

**Responses of two semiarid conifer tree species to reduced precipitation and warming  
reveal new perspectives for stomatal regulation**

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## Abstract

Relatively anisohydric species are predicted to be more predisposed to hydraulic failure than relatively isohydric species, as they operate with narrower hydraulic safety margins. We subjected co-occurring anisohydric *Juniperus monosperma* and isohydric *Pinus edulis* trees to warming, reduced precipitation, or both, and measured their gas exchange and hydraulic responses. We found that reductions in stomatal conductance and assimilation by heat and drought were more frequent during relatively moist periods, but these effects were not exacerbated in the combined heat and drought treatment. Counter to expectations, both species exhibited similar  $g_s$  temporal dynamics in response to drought. Further, whereas *P. edulis* exhibited chronic embolism, *J. monosperma* showed very little embolism due to its conservative stomatal regulation and maintenance of xylem water potential above the embolism entry point. This tight stomatal control and low levels of embolism experienced by juniper refuted the notion that very low water potentials during drought are associated with loose stomatal control and with the hypothesis that anisohydric species are more prone to hydraulic failure than isohydric species. Because direct association of stomatal behavior with embolism resistance can be misleading, we advocate consideration of stomatal behavior relative to embolism resistance for classifying species drought response strategies.

**Keywords:** drought, increased temperature, global change, mortality, iso- vs. anisohydric behavior, stomatal conductance, hydraulic conductivity, hydraulic failure, carbon starvation.

## Introduction

Drought-induced forest mortality has become a major focus of attention in plant ecological research (Allen et al. 2010). Plant responses to drought include adjustments at different timescales and have been characterized according to different schemes (e.g. Chaves et al. 2003; Maseda & Fernández 2006; Choat et al. 2012). A useful framework classifies plants based on stomatal regulation of leaf water potential in response to changes in atmospheric moisture demand and soil water supply (isohydric vs. anisohydric species; Stocker 1956; Larcher 1975; Jones 1998; Tardieu & Simonneau 1998). The iso- vs. anisohydric dichotomy has been given a central role in theories explaining the physiological causes of drought-induced mortality, and has been proposed as a predictive trait of the specific underlying mechanism of mortality (McDowell et al. 2008, Plaut et al. 2012).

Relatively isohydric plants respond quickly to declining water availability and rising atmospheric moisture demand by closing their stomata in order to control water losses and avoid excessively low leaf water potentials that could cause cavitation and, ultimately, hydraulic failure. The hypothesized cost to this strategy is a negative carbon balance due to an inability to maintain photosynthetic rates during drought (McDowell et al. 2008; Galiano et al. 2011). Moreover, when declining water availability is accompanied by elevated temperature, increased respiration rates could raise the likelihood of carbon starvation (Adams et al. 2009, 2013). At the other end of the continuum, anisohydric plants show less strict stomatal regulation in response to drought, and their leaf water potentials more directly track the fluctuations in soil water availability and atmospheric moisture demand. In these species, xylem may operate with narrower safety margins (the difference between the minimum xylem pressure a stem experiences and the pressure at which it would lose some fraction of its hydraulic conductivity, e.g. 50% loss), and they have been hypothesized to be exposed to greater risk of hydraulic failure than isohydric plants (McDowell et al. 2008). Although recent research shows that carbon starvation and hydraulic failure are highly interrelated processes that should be studied concurrently (McDowell 2011; Sala et al. 2012; Sevanto et al. 2014), the link between stomatal regulation and the likelihood of different modes of mortality is still an open question with important implications for our ability to understand and model plant drought responses (McDowell et al. 2013).

The use of the iso-/aniso-hydric dichotomy to characterize drought responses in general and the process of drought-induced mortality in particular can be problematic for several reasons. Firstly, this categorization reflects two theoretical extremes, while the stomatal behavior of many plants is better represented as occurring along a spectrum between these two endpoints (Klein 2014), and it is likely to be more flexible than implied by a dichotomic classification. Several studies have shown that the iso- vs. aniso-hydric characterization may vary within species as a function of soil water availability (Franks et al. 2007; Domec and Johnson 2012; Zhang et al. 2011). Secondly, the iso-/aniso-hydric classification, which was originally proposed in the context of short-term diurnal stomatal responses, rests on the frequently untested assumption that a rapid stomatal response implies the maintenance of relatively constant leaf water potentials over much longer periods. This is not necessarily the case, depending on the relative vulnerability of stomata and xylem to declining water potentials (Martínez-Vilalta et al. 2014). The use of iso-/aniso-hydric in this context may conflate species differences in stomatal regulation behavior with differences in resistance to drought-induced embolism, which calls for alternative ways of comparing stomatal responses across species (Klein 2014). In addition, different mechanisms of stomatal closure in conifers (based on high abscisic acid concentrations vs. very low leaf water potentials; Brodribb and McAdam 2013; Brodribb et al. 2014) may complicate the relationship between stomatal regulation and leaf water potential dynamics across species.

Most studies use the relationship between stomatal conductance and either leaf water potential or vapor pressure deficit (VPD) to characterize stomatal behavior. Some studies have shown that higher stomatal sensitivity in response to drying soil (Zhao et al. 2013) or narrower carbon safety margins, defined as the difference between leaf water potential when growth is zero and leaf water potential when net photosynthesis is zero (closed stomata, Mitchell et al. 2014), can lead plants to a negative carbon balance and faster depletion of their reserves. At the same time, the same stomatal conductance at a given water potential or VPD value may have very different implications depending on the xylem hydraulic vulnerability of the species and fails to account for the fact that leaf water potentials (particularly midday values) are affected by the vulnerability to embolism. The hydraulic connectivity to the soil is another key factor that can complicate the interpretation of correlations between stomatal conductance and water potential or VPD. Plaut et al. (2012) and Sevanto et al. (2014) demonstrated that pre-dawn leaf water potentials may not reflect soil moisture content during drought in piñon pine (*Pinus edulis*) due to plant hydraulic isolation from the soil. Finally, studies of drought responses in plants have frequently focused on soil water availability as the major

stress driver. These studies have shown that stomatal sensitivity (timing of closure during drought) and the magnitude of decrease in hydraulic conductivity under similar drought conditions ranges widely between and within species, depending on hydraulic architecture and root properties (Rogiers et al. 2011; Will et al. 2013). However, drought has two components: reduced soil water availability and increased atmospheric water demand, and stomata respond to both. Vapor pressure deficit increases non-linearly with temperature, generally increasing atmospheric water demand and transpiration at a given stomatal conductance ( $g_s$ ) (Oren et al. 1999, Breshears et al. 2013). As a result, warming has been shown to exacerbate the effects of drought (Williams et al. 2013). This is important in the context of climate change, because the confidence in future projections is much higher for temperature and VPD than for rainfall and soil water content (IPCC, 2013) and, hence, changes in the former may be more reliable drivers to predict changes in vegetation.

In the present study, we used an ecosystem-scale experiment in a piñon-juniper woodland (dominated by *Pinus edulis* and *Juniperus monosperma*) in northern New Mexico, USA, to test the hypothesis that relatively isohydric plants are less prone to hydraulic failure than relatively anisohydric plants because of their earlier stomatal closure during drought. Experimental treatments were used to simulate different climatic conditions including ambient, reduced precipitation, increased temperature and the combination of both. Piñon-juniper woodlands have been a model system to study drought-related mortality (Breshears et al. 2005, 2009a; McDowell et al. 2008, Plaut et al. 2012, 2013; Limousin et al. 2013). *P. edulis* has been characterized as a relatively isohydric species and *J. monosperma* as relatively more anisohydric (West et al. 2007; Plaut et al. 2012). Additionally, *P. edulis* is more vulnerable to drought-induced xylem embolism than *J. monosperma* (Linton et al. 1998, Wilson et al. 2008). Our main objectives here were to: (1) determine the individual and combined effects of temperature and soil moisture on plant hydraulics and gas exchange of coexisting *P. edulis* and *J. monosperma*, and (2) analyze stomatal regulation of these two plant species with contrasting hydraulic resistance, and how this regulation relates to their differential vulnerability to drought. We expect the effects of increased temperature (Heat) and reduced precipitation (Drought) to be additive (or even multiplicative), so that the combined treatment (Heat & Drought) would have the greatest impact on plant performance, with lower stomatal conductance and photosynthesis rates and higher embolism levels. Differences in stomatal regulation between species will differ depending on whether we express stomatal conductance as a function of absolute water potential, as a function of the distance to a “dangerous” plant water potential (e.g., hydraulic safety margin), or directly as a function of percent loss of hydraulic conductivity

in the xylem. We assessed the relevancy of these three representations for understanding plant responses to drought in the context of predicting tree mortality. Finally, per McDowell et al. (2008) we hypothesize that the anisohydric *J. monosperma* will be more prone to hydraulic failure than the isohydric *P. edulis*, and this will be reflected in narrow safety margins.

## Material and Methods

### *Site description and experimental design*

The study was conducted at the Los Alamos Survival/Mortality Experiment located on Frijoles Mesa at 2175 m a.s.l. in Los Alamos County, New Mexico (35°49'5"N 106°18'19"W). Mean annual temperature (25-year mean 1987-2011) is 9.2 °C, January being the coldest month (-2°C on average) and July the warmest month (20°C). Mean annual precipitation (1987-2012) is 415 mm of which roughly 50 % falls during the North American Monsoon season from July to September (Los Alamos Weather Machine <http://environweb.lanl.gov/weathermachine/>). The site is dominated by piñon pine (*Pinus edulis* Engelm.) and juniper (*Juniperus monosperma* (Engelm.) Sarg.); shrubby gambel oak (*Quercus gambelii* Nutt.) and an occasional ponderosa pine (*Pinus ponderosa* C. Lawson) occurs in the vicinity. Soils are Hackroy clay loam derived from volcanic tuff with a typical profile of 0 to 8 cm of sandy loam, 8 to 35 cm of clay and 35 to 150 cm Bedrock (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture <http://websoilsurvey.nrcs.usda.gov/>). Soil depth at the site ranges from 40 to 80 cm.

A manipulative experiment was established at the site in spring of 2012 using open top chambers and a drought structure to impose the treatments. A total of 63 trees, 32 *J. monosperma* and 31 *P. edulis*, were randomly selected and assigned to one of the five treatment combinations : (1) control (C), with no heating and no precipitation exclusion; (2) control chamber (CC), trees located inside open top chambers with temperature regulated to match outside air temperature and no rain exclusion; (3) drought (reduced precipitation, D), with ~45 % precipitation interception; (4) heat (H), with a chronic temperature increase of ~5 °C; and (5) heat & drought (HD), both treatments at the same time. On average, trees in the D and HD treatments were located 10.6 m and 11.0 m from the nearest edge of the drought structure, respectively. This equals a distance of 4.9 and 4.1 times tree height for each treatment. All the trees in C and D treatments lacked chambers, whereas open top chambers were installed surrounding CC, H and HD trees. In total, 18 chambers of different sizes were built, with some chambers including multiple trees (up to five) when the

spatial arrangement of trees did not allow building of separate chambers. Ambient temperature was monitored on site (sensors: CS215 Temperature and Relative Humidity Probe, Campbell Scientific, Logan, UT, USA) and used as a reference for chamber temperature control. Similar sensors were installed inside each chamber at two heights, 1 m and 2/3 of canopy height; and chamber temperature was determined from the average. Precipitation exclusion for D and HD treatments was accomplished by means of thermoplastic polymer troughs covering ~45 % of total plot area. Start date for all treatments was June 11<sup>th</sup> 2012.

Five to nine individuals per species and treatment were monitored, with size ranging from 0.5 to 5.5 m tall and 1 to 5 m of canopy width. Physiological data were collected monthly during spring to fall 2012 and 2013 with a total of seven campaigns per year. The two first physiological campaigns of 2012 were carried out before the treatments began. Meteorological data were recorded using a Campbell Scientific CR1000 datalogger at a maximum frequency of every 30 minutes throughout the experimental period for all parameters except precipitation, which was measured continuously. Meteorological sensors included an AIO 102778 Weather Sensor, (Climatronics, Bohemia, NY, USA) for air temperature, relative humidity, wind speed and direction and barometric pressure, Campbell Scientific CS215 for additional measurements of air temperature and relative humidity, LI-200S Pyranometer (Li-Cor, Lincoln, NE, USA) for global radiation, Li-Cor LI-190SB Quantum Sensor for photosynthetically active photon flux density, and TR-525USW-R3 Tipping Bucket Rain Gauge (Texas Electronics, Dallas, TX, USA). Soil water content (SWC) was measured periodically at 10 to 60 cm depth using Diviner 2000 probes (Sentek Sensor Technologies, Stepney, SA, Australia). The SWC values reported here are the averages for the top 40 cm of soil, as this was the minimum soil depth in the study area.

#### *Water potentials and leaf gas exchange*

All sampling campaigns lasted two consecutive days during which water potentials and gas exchange were measured for all trees. Two twigs per tree were collected at two times: before sunrise to measure predawn water potential ( $\psi_{pd}$ ) and between 11:30 and 13 h (solar time) on the same day to measure midday water potential ( $\psi_{md}$ ). Twigs were immediately placed in plastic bags and stored in a refrigerator until they were measured (within 1-2 hours) at the field site using a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA). Daily change in water potential ( $\Delta\psi$ ) was calculated as the difference between  $\psi_{pd}$  and  $\psi_{md}$ . Instantaneous determinations of leaf stomatal conductance ( $g_s$ ) and net assimilation rate ( $A_N$ )

were conducted using a Li-Cor LI-6400 infrared gas-exchange analyzer system. These measurements were carried out at mid-morning, when highest stomatal conductance could be expected, under the following conditions: 380 ppm of CO<sub>2</sub> concentration (as the average of ambient air fluctuation between 360-400 ppm), 1500 μmol m<sup>-2</sup> s<sup>-1</sup> light-saturating photosynthetic photon flux density (PPFD), block temperature fixed to 20 or 25 °C depending on the air temperature (to reduce the temperature gradient between inside and outside the leaf chamber), and relative humidity on full scrub (as ambient air humidity was very low and this procedure allowed greater stability). Environmental conditions outside the chambers during measurement varied between 13 and 33 °C for temperature and 750 - 1800 μmol m<sup>-2</sup> s<sup>-1</sup> for PPFD. Measurements were taken once steady state gas exchange had been maintained for at least 2 min, on sun-exposed shoots on the southern hemisphere of the canopy. Leaf area of measured foliage was determined using a Li-Cor LI-3100C area meter and used to correct gas exchange rates.

#### *Percentage loss of hydraulic conductivity*

Percentage loss of xylem hydraulic conductivity was estimated from the vulnerability curves measured in a subset of our experimental trees (n=3 per species per treatment) from August to October 2013 (14-16 months after treatments began) in a companion study (Zeppel et al., in prep; Supporting Information Figure S1). Branches were cut in the field and wrapped in plastic bags. Once in the laboratory they were cut under water and allowed to rehydrate in a refrigerator for 24 hours before processing to avoid any potential artifacts (Wheeler et al. 2013). Water potentials after rehydration were roughly 0.02 MPa higher than predawn water potentials, indicating xylem integrity. Vulnerability curves were generated using the air injection method (Cochard et al. 1992) and fitted with a Weibull function (Neufeld et al. 1992):

$$PLC = 100 - 100 \cdot e^{[-(P_w/a)^b]} \quad (1)$$

where *PLC* is the percentage loss of hydraulic conductivity, *P<sub>w</sub>* is the applied pressure that corresponds to the negative value of the plant water potential, and *a* and *b* are fitted parameters. From equation (1) we can estimate: 1) the water potential corresponding to 50 % loss of conductivity ( $\psi_{50} = -b$ ); 2) the air-entry point,  $\psi_e$ , an estimation of the xylem tension at which pit membranes are overcome and embolism starts to spread (Domec and Gartner 2001); and 3) the hydraulic failure threshold,  $\psi_{max}$ , an estimate of the maximum tension of the xylem before failing and becoming non-conductive.  $\psi_e$  and  $\psi_{max}$  were estimated following Domec and Gartner (2001) and are linear approximations of the applied pressures at the air entry point and at complete



embolism respectively. In our study, they correspond to average PLC values of approximately 10 and 90 %, respectively. As no significant treatment effects were detected at the species level, all treatments' level data were pooled to make composite vulnerability curves for each species. Therefore, average values of  $a$  and  $b$  by species were used in this study to estimate PLC from  $\psi_{md}$  values measured over the study period. In order to characterize hydraulic safety margins ( $\psi_{sf}$ ), we subtracted  $\psi_e$  from  $\psi_{md}$  (Meinzer et al. 2009). Positive  $\psi_{sf}$  values indicate that the  $\psi_{md}$  of the sample is above  $\psi_e$  and therefore PLC would be expected to be  $\sim 0$  %. Negative values indicate the likely presence of embolism; the more negative the value the greater the level of embolism. The results were qualitatively identical if other definitions of safety margins were used, e.g.  $\psi_{50}$  or  $\psi_{max}$  instead of  $\psi_e$  (data not shown).

### *Statistical analysis*

We used general linear mixed models to study the time series of  $\psi_{pd}$ ,  $\psi_{md}$ ,  $\psi_{sf}$ ,  $A_N$ ,  $g_s$ , PLC and SWC. Sampling date, heating (yes or no), drought (yes or no) and their interaction were used as explanatory variables in the fixed part of the models. Similar models were used to study the response of these variables ( $\psi_{pd}$ ,  $\psi_{md}$ ,  $\psi_{sf}$ ,  $A_N$ ,  $g_s$  and PLC) to soil water content. Tree nested into chamber was included as a random factor in all statistical models. For this purpose all outside chamber trees were considered to be in the same (fictitious) chamber (no chamber). Similar mixed models with drought, heating and their interaction as fixed factors were used to assess the relationships between response variables:  $\Delta\psi$  versus  $\psi_{pd}$ ,  $g_s$  versus  $\psi_{sf}$  and  $g_s$  versus PLC. When analyzing the relationship between  $g_s$  and  $\psi_{sf}$  and PLC we only used data from campaigns for which PPFD  $> 1250 \mu\text{mol m}^{-2} \text{s}^{-1}$  and air temperature  $> 18 \text{ }^\circ\text{C}$ , to avoid  $g_s$  depression due to suboptimal light and temperature conditions.

Prior to all analyses, data were log or square root transformed to achieve normality whenever required (see Supporting Information, Tables S1-S13). A different model was fitted for each species in all statistical tests except for seasonal variation of SWC where both species were considered together. Our model selection procedure always started from the saturated model and progressively removed the variables with the lowest explanatory power until the minimal adequate model with the lowest Akaike Information Criterion (AIC) was obtained. Models within two AIC units of the best fitting model were considered equivalent in terms of fit and the simplest one was selected. All analyses were carried out using the R Statistical Software version

3.0.2 (R Development Core Team, 2012), using the function *lme* of the *nlme* package for Linear and Nonlinear Mixed Effects Models.

## Results

Over the study period, temperature was above the 25 year average and precipitation varied considerably between studied years (Figure 1). Annual precipitation in 2012 and 2013 was 226 and 426 mm, respectively, compared with the 25 year average of 415 mm. 2013 was wetter than 2012 but 30 % of rainfall was concentrated in just one week of September (Figure 1c) thus most sampling conducted in 2013 was also during a relatively dry period compared to historical conditions. Soil moisture varied between 3.8 and 43.4 % in control treatments, with an average around 18%. A large peak in SWC was observed after the heavy rains of September 2013 (Figure 1d). Winters were cold with sporadic snowfalls from November to April. The two growing seasons studied were warm with average temperature (May - October) of 20.2 and 19.0 °C in 2012 and 2013, respectively, compared with the 15.7 °C 25 year average for these months. High temperature and evaporative demand occurred in the dry pre monsoon period, with maximum values around 35 °C and 4 kPa (Figure 1a, b). Treatment trees showed a consistently lower SWC than controls but differences among treatments were relatively small due in part to the regional dry conditions. Only the interaction between heating and date was significant ( $P < 0.001$ ), although distinctive periods with significant treatment effects could not be identified (Figure 1d). There were no differences in temperature and VPD between C and CC treatments ( $P = 0.26$  and  $0.19$ , respectively). Daily average temperature was roughly 4.4 °C higher in H and HD chambers than in D and controls (Figure 1a). Higher temperatures in H and HD treatments were also reflected in an increased evaporative demand of about 0.54 kPa daily mean from May to October (Supporting Information, Figure S2).

Overall, H and D treatments resulted in lower  $\psi_{pd}$ ,  $\psi_{sf}$ ,  $g_s$  and  $A_N$ , but the differences were not significant for all dates (Figures 2 and 3). In general the differences were clearer (and significant) during moderately wet periods (spring, autumn), whereas they decreased under extremely dry conditions or under extremely wet periods such as September 2013. Between species, drought effects were more noticeable (more dates with significant differences) in *J. monosperma* than in *P. edulis* (Table 1). Significant effects ( $P < 0.05$ ) were more frequently associated with drought than heat treatments, particularly in *J. monosperma* (Supporting Information Tables S1-S5). Most of the differences detected between treatments for PLC over

time in *J. monosperma* have little biological significance since PLC was typically less than 7% (except June 2013, Figure 2c). Interestingly, the interaction between the sampling date and drought and warming, HD treatment, had no additional effect on *P. edulis* for any of the variables studied (i.e., its effect was not different from that of D or H treatments alone), and it only affected the interaction between sampling date and  $\psi_{pd}$ ,  $A_N$ , and  $g_s$  ( $P < 0.05$ ) for *J. monosperma*. In some cases plants subjected to the combined HD treatment were closer to the controls (C + CC) than either D or H plants (Figures 2, 3).

Neither drought, nor the combined treatment had any significant effect on the physiological variables' ( $\psi_{pd}$ ,  $\psi_{sf}$ , PLC,  $A_N$  and  $g_s$ ) responses to SWC in any of the species. Only heated *P. edulis* trees showed lower  $\psi_{pd}$  for a given SWC (P-value of intercept and slope  $< 0.05$ , Supporting Information Table S6; see also Figure S3a). Stomatal conductance and  $A_N$  were also affected by heat in both species (Supporting Information Table S11; Figure S3d,e).

Predawn water potentials ( $\psi_{pd}$ ) tracked the changes in environmental conditions, particularly SWC and VPD (Figure 2), and the average values ranged from -0.9 to -6.6 MPa in *J. monosperma* and from -0.9 to -2.7 MPa in *P. edulis*.  $\psi_{pd}$  were highest in spring and autumn, and plants achieved the most negative water potentials in the dry pre-monsoon season. In 2013, spring was drier than autumn, and it was reflected in the  $\psi_{pd}$  of both species (Figure 2a). Midday water potentials ( $\psi_{md}$ ) were correlated with  $\psi_{pd}$  in both species ( $R^2_{juniper} = 0.87$  and  $R^2_{piñon} = 0.23$ ,  $P < 0.001$  in both cases).

Vulnerability curves measured in this study showed higher hydraulic resistance at all pressures in *J. monosperma* than *P. edulis* with an average  $\psi_{50}$  of -10.8 MPa and -4.4 MPa respectively (Supporting Information Figure S1). When  $\psi_{md}$  values were related to air-entry water potentials, the corresponding hydraulic safety margins ( $\psi_{sf}$ ) reflected similar temporal dynamics between species but very different absolute values:  $\psi_{sf}$  was always positive for *J. monosperma* (implying PLC  $< 10\%$  in this species), whereas it was negative throughout the study period for *P. edulis* (Figure 2b; note that the scale is different for each species). These safety margins result in a PLC range from 10 to 40 % in *P. edulis* whereas *J. monosperma* shows barely detectable hydraulic conductivity losses except during the driest season in 2013 (Figure 2c). In *J. monosperma*  $\psi_{pd}$  and  $\psi_{sf}$  remained nearly constant at high soil moisture levels and dropped sharply when SWC decreased below 20 %. A similar pattern was shown by *P. edulis*, although the decline was less steep in this species (Supporting Information Figure S3a, b; Tables S6-S7).

Stomatal conductance ( $g_s$ ) dynamics were consistent with  $\psi_{pd}$  patterns for the two species. However, in spite of large differences in absolute  $\psi_{pd}$  values between species, the isohydric *P. edulis* and the anisohydric *J. monosperma* showed similar seasonal stomatal behavior, closing their stomata at similar times in early summer under dry conditions and showing a fast recovery after monsoon rains (Figure 3a). Despite similar temporal patterns, absolute  $A_N$  values were slightly higher in *J. monosperma* than *P. edulis*, as shown by the fact that the slope of the regression between *P. edulis* and *J. monosperma*  $A_N$  values was slightly, but significantly, lower than one (95% confidence interval = 0.81 - 0.97); see Supporting Information Figure S4). However, this difference declined during relatively dry periods, and  $A_N$  rapidly approached  $\sim 0$  in both species as drought developed (Figures 3b; S4). Similar to  $A_N$ , absolute  $g_s$  values were slightly lower in *P. edulis* (Figure S5, Supporting Information); and in this case the difference between species was greater in heated trees ( $P < 0.05$ ). Net assimilation rate and stomatal conductance appeared sensitive to SWC over the entire range of SWC variation, but the decline in  $A_N$  and  $g_s$  accentuated when SWC dropped below 20 % (Supporting Information Figures S3d and e; Tables S9-10).

The difference between predawn and midday water potentials ( $\Delta\psi$ ), a measure of the water potential reduction through the plant associated with whole-plant water transport at midday, decreased in both species in response to drying soil (as indicated by declining  $\psi_{pd}$ ) ( $R^2_{\text{juniperus}}=0.34$  and  $R^2_{\text{pinus}}=0.64$ ,  $P < 0.001$  in both cases; Figure 4, Supporting Information Table S11).  $\Delta\psi$  decline in *P. edulis* was steeper than in *J. monosperma*. However, this relationship was unaffected by treatments in either species.

Stomatal conductance was also examined as a function of hydraulic measures for those dates with optimum values of PPF and temperature (Figure 5). In *J. monosperma*,  $g_s$  decreased steeply with narrowing  $\psi_{sf}$  ( $P < 0.001$ ,  $R^2=0.67$ ; Figure 5a, Supporting Information Table S12), and this relationship was similar across treatments. A similar behavior was observed in *P. edulis*, but in this case  $\psi_{sf}$  explained less than 10% of stomatal conductance variability ( $P < 0.001$ ,  $R^2=0.07$ ). For this species the only significant treatment effect indicated that droughted (D) plants had slightly lower  $g_s$  for a given  $\psi_{sf}$  than controls ( $P < 0.05$ ; Figure S6a). There was a strong negative relationship between stomatal conductance and percent loss of xylem hydraulic conductivity (PLC) for *J. monosperma* across treatments ( $R^2=0.75$ ; Supporting Information Table S13), although the range of variation of  $g_s$  occurred within a mere 10 % variation in PLC (from 0 to 10 %; Figure 5b). *P. edulis* drought (D) trees showed lower  $g_s$  values when PLC was 0 ( $P$ -value of intercept difference  $< 0.05$ ). A clear reduction of stomatal conductance with increasing PLC was also observed in *P. edulis*, although in this case the

model explained only 7% of variance in  $g_s$  ( $P < 0.05$ ,  $R^2 = 0.07$ ). The previous results implied that, although stomatal closure occurred at much higher (less negative) water potentials in *P. edulis* (as expected), hydraulic safety margins were always wider in *J. monosperma* (Figure 2b), even under extremely dry conditions, due to the high sensitivity of its stomata to increasing PLC.

## Discussion

### *Effects of drought and warming on plant hydraulics and gas exchange*

Stomatal and hydraulic responses to heat and reduced precipitation treatments clearly illustrate that low water availability and an increase in temperature can have a negative impact on tree performance regardless of the species' strategy to face drought. As expected, drought and heat treatments impacted plant performance resulting in lower water potentials,  $g_s$  and  $A_N$  rates than controls (Fig. 2 and 3). Treatment effects were not always significant and differences in SWC were small due to regionally dry conditions in 2012 and 2013. But,  $\psi_{pd}$  was lower in D and HD trees relative to the controls, supporting the effectiveness of the drought treatment and suggesting that similar SWC may simply reflect higher water use in the control treatments.

Significant differences between treatments were more frequent under moderate ambient temperatures and water availability, likely because even control trees were severely water-limited throughout much of the study period such that treatment effects often disappeared during the driest part of the year (cf. Zhao et al. 2013) and conversely, even drought treatments had abundant water during periods of anomalously high precipitation (SWC ~40 %), such as in September 2013. This is in agreement with a previous study conducted in a nearby area (Mesita del Buey, ~8 km distant) showing that water is not available for plant extraction at SWC < 18 % on the clay loam portion of the soil (Breshears et al. 2009b). This result is also consistent with the threshold-like responses to SWC observed for some physiological parameters in this study for SWC ~20 % (Supporting Information Figure S3). Interestingly, measured SWC fluctuated around 18 %, close to this SWC threshold, for most of the study period and regardless of treatment (Figure 1d).

The combined treatment, heat and drought, is the most realistic scenario according to IPCC (2013) projections of increasing drought (in terms of frequency and intensity) and warmer temperatures. Our hypothesis was that this treatment would have the largest impact on plants' performance. However, the interaction between drought and heat did not exacerbate stress beyond that experienced by trees in either

the heat or drought treatments and thus we reject our initial hypothesis. This is likely because homeostatic regulation of water use resulted in all experimental treatments reaching the SWC threshold of 20% at approximately the same times (Figures 1d and S3), suggesting that immediate synergistic effects on plants hydraulics and stomatal control in response to an increase in temperature and a lower level of soil water content cannot be assumed. Other studies accounting for the effects of both temperature and water availability have shown that high VPD associated with warmer temperatures intensifies the effects of drought (Williams et al. 2013, Will et al. 2013, Duan et al. 2014), and that the soil moisture content at which whole plant C balance became negative increases with temperature (Adams et al. 2009, Zhao et al. 2013). It remains to be established to what extent the lack of interaction between reduced precipitation and warming observed in our study is due to the extremely dry ambient conditions during our study period. This interaction may be easier to detect in moister environments or in other variables not measured here.

*Anisohydric species are not necessarily more prone to hydraulic failure*

The marked seasonality of temperature and precipitation in the study area, together with our study treatments, provided a wide range of environmental conditions, from reasonably favorable with elevated soil water content and moderate temperature to extremely dry conditions under low soil water content and high temperatures. Over this seasonal and experimental drought gradient *P. edulis* water potentials showed the expected isohydric behavior, with relatively low temporal variation in  $\psi_{pd}$ , whereas *J. monosperma* presented a typical anisohydric pattern (Figure 2a, West et al. 2007, Plaut et al. 2012, Limousin et al. 2013). This contrasting behavior in terms of water potential dynamics did not result in clear differences in the temporal dynamics of  $g_s$  or  $A_N$  between species (Figure 3). Stomata closed and constrained  $A_N$  at similar times and under similar environmental conditions in both species, albeit at very different leaf water potentials (Figures 3 and 5). This difference is likely related to different mechanisms of stomatal closure in the two conifers studied here. Whereas stomatal closure in pines seems to be characterized by high abscisic acid (ABA) concentrations, in junipers stomatal closure may occur at relatively low ABA levels, presumably due to low guard cell turgor associated with their particularly negative leaf water potentials (Brodribb and McAdam 2013; Brodribb et al. 2014). Interestingly, our observations coupled with these different mechanisms of stomatal closure imply that low water potentials are not necessarily associated with loose stomatal regulation, one of the key assumptions of the iso-/anisohydric paradigm. Although  $g_s$  and  $A_N$  were slightly higher in the relatively anisohydric *J.*

*monosperma* than in *P. edulis* (Supporting Information Figures S4, S5), it is unclear whether this small difference has important biological implications. Our results are consistent with previous studies showing that gas exchange of anisohydric species is not necessarily less constrained than isohydric species during drought (Quero et al. 2011), calling into question the generality of the hypothesis that anisohydric species are less prone to the carbon starvation process (McDowell et al. 2008).

Surprisingly, tight control of  $\psi_{md}$  and earlier response to drying soil in *P. edulis* (steeper relationship between  $\psi_{md}$  and  $\psi_{pd}$ ) was not enough to avoid excessive hydraulic tensions that cause embolism in this species (Sperry et al. 2002; McDowell et al. 2008). In fact  $\psi_{md}$  was beyond the air entry point ( $P_e$ , Meinzer et al. 2009) throughout the entire study period in *P. edulis*, implying chronic embolism, whereas hydraulic safety margins were always positive for *J. monosperma*, indicating avoidance of embolism (Figure 2b, 5a). Two main causes explain the contrasting pattern of embolism we observed in these two species over the course of the study. Firstly, *J. monosperma* is highly resistant to cavitation (low  $\psi_{50}$ , Supporting Information Figure S1), as reported previously for this species (Linton et al. 1998; Wilson et al. 2008, Plaut et al. 2012), and is well-recognized for its greater drought tolerance and survival ability during a previous severe drought that caused widespread mortality of co-occurring *P. edulis* (Breshears et al. 2005, 2009a). Secondly, although stomatal closure occurs at much lower water potentials in *J. monosperma* than in *P. edulis*, when stomatal response is presented as a function of hydraulic safety margins (Figure 5a) or PLC (Figure 5b), it becomes clear how conservative stomatal behavior actually is in juniper. Our results show that in *J. monosperma*, stomatal conductance is curtailed as soon as xylem tensions approached the air entry point ( $P_e$ ), avoiding any significant xylem cavitation. This result is not consistent with the hypothesis that anisohydric species will risk embolism during drought and thus are more likely to experience hydraulic failure than isohydric plants (McDowell et al. 2008).

More generally, our results challenge the notion that the regulation of leaf water potential per se can be used to establish the most likely physiological mechanism of drought mortality in these conifers. *P. edulis* and *J. monosperma* have long been recognized as examples of isohydric and anisohydric trees (Lajtha and Barnes 1991, West et al. 2007, Plaut et al. 2012). More recently, the differing stomatal behavior of these two species has been used to hypothesize that isohydric species (piñon) are more likely to experience carbon starvation than anisohydric species (juniper), which are more likely have hydraulic failure at mortality than isohydric species (McDowell et al. 2008, Breshears et al. 2009a). However the iso vs. anisohydric

characterization of plant species is typically based on a descriptive observation of stomatal response or leaf water potential dynamics without regard to species differences in embolism resistance (Linton et al. 1998, Wilson et al. 2008, Supporting Information Figure S1) or other relevant plant attributes related to stomatal closure mechanisms (Brodribb and McAdam 2013; Brodribb et al. 2014), maintenance of turgor in leaves (Meinzer et al. 2014) or phloem transport (Nikinmaa et al. 2013; Sevanto et al. 2014). Our findings demonstrate that conflation of stomatal behavior with these other elements, and particularly with embolism resistance in *P. edulis* and *J. monosperma*, has misled hypothesis development for drought-induced mortality in trees. Consequently, iso- vs. anisohydric characterization of stomatal behavior, as typically based on observations of leaf water potential dynamics and/or stomatal conductance, should not form the basis of assumptions or hypotheses for the physiological mechanism of drought-induced mortality among tree species. Instead, we advocate consideration of stomatal behavior relative to embolism resistance for predicting the physiological process by which trees die from drought (cf. Klein 2014; Martínez-Vilalta et al. 2014).

In conclusion, our study highlights the complexity of characterizing stomatal regulation and the importance of how variables are expressed when assessing the physiological implications of such regulation. We examined stomatal responses through a wide range of drought conditions in two model species, the relatively isohydric *P. edulis* and relatively anisohydric *J. monosperma* and conclude that the latter can be considered to have either a lower stomatal control (the classical view, if  $g_s$  is related to absolute water potentials), a higher stomatal control (if  $g_s$  is related to hydraulic safety margins) or a similar behavior (if the temporal dynamics of  $g_s$  or its response to SWC are considered) compared to the former species. This discrepancy arises from the fact that the original classification between iso- vs. anisohydric plants is based strictly on their capacity to regulate leaf water potentials (Stocker, 1956; Jones, 1998; Tardieu & Simonneau, 1998). Nonetheless, this classification fails to account for the large differences in vulnerability to embolism among species (Choat et al. 2012; Ogasa et al. 2013) and within species (Anderegg 2014). Moreover, different species may operate over very different ranges of water potentials even under similar environmental conditions, reflecting differences in stomatal closure mechanisms (Brodribb et al. 2014), rooting depth, xylem anatomy (Zeppel et al. in prep), leaf turgor regulation (Meinzer et al., 2014) and phloem transport (Nikinmaa et al. 2013). These distinctions in hydraulic behavior are important and we show that they have very relevant implications for how we understand the mechanism of drought-induced mortality in iso- vs. anisohydric species (cf. McDowell et al. 2008). We argue that a more integrative approach incorporating stomatal and



xylem responses with declining water potential (cf. Klein 2014; Martínez-Vilalta et al. 2014) would be a step forward in classifying drought response strategies and predicting physiological mechanisms of mortality.

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### References

- Adams H.D., Guardiola-Claramonte M., Barron-Gafford G.A., Villegas J.C., Breshears D.D., Zou C.B., ..., Huxman T.E. (2009) Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* 106: 7063–6
- Adams H.D., Germino M.J., Breshears D.D., Barron-Gafford G.A., Guardiola-Claramonte M., Zou C.B. & Huxman T.E. (2013) Nonstructural leaf carbohydrate dynamics of *Pinus edulis* during drought-induced tree mortality reveal role for carbon metabolism in mortality mechanism. *New Phytologist* 197: 1142–51
- Allen C.D., Macalady A.K., Chenchouni H., Bachelet D., McDowell N.G., Vennetier M., ..., Cobb N. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660–684
- Anderegg W.R.L. (2014) Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. *New Phytologist*. In press.
- Breshears D.D., Cobb N.S., Rich P.M., Price K.P., Allen C.D., Balice R.G., ..., Meyer C.W. (2005) Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* 102: 15144–8

Breshears D.D., Myers O.B., Meyer C.W., Barnes F.J., Zou C.B., Allen C.D., ..., Pockman W.T. (2009a) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. *Frontiers in Ecology and the Environment* 7: 185–189

Breshears D.D., Myers O.B. & Barnes F.J. (2009b) Horizontal heterogeneity in the frequency of plant-available water with woodland intercanopy - canopy vegetation patch type rivals that occurring vertically by soil depth. *Ecohydrology* 519: 503-519.

Breshears D.D., Adams H.D., Eamus D., McDowell N.G., Law D.J., Will R.E., ..., Zou C.B. (2013) The critical amplifying role of increasing atmospheric moisture demand on tree mortality and associated regional die-off. *Frontiers in Plant Science* 4: 266

Brodribb T.J. & McAdam S.A.M (2013) Abscisic acid mediates a divergence in the drought response of two conifers. *Plant Physiology* 162: 1370-1377

Brodribb T.J., McAdam S.A.M., Jordan G.J. & Martins S.C.V. (2014) Conifer species adapt to low-rainfall climates by following one of two divergent pathways. *Proceedings of the National Academy of the United States of America* 111: 14489-14493.

Chaves M.M., Maroco J.P. & Pereira J.S. (2003) Review : Understanding plant responses to drought — from genes to the whole plant. *Functional Plant Biology* 30: 239–264

Choat B., Jansen S., Brodribb T.J., Cochard H., Delzon S., Bhaskar R., ..., Zanne A.E. (2012) Global convergence in the vulnerability of forest to drought. *Nature* 491: 752-755

Cochard H., Cruziat P. & Tyree M.T. (1992) Use of Positive Pressures to Establish Vulnerability Curves. *Plant Physiology* 100: 205–209

Domec J-C & Gartner B.L. (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. *Trees* 15: 204–214

Domec J-C & Johnson D.M. (2012) Does homeostasis or disturbance of homeostasis in minimum leaf water potential explain the isohydric versus anisohydric behavior of *Vitis vinifera* L. cultivars? *Tree Physiology* 32: 245–8

Duan H., Amthor J.S., Duursma R.A., O'Grady A.P., Choat B., Tissue D.T. (2013) Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated CO<sub>2</sub> and elevated temperature. *Tree Physiology* 33: 779-792.

Franks P.J., Drake P.L. & Froend R.H. (2007) Anisohydric but isohydrodynamic: seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance. *Plant, Cell and Environment* 30: 19–30

Galiano L., Martínez-Vilalta J. & Lloret F. (2011) Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytologist* 190: 750–9

Jones H.G. (1998) Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* 49: 387–398

Klein T. (2014) The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. *Functional Ecology*. In Press. doi: 10.1111/1365-2435.12289

Lajtha K. & Barnes F.J. (1991) Carbon gain and water use in pinyon pine-juniper woodlands of northern New Mexico: field versus phytotron chamber measurements. *Tree physiology* 9: 59–67

Larcher W. (1975) *Physiological plant ecology: ecophysiology and stress physiology of functional groups*. 1st ed. Springer-Verlag, Berlin

Limousin J-M, Bickford C.P., Dickman L.T., Pangle R.E., Hudson P.J., Boutz A.L., ..., McDowell N.G. (2013) Regulation and acclimation of leaf gas exchange in a piñon-juniper woodland exposed to three different precipitation regimes. *Plant Cell and Environment* 36: 1812–25

Linton M.J., Sperry J.S. & Williams D.G. (1998) Limits to water transport in *Juniperus osteosperma* and *Pinus edulis*: implications for drought tolerance and regulation of transpiration. *Functional Ecology* 12: 906–911

Martínez-Vilalta J., Poyatos R., Aguadé D., Retana J. & Mencuccini M. (2014) A new look at water transport regulation in plants. *New Phytologist* 204: 105–15

Maseda P.H. & Fernández R.J. (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *Journal of Experimental Botany* 57: 3963–77

McDowell N.G., Pockman W.T., Allen C.D., Breshears D.D., Cobb N., Kolb T., ..., Yezzer E.A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178: 719–39

McDowell N.G. (2011) Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* 155: 1051–9

McDowell N.G., Fisher R.A., Xu C., Domec J.C., Hölttä T., Mackay D.S., ..., Pockman W.T. (2013) Evaluating theories of drought-induced vegetation mortality using a multimodel-experiment framework. *New Phytologist* 200:304-321

Meinzer F.C., Johnson D.M., Lachenbruch B., McCulloh K.A. & Woodruff D.R. (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology* 23: 922–930

Meinzer F.C., Woodruff D.R., Marias D.E., McCulloh K.A. & Sevanto S. (2014) Dynamics of leaf water relations components in co-occurring iso- and anisohydric conifer species. *Plant Cell and Environment* 2014

Mitchell P.J., O'Grady A.P., Tissue D.T., Worledge D. & Pinkard E.A. (2014) Co-ordination of growth, gas exchange and hydraulics define the carbon margin in tree species with contrasting drought strategies. *Tree Physiology* 34: 443-458

Neufeld H.S., Grantz D.A., Meinzer F.C., Goldstein G., Crisosto G.M. & Crisosto C. (1992) Genotypic variability in vulnerability of leaf xylem to cavitation in water-stressed and well-irrigated sugarcane. *Plant Physiology* 100: 1020–8

Nikinmaa E., Hölttä T., Hari P., Kolari P., Mäkelä A., Sevanto S. & Vesala T. (2013) Assimilate transport in phloem sets conditions for leaf gas exchange. *Plant Cell and Environment* 36: 655–69

Ogasa M., Miki N.H., Murakami Y. & Yoshikawa K. (2013) Recovery performance in xylem hydraulic conductivity is correlated with cavitation resistance for temperate deciduous tree species. *Tree Physiology* 33: 335–44

Oren R., Sperry J.S., Katul G.G., Pataki D.E., Ewers B.E., Phillips N. & Schäfer K.V.R. (1999) Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant Cell and Environment* 22: 1515–1526

Plaut J.A., Yezzer E.A., Hill J., Pangle R., Sperry J.S., Pockman W.T. & McDowell N.G. (2012) Hydraulic limits preceding mortality in a piñon-juniper woodland under experimental drought. *Plant Cell and Environment* 35: 1601–17

Plaut J.A., Duncan Wadsworth W., Pangle R., Yezzer E.A., McDowell N.G. & Pockman W.T. (2013) Reduced transpiration response to precipitation pulses precedes mortality in a piñon-juniper woodland subject to prolonged drought. *New Phytologist* 200: 375–387

- Quero J.L., Sterck F.J., Martínez-Vilalta J. & Villar R. (2011) Water-use strategies of six co-existing Mediterranean woody species during a summer drought. *Oecologia* 166: 45–57
- Rogiers S.Y., Greer D.H., Hatfield J.M., Hutton R.J., Clarke S.J., Hutchinson P.A. & Somers A. (2012) Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. *Tree Physiology* 32: 249–61
- Sala A., Woodruff D.R. & Meinzer F.C. (2012) Carbon dynamics in trees: feast or famine? *Tree Physiology* 32: 764–775
- Sevanto S., McDowell N.G., Dickman L.T., Pangle R. & Pockman W.T. (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant Cell and Environment* 37: 153–161
- Sperry J.S., Hacke U.G., Oren R. & Comstock J.P. (2002) Water deficits and hydraulic limits to leaf water supply. *Plant, Cell and Environment* 25: 251–263
- Stoker O. (1956) Die Abhängigkeit des transpiration von den umweltafaktoren. In: Encyclopedia of Plant Physiology, vol III Water Reactions of Plants (Ed. by W. Ruhland), pp 436–488. Springer-Verlag, Berlin.
- Tardieu F. & Simonneau T. (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours Franc. *Journal of Experimental Botany* 49: 419–432
- West A.G., Hultine K.R., Jackson T.L. & Ehleringer J.R. (2007) Differential summer water use by *Pinus edulis* and *Juniperus osteosperma* reflects contrasting hydraulic characteristics. *Tree Physiology* 27: 1711–20
- Wheeler J.K., Hugget B.A., Tofte A.N., Rockwell F.E. & Holbrook N.M. (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant, Cell and Environment* 36: 1938–1949.
- Will R.E., Wilson S.M., Zou C.B. & Hennessey T.C. (2013) Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest-grassland ecotone. *New Phytologist* 200: 366–74
- Williams A.P., Allen C.D., Macalady A.K., Griffin D., Woodhouse C.A., Meko D.M., ..., McDowell N.G. (2013) Temperature as a potent driver of regional forest drought stress and tree mortality. *Nature Climate Change* 3: 292–297
- Willson C.J., Manos P.S. & Jackson R.B. (2008) Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus *Juniperus* (Cupressaceae). *American Journal of Botany* 95: 299–314

Zhang Y., Oren R. & Kang S. (2011) Spatiotemporal variation of crown-scale stomatal conductance in an arid *Vitis vinifera* L. cv. Merlot vineyard: direct effects of hydraulic properties and indirect effects of canopy leaf area. *Tree Physiology* 32: 262–79

Zhao J., Hartmann H., Trumbore S., Ziegler W. & Zhang Y. (2013) High temperature causes negative whole-plant carbon balance under mild drought. *New Phytologist* 200: 330–339

**Table 1.** Number of days with significant treatment effects from drought (D) and heat (H) to controls (C&CC) or their interaction (HD) relative to H and D (not to controls), on predawn water potential ( $\psi_{pd}$ ), hydraulic safety margin ( $\psi_{sf}$ ), Percent loss of hydraulic conductivity (PLC), stomatal conductance ( $g_s$ ), and net assimilation rate ( $A_N$ ). The + and - symbols indicate positive and negative effects, and *n.s.* indicates non-significant effects.

Variable \ Treatment	<i>J. monosperma</i>			<i>P. edulis</i>		
	D	H	HD	D	H	HD
$\psi_{pd}$	9-	4-	0	6-	4-	n.s.
$\psi_{sf}$	8-	3-	n.s.	2-	1-	n.s.
PLC	7+	3+	n.s.	2+	1+	n.s.
$g_s$	5-	1-/1+	0	7-	5-	n.s.
$A_N$	6-	2-	0	5-	5-	n.s.

**Figure 1.** Meteorological conditions during the course of the experiment from June 2012 to October 2013.

Panels: a) temperature; b) vapor pressure deficit (VPD) cumulative precipitation; c) cumulative precipitation and d) soil water content (SWC). In panels (a) and (b) solid lines represent daily mean values of the air outside the chambers (no heating) (C&D), inside control chambers (CC), and inside heated chambers (HD) (H omitted due to overlapping); whereas shaded lines indicate maximum and minimum daily values. Panel c shows cumulative precipitation at the SUMO meteorological station (ambient) and as calculated in the reduced rainfall treatments (D& HD). Panel d shows the average SWC from 0 to 40 cm depth. Different colors indicate different treatments. Vertical lines indicate heating treatment beginning in panels a and b, and the first rainfall interception date in c and d.

**Figure 2.** Time series of (a) predawn water potentials ( $\psi_{pd}$ ), (b) hydraulic safety margin ( $\psi_{sf}$ ) and (c) percentage loss of hydraulic conductivity (PLC) in branches of *P. edulis* and *J. monosperma* during the experimental period of 2012 and 2013. PLC was estimated from  $\psi_{md}$  by hydraulic vulnerability curve (Supporting Information Fig. S1). Means and standard errors are shown. N varies from five to 13 depending on treatment and species. Vertical dashed lines indicate the date the treatments began and in Fig. 3b red horizontal lines show the point at which  $\psi_{md}$  achieves the air-entry point,  $\psi_e$ . Asterisks indicate significant differences between treatments (H & D) and controls for a given date (\*: 0.01<P<0.05, \*\*: 0.001<P<0.01, \*\*\*: P<0.001).

**Figure 3.** Time series of (a) stomatal conductance ( $g_s$ ) and (b) net assimilation rate ( $A_N$ ) in leaves of *P. edulis* and *J. monosperma* during the experimental period of 2012 and 2013. Means and standard errors are shown. N varies from five to 13 depending on treatment and species. Vertical dashed lines indicate the date the treatments began. Asterisks indicate significant differences between treatments (H & D) and controls for a given date(\*: 0.01<P<0.05, \*\*: 0.001<P<0.01, \*\*\*: P<0.001).

**Figure 4.** Relationship between the predawn to midday difference in water potential ( $\Delta\psi$ ) and predawn water potential ( $\psi_{pd}$ ) in branches of *P. edulis* and *J. monosperma* (solid grey triangles and black circles, respectively). Data corresponds to values measured in all trees during the different campaigns carried out through the experiment (N=435 and 419 for *J. monosperma* and *P. edulis* respectively). Data from different treatments are pooled together. Power regression fits are depicted for both species.



**Figure 5.** Relationship between stomatal conductance ( $g_s$ ) and hydraulic safety margin ( $\psi_{sf}$ ; a) and between  $g_s$  and percentage loss of hydraulic conductivity (PLC; b) in *P. edulis* and *J. monosperma* (solid grey triangles and black circles, respectively). In Fig. 5a, the vertical dashed line indicates the point at which the  $\psi_{md}$  reaches the air-entry point,  $\psi_e$ . Data correspond to values measured in all trees during the different campaigns carried out through the experiment (N=280 and 269 for *J. monosperma* and *P. edulis*, respectively). Solid lines are species-specific linear models for juniper (black) and piñon (grey).









