Tree water dynamics in a drying and warming world

Short running title: Future tree water dynamics

Charlotte Grossiord[1]*, Sanna Sevanto[1], Isaac Borrego[1], Allison M. Chan[1], Adam D. Collins[1], Lee T. Dickman[1], Patrick J. Hudson[2], Natalie McBranch[1], Sean T. Michaletz[1], William T. Pockman[2], Max Ryan[1], Alberto Vilagrosa[3], Nate G. McDowell[4].

[1]Earth and Environmental Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

[2]Department of Biology, MSC03 202, University of New Mexico, Albuquerque, NM 87131-0001, USA

[3]Fundación CEAM, Joint Research Unit University of Alicante – CEAM, Univ. Alicante, PO Box 99, 03080 Alicante, Spain

[4]Atmospheric Sciences & Global Change Division, Pacific Northwest National Laboratory, Richland, WA 99352, USA

*Correspondence to: Charlotte Grossiord, Email: cgrossiord@lanl.gov

Abstract

      Disentangling the relative impacts of precipitation reduction and vapor pressure deficit (VPD) on plant water dynamics and determining whether acclimation may influence these patterns in the future is an important challenge. Here, we report sap flux density (F_D), stomatal conductance (G_s), hydraulic conductivity (K_L) and xylem anatomy in piñon pine (*Pinus edulis*) and juniper (*Juniperus monosperma*) trees subjected to five years of precipitation reduction, atmospheric warming (elevated VPD) and their combined effects. No acclimation occurred under precipitation reduction: lower G_s and F_D were found for both species compared to ambient conditions. Warming reduced the sensibility of stomata to VPD for both species but resulted in the maintenance of G_s and F_D to ambient levels only for piñon. For juniper, reduced soil moisture under warming negated benefits of stomatal adjustments and resulted in reduced F_D, G_s and K_L. Although reduced stomatal sensitivity to VPD also occurred under combined stresses, reductions in G_s, F_D and K_L took place to similar levels as under single stresses for both species. Our results show that stomatal conductance adjustments to high VPD could minimize but not entirely prevent additive effects of warming and drying on water use and carbon acquisition of trees in semi-arid regions.

Keyword index: acclimation, hydraulics, *Juniperus monosperma*, megadrought, *Pinus edulis*, sap flux, transpiration, stomatal conductance, vapor pressure deficit, xylem anatomy

Introduction

Climate change is expected to cause greater stress on forests by superimposing more extreme droughts (i.e. extended periods of low precipitation) on elevated evaporative demand (i.e. vapor pressure deficit or *VPD*) due to warming (Allen et al. 2015). However, uncertainties about the role of *VPD vs.* water availability on plant function, particularly transpiration (*E)* during periods of water stress, are currently large (McDowell & Allen 2015, Novick et al. 2016). Plants are known to have a wide range of strategies to deal with drought and elevated *VPD* resulting from higher temperature, including physiological (e.g. stomatal control on *VPD*) and structural acclimation (e.g. growing deeper roots) (Chaves et al. 2002). Thus, while reduced precipitation and rising *VPD* and temperature could increase plant stress in the future (Trenberth et al. 2014), active acclimation may partially or completely mitigate impacts of climate change. Disentangling the relative impacts of precipitation reduction and rising *VPD* on plant functions and determining if long-term climate acclimation could influence plant water and carbon dynamics is critical both to fundamental understanding of plant function (Oren et al. 1999) and for improved prediction of climate impacts on terrestrial ecosystems (Allen et al. 2015).

E is the driving force for water movement through the plant and is defined as a function of stomatal conductance (g_s) and *VPD* (i.e. the difference between actual and saturated air vapor pressures). The regulation of *E* under adverse conditions depends on water available for root uptake, efficiency of the hydraulic system, and regulation of stomatal aperture at the leaf-atmosphere interface (reviewed in Bréda et al. 2006). When soil moisture drops with relatively unchanged *VPD*, plants regulate g_s to avoid damage to the hydraulic system that could impair water transport to the leaves (Sperry et al. 1998, Cochard et al. 2002), but at the cost of carbon acquisition. This relative drop in g_s partially depends on plant stomatal behavior that ranges along a continuum from anisohydric to isohydric strategies

(Tardieu & Simonneau 1998; Franks et al. 2007; Klein 2014; Martinez-Vilalta et al. 2014; Martinez-Vilalta & Garcia-Forner 2016). Furthermore, the integrity of the hydraulic system regulates the plant's capacity to supply water to the canopy during dry periods. Alterations of the hydraulic system can take place at multiple places along the soil to leaf pathway during drought, including the soil-root interface and the xylem tissues (Sperry et al. 1998). Xylem hydraulic conductivity (K_h) directly reflects these alterations, and reductions in K_h are usually observed during natural and experimental precipitation reduction (Sperry et al. 1998, Whitehead 1998, Maherali & DeLucia 2000, Ladjal et al. 2005, Medeiros and Pockman 2011, Fonti & Jansen 2012). To reduce vulnerability of the hydraulic system, plants may acclimate to increasing aridity by producing conducting elements with reduced lumen diameters (Sperry and Hacke 2004, Fonti & Jansen 2012), by increasing secondary wall thickness (Hacke et al. 2001, Pitterman et al. 2006), or both. Though such physiological and anatomical responses are well documented, the degree of active acclimation in hydraulic traits that could influence tree water and carbon relations during extreme drought events remains largely unknown.

Contrary to precipitation reduction, the influence of warming, and its direct effect on *VPD*, has often been overlooked in hydrological studies even though *VPD* is a major driver of ecosystem water and carbon fluxes. Despite the primary role of soil moisture in drought-induced forest decline (McDowell et al. 2008), atmospheric dryness has also been identified as a major contributor of drought-induced mortality processes (Eamus et al. 2013, Williams et al. 2013). Predicting *VPD* effects on water dynamics is more complex than predicting precipitation effects because of its dual and conflicting impacts on g_s and E. Increasing temperature and *VPD* enhances the driving force for water loss per stomatal aperture while simultaneously inducing stomatal closure to minimize water loss to an increasingly desiccating atmosphere (Jarvis & McNaughton 1986, Monteith 1995). Furthermore, the

extent to which stomata could respond to rising *VPD* may be influenced by long-term active

acclimation, modifying the stomatal sensitivity to evaporative demand (Marchin et al. 2016).

For instance, plants could modify the synthesis of chemical signals that induce stomatal

closure to high *VPD* in order to maintain g_s during heat waves. Plants could thereby maintain

high carbon acquisition for growth and defense functions against the increasing intensity and

frequency in herbivore and pathogen damage expected under a global warming scenario

(Kurz et al. 2008). In a recent study, Will et al. (2013) showed that a *VPD* increase of 45%,

due to 3°C temperature rise, induces higher *E* during the growing season. Similarly, Duan et

al. (2014) found that plants exposed to a 75% increase in *VPD* (i.e. +4°C) had higher water

use rates than ambient plants under high soil water availability. In both studies, higher water

use eventually lead to a more rapid depletion of soil moisture for warmed plants, and thus,

earlier exposure to drought stress during the growing season. Whether physiological

acclimation to high *VPD* occurred in these experiments remains unclear, particularly as they

were short-term studies on young individuals.

The relative impacts of low precipitation *vs.* high atmospheric demand and

temperature are particularly difficult to disentangle as they often occur concurrently in natural

conditions (Dai 2013, Williams et al. 2013). To improve our understanding and predictions of

plant responses to future climate it is essential to separate the independent and interactive

effects of these climatic drivers. Manipulative field experiments are important tools to

decipher plant community responses to possible climate change (Knapp et al. 2016).

However, most of our knowledge is based on studies with potted plants or seedlings under

experimental field conditions, which may not represent the true responses of mature forests

(Poorter et al. 2012).

In a semi-arid woodland, we subjected mature piñon pine (*Pinus edulis*) and one-seed

juniper (*Juniperus monosperma*) trees to multi-year precipitation reduction (~45%),

atmospheric warming (+4.8°C) and their simultaneous effects. These values were selected to match projected conditions for 2100 under a business-as-usual scenario in our study region (Pachauri et al. 2014). After four years of treatment exposure, we increased precipitation reduction to ~90% to simulate the effect of a "megadrought" as is likely to occur in this region within the next 50 years (Cook et al. 2015). We report here the responses of E, canopy-level stomatal conductance (G_s), K_h and xylem anatomy, and examine their relationships with precipitation reduction and increased VPD. Our objectives were to determine the relative effects of reduced precipitation $vs.$ high VPD and temperature on water dynamics of trees, as has been done theoretically and using eddy covariance data (McDowell & Allen 2015, Novick et al. 2016, respectively), but here for the first time using a cause-and-effect manipulative study. Further, we sought to determine the role of active acclimation processes on these responses. We hypothesized that if no active acclimatory responses occur (i.e. at the rooting-, hydraulic- or stomatal-level):

(1) higher VPD (induced by atmospheric warming) would increase E relative to ambient conditions under high soil moisture periods (i.e. spring and autumn), and reduce E during the late spring and summer because of a more rapid depletion of soil moisture (i.e. quicker exhaustion),

(2) precipitation reduction would reduce E relative to ambient conditions throughout the growing season (i.e. reduced availability),

(3) the combination of VPD rise and precipitation reduction would reduce E relative to single stresses throughout the growing season because of the combination of higher water use and lower water availability (i.e. reduced availability and quicker exhaustion),

(4) E responses to the treatments would vary between species due to different functional characteristics with more rapid reductions in E during the late spring and summer in isohydric piñon trees than relatively anisohydric juniper in all treatments,

(5) deviations in expected E responses to the treatments would be related to stomatal (G_s), xylem hydraulic (K_h) or anatomic acclimation processes that could maintain E responses similar to ambient levels in order to conserve high carbon acquisition rates (e.g. changes in xylem anatomy increasing the hydraulic resistance (increased K_h) in the warming treatment and resulting in similar E under higher VPD conditions).

Material and methods

Site description

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 a.s.l). The site is characterized by Hackroy clay loam soils derived from volcanic tuff (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, http://websoilsurvey.nrcs.usda.gov) with a soil depth ranging from 40 to 55 cm (Grossiord et al. 2016a). The vegetation is dominated by piñon pine (*Pinus edulis* Engelm.) and one-seed juniper (*Juniperus monosperma* (Engelm.) Sarg.). Grasses, cacti and other tree species such as Gambel oak (*Quercus gambelli* Nutt.) can be found in inter-canopy spaces but they do not contribute significantly to total stand biomass. The climate is semi-arid, with a mean annual temperature of 10.1°C (1987-2015 mean) and a mean annual precipitation of 360 mm (1987-2015 mean), with about 50% falling during the North American Monsoon season from July to September (Los Alamos Weather Machine, http://environweb.lanl.gov/weathermachine).

In June 2012, open-top chambers increasing air temperature by ~4.8°C and a precipitation exclusion structure consisting of clear polymer troughs to reduce incoming precipitation by 45%, were installed at the site (i.e. covering ~45% of the land surface area, Pangle et al. 2012; Adams et al. 2015). We randomly selected 64 trees for the experiment (32

juniper and 32 piñon pine trees, >3 cm diameter at breast height). Mean tree age was 56 ± 5 years and 79 ± 7 years for piñon and juniper, respectively (measured from tree cores). Tree height ranged between 1.5 m and 4.5 m. The trees were assigned to five treatments (5-6 trees per treatment and per species): ambient for trees in ambient temperature and precipitation (A), chamber control for trees situated inside chambers with temperature regulated to match ambient temperature (CC), heat for trees inside chambers where temperature was maintained at 4.8°C above ambient temperature (H), precipitation reduction for trees located within the precipitation exclusion structure (PR), and heat and precipitation reduction for trees where both treatments were applied simultaneously (H+PR). Chamber footprints ranged from 6 m^2 to 20 m^2 and contained between one and five trees located at a minimum distance of 1.5 m from the chamber boundary. The selected trees in the precipitation reduction treatment were located at least 10m from the border of the precipitation exclusion structure (equivalent to two times the height of the tallest tree in the PR treatment). Previous studies conducted at this site found no differences between trees in the A and CC treatments for physiological responses such as assimilation, stomatal conductance, phenology or water uptake depth (Adams et al. 2015, Garcia-Forner et al. 2016, Grossiord et al. 2016a, b), suggesting no indirect effect of the chambers (e.g. change in windspeed, change in incoming radiation or change in soil moisture). We thus pooled measurements from the two treatments together for statistical analyses (i.e. A treatment).

In April 2016, we increased the coverage of the precipitation exclusion structure to 90% by adding additional clear polymer troughs. This paper only reports data from the growing season of 2016 (i.e. March-September). Thermal bubble insulation was installed underneath the polymer troughs and portable blower fans (TE-CF2421, Triangle, Jacksonville, AR, USA) were placed throughout the PR and H+PR treatment to avoid temperature excess under the precipitation exclusion structure. Soil temperature was

measured continuously (RT-1, Decagon Devices Inc, Pullman, WA, USA) over a 0-30 cm depth at the base of each tree. Mean daily soil temperature under the structure was on average $1.4°C \pm 0.9°C$ higher than ambient conditions (Fig. S1), which is comparable to another rainfall manipulation study located ~150km distant (Pangle et al. 2012). Climatic conditions were measured continuously and recorded by a weather station at the site. Atmospheric temperature and relative humidity were measured in all chambers and used for controlling the industrial-scale air-conditioning units that controlled chamber temperature (Fig. S1). The mean daily temperature in the heated chambers (H and H+PR treatments) was $4.5 \pm 0.9C°$ higher than ambient conditions in 2016, leading to a mean daily increase in *VPD* of 68% (i.e. + 0.9-2.3 kPa, Fig. 1) (see Grossiord et al. 2016a, b for 2012-2015 climatic data). We estimated the daily relative extractable water (*REW*, unitless) over the whole root zone at our site for each treatment using a forest water balance model (see SI & Grossiord et al. 2016a) (Fig. 1). The water balance model has been tested at our study site using leaf predawn water potential and soil moisture data (Grossiord et al. 2016a). *REW* represents the ratio between available soil water and maximum extractable water and varies between 1 (i.e. field capacity) and 0 (i.e. permanent wilting point) (Granier et al. 1999).

Sap flux measurements

The thermal dissipation method (Granier 1987) was used to measure the sap flux density of trees (F_D, L dm^{-2} h^{-1}). In October 2015, two 20 mm-long probes (Plant Sensors, Nakara, Australia) were installed under the bark and the cambium at 0.8 to 1.3 m aboveground with a 10 cm vertical spacing between probes. This height was necessary to minimize thermal disturbance from ground heating. All sensors were covered with reflective insulation to reduce the risk of direct sunlight causing thermal gradients. Each sensor was supplied with 0.2 W of constant power. Data were collected every 10 s with a CR1000 data

logger (Campbell Scientific Inc, Leicestershire, UK), and 30 min averages were recorded. F_D was calculated following the equation proposed by Granier (1987). The empirically derived coefficients in the Granier equation may introduce error in F_D calculations, although errors resulting from non-species-specific calibration are generally greater for ring-porous than diffuse-porous or tracheid-bearing species such as piñon and juniper (Bush et al. 2010). We selected only dominant trees for the sap flow measurements, thus 42 trees were equipped with probes. For each species and each treatment, the number of trees measured and included in the analyses was 21 for piñon (n=8 for A, n=4 for H, n=5 for PR, n=4 for H+PR) and 21 for juniper (n=8 for A, n=5 for H, n=4 for PR, n=4 for H+PR).

In October 2016, we sampled one wood core just above the sensors of each tree to determine the tree-specific sapwood thickness using either visual discoloration (piñon) or cresyl violet dye (juniper). For most trees, sapwood thickness was < 20 mm (i.e. less than the sensor length), so we applied the correction proposed by Clearwater et al. (1999) for such situations. For seven piñon trees, sapwood thickness was at maximum 0.8 mm longer that the probes, representing less than 5% of the sensor length so no correction was applied. During the measurement period, sap flux signals (mV) were stable during the night and from night to night (Fig. S2), showing that there was little to no occurrence of nighttime transpiration. This was consistent with the fact that the *VPD* at night was relatively low even in the heated treatments (between 0.03 kPa and 1.40 kPa, Fig. S2).

Canopy-level stomatal conductance

To determine whether stomatal-level acclimation occurred in response to the treatments, we calculated canopy-level stomatal conductance (G_s) for individual trees using a simplified inversion of the Penman-Monteith model (Monteith & Unsworth 1990). This

model assumes large boundary layer conductance, low water storage above the sap flux sensor and no vertical *VPD* gradients within the canopy (Ewers & Oren, 2000). As the study site is characterized by high winds (i.e. large boundary layer conductance), an open (leaf area index of 1.5 m^2 m^{-2}) and short canopy (approx. canopy height of 1.5 m), we expect that the conditions were matched in our study. Using whole-tree transpiration per unit leaf area (E_L in kg H_2O m^{-2} leaf s^{-1}) and *VPD*, canopy-level stomatal conductance to water vapor for individual trees (G_s, m s^{-1}) was calculated as:

$$G_S = \frac{K_G(T_A)E_L}{VPD}$$

where K_G is the conductance coefficient as a function of temperature ($115.8 \pm 0.4236T_A$, kPa m^3 kg^{-1}) and accounts for temperature effects on the psychrometric constant, latent heat of vaporization, specific heat of air at constant pressure and air density, and T_A is the air temperature (°C) (Phillips & Oren, 1998). E_L of each tree was calculated by multiplying F_D by the total sapwood area, and dividing by total leaf area (see SI). G_s was converted to mmol m^{-2} s^{-1} using site-specific atmospheric pressure (77.1 kPa) and T_A (Pearcy et al. 1989).

Hydraulic conductivity and xylem anatomy

In September 2016, we cut a 20 cm-long branch (diameter between 2-5 mm) from each tree and sealed it in a humid plastic bag. The samples were transported to the University of New Mexico where they were refrigerated until they were measured (within 24 h). Samples were submerged in DI water, and trimmed to ~3 cm in length, to remove distal embolized conduits. We inserted the samples into a steady state flow meter to measure hydraulic conductance, K, kg s^{-1} MPa^{-1} (see Hudson et al. 2010 for full description of methods). The hydraulic head pressure was supplied by a gas tank, and maintained at 0.07 MPa, and we used degassed 20mM KCl solution as a sap surrogate. We calculated stem

hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}) by multiplying K by the length of sample. Sapwood cross-sections were measured for each sample to normalize K_h at the tissue level (K_S, sapwood area-specific hydraulic conductivity, kg m^{-1} s^{-1} MPa^{-1}). Sapwood area was measured from cross sections taken at the sample's distal end. Sections were photographed at 10x magnification using a dissecting microscope (Carl Zeiss MicroImaging, Gottingen, Germany). We used ImageJ software to determine sapwood area (SA) from images by subtracting pith area from total cross section area. Distal leaf area for each sample was used to normalize K_h at shoot level (K_L, leaf area-specific hydraulic conductivity, g m^{-1} s^{-1} MPa^{-1}). Leaf area was measured using a scanner and ImageJ software. To calculate SA:LA, SA was divided by LA and multiplied by 10^4 (Brodribb & Field 2000).

Hand sections were obtained from branch hydraulic conductance samples, stained with Safranin-O, and photographed at 400x magnification on a compound microscope (Zeiss AxioImager M1, Zeiss) (Fig. S3). Early-wood tracheids from latest growth rings were measured, as these tracheids are responsible for the majority of water transport (Domec & Gartner 2002, Bouche et al. 2014). Lumen area (A_{Lumen}) and adjacent anticlinal walls thickness (T_W) were traced using ImageJ. Between 30 and 50 tracheids were measured from each sample. The hydraulically weighted lumen diameter, D_H, was calculated as:

$$D_H = 2\left(\frac{\Sigma r^5}{\Sigma r^4}\right)$$

where r is the lumen radius, in microns (Sperry and Hacke 2004). Conduit thickness to span ratio $(T_W/b)^2$ was calculated as the square of the ratio of adjacent double wall thickness to lumen diameter (Hacke et al. 2001).

Statistical analyses

All analyses were performed using the software R (3.2.1, R Development Core Team 2015).

Responses of F_D, G_s, K_L, $SA{:}LA$, A_{Lumen}, T_W, D_H and $(T_W/b)^2$ to precipitation reduction or atmospheric warming was determined through mixed linear, random intercepts models where species, heating (yes or no), drought (yes or no) and season (i.e. DOY 60 to 140=spring, 141 to 214 = summer and 215 to 262 = late summer/recovery period after monsoon events, for F_D and G_s only) 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 (heating, drought, season, species and their interactions) and we progressively removed the variables with the lowest explanatory power until the minimal model with the lowest Akaike Information Criterion (AIC) that still included the drought:heating interaction was obtained. Post-hoc analysis was performed with Tukey's HSD test. R-square (r^2) was obtained for linear mixed effects models following Nakagawa & Schielzeth (2013) and adapted by Jon Lefcheck (http://jonlefcheck.net/2013/03/13/r2-for-linear-mixed-effects-models/). An alpha critical value of α=0.05 was used to determine statistical significance. Statistical analyses were performed using the package *nlme* for Linear mixed effects models.

Logarithmic equations were fitted to G_s and *VPD* relationships of individual trees as the response of G_s to *VPD* can be isolated and analytically expressed in logarithmic form (Oren et al. 1999):

$$G_s = G_{sref} - m\ln(VPD)$$

where G_{sref} is a reference surface conductance at $VPD = 1$ kPa, and m is the stomatal sensitivity of G_s in response to $\ln(VPD)$ (Oren et al. 1999). The relationships were fitted using the package *nls* for non-linear regressions.

Following Novick et al. (2016)'s approach to assess *VPD vs.* soil moisture limitations on stomatal conductance, we extracted G_{sref} and *m* factors for each tree from the fitted relationships. G_{sref} is expected to decline with reduced precipitation and therefore accounts for moisture effects on G_s (Granier et al. 2000). The *m* parameter describes limitations imposed by *VPD* and thus accounts for evaporative demand effects on G_s (Novick et al. 2016). G_{sref} and *m* factors were used to analyze stomatal acclimation processes to precipitation reduction and *VPD* rise, however care was taken in interpretations of these acclamatory responses as G_{sref} and *m* factors can be correlated statistically and biologically (Oren et al. 1999). Differences in G_{sref} and *m* between treatments were determined for each species using similar model selection procedure as described above.

Results

Sap flux measurements

Sap flux density (F_D) varied strongly along the growing season for both species (Fig. 1 & Table S1). Following reductions in relative extractable soil water (*REW*), F_D dropped progressively in spring for both species (Fig. 1). At the start of the summer, F_D increased for both species following rain events that recharged *REW* (Fig. 1). F_D then dropped rapidly during the summer months as *REW* reached low values (Fig. 1). After monsoon events recharged *REW* in August, F_D increased and reached values close to the start of the summer (Fig 1).

F_D did not differ between the two species but varied in response to the treatments (Table S1). Both species were affected by precipitation reduction with lower F_D in the PR (i.e. precipitation reduction only) and H+PR (i.e. heat+precipitation reduction) treatments compared to ambient trees (Fig. 1 & Table S1), suggesting no or insufficient adjustments that

could maintain sap flux density under reduced soil moisture conditions. Similarly, high VPD (i.e. warming) reduced juniper F_D (i.e. H treatment) throughout the growing season (Fig. 1 & Table S1), also suggesting no or insufficient acclimation to temperature rise. Although warming also influenced F_D for piñon, the effect varied between seasons (i.e. H treatment, Fig. 1 & Table S1). During periods of high REW, F_D in warmed piñon trees was similar or even higher (i.e. after monsoon) than ambient trees (Fig. 1). When REW dropped in spring and during the summer, F_D in warmed piñon trees was lower than for ambient trees (Fig. 1).

Canopy stomatal conductance

We observed a typical logarithmic G_S response to VPD in all species and all treatments (Fig. 2). For both species, we found that precipitation reduction reduced the reference G_S (G_{Sref}) (i.e. PR and H+PR treatments) (Figs. 2 & 3, Table S2), suggesting soil water limitation to canopy conductance under precipitation reduction (Novick et al. 2016). For juniper G_{Sref} was reduced by warming as well (i.e. H treatment, Table S2), suggesting that soil water limitation also occurred under high VPD conditions even though incoming precipitation was the same as in the ambient treatment (Novick et al. 2016). For warmed piñons G_{Sref} was similar to ambient trees (Figs. 2 & 3, Table S2) indicating no soil moisture limitation in this treatment. For both species, we found that precipitation reduction and warming reduced the *m* factor (Figs. 2 & 3, Table S3) (i.e. H, PR and H+PR treatments), suggesting decreased stomatal sensitivity to VPD (Novick et al. 2016).

Hydraulic conductivity and wood anatomy

Leaf area-specific hydraulic conductivity (K_L) was similar in juniper and piñon but the two species responded differently to the treatments (Fig. 4 & Table S4). For juniper, warming reduced K_L in the H treatment (Fig. 4) suggesting hydraulic impairments, but precipitation

reduction had no effect. For piñon reduced precipitation induced impairments of the hydraulic system and resulted in reduced K_L in the PR and H+PR treatments compared to ambient trees but warming had no effect (Fig. 4). No difference in branch-level allocation ratios of sapwood area to leaf area (*SA:LA*) was found between species or in response to the treatments (Fig. 4 & Table S5), suggesting no adjustments in leaf area in response to warming and precipitation reduction.

Wood anatomical traits were not significantly different between species (Tables S6, S7, S9), apart for wall thickness (T_W) that was higher in piñon (Table S8). Neither warming nor reduced precipitation influenced the lumen area (A_{Lumen}, Table S6 & Fig. 4), the hydraulically weighted lumen diameter (D_H, Table S7) or the conduit thickness to span ratio in either species ((T_W/b)^2, Table S9), indicating no xylem anatomical adjustments to the treatments. Reduced precipitation however significantly influenced T_W for piñon, with significantly lower values in the PR treatment (Fig. 4). Warming had no influence on T_W (Fig. 4).

Discussion

The recent increase in drought-associated tree mortality is the consequence of two abiotic variables associated with climate change: reduced precipitation and elevated evaporative demand driven by temperature rise (Allen et al. 2015, Williams et al. 2013). Here we show that although different tree species show contrasting but active acclimation capacities to higher *VPD*, acclimation to warming and high evaporative demand could prevent additive effects of warming and drying in the future. Soil water limitations under simultaneous warming and precipitation reduction will reduce water dynamics for both tree functional types in the future (Fig. 1), but these reductions may not be exacerbated relative to reductions under current extreme drought events, as simulated in the present study.

Hydrological responses to precipitation reduction

Our study showed that precipitation reduction would induce impaired physiology for isohydric and anisohydric tree species. Following our initial hypotheses, sap flux density (F_D) was reduced in both species throughout the growing season under precipitation reduction (Fig. 1), suggesting no occurrence of active acclimation processes at the stomatal-, hydraulic- or root-level in response to extreme precipitation reduction, or at least insufficient acclimation to compensate for the decrease in available water resources (Grossiord et al. 2016a).

At the stomatal-level, no active acclimation to soil moisture was found. Precipitation reduction strongly decreased G_{sref} in both species (Figs. 2 & 3) suggesting no active processes that would influence tree water relations at the time scale of this study. Reductions in leaf turgor resulting from the low water potentials in this treatment (Grossiord et al. 2016a, b) probably explain this passive stomatal closure response to a reduction in soil moisture (Kramer 1988) (Fig. 1). Similarly, no active acclimation in xylem hydraulics or anatomical traits seemed to have taken place under reduced precipitation. Juniper showed no changes in any of these traits, while piñon had reduced K_L and T_W (Fig. 4). Instead of suggesting acclimation that could increase hydraulic resilience to precipitation reduction, a decrease in K_L points to higher risk of xylem embolism, particularly as no other anatomical changes were associated (Fig. 4). Previous studies have also reported lower K_L, potentially through higher embolism, for piñon and other isohydric species under natural or experimental precipitation reduction (Hereş et al. 2014, Pangle et al. 2015). Reductions in T_W under prolonged water stress may also indicate reduced carbon allocation to hydraulic safety for piñon trees (Mitchell et al. 2013).

Active acclimation processes that could occur in response to precipitation reduction but that were clearly not sufficient to maintain high rates of F_D (Fig. 1), and carbon assimilation (Grossiord et al. 2016b), include shifts in soil moisture uptake. Indeed, the development of deeper roots could enable the absorption of water from greater depths where moisture content is usually higher during dry periods. In a previous study at the same site, we found a shift towards deeper water sources during the summer for juniper, or after monsoon events for piñon trees subjected to precipitation reduction (Grossiord et al. 2016a). However, our findings show that these shifts were apparently not sufficient to compensate for the 90% reduction in precipitation.

Overall, it appears that active acclimation processes are not likely to occur in response to precipitation reduction, or at least they may not be sufficient to maintain water and carbon dynamics under prolonged precipitation reduction and during extreme events. However it must be noted that the study trees were subjected to 45% precipitation reduction for four years before being subjected to a 90% reduction for one growing season only. Active acclimation processes, particularly structural changes, may take longer to develop than our treatment exposure time, especially as our species have slow growth rates. The projected 50% reduction in precipitation by 2100 (Pachauri et al. 2014) and the decade-long "megadroughts" expected to occur in this region within the next 50 years (Cook et al. 2015) could likely result in reduced physiological and hydraulic performance, independent of tree functional type.

Hydrological responses to atmospheric warming

Atmospheric warming, and thus higher *VPD*, had contrasting effects on the hydrological responses of the studied species. We hypothesized that higher *VPD* and temperature would increase F_D during periods of high soil water availability (i.e. spring and autumn), but that F_D

would be lower and decline more rapidly during the summer compared to ambient conditions due to quicker water exhaustion in the soil profile. Indeed, previous studies have found that warming between 1.6 and 5°C increases transpiration between 11% and 74% (Kellomäki & Wang 1998, Van Herk et al. 2011, Duan et al. 2014, Wieser et al. 2015), and that reductions in F_D are proportional to the rate of soil moisture exhaustion during the summer (Duan et al. 2014, Will et al. 2013). Interestingly, this response was not observed at our site: atmospheric warming resulted in lower F_D for juniper, including during periods of high soil moisture (Fig. 1); and similar or slightly reduced F_D (i.e. during drying periods) for piñon (Fig. 1). These responses suggest water limitations for juniper and stomatal acclimation to elevated VPD for piñon.

The reduction in stomatal sensitivity of G_S to changes in VPD (the m factor) and the absence of changes in G_{Sref} demonstrated by piñon trees growing under higher VPD (Fig. 3) suggests active stomatal acclimation to warming (Fig. 5), particularly as trees were thereby able to maintain high F_D rates and thus carbon acquisition under drier atmospheric conditions (Fig. 1). Other studies have also reported stomatal adjustment to VPD that enable maintenance of water and carbon relations (Nejad & van Meeteren 2008; Sermons et al. 2012; Will et al. 2013; Carins Murphy et al. 2014), and which has also been related to long-term rise in growth temperature (Sermons et al. 2012). This active stomatal process could involve osmotic adjustments whereby a higher concentration of solutes in symplasts helps maintain turgor (Bartlett et al. 2012; Meinzer et al. 2014). Alternatively, this adaptive response could be driven by differences in stomatal size and density (Luomala et al. 2005). No other acclamatory responses were evident for piñon in this treatment as K_L values (Fig. 4), anatomical structures (Fig. 4) and water uptake depth (Grossiord et al. 2016a) were consistent between H and A trees. Thus, only stomatal-level adaptive responses were responsible for the observed maintenance of water dynamics under elevated VPD for piñon trees.

A reduction in the *m* factor was also found for warmed juniper trees (Fig. 3), again suggesting reduced stomatal sensibility to air dryness and thus stomatal acclimation to warming and elevated *VPD* (Fig. 5). However, stomatal adjustments to *VPD* did not result in the maintenance of G_s (Fig. 2), and reduction in G_{Sref} also occurred under warming (Fig. 3) indicating that patterns were also partially driven by soil moisture limitations in this treatment (Novick et al. 2016) even though precipitation was similar to ambient conditions. Correlations between G_{Sref} and *m* may complicate interpretations of the drivers of these changes (Oren et al. 1999). However, additional results confirm that reductions in G_{Sref} were driven by reduced soil moisture. Indeed, previous observations at this site showed significant reductions in predawn leaf water potential for heated juniper trees (Grossiord et al. 2016a) supporting that trees experienced water stress and substantial risk of embolism. Furthermore, lower K_L for heated trees relative to ambient trees (Fig. 4) suggests that juniper trees growing in warmer conditions could have experienced some degree of xylem cavitation as no shifts in anatomical traits or sapwood area:leaf area ratios that could have reduced K_L occurred concurrently (Fig. 4). Higher water stress under warmed conditions may have arisen from changes in water sources. In a previous study at the same site, juniper trees were found to shift their water sources to more shallow horizons when subjected to warming, thereby reducing available soil water content relative to ambient conditions (Grossiord et al. 2016a). This belowground response may be partially related to the higher respiration losses of juniper under warming (A. Collins, unpublished data), and lower carbon availability to maintain a deep rooting system. Thus, even though incoming precipitation was similar to ambient conditions, trees experienced analogous drought stress to the 90% precipitation reduction scenario (i.e. similar reductions in F_D in the warmed and drought treatments, Fig. 1).

Overall, our results reveal that under projected warming, reduced stomatal sensitivity to *VPD* rise may occur in both isohydric and anisohydric functional types. For relatively isohydric

species such as piñon, stomatal adjustments to *VPD* may lead to similar water and carbon dynamics under higher evaporative demand because trees would maintain stomata relatively open under drier atmospheric conditions. However, for relatively anisohydric species such as juniper, temperature could cause soil water stress (through shifts in rooting depth, Grossiord et al. 2016a), which would negate stomatal-level adjustments to high *VPD*. Consequently, projected warming of 5°C by 2100 (Pachauri et al. 2014) could result in deterioration of water dynamics for anisohydric tree species, independent of precipitation regimes. We however need to acknowledge that the trees in our study were exposed to high *VPD* and warming simultaneously. Previous studies have found that warming only (i.e. at constant *VPD*) could also induce physiological and structural acclimation and modify water and carbon relations (e.g. increased wood density and reduced hydraulic conductivity) (Thomas, Montagu & Conroy 2004). Consequently, although the design of our study represents a realistic scenario of future climate (Kumagai et al. 2004), it does not allow us to address tree responses to increasing *VPD* only.

Hydrological responses under simultaneous stresses

The comparison between responses in individual treatments and in the combined treatment enabled us to disentangle the driver of F_D dynamics under projected warming and precipitation reduction. We found that both species had lower F_D under multiple stresses suggesting that acclimation to both factors did not occur and that future climatic conditions will negatively impact their functioning. However, contrary to our initial hypothesis, an exacerbated response in F_D reductions due to an additive effect of warming and reduced precipitation was not observed (Fig. 1). This suggests that trees may have acclimated to one stress only, i.e. reduced precipitation or elevated *VPD* and that only one abiotic factor had a decisive effect on F_D reductions.

For piñon, we observed a reduction in stomatal sensitivity to VPD (i.e. m factor) and a concurrent reduction in G_{sref} under simultaneous stresses (Figs. 2 & 3), indicating active stomatal acclimation to VPD rise through a weaker stomatal control in dry air conditions (Fig. 6) but also a soil moisture limitation. Additionally, lower K_L was found for piñon trees in this treatment, similar to responses under precipitation reduction only (Fig. 4). Previous observations at this site also found a shift of water sources in this treatment and under precipitation reduction alone (Grossiord et al. 2016a). When both stresses act together, it seems that soil moisture limitations through reduced precipitation have a detrimental effect on piñon water and carbon dynamics (Grossiord et al. 2016a, b). However, our results show that stomatal-adjustments to VPD rise may minimize restrictions associated to dry air on tree water dynamics. Thus, under projected warming and drying, F_D reductions in piñon trees may not be worse than reductions currently observed during extreme precipitation events.

For juniper, we also found a reduction in stomatal sensitivity to VPD and a simultaneous reduction in G_{sref} (Figs. 2 & 3), suggesting adjustments to VPD rise (Fig. 6) but not to precipitation reduction as trees still experienced reduced soil moisture (i.e. reduction in G_{sref}). No other acclamatory shifts in xylem anatomy or in K_L were found under combined stresses (Fig. 4) indicating that juniper's reaction to multiple stresses is primarily driven by reduced soil moisture, similar to piñon. However, at the same site we found evidence that juniper trees subjected to multiple stresses shifted their water uptake to more superficial horizons (Grossiord et al. 2016a). This suggests that water stress was also the consequence of a belowground process driven by higher temperature rather than only by precipitation reduction. Structural acclimation to precipitation reduction (i.e. deeper water uptake) could have been lost under simultaneous warming because adjusting to one stressor (i.e. stomatal adjustments to VPD rise) may come at the cost of misadjusting to another (Holmgren et al. 1997, Niinemets & Valladares 2006, Reyer et al. 2013). Under a drying and warming climate,

higher temperature may therefore contribute to an increasing drought exposure risk for juniper because of temperature-related belowground processes that cannot be compensated for by the reduced stomatal sensitivity to *VPD*. Future experiments are needed to bring more light to juniper's carbon allocation patterns in response to temperature rise in order to test this hypothesis.

Conclusions & consequences for global predictions

The main results of the present study reinforces the idea that forest responses to climate change will depend on many biotic and abiotic factors, including changes in temperature and precipitation regimes, and the degree or capacity of acclimation of trees to a novel climate. Our observations from a mature forest ecosystem suggest that in semi-arid regions such as our study region, rapid adjustment processes at the stomatal-level could occur in response to long-term rise in evaporative demand, independent of plant functional type. Even if reduction in precipitation will still drastically impair the water and carbon dynamics of trees in the future, stomatal-adjustments to *VPD* will prevent an additive effect of warming and drying and not translate to an exacerbated impact of air and soil dryness on tree functions in semi-arid regions. Consequently, reductions in tree water use may be identical in the future to those under current extreme drought events. Our projections of species distributions and functioning may thus be biased as our observations diverge from current conceptual understanding.

Indeed, previous findings suggested that warming superimposed on drought is going to exacerbate the drought stress experienced by trees and could lead to more frequent drought-associated mortality events (Allen et al. 2015, García de la Serrana et al. 2015, Williams et al. 2013). However, after a period of five years under warmer (+ ≈5°C) and drier (-50%

precipitation) conditions, we observed no mortality or significant decline at our study site.

The observed active stomatal adjustments to high *VPD* that enabled the maintenance of transpiration (and carbon acquisition, Grossiord et al. 2016a) under drier and warmer conditions similar to under single stresses could partially explain this response. Furthermore, higher mortality risks under the "hotter drought" concept have also been associated to increasing bark-beetle infestation and wildfires (Allen et al. 2015, Williams et al. 2013), which did not occur at our site. In the absence of these two factors and in already warm and dry regions, our results show that precipitation reduction could have similar effects on tree water use regardless of whether they are accompanied by warmer temperatures. Thus, management options that would sustain forest ecosystems in semi-arid regions should preferably aim at reducing climate-related disturbances like insect outbreaks and wildfires rather than only improving water resources. Additionally, instead of allowing functional shifts in response to temperature and precipitation reduction, models should incorporate the actual response of trees under a multiple stress scenario as short-term acclimation processes (i.e. at the stomatal-level) may compensate for the additive effects of abiotic stresses.

Our results also illustrate that some functional groups might be more susceptible to long-term increases in evaporative demand and warming than others. Relatively isohydric species such as piñon seem able to acclimate at the foliar-level to *VPD* and temperature rise and thereby maintain transpiration and carbon acquisition rates as long as water availability remains high. Thus, under a global warming scenario, isohydric species may be able to maintain carbon acquisition during periods of high precipitation and soil moisture (i.e. spring and autumn). These results expand on previous predictions suggesting higher mortality risk for isohydric trees compared to anisohydric ones through stronger stomatal sensitivity to drought and higher carbon starvation risk (McDowell et al. 2008, Sevanto et al. 2014). Although no mortality was observed in this study, the results suggest that juniper, more than piñon, will be

vulnerable to climate warming. Indeed, for relatively anisohydric species such as juniper, we found that foliar-level acclimation to *VPD* may be cancelled out by higher respiratory losses and an overall negative carbon balance (Collins et al., unpublished data). An unfavorable carbon budget may impair juniper's ability to maintain its deep rooting system (Grossiord et al. 2016a), thereby exposing trees to higher soil moisture stress, particularly in semi-arid climates. Consequently our results suggest that management practices aimed at improving soil water resources may have little impact over anisohydric species in the future, particularly in already water-limited ecosystems.

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Figure legends

Figure 1. Daily relative extractable water (*REW*, unitless), precipitation (mm), mean daily vapor pressure deficit (*VPD*, kPa), mean daily sap flux density (F_D, L dm^{-2} h^{-1}) for juniper and piñon for each treatment (A = ambient, H = heat, PR = precipitation reduction and H+PR = heat+precipitation reduction) during the 2016 growing season (i.e. March-September). *VPD* in the PR treatment is not represented here as it is the same as in the A treatment.

Figure 2. Relationships between mean daily canopy-level stomatal conductance (G_s) and vapour pressure deficit (*VPD*) for juniper and piñon trees in each treatment (A = ambient, H = heat, PR = precipitation reduction and H+PR = heat+precipitation reduction), and fitted logarithmic relationships between G_S and *VPD* (Oren et al. 1998) for each treatment.

Figure 3. Mean of stomatal sensitivity to vapor pressure deficit (*m* factor unitless) and reference canopy stomatal conductance (G_{Sref}) for juniper and piñon in each treatment (A = ambient, H = heat, PR = precipitation reduction and H+PR = heat+precipitation reduction), with standard errors of the mean. Asterisks denote significant differences between treatments (H, PR and H+PR) and control (A) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$) using post-hoc analyses. Following Novick et al. (2016), G_{sref} accounts for G_s sensitivity to moisture and the *m* parameter accounts for G_s sensitivity to evaporative demand.

Figure 4. Mean of leaf area-specific hydraulic conductivity (K_L), sapwood area:leaf area ratio (*SA:LA*), lumen area (A_{Lumen}) and adjacent anticlinal walls thickness (T_W) for juniper (J) and piñon (P) in each treatment (A = ambient, H = heat, D = drought and HD = heat+drought), with standard errors of the mean. Asterisks denote significant differences between treatments

(D, H and HD) and control (A) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$) using post-hoc analyses.

Figure 5. Variations in *VPD* and *REW* along the growing season under ambient (i.e. blue lines) and warming (i.e. red lines) conditions (a), the resulting hypothetical relationships between stomatal conductance (G_s) and vapor pressure deficit (*VPD*) under ambient (i.e. blue lines) and warming (i.e. red lines) conditions (b), and their respective consequences on the seasonal variations in F_D (c) for piñon and juniper trees. The responses are shown in conditions of absence of stomatal acclimation or presence of active stomatal acclimation to high *VPD*. If no acclimation to *VPD* occurs in trees subjected to warming then the sensitivity of G_S to *VPD* (i.e. *m* factor, Novick et al. 2016) would remain the same as in ambient trees. However trees would be exposed to higher *VPD* throughout the growing season, inducing higher F_D at the start of the growing season (i.e. when *VPD* is still relatively low) and lower F_D through stomata closure during the warmest period of the growing season (i.e. when *VPD* is high). An active acclimation process to high *VPD* (e.g. changes in chemical signals like abscisic acid synthesis or stomatal density) could modify G_S sensibility to *VPD* by rendering stomata less reactive to stimuli that would otherwise provoke stomatal closure (i.e. to maintain stomata open under drier air conditions and conserve carbon acquisition). Consequently, trees could express higher F_D at the beginning of the growing season (i.e. when *VPD* is still relatively low) and maintain higher F_D than ambient trees during the warmest period of the growing season (i.e. when *VPD* is high). Because of piñon's isohydric stomatal strategy, the stomatal closure point at high VPD ($G_S = 0$) would be reached earlier than for juniper (McDowell et al. 2008). Consequently F_D reductions during the growing season should be more rapid than for anisohydric juniper trees. For representation purposes,

indirect effects of warming on REW were not taken into account (i.e. higher soil evaporation or more rapid soil moisture depletion due to higher F_D).

Figure 6. Variations in VPD and REW along the growing season under ambient (i.e. blue lines) and warming + precipitation reduction (i.e. red lines) conditions (a), the resulting hypothetical relationships between stomatal conductance (G_s) and vapor pressure deficit (VPD) under ambient (i.e. blue lines) and warming + precipitation reduction (i.e. red lines) conditions (b), and their respective consequences on the seasonal variations in F_D (c) for piñon and juniper trees. The responses are shown in conditions of absence of stomatal acclimation or presence of active stomatal acclimation to high VPD. Under warming and reduced precipitation conditions, if no acclimation to VPD occurs, the sensitivity of G_S to VPD would remain the same as in ambient trees but reduced soil moisture (induced by reduced precipitation) would result in lower reference G_S (i.e. G_{sref}, Novick et al. 2016). Consequently trees would have higher F_D at the beginning of the growing season (i.e. when VPD is still relatively low and REW relatively high) and quicker reduction in F_D during the summer compared to trees subjected to warming only (combination of high VPD and more rapid drop in REW). If active acclimation occurs, trees subjected to warming and precipitation could express higher F_D at the start of the growing season (i.e. when VPD is still relatively low and REW relatively high) and demonstrate a less marked drop in F_D during the warmest period of the growing season (i.e. when VPD is high and REW is low). Similarly as under warming conditions, piñon's isohydric stomatal strategy would result in the stomatal closure point at high VPD ($G_S = 0$) to be reached earlier than for juniper (McDowell et al. 2008). Consequently F_D reductions during the growing season should be more rapid than for anisohydric juniper trees. For representation purposes, indirect effects of warming on REW

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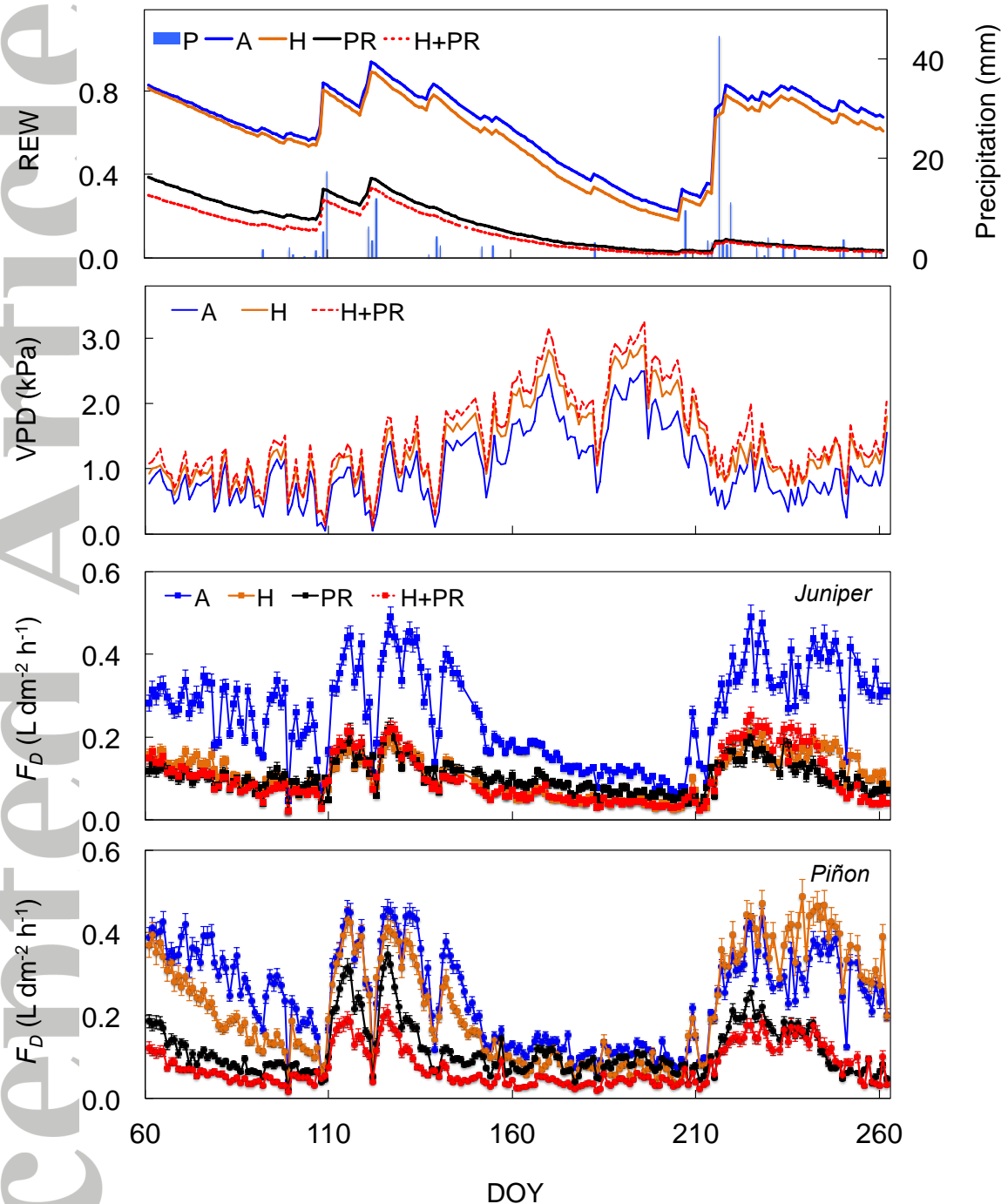
Figures

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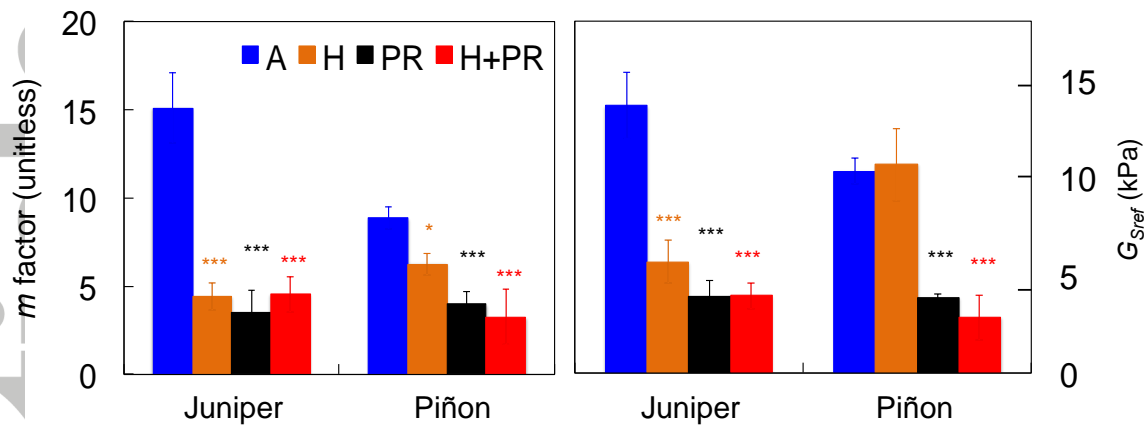logarithmic relationships between G_S and *VPD* (Oren et al. 1998) for each treatment.

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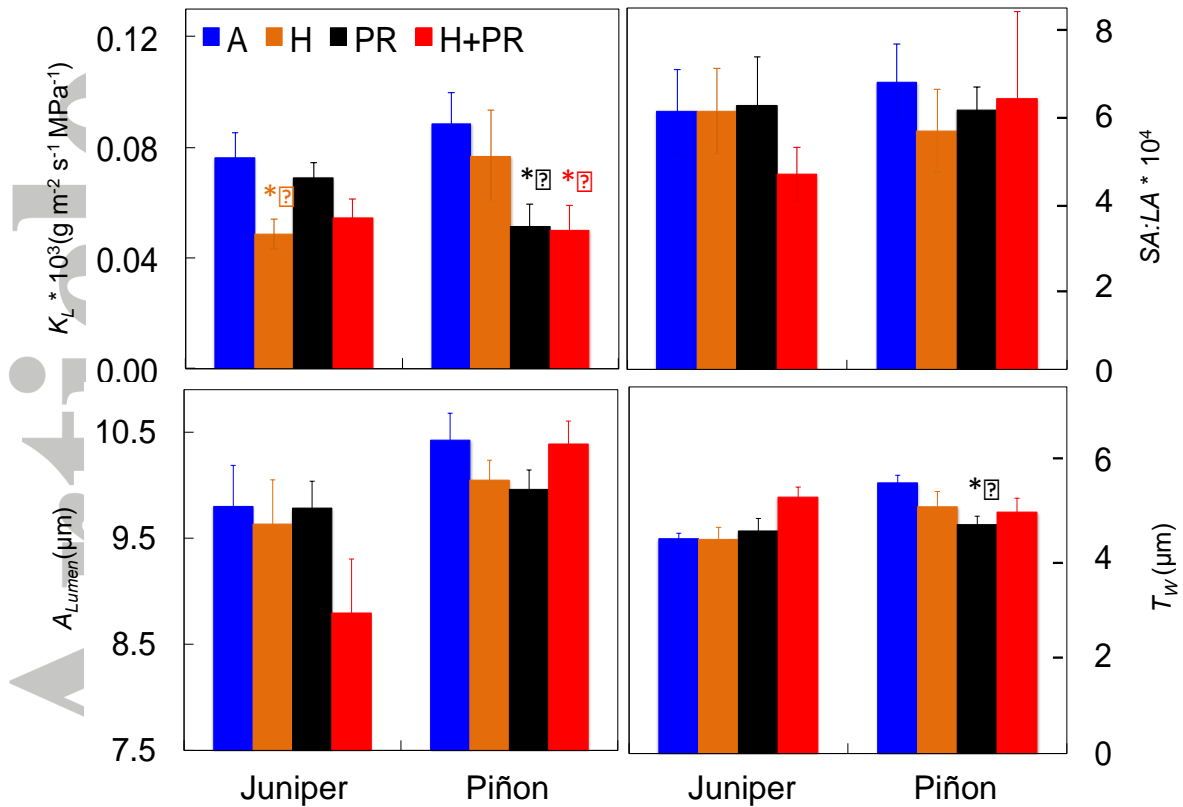
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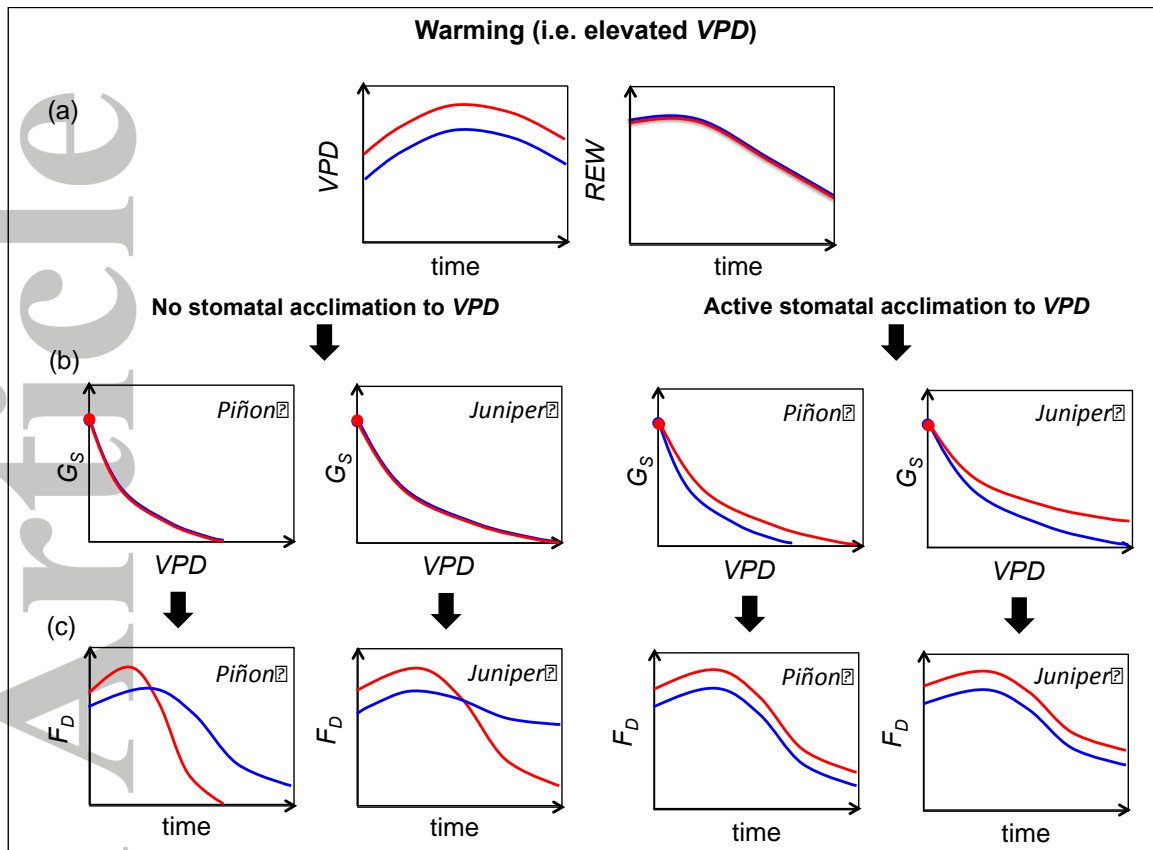
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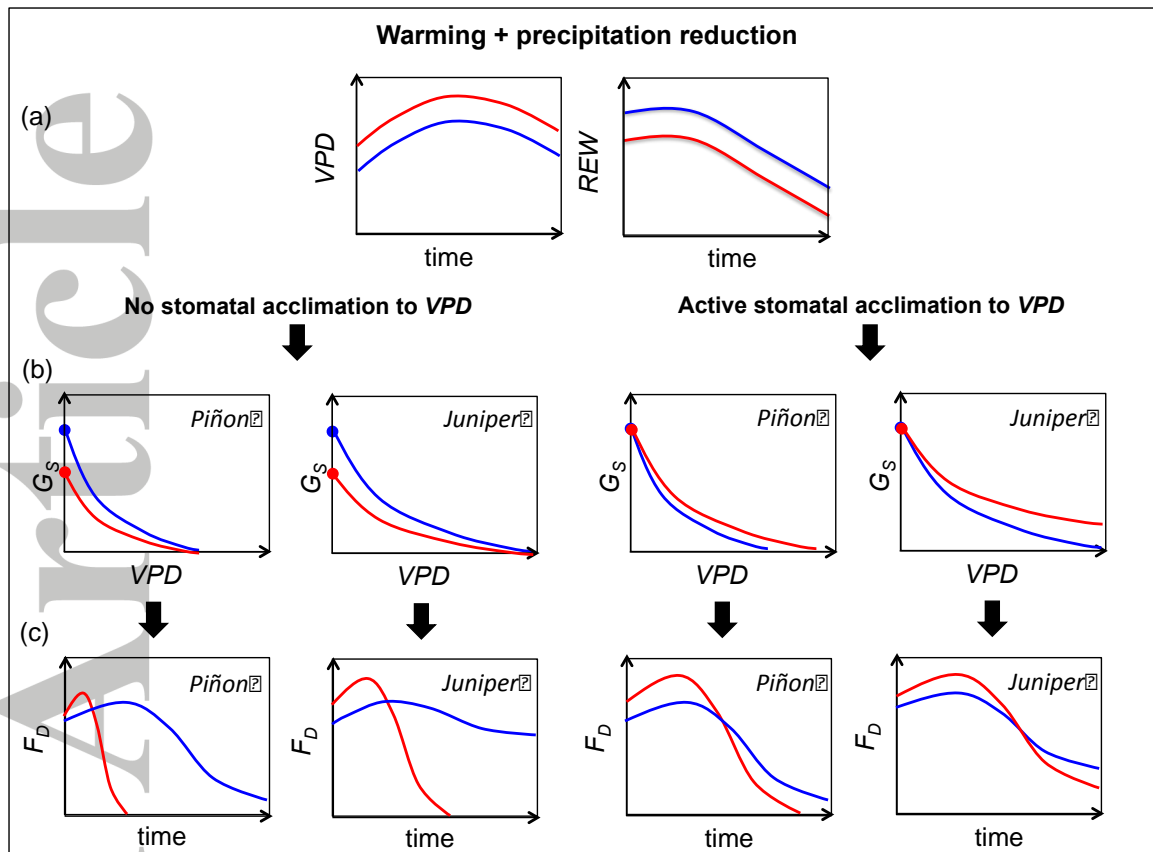
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SUMMARY STATEMENT

Disentangling the relative impacts of precipitation reduction and vapor pressure deficit (VPD) on plant water dynamics and determining whether acclimation may influence these patterns in the future is an important challenge.

Here, we report sap flux density (F_D), stomatal conductance (G_s), hydraulic conductivity (K_L) and xylem anatomy in piñon pine (*Pinus edulis*) and juniper (*Juniperus monosperma*) trees subjected to five years of precipitation reduction, atmospheric warming (elevated VPD) and their combined effects.

Our results show that stomatal conductance adjustments to high VPD could minimize but not entirely prevent additive effects of warming and drying on water use and carbon acquisition of trees in semi-arid regions.