

# Warming combined with more extreme precipitation regimes modifies the water sources used by trees

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## Summary

- The persistence of vegetation under climate change will depend on a plant's capacity to exploit water resources. We analyzed water source dynamics in piñon pine and juniper trees subjected to precipitation reduction, atmospheric warming, and to both simultaneously.
- Piñon and juniper exhibited different and opposite shifts in water uptake depth in response to experimental stress and background climate over 3 yr. During a dry summer, juniper responded to warming with a shift to shallow water sources, whereas piñon pine responded to precipitation reduction with a shift to deeper sources in autumn. In normal and wet summers, both species responded to precipitation reduction, but juniper increased deep water uptake and piñon increased shallow water uptake.
- Shifts in the utilization of water sources were associated with reduced stomatal conductance and photosynthesis, suggesting that belowground compensation in response to warming and water reduction did not alleviate stress impacts for gas exchange.
- We have demonstrated that predicted climate change could modify water sources of trees. Warming impairs juniper uptake of deep sources during extended dry periods. Precipitation reduction alters the uptake of shallow sources following extended droughts for piñon. Shifts in water sources may not compensate for climate change impacts on tree physiology.

## Introduction

Models of climate change predict a global increase in atmospheric temperatures between 1.1 and 6.4°C by 2100 (Meehl *et al.*, 2007). Precipitation projections are less certain, but variation in precipitation amounts and its seasonality are expected to be maintained or accentuated, and future droughts will be superimposed on warmer conditions (Allen *et al.*, 2015; McDowell *et al.*, 2015). Warmer temperatures are thus expected to amplify drought intensity and duration (Trenberth & Fasullo, 2013; Will *et al.*, 2013; Williams *et al.*, 2013). These unprecedented climatic changes will have a profound impact on ecosystem water balances and plant persistence if they do not acclimate to these novel conditions (Nicotra *et al.*, 2010).

Interspecific variation in belowground rooting strategies is critical for plant water access, canopy transpiration and carbon assimilation. Trees often depend on access to deep and moist soil layers to withstand seasonal heat waves and droughts (Valentini *et al.*, 1992; David *et al.*, 2007; Eggemeyer *et al.*, 2009; Rossatto *et al.*, 2012). Water uptake patterns can also vary interannually following seasonal fluctuations in precipitation (Snyder & Williams, 2003; Klein *et al.*, 2014; Barbeta *et al.*, 2015; Voltas *et al.*, 2015). This ability to modify water source use based on available soil moisture depends on the depth and distribution of

functional roots (Ehleringer & Dawson, 1992). A dimorphic rooting system, in which roots are distributed in shallow and deep soils, can enable the uptake of seasonal rain and simultaneously allow the extraction of water from deeper soils that have been charged during previous seasons (Ehleringer & Dawson, 1992; Dawson & Pate, 1996). In the context of climate change, species that have adapted to recurrent droughts by maintaining this characteristic rooting system may have a competitive advantage over species with static access to limited soil layers. In order to accurately predict species dynamics, the determination of how water sources will change under projected climate is critical (West *et al.*, 2008), in particular to enable improved model assumptions about the depth of water acquisition in response to climate change (Zeng, 2001; Huxman *et al.*, 2005; Rodríguez-Iturbe & Porporato, 2005; Javaux *et al.*, 2013).

As one of the most widely distributed ecosystems in the southwest USA, piñon–juniper woodlands are a vital natural resource (Miller & Wigand, 1994). This ecosystem is particularly at risk with anticipated changes in climatic conditions within its distributional range (Allen & Breshears, 1998; Breshears *et al.*, 2005). In recent decades, severe drought events have caused extensive mortality of piñon pine (*Pinus edulis*), whereas co-occurring juniper trees (*Juniperus monosperma*) have largely survived, suggesting a different drought tolerance between the two species

(Breshears *et al.*, 2005; Williams *et al.*, 2010). Many studies on the responses of piñon and juniper to drought have demonstrated that the two species differ in their water relations (e.g. McDowell *et al.*, 2008; Plaut *et al.*, 2012, 2013; Gaylord *et al.*, 2013; Limousin *et al.*, 2013). For instance, piñon has higher transpiration rates than juniper during periods of high soil moisture (West *et al.*, 2008). Piñon is more vulnerable than juniper to embolism (Linton *et al.*, 1998; Pockman & Sperry, 2000; Garcia-Forner *et al.*, 2016), and limits transpiration more rapidly with declining soil water potential (Lajtha & Barnes, 1991; Garcia-Forner *et al.*, 2016).

An analysis of the stable isotope composition of water provides a powerful, reliable and nondestructive method to study plant water uptake dynamics. Typically, no fractionation occurs during water absorption by the roots (Zimmermann *et al.*, 1968; Dawson & Ehleringer, 1993; but see Ellsworth & Williams, 2007); therefore, the isotope composition of water in the xylem reflects the mean isotope value of water extracted by functional roots from the soil. Evaporation at the surface of the soil causes oxygen and deuterium enrichment near the soil surface (Supporting Information Fig. S1) that commonly decreases exponentially with soil depth (Fig. S2; Barnes & Allison, 1988; Ehleringer & Dawson, 1992). The comparison of xylem water and soil water from different depths reveals the mean depth from which water originates, when a sufficient gradient in soil water isotope values is present. Using the stable isotope composition of water, previous work has demonstrated that piñon and juniper trees differ in their water uptake patterns, with both having dimorphic rooting systems (Williams & Ehleringer, 2000), but with juniper typically more deeply rooted than piñon (West *et al.*, 2007a,b). Although the water dynamics of piñon and juniper trees have been addressed previously, to our knowledge, there have been no studies on the long-term water uptake patterns of trees in response to the novel conditions anticipated with climate change.

We examined the seasonal variation in water uptake depth in piñon and juniper trees exposed to a 3-yr reduction in precipitation (−45%), atmospheric warming (+4.8°C), and both stressors acting simultaneously using seasonal variation in xylem water and soil water isotopic values. Predawn leaf water potential, maximum CO<sub>2</sub> assimilation and stomatal conductance were measured simultaneously to determine how the variation in water sources affected water availability, water use and carbon assimilation rates. We hypothesized the following: that both species would respond to precipitation reduction or to warming by taking up water from deeper sources relative to trees in ambient conditions as a result of increased soil water depletion; that warming combined with precipitation reduction would result in a deeper water uptake relative to trees exposed to either stressor alone because of the increased soil water depletion; and that access to deeper water sources would compensate for treatment-induced reductions in shallow soil water, and thus be reflected by homeostatic leaf-level physiology of manipulated trees relative to ambient conditions.

## Materials and Methods

### Study site

The study was conducted at the Los Alamos Survival-Mortality (SUMO) experiment located in Los Alamos County, New Mexico (35.49°N, 106.18°W, 2175 m above sea level, asl). The site is characterized by Hackroy clay loam soils derived from volcanic tuff with a soil depth of 40–55 cm (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, <http://websoilsurvey.nrcs.usda.gov>). The vegetation is dominated by piñon pine (*Pinus edulis* Engelm.) and juniper (*Juniperus monosperma* (Engelm.) Sarg.). Grasses, cacti and other tree species, such as Gambel oak (*Quercus gambelli* Nutt.), can be found in intercanopy spaces, but do not contribute significantly to the total stand biomass. The mean temperature during the growing season is 17.4°C (1987–2011 mean) and the mean annual precipitation is 415 mm (1987–2012 mean), with a mean precipitation of 240 mm during the growing season (i.e. April to October) (Los Alamos Weather Machine, <http://environ-web.lanl.gov/weathermachine>).

In June 2012, a manipulative experiment was initiated at the site, consisting of open-top chambers that increased air temperature by 4.8°C and a precipitation exclusion structure consisting of clear polymer troughs that reduced precipitation by 45%. In total, 64 trees were selected for the experiment (32 juniper and 32 piñon pine trees). The mean tree ages were  $56 \pm 5$  and  $79 \pm 7$  yr for piñon and juniper, respectively. Selected trees were assigned to five treatments: ambient temperature and precipitation; chamber control for trees located inside chambers with temperature regulated to match ambient temperature; heat for trees inside chambers in which the temperature was maintained at 4.8°C above ambient temperature; drought for trees located within the precipitation exclusion structure; and heat + drought for trees in which both treatments were applied simultaneously. Tree height ranged between 0.5 and 4.5 m. Chamber footprints ranged from 6 to 20 m<sup>2</sup> and contained between one and five trees located at a minimum distance of 1.5 m from the chamber boundary. Although some roots of trees inside the chambers may have extended beyond the chamber boundary, we expected the majority of roots to be growing inside the chambers. The lateral roots of these species typically do not extend beyond 1.5 times tree height (maximum tree height in chambers is 3.5 m), and the majority are usually found in close proximity to the bole (Gottfried *et al.*, 1995). The selected trees in the drought treatment were situated at a minimum of 10 m from the border of the precipitation exclusion structure (equivalent to twice the height of the tallest tree in the drought treatment) to ensure that the majority of roots were subjected to the drought treatment. Climatic conditions were measured continuously and recorded with two weather stations located on site. Atmospheric temperature and relative humidity were measured in all chambers and used to control the industrial-scale air-conditioning units that controlled chamber temperature. Soil water content (%) was measured manually between 0 and 70 cm depth using Diviner 2000 probes at 66 locations covering all treatments (Sentek Sensor Technologies,

Stepney, SA, Australia; Fig. S3). In addition, we characterized soil drought intensity by estimating the daily relative extractable water in the soil over the whole root zone (REW, unitless) for the 2013–2015 period (Fig. S4, see Notes S1 for more details). More details on the study site are provided by Adams *et al.* (2015) and Garcia-Forner *et al.* (2016). Climatic conditions for the whole study period are presented in Fig. S4.

### Water uptake patterns

We quantified seasonal variation in water uptake over 3 yr (2013, 2014 and 2015) using twig and soil samples collected on three dates per year that represented contrasting soil water conditions: April (i.e. usually corresponding to wet soil conditions), June (i.e. corresponding to the driest soil conditions) and September (i.e. usually recovery transition from dry to wet). For each date, the stable isotope ratios of hydrogen and oxygen in each water sample were determined (see later) and expressed in standard delta notation ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , respectively, ‰). For the plants, the xylem water of a 5–10-cm-long branch sample from each tree was sampled between 05:00 and 07:00 h solar time. Samples were taken from healthy branches using clippers. The samples were immediately sealed in airtight vials, the vial lid was wrapped with parafilm and the vial was placed in cool conditions to avoid evaporation. On the same day as the twig sampling, soil samples were collected every 5 cm at eight depths (0–5, 5–10, 10–15, 15–20, 20–25, 30–35, 40–45 and 50–55 cm) using a soil corer driven by an electric hammer (PN425; JMC Soil Samplers, Newton, IA, USA). As differences in water input and soil evapotranspiration between soils under the precipitation exclusion structure and in ambient conditions can induce differences in isotopic values of water isotopes, soils were repeatedly sampled at four positions in the drought treatment near the drought and drought + heat trees, and at four positions in the ambient treatment near the ambient, heated and chamber control trees, that were selected at the start of the study. The soil samples were placed directly in vials, sealed with a lid and parafilm, and stored in cool conditions. As the depth of the tuff bedrock is variable at our study site (between 40 and 55 cm), the maximum depth of soil sample collection (i.e. 50–55 cm) varied by date and position.

Water from xylem and soil samples was extracted using a custom-made cryogenic vacuum distillation system housed in the Center for Stable Isotope Biogeochemistry at the University of California (Berkeley, CA, USA). The extraction time was 60 min for both xylem and soil samples. We followed the guidelines of West *et al.* (2007a,b) based on Ehleringer *et al.* (2000), who tested a diversity of plant and soil materials for extraction times, and found that 60 min was sufficient in all cases. Our soil type was also included in this test and the extraction time was demonstrated to be sufficient, even though clay loam soils have been shown to be problematic in the past. In addition, a wide range of soils have been extracted with various water retention characteristics for periods ranging from 45 min to 6 h and, in all cases, 60 min was sufficient to extract 99.998% of the water (see Oshun *et al.*, 2016).

The entire extraction system was first frozen with liquid nitrogen ( $-176^\circ\text{C}$ ) and evacuated under 4 atm of negative pressure (vacuum) to test for leaks before each sample was extracted. After this, a sample was placed in the extraction system, frozen with liquid nitrogen and the system was evacuated once again. After this, the sealed sample vessels were submerged in boiling water at  $100^\circ\text{C}$ , whilst a collection vessel was submerged in liquid nitrogen allowing the extraction to proceed. After the extraction, water samples were transferred into cap-crimp 2-ml vials and stored at  $-2^\circ\text{C}$  until analyses.  $\delta^2\text{H}$  of each sample was determined on a dual inlet (DI) using a hot chromium reactor unit (H/Device™) interfaced with a Thermo Delta Plus XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).  $\delta^{18}\text{O}$  of each sample was analyzed by continuous flow using a Thermo Gas Bench II (Thermo Fisher Scientific) interfaced to a Thermo Delta Plus XL mass spectrometer. The long-term precision values ( $n > 50\,000$  known test samples run since 2001) of these isotopic analyses are 0.18‰ and 0.06‰ for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , respectively.

### Quantification of water sources

A Bayesian mixing model was used to quantify the contribution of potential water sources for trees of each species and for each sampling date. In addition, the mixing model was used to quantify contributions of water sources for trees in the different treatments when significant differences in xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  were found among treatments.

For this purpose, we used the package SIAR in R (Parnell *et al.*, 2010). Three sources of water were considered to contribute significantly to tree water use, and were thus included in the model: soil water that has evaporated from either shallow or deep soil layers (0–15 and 15–55 cm) and water stored in the cracks of the bedrock that is not subjected to evaporation (i.e. bedrock water). We assumed no isotopic fractionation during water uptake (Ehleringer & Dawson, 1992). One hundred thousand iterations were run separately for each sampling date and the first 50 000 were discarded to obtain the most likely contribution of each source for each species on each sampling date.

The volcanic tuff at our site is fractured (Trainer, 1974), which allows tree roots to grow into the bedrock (Tierney & Foxx, 1982; Reneau *et al.*, 1995; Newman *et al.*, 1997). To account for bedrock water use, we used the long-term average isotopic values of precipitation during winter (November–March) in the mixing model, as individual values for the studied years were not available. Indeed, under conditions of low evaporative demand, winter precipitation penetrates into deep soil layers and bedrock cracks (Earman *et al.*, 2006), and could be a substantial water source for trees at our study site during the summer (Newman *et al.*, 2006). The isotopic value of this deeper moisture source was kept constant over the course of the year as it was shown not to be subject to evaporation at a nearby similar site (Newman *et al.*, 1997, 2010). Monthly means of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of precipitation were obtained from the Boulder station (40.00°N, 105.26°W, 1660 m asl) in Colorado, USA. No post-processing or weighting of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of precipitation data was applied. This station was selected from the Global Network of Isotopes in

Precipitation as it was the closest station that included monthly measurements for several years (2009–2013), and relative humidity and atmospheric temperatures were representative of the conditions at our experimental site. To ensure correct representation, point measurements of isotopic values of precipitation obtained close to our study site were compared with the data from the Boulder station and yielded very similar patterns (Earman *et al.*, 2006; Shim *et al.*, 2013) (Fig. S5).

### Leaf water potential and gas exchange

We measured predawn leaf water potential ( $\Psi_{PD}$ , MPa) during all sampling dates on all trees over the 2013–2015 period. Two branch samples from each tree were taken before sunrise, placed in plastic bags and stored in a refrigerator until they were measured within 1 h at the field site with a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA).

On the same dates, we measured maximum  $\text{CO}_2$  assimilation ( $A_{\text{Max}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and leaf stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) on one current-year, sun-exposed shoot from each tree (Li-Cor Environmental, Lincoln, NE, USA). Measurements were carried out using a Li-Cor LI-6400 infrared gas analyzer system. The measurements took place in the morning when the highest stomatal conductance was expected. Measurements were conducted with the following settings using the  $2 \times 3$  LED chamber: 380 ppm of reference  $\text{CO}_2$  concentration,  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  light-saturating photosynthetic photon flux density, block temperature at  $20^\circ\text{C}$  or  $25^\circ\text{C}$  to match ambient air temperature, and relative humidity on full scrub. Leaf temperature was measured using the energy balance. The projected leaf area of the measured foliage was determined using a Li-Cor LI-3100C area meter and was used to correct  $A_{\text{Max}}$  and  $g_s$ .

### Statistical analyses

All analyses were performed using the software R (v.3.2.1; R Core Team, 2015). We analyzed differences in xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  between species using mixed linear, random slope models in which species, year and season (i.e. spring, summer and autumn) were used as fixed effects. Responses of xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of each species to precipitation reduction, atmospheric warming and the combination of the treatments were also analyzed using mixed linear, random slope models in which year, season (i.e. spring, summer and autumn), heating (yes or no) and drought (yes or no) were used as fixed effects. For all tests, the individual trees nested in the chambers were input as random effects. The model selection procedure started with all variables, and progressive removal of the variables with the lowest explanatory power was performed until the minimal model with the lowest Akaike information criterion (AIC) was obtained. Similar models were used to determine differences in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of soil water in which year, season and depth were used as explanatory factors in the fixed part of the model. We also used similar mixed models to assess the relationships between xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , and mean soil water content in shallow soil layers

(0–15 cm) and deep soil layers (15–55 cm), tree water access ( $\Psi_{PD}$ ) and gas exchange ( $A_{\text{Max}}$  and  $g_s$ ).

*Post-hoc* analysis was performed with Tukey's honestly significant difference (HSD) *post-hoc* test.  $r^2$  was obtained for linear mixed effects models following Nakagawa & Schielzeth (2013) and adapted by Jon Lefcheck (<http://jonlecheck.net/2013/03/13/r2-for-linear-mixed-effects-models/>).  $\alpha = 0.05$  was used to determine statistical significance. Statistical analyses were performed using the package *nlme* for linear mixed effects models.

No significant differences in climatic parameters were found between ambient conditions and control chambers ( $P = 0.760$  for mean air temperature,  $P = 0.997$  for relative humidity and  $P = 0.999$  for REW). In addition, we found no differences between xylem isotopes in ambient and chamber control conditions, and between soil water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  from soil samples collected under the drought exclusion structure and in ambient conditions (Fig. S6); we thus pooled these measurements together to increase the number of samples and thus statistical power.

## Results

### Climate under ambient conditions

All 3 yr were characterized by contrasting climatic conditions and soil water availability (i.e. REW: 2013 < 2014 < 2015, Fig. S4). Precipitation amounts during the growing season were 127, 240 and 280 mm for 2013, 2014 and 2015, respectively. Compared with the 20-yr mean, growing season precipitation decreased by 47% in 2013 (i.e. dry year), was equal in 2014 (i.e. normal year) and increased by 17% in 2015 (i.e. wet year). Soil water content was typically high in the spring, except in 2013 (Figs S3, S4). Soil water availability was typically low in the summer, except for 2015, where soil water availability remained high throughout the summer (Figs S3, S4). The autumn sampling date was always characterized by high soil water content as precipitation during the monsoon period (i.e. August–September) increased soil water (Figs S3, S4).

### Stable water isotopes in xylem and soil water

**Soil water** For most sampling dates, the profile of soil water isotopic values ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) was characterized by more positive isotopic values in shallow soil layers and more negative values in deeper soil layers (e.g. Barnes & Allison, 1988;  $P < 0.001$ , Figs S2 and S7–S9 for individual dates). In spring 2015, an inverse curve was found with depleted values in shallow soil layers and enriched values in deeper soil layers (Fig. S9). This inverse pattern could be explained by shallow infiltration of spring precipitation events characterized by negative isotopic values (Newman *et al.*, 1997). Despite the significant difference between shallow and deep soil layers for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , for most dates, no significant differences were found among isotopic values in the first three soil layers (0–5, 5–10 and 10–15 cm) and among the deepest soil layers (15–20, 20–25, 30–35, 40–45 and 50–55 cm) (Figs S7–S9). Soil water isotopic values varied significantly among sampling dates ( $P < 0.001$ ).



**Xylem water Differences between treatments.** Warming and precipitation reduction both impacted the xylem water isotopic values of juniper trees, but these impacts were dependent on the year and season (Table S1). Across all years, we found more positive  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for juniper in the heat and heat + drought treatments compared with ambient trees during the summer (Fig. 1). More specifically, during the dry 2013 summer, juniper trees subjected to warming and the combination of warming and precipitation reduction exhibited more positive  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values than ambient trees (Fig. 2), suggesting a higher reliance on superficial water sources. Results from the mixing model confirmed that the contribution of superficial soil water was 23% and 28% higher for trees exposed to warming and the two stressors simultaneously, whereas the contribution of bedrock water was 20% and 26% lower, respectively, in these two treatments, in comparison with ambient trees (Fig. 3). However, during a more typical summer in 2014, more negative  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values were found in the drought treatment relative to ambient trees (Fig. 2), consistent with greater reliance on deeper water sources. The mixing model revealed that the contribution of bedrock water was 35% higher than for ambient trees at this same date (Fig. 3).

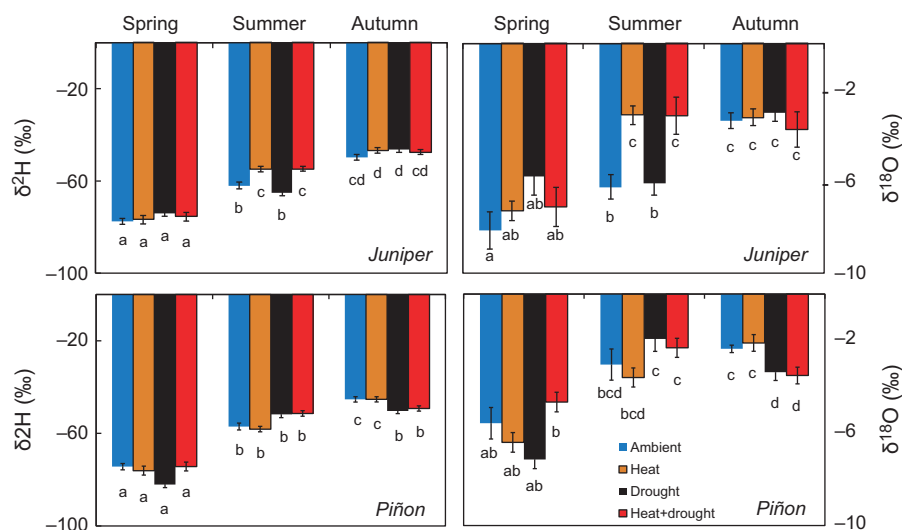
For piñon, only our precipitation reduction treatment influenced the xylem water isotopic values, but this effect was dependent on the year and season (Table S2). Across all years, we found more negative  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for trees in the drought and drought + heat treatments than for ambient trees in autumn (Fig. 1). More specifically, during autumn sampling in the dry year of 2013, more negative  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values were found in the drought and heat + drought treatments compared with ambient trees (Fig. 4), consistent with higher reliance on deep water sources. For this date, the contribution of deep soil water was 7% and 9% higher in these trees compared with ambient trees (Fig. 3). However, during the wet summer of 2015, more positive  $\delta^2\text{H}$  values were found in the drought and heat + drought treatments when compared with ambient trees (Fig. 4). Results from the mixing model showed

that, for this date, the contribution of shallow soil water was 25% and 24% higher for drought and heat + drought trees compared with ambient trees (Fig. 3). For the other sampling dates, no differences were found between treatments for juniper and piñon.

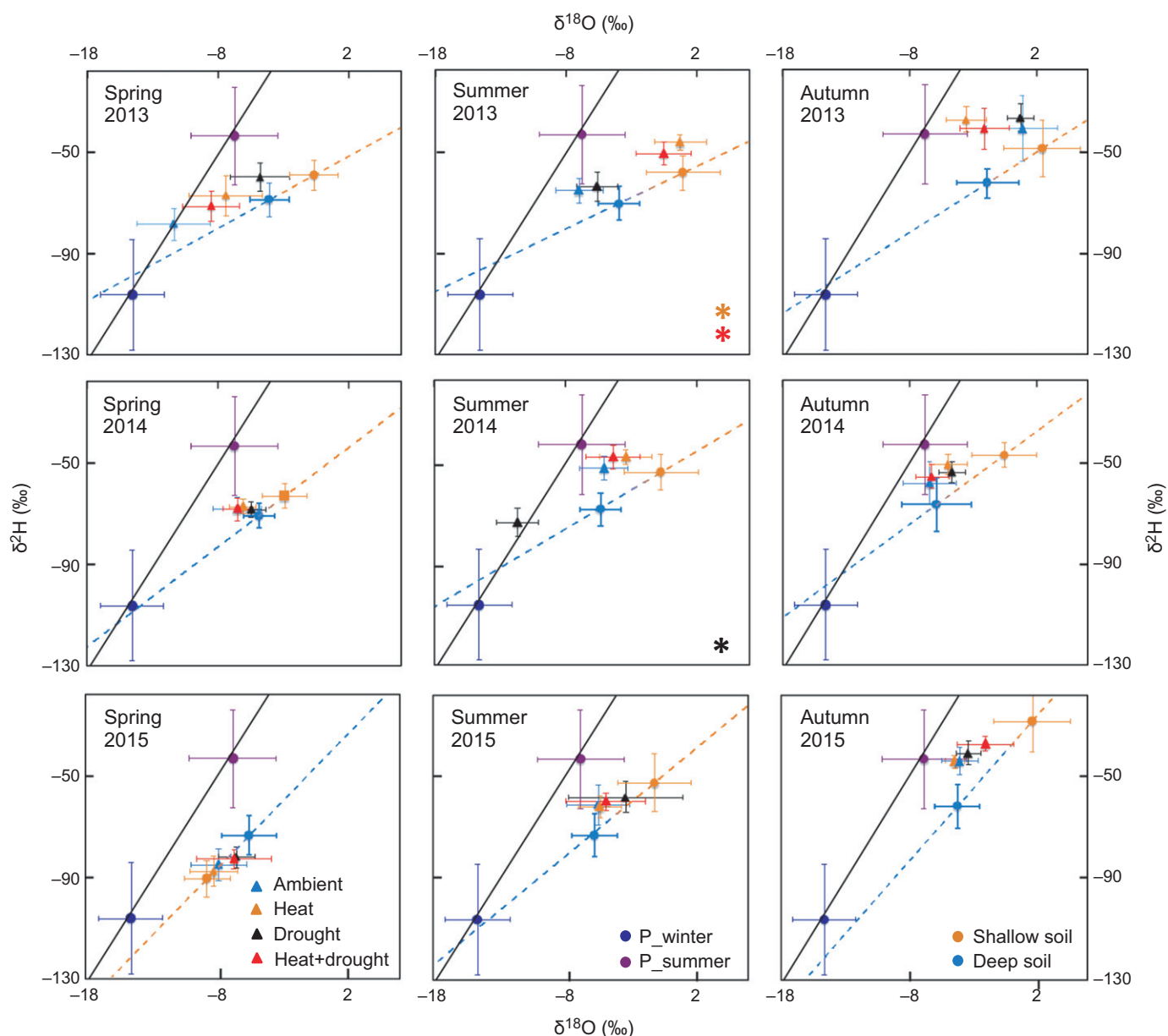
### Relationship between xylem water isotopic values, soil water availability and gas exchange rates

For juniper, we found a significant effect of soil water availability in shallow soil layers on xylem water isotopes ( $P=0.002$  and  $P<0.001$  for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , respectively). The strong positive relationships suggest that juniper takes up water from progressively deeper soil layers as soil water availability decreases in shallow soils (Fig. 5a,b). We also found strong relationships between xylem water isotopes and  $g_s$  ( $P=0.006$  and  $P=0.004$  for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , respectively) and  $A_{\text{Max}}$  ( $P=0.010$  for  $\delta^2\text{H}$ ). These significant relationships suggest that, as shallow soils dry and trees take up water from progressively deeper soil layers, gas exchange rates decrease (Fig. 5c,d). No relationship between soil water availability,  $\Psi_{\text{PD}}$ ,  $g_s$  or  $A_{\text{Max}}$ , and xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , was found for piñon.

For both species, when significant differences in water isotopes occurred among treatments during specific sampling dates, we tested for significant relationships between water isotopes and gas exchange. For juniper during the dry summer of 2013, a significant negative relationship was found between xylem  $\delta^{18}\text{O}$  and  $\Psi_{\text{PD}}$ ,  $A_{\text{Max}}$  and  $g_s$  (Fig. S10a–c), suggesting that trees possessed a bias towards shallower water uptake in the heat and heat + drought treatments compared with ambient trees, and this resulted in higher drought stress and lower gas exchange rates. Similarly, a significant positive relationship was found for juniper between  $\delta^{18}\text{O}$  and  $A_{\text{Max}}$  during summer 2014 (Fig. S10d), suggesting that, as trees became more reliant on deeper water in the drought compared with ambient treatment, this resulted in lower overall assimilation rates. For piñon, a significant positive relationship was found between xylem water



**Fig. 1** Mean xylem water isotopic values ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , ‰) of juniper and piñon for each treatment for the three sampling dates in 2013, 2014 and 2015 with  $\pm$  SE. Letters denote significant differences between sampling dates and treatments for a given isotope.

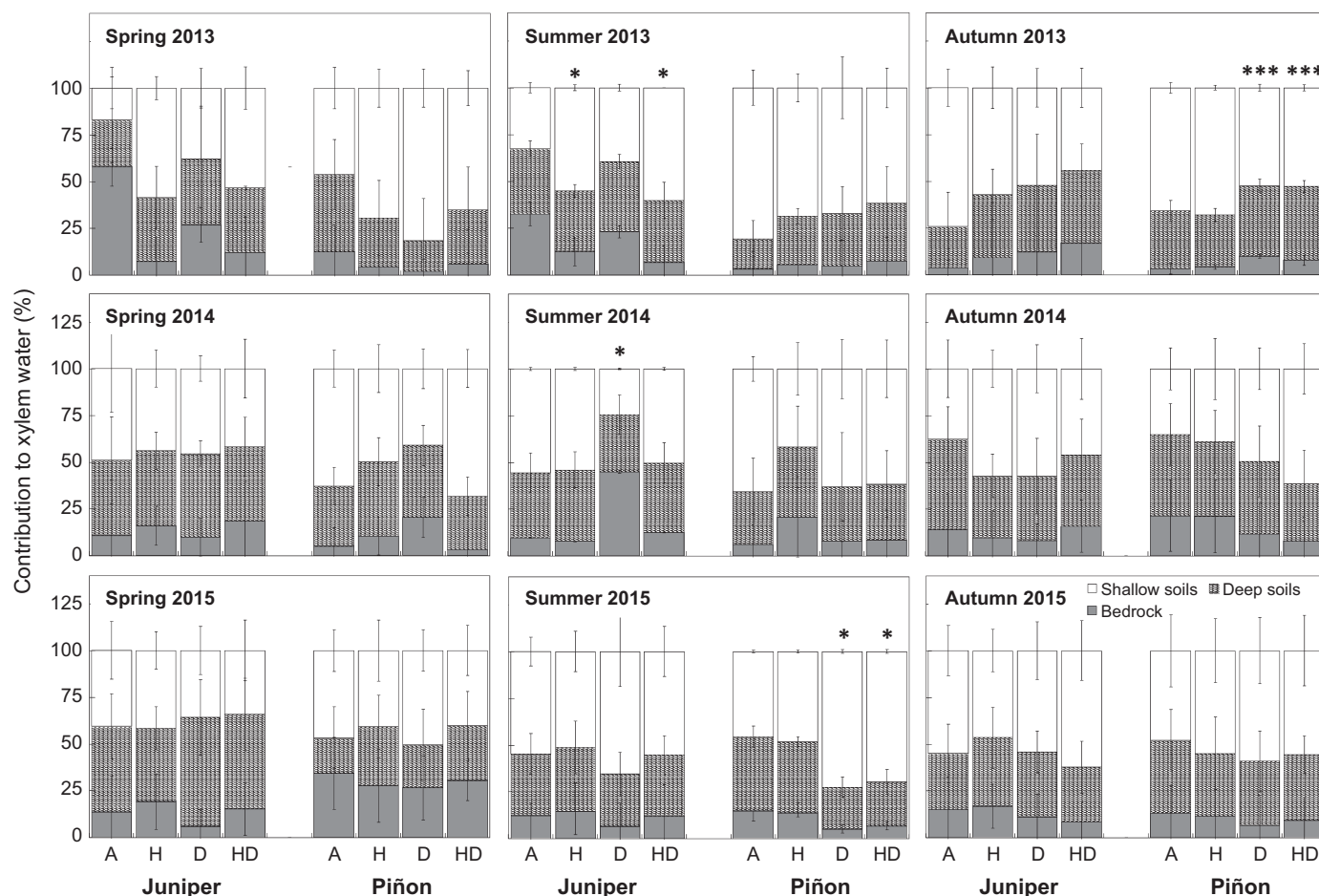


**Fig. 2** Xylem water isotopic values ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , ‰) of juniper in each treatment, isotopic values of shallow (0–15 cm) and deep (15–50 cm) soil layers and isotopic values of mean winter (P\_winter) and summer (P\_summer) precipitation with  $\pm$  SD for each date. The dashed and solid lines represent the local soil water line and local meteoric water line (LMWL) established using multiple years (Vuataz, 1986), respectively. The LMWL represents the linear variation of the isotopic composition of precipitation events in the study area. When evaporation occurs,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values increase and plot to the right of the meteoric water line. The slope of the evaporation line is also lower than that of the meteoric line because the  $^{18}\text{O}$  and  $\delta^2\text{H}$  values are subject to different amounts of fractionation during evaporation. Asterisks in the right lower corner denote significant differences between treatments and control for a given date (\*,  $P < 0.05$ ).

isotopic values and  $\Psi_{\text{PD}}$  ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ),  $A_{\text{Max}}$  ( $\delta^2\text{H}$ ) and  $g_s$  ( $\delta^2\text{H}$ ) during autumn 2013 (Fig. S11a–c), suggesting that a deeper water uptake of trees in the drought and heat + drought treatments resulted in lower water availability and gas exchange rates. During the wet summer of 2015, a significant negative relationship was found between  $\delta^2\text{H}$  and  $A_{\text{Max}}$  and  $g_s$  for piñon (Fig. S11d,e), suggesting that a shallower water uptake of trees in the drought and heat + drought treatments resulted in lower gas exchange rates.

#### Differences between species and seasonal variations in water sources

Xylem water isotopic values varied between  $-105.1\text{‰}$  and  $-27.4\text{‰}$  for  $\delta^2\text{H}$ , and between  $-16.6\text{‰}$  and  $6.4\text{‰}$  for  $\delta^{18}\text{O}$ . We found significant differences between species depending on the year and season for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  (Table S3). *Post-hoc* analysis revealed that, for most spring and summer dates, xylem water isotopic values were more positive for piñon than for juniper,



**Fig. 3** Mixing model results showing mean percentage of contribution to xylem water of unevaporated summer precipitation, shallow soil layers (0–15 cm), deep soil layers (15–55 cm) and water in the fractured bedrock, with  $\pm$  SE, for juniper and piñon for each date. Asterisks denote significant differences between treatments and control in  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  for a given date (\*,  $P < 0.05$ ; \*\*\*,  $P < 0.0001$ ).

suggesting more shallow water uptake (Fig. S12, see Supporting Information for results of specific dates, Notes S2).

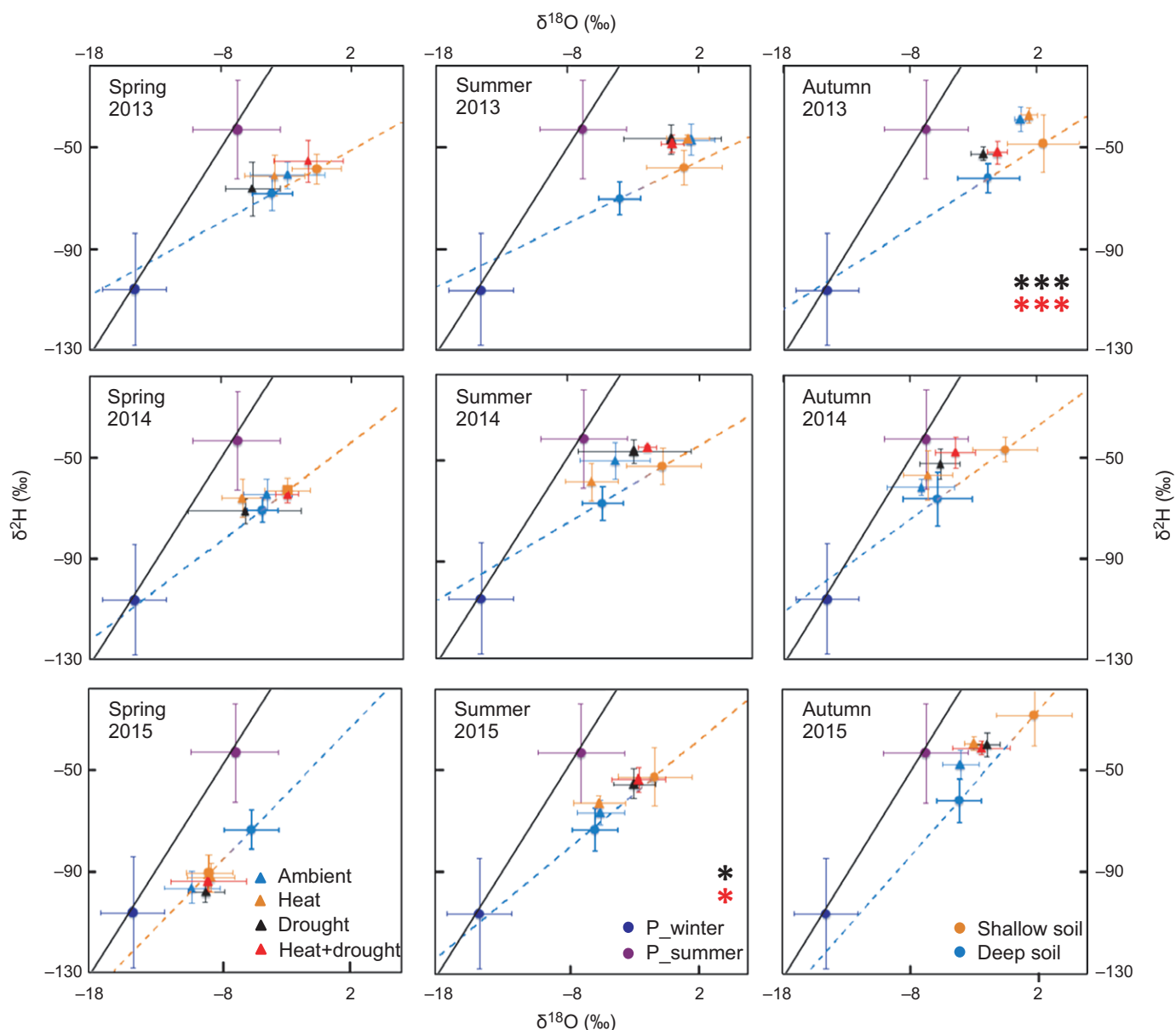
Xylem water isotopes of both species varied across years and seasons (Tables S1, S2). The most negative isotopic values for juniper in 2013 and 2014 were found during the summer, suggesting the deepest water uptake during this period (Fig. S13). During these dates, some xylem isotopic values of juniper were more enriched than the local meteoric water line (Fig. S13). This could be related to isotopic enrichment associated with cuticular water loss (Dawson & Ehleringer, 1993), or interannual variability in isotopic values of precipitation that differ from the local meteoric water line, established using multiple years. In 2015, more negative values were found in spring for juniper, suggesting more shallow water uptake compared with summer and autumn (Fig. S9). During all years, the most negative values for piñon were found during the spring (Fig. S13), suggesting the deepest water uptake during this period. In spring 2015, the more negative values of piñon suggest a more shallow water uptake compared with the summer and autumn sampling based on the inverse soil water isotope curve found for this date (Fig. S9). More depleted precipitation in spring 2015 relative to summer and autumn 2015 could also partially explain this pattern.

Using the isotopic values of xylem and soil water, significant variation in the contribution of the different water sources was found for both species throughout the growing season (Fig. S14). In spring, both species took up a high proportion of water originating from shallow soil layers (44% and 62% for juniper and piñon, respectively). During the summer, juniper took up a higher proportion of water originating from deep soil layers (42%) and bedrock (34%), whereas piñon xylem water still predominantly originated from shallow soil layers (65%). In autumn, both tree species had the highest contribution of xylem water originating from shallow soil layers (59% and 54% for juniper and piñon, respectively). Because our water source values were close to each other, large standard deviations (SDs) are associated with our results (Fig. S14) and care should be taken during interpretation.

## Discussion

### Response of water uptake depth to single stressors and implications for physiology

When subjected to reduced precipitation, juniper exhibited depleted xylem water isotopic values relative to ambient trees

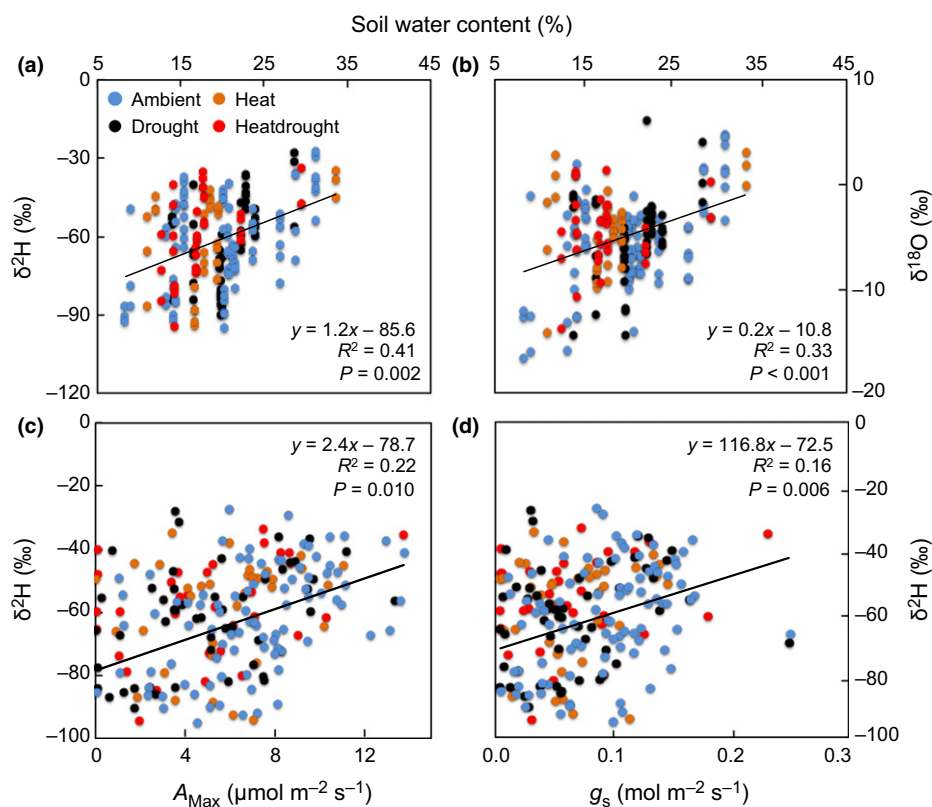


**Fig. 4** Xylem water isotopic values ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , ‰) of piñon in each treatment, isotopic values of shallow (0–15 cm) and deep (15–50 cm) soil layers and isotopic values of mean winter ( $P_{\text{winter}}$ ) and summer ( $P_{\text{summer}}$ ) precipitation, with  $\pm$  SD, for each date. The dashed and solid lines represent the local soil water line and the local meteoric water line (LMWL) established using multiple years (Vuataz, 1986), respectively. The LMWL represents the linear variation of the isotopic composition of precipitation events in the study area. When evaporation occurs,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values increase and plot to the right of the meteoric water line. The slope of the evaporation line is also lower than that of the meteoric line because the  $^{18}\text{O}$  and  $\delta^2\text{H}$  values are subject to different amounts of fractionation during evaporation. Asterisks in the right lower corner denote significant differences between treatments and control for a given date (\*,  $P < 0.05$ ; \*\*\*,  $P < 0.0001$ ).

during average summer conditions (i.e. 2014) (Fig. 2), consistent with a higher reliance on deeper water sources (Fig. 3). Juniper thus seems to be able to maintain the ability to increase its water uptake depth when it is subjected to long-term precipitation reduction. This experimental result supports the common (yet rarely tested) assumption that trees can increase their carbon allocation to roots growing deeper in the soil under conditions of soil water depletion (Schenk & Jackson, 2002; Bréda *et al.*, 2006). Conversely, when subjected to warming alone during very dry summer conditions (i.e. 2013), juniper trees modified their water

uptake depth, showing a higher reliance on shallow soil water (Fig. 3). As warming can increase evapotranspiration and thus reduce soil water in dry ecosystems (Will *et al.*, 2013; Williams *et al.*, 2013), we expected to observe a similar response to trees subjected to precipitation reduction (i.e. deeper water uptake relative to ambient trees). Our results, however, indicate that, during these very dry conditions, no differences in soil water content occurred between warmed and ambient conditions at most depths (Fig. S3, i.e. summer 2013). This suggests that warming negatively influences water uptake patterns of juniper by reducing





**Fig. 5** Relationships between xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , and (a, b) mean soil water content in shallow soil layers (0–15 cm), (c) maximum  $\text{CO}_2$  assimilation ( $A_{\text{Max}}$ ) and (d) stomatal conductance ( $g_s$ ) for juniper across all sampling dates. For each relationship, the equation,  $R^2$  and  $P$  values are given in the right corner. For piñon, no significant relationship was found.

its access to deep water sources and increases its reliance on shallow soil water during dry summers. This pattern may be related to higher respiration rates in the warming treatment and thus lower carbon available for root growth.

Piñon also modified which water sources it used when subjected to precipitation reduction, but this change occurred during the recovery period (i.e. autumn) following an extended and intense dry summer (i.e. 2013). Trees in this treatment showed xylem isotopic values consistent with a deeper water uptake (Fig. 4) and a lower contribution of shallow soil resources relative to ambient trees during the recovery period (Fig. 3). This result is consistent with less reversible embolism, or even root death, in surface roots relative to deep roots in the precipitation reduction treatment (Williams & Ehleringer, 2000; West *et al.*, 2007b), such that surface roots need to regrow before conducting water, whereas deep roots are more immediately capable of absorption and conductance of water during the recovery period. By contrast, during a relatively wet summer in 2015, piñon trees subjected to precipitation reduction exhibited shallower water uptake (Figs 3, 4). Interestingly, when subjected to warming alone, piñon trees did not differ in their water uptake pattern compared with ambient treatment trees. As piñon maintains a higher fraction of shallow water uptake than juniper, we could expect piñon to be more greatly affected by warming. Shallow roots could experience greater temperature extremes that may eventually lead to the inhibition of root activity (Williams & Ehleringer, 2000), particularly under warmed conditions. In several warm sites, high soil temperature has been shown to affect shallow root activity and mortality (Hendrick & Pregitzer, 1993)

and to inhibit root elongation (Drennan & Nobel, 1998). Changes in atmospheric temperatures of *c.* 5°C may not greatly affect soil water availability in our semi-arid climate. Indeed, changes in soil water content at our site seem to indicate that temperature rise, and thus higher evapotranspiration from soils and plants, may diminish soil water content during relatively wet conditions (Fig. S3, i.e. 2015).

By extension, it therefore seems possible that global warming could affect the ‘root foraging’ capacity of juniper by restricting or even reducing water uptake by roots from deep moisture sources during intense droughts. Conversely, our results suggest that piñon may not modify its water source use in response to warming, suggesting that water availability may instead be the major driver of plastic response for this species. Interestingly, nearly any change in water uptake depth of trees subjected to single stressors relative to ambient trees, be it movement to shallower or deeper water, is associated with a reduction in predawn leaf water potential and gas exchange (Figs S10, S11). Thus, although these shifts may be both a necessary and/or adaptive response to a change in imposed climate, they are still insufficient to fully mitigate the consequences on tree physiology.

#### Response of water uptake depth to warming superimposed on precipitation reduction and implications for physiology

For juniper, we found that trees subjected to simultaneous precipitation reduction and warming during a very dry summer exhibited a similar water uptake pattern to trees subjected to

warming alone (Fig. 2): a shift towards the use of shallower moisture sources (Fig. 3). Conversely, piñon trees subjected to both stressors exhibited similar patterns to trees subjected to precipitation reduction alone: a reduction in shallow water uptake recovery after an intense drought (Fig. 4) and a shallower water uptake during relatively wet conditions (Fig. 4). Thus, warming could have a decisive effect on water uptake patterns of juniper, whereas precipitation reduction could play a larger role for water uptake patterns of piñon in the future. Piñon is usually considered to be more susceptible than juniper to drought, and could thus be more responsive to changes in soil water availability. Conversely, as juniper has a high drought tolerance and plasticity in water uptake depth, these trees might instead be more responsive to novel changes in temperature. This could occur, for example, via stomatal responses to vapor pressure deficit (VPD) (S. Sevanto, unpublished) or temperature effects on respiration, both of which would influence plant total carbon balance. As when trees were exposed to single stressors, we found that, when exposed to simultaneous stressors, the significant changes in water sources also resulted in reduced water potentials and gas exchange rates, independent of the season or the year (Figs S10, S11).

Counter to our expectation, we found no indication for an exacerbated response in adaptive water uptake patterns under relatively drier conditions imposed by a simultaneous reduction in precipitation and atmospheric warming. This observation is in accordance with previous findings from the same experiment in which no evidence of exacerbated stress was found under simultaneous warming and precipitation reduction (Adams *et al.*, 2015; Garcia-Forner *et al.*, 2016; Grossiord *et al.*, 2016). This work thus suggests that the effects of these levels of precipitation reduction and warming are probably not additive in these tree species and under our semi-arid conditions.

### Differences in water uptake dynamics between species

Overall, we found that increased temperature and reduced precipitation caused piñon pine and juniper to shift water uptake depth, but with contradictory responses. Despite these different responses, neither species was able to compensate for drought and temperature stress through shifts in water uptake depth. Species differences in water use and depth of water uptake in co-occurring piñon and juniper have been studied previously, and our results from the ambient treatment were mostly consistent with this previous work. In our study, xylem water isotopic values during the driest period of the growing season indicated that juniper made a greater use than piñon of deep water sources (Figs 3, S12–S14), consistent with previous water use studies (Flanagan *et al.*, 1992; West *et al.*, 2007a,b; but see Williams & Ehleringer, 2000). These results are consistent with observations that *P. edulis* is more dependent than juniper on the availability of summer rainfall that only reaches shallow soil layers (Pendall *et al.*, 1999; West *et al.*, 2007a,b, 2008), and findings that juniper has a deeper rooting system than piñon (Tierney & Foxx, 1982; Foxx & Tierney, 1987; Newman *et al.*, 2010). We also observed in spring that xylem water isotopic values for both species suggested that water uptake mostly originated from shallow

resources (Figs 3, S13, S14), and that species differences in water uptake depth developed during the summer, a seasonal shift also observed previously (West *et al.*, 2007a). However, unlike West *et al.* (2007a), we did not observe these differences persisting into autumn, but instead both species showed similar xylem water isotopic values following the growing season, corresponding to a mixture of summer precipitation from shallow and deep soil layers that varied by year in our study (Fig. 3), similar to results for Mediterranean *Pinus halepensis* (Volas *et al.*, 2015). Xylem water isotopic values of some juniper trees, especially in spring 2013, indicated water sources consistent with water stored in bedrock fractures (Figs 3, S13, S14), in contrast with previous findings that bedrock contributed little to transpiration sources in juniper (West *et al.*, 2007a). The higher bedrock contribution to transpiration at our site could be related to the high porosity of the bedrock (*c.* 40%, B. D. Newman, unpublished), indicating a high storage capacity.

### Conclusions

Overall, this work suggests that warmer conditions superimposed on more extreme precipitation regimes will probably modify the utilization of water resources for both species in the future. Both tree species were found to modify their water uptake dynamics in response to these two climatic stressors, but they did so differently, with piñon responding more to drought and juniper to increased temperatures. Climate warming combined with more infrequent precipitation could have a decisive effect on water uptake patterns of juniper through alteration of its plastic ability to modify water extraction depth throughout the growing season. Conversely, the drier conditions driven by climate change could alter the extension and activity of shallow piñon roots during the growing season, reducing the uptake of water from summer rain events, and potentially altering water uptake patterns during following years through changes in root uptake efficiency. Independent of the primary stressor driving these changes in water sources, this study demonstrates that acclimation of water uptake dynamics may not increase the resistance of forests to the extreme conditions anticipated for the future.

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### Author contributions

N.G.M. planned and designed the research. C.G., S.S., H.D.A., A.D.C., L.T.D., E.A.S. and N.G.M. conducted the fieldwork. C.G. analyzed the data. C.G., S.S., T.E.D., H.D.A., A.D.C., B.D.N., L.T.D., E.A.S. and N.G.M. wrote the manuscript.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Soil water isotopic values for shallow and deep soil layers.

**Fig. S2** Mean soil water isotopic values for each soil depth.

**Fig. S3** Mean soil water content along the soil depth.

**Fig. S4** Climatic and soil water conditions.

**Fig. S5** Relationship between oxygen isotopic measurements of precipitation measured at the Boulder station in Colorado and obtained close to our study site.

**Fig. S6** Mean soil water isotopic values in ambient conditions and under the precipitation exclusion structure.

**Figs S7–S9** Profile of soil water isotopic values across all treatments during spring, summer and autumn 2013–2015.

**Fig. S10** Relationships between xylem water  $\delta^{18}O$  values and predawn leaf water potential, maximum  $CO_2$  assimilation and stomatal conductance.

**Fig. S11** Relationships between xylem water  $\delta^2H$  values and predawn leaf water potential, maximum  $CO_2$  assimilation and stomatal conductance.

**Fig. S12** Mean xylem water isotopic values of juniper and piñon for each date.



**Fig. S13** Xylem water isotopic values of juniper and piñon in all treatments.

**Fig. S14** Mixing model results showing mean percentage of contributions to xylem water.

**Tables S1, S2** Summary of the linear mixed model of xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for juniper and piñon

**Table S3** Summary of the linear mixed model of xylem water  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for both species

**Notes S1** Soil water characteristics.

**Notes S2** Results of xylem water.

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