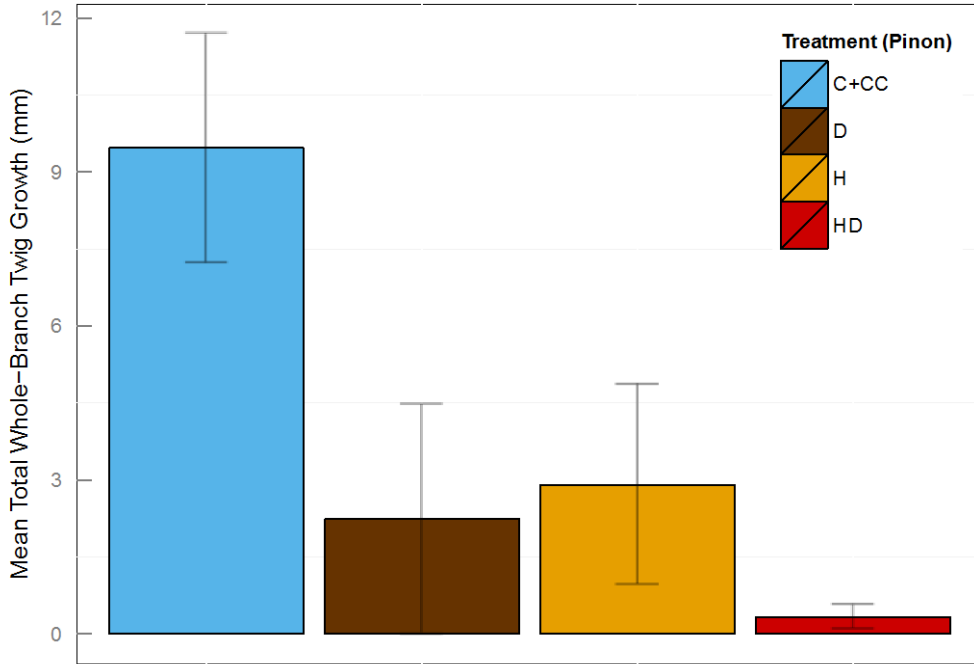


Figure S4. Mean whole-branch twig growth. Error bars represent +/- standard error of the mean.

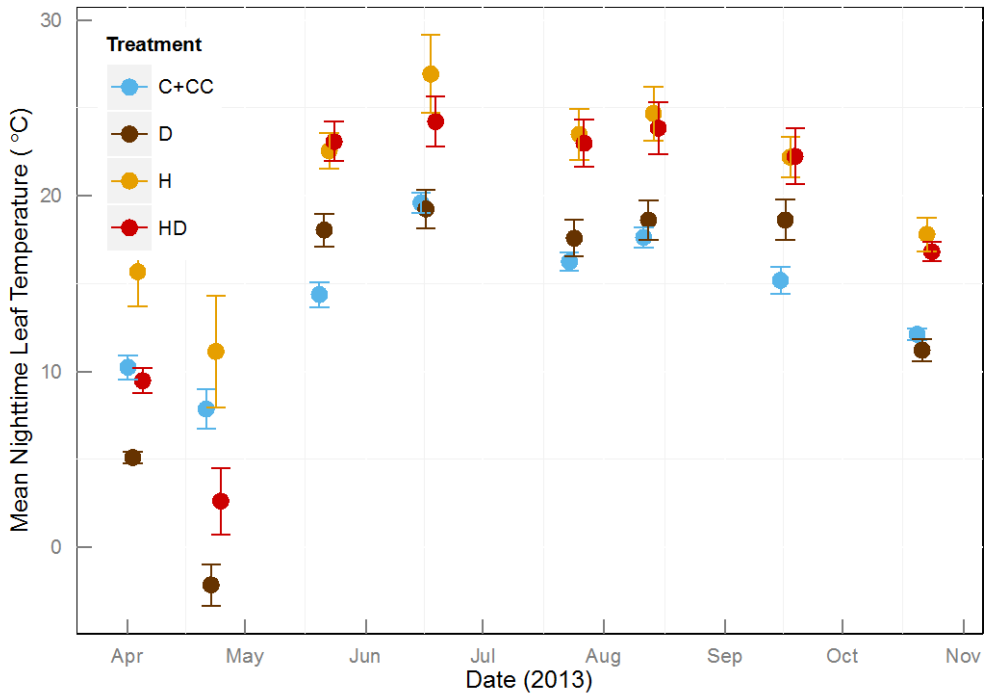


Figure S5. Mean nighttime foliar temperature over the measured growing season for both species. Error bars indicate +/- standard error of the mean.

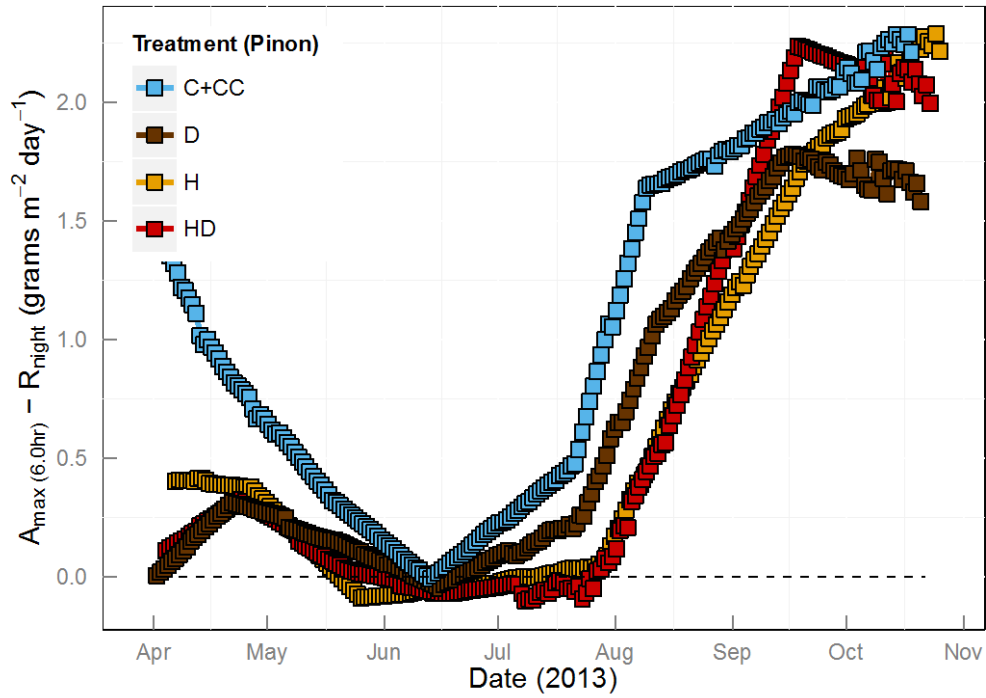


Figure S6a. Piñon mean of A-R over the growing season. Error bars are omitted for clarity. Values below zero indicate that for a given treatment on a given day, mean R exceeds A.

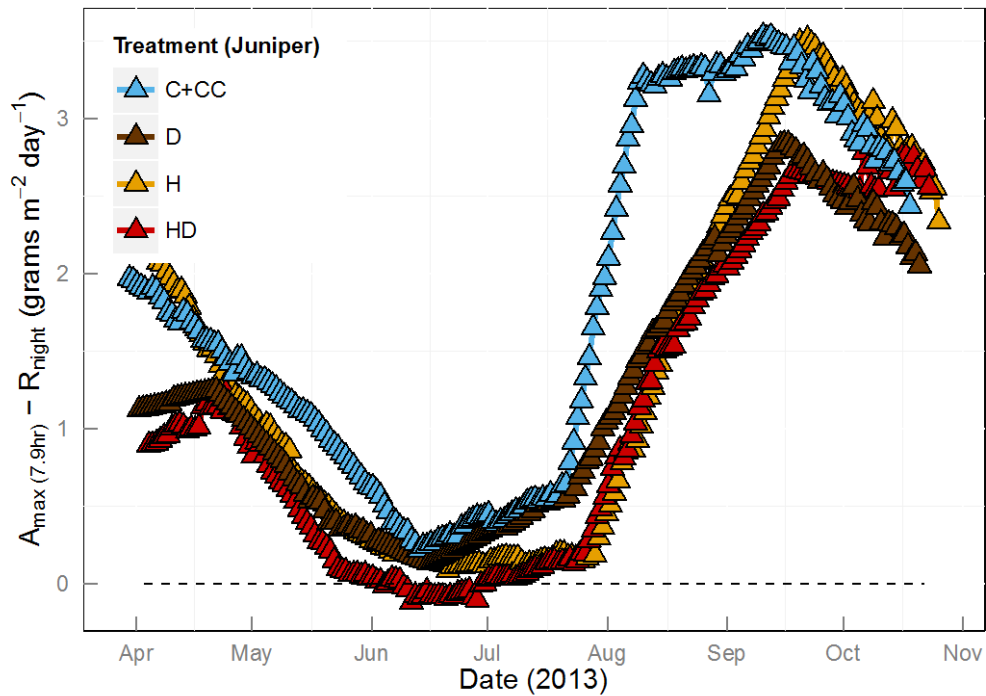


Figure S6b. Juniper mean of A-R over the growing season. Error bars are omitted for clarity. Values below zero indicate that for a given treatment on a given day, mean R exceeds A.

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