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Stomatal behaviour and stem xylem traits are coordinated for woody plant species under exceptional drought conditions

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Abstract

Isohydry (maintenance of plant water potential at the cost of carbon gain) and anisohydry (gas exchange maintenance at the cost of declining plant water status) make up two ends of a stomatal drought response strategy continuum. However, few studies have merged measures of stomatal regulation with xylem hydraulic safety strategies based on in situ field measurements. The goal of this study was to characterize the stomatal and xylem hydraulic safety strategies of woody species in the biodiverse Mediterranean-type ecosystem region of California. Measurements were conducted in situ when California was experiencing the most severe drought conditions in the past 1,200 years. We found coordination among stomatal, hydraulic, and standard leaf functional traits. For example, stem xylem vulnerability to cavitation (P_{50}) was correlated with the water potential at stomatal closure (P_{close}); more resistant species had a more negative water potential at stomatal closure. The degree of isohydry–anisohydry, defined at $P_{close} - P_{50}$, was correlated with the hydraulic safety margin across species; more isohydric species had a larger hydraulic safety margin. In addition, we report for the first time P_{close} values below -10 MPa. Measuring these traits in a biodiverse region under exceptional drought conditions contributes to our understanding of plant drought responses.

KEYWORDS

anisohydry, California, chaparral, coastal sage scrub, hydraulic safety margin, isohydry, Mediterranean-type ecosystem, stomatal conductance, water potential

1 | INTRODUCTION

Mediterranean-type ecosystems (MTEs) are biodiversity hotspots and conservation priorities (Cowling, Rundel, Lamont, Arroyo, & Arianoutsou, 1996). Their characteristic cool, wet winters and warm, dry summers result in an annual summer-drought that stresses the water status of vegetation. High interannual rainfall variability also leads to periodic extended drought, as there can be consecutive years with below average precipitation. These variable climatic conditions also mean that MTEs may be climate change hotspots (Diffenbaugh, Giorgi, & Pal, 2008; Field, Barros, Mach, & Mastrandrea, 2014). Already, drought in conjunction with warmer temperatures due to climate change is leading to global change-type drought, which has been linked to worldwide regional forest mortality events (Allen, 2009;

Breshears et al., 2005). In fact, the 2012–2014 drought in California was the most intense drought in the past 1,200 years (Griffin & Anchukaitis, 2014). A recent study conducted in a southern California native shrubland found that shallow-rooted species with cavitation resistant xylem experienced the highest mortality levels due to drought, whereas deeply rooted species with xylem vulnerable to cavitation had lower mortality levels (Venturas et al., 2016). This differential mortality among co-occurring species due to varying drought survival strategies (Pivovarovff, Pasquini, et al., 2016) may result in changes in community composition and ultimately biodiversity loss. However, predicting mortality remains a challenge for vegetation models due to wide variation in premortality physiological changes among species (Anderegg et al., 2016; Garcia-Forner, Sala, Biel, Save, & Martinez-Vilalta, 2016; McDowell, 2011; McDowell

et al., 2008; Pivovarovoff, Pasquini, et al., 2016; Sevanto, Xu, & Way, 2016).

Recent work on plant mortality due to drought has focused on hydraulic strategies of woody plant species (Allen et al., 2010; Garcia-Forner, Adams, et al., 2016; McDowell, 2011; McDowell et al., 2008; Sevanto, McDowell, Dickman, Pangle, & Pockman, 2013; Skelton, West, & Dawson, 2015), with significant attention given to stomata. Stomata regulate the exchange of carbon dioxide (CO_2) and water between plants and the atmosphere. However, this poses a dilemma for plants because when stomata open to allow CO_2 uptake, water is lost, which can lead to dangerous water deficits that approach hydraulic dysfunction thresholds. In spite of this, strategic stomatal behaviour can balance this contradictory CO_2 demand versus water supply predicament. Specifically, isohydry versus anisohydry make up two ends of a stomatal regulation strategy continuum (Klein, 2014; Tardieu & Simonneau, 1998). Isohydric species close their stomata before water potentials become dangerously low, leading to relatively unchanging water potentials regardless of environmental conditions, such as low soil moisture availability or high vapour pressure deficit. Anisohydric species, on the other hand, keep their stomata open at the onset of drought, even when water potentials decline and the risk of hydraulic failure increases.

In a current review of stomatal behaviour and water potential regulation, Martínez-Vilalta and Garcia-Forner (2017) examined three different definitions of isohydry–anisohydry. The first definition characterizes isohydry based on minimum seasonal midday water potentials, the second definition on seasonal variability of midday water potentials, and the third on the relationship between predawn and midday water potentials. Each definition has its own advantages and limitations (please see Martínez-Vilalta & Garcia-Forner, 2017, for more detail). One of the major limitations common among all three definitions is the lack of integrating stem xylem vulnerability to cavitation, a critical drought response trait that has been implicated in plant mortality. Skelton et al. (2015) defined a new isohydry–anisohydry framework that merged stomatal and xylem hydraulic strategies, expressed as the difference between the water potential at stomatal closure versus the water potential at 50% loss of stem hydraulic conductivity. Although stems and leaves are seemingly separate organs, they represent a coordinated pathway along the soil–plant–atmosphere continuum. Many studies only focus on one organ or the other. Hence, merging the roles that leaves and stems play in drought strategies (Pivovarovoff, Sack, & Santiago, 2014) improves our understanding of plant responses to a changing climate.

The goal of this study was to characterize the coordination of stomatal and stem xylem traits in the biodiverse MTE region of California using in situ field-based measurements. In situ field-based measurements have an advantage over using excised samples in lab-based measurements, such as benchtop dehydration, as samples in the field are intact and hence not decoupled from whole-plant responses. At the time of this study (July 2014–May 2015), California was experiencing the most severe drought conditions in the past 1,200 years (Griffin & Anchukaitis, 2014). From a single field site, we included chaparral, coastal sage scrub, and riparian vegetation types to cover a broad range of species and life history strategies. Chaparral species studied here were sclerophyllous evergreen shrubs and trees.

Coastal sage scrub is an endangered ecosystem (Castellanos & Mendoza, 1991), including shallow-rooted and drought deciduous species. Riparian species establish in moist habitats near rivers or streams. Our study question was, Are stomatal and stem xylem traits coordinated in woody angiosperm species across vegetation types? To answer this question, we first characterized the stomatal strategy of each study species along the isohydric–anisohydric continuum, using the Skelton definition ($P_{\text{close}} - P_{50}$). We hypothesized that the stomatal strategy of species would be grouped by vegetation type, with riparian species being strongly isohydric as they would maintain high water potentials across seasons, coastal sage scrub being intermediate as the deciduous nature of these species would allow them to shut down during the summer drought to avoid a detrimental water status, and chaparral species being strongly anisohydric as they continue to function despite warm and dry seasonal conditions. Second, we tested for coordination among measured leaf and stem traits. We hypothesized that stomatal and xylem traits were coordinated in this diverse plant community and that the degree of isohydric–anisohydric behaviour would be associated with the magnitude of the hydraulic safety margin of a species. Finally, additional standard leaf functional traits were measured, including leaf mass per area and cuticular conductance, to evaluate their coordination with isohydric versus anisohydric strategies.

2 | MATERIALS AND METHODS

2.1 | Field site and study species

Fieldwork was conducted every 2 months from July 2014 to May 2015 at the Santa Margarita Ecological Reserve (33°28'N, 117°09'), located at the border between Riverside and San Diego counties in southern California, USA. With a Mediterranean-type climate, the mean temperature is 16.3°C and the mean rainfall is 33 cm, typically occurring during the winter months between November and March (Figure 1a). However, during the time of this study, the majority of California was experiencing “Extreme” to “Exceptional” drought conditions, the most severe drought classifications (University of Nebraska-Lincoln, 2017). This study focused on 17 commonly occurring woody species (Table 1), from chaparral, coastal sage scrub, and riparian vegetation types.

2.2 | Stomatal conductance

Stomatal conductance was measured every 2 months using a leaf porometer (SC-1, Decagon, Pullman, WA, USA). The leaf porometer was calibrated at the start of and throughout each measurement day. Measurements were taken on recently mature leaves between 07:00 and 15:00 in “rounds.” For each round, we took a stomatal conductance and paired leaf water potential measurement for each species (Table 1). We completed a full round before repeating a species and each round was performed on a different individual. We measured between 3 and 7, but typically 6, rounds a day. When deciduous species completely lost their leaves, they were omitted from those days' measurements. Maximum daily stomatal conductance (g_s) was calculated as the average of the three highest measurements for a species

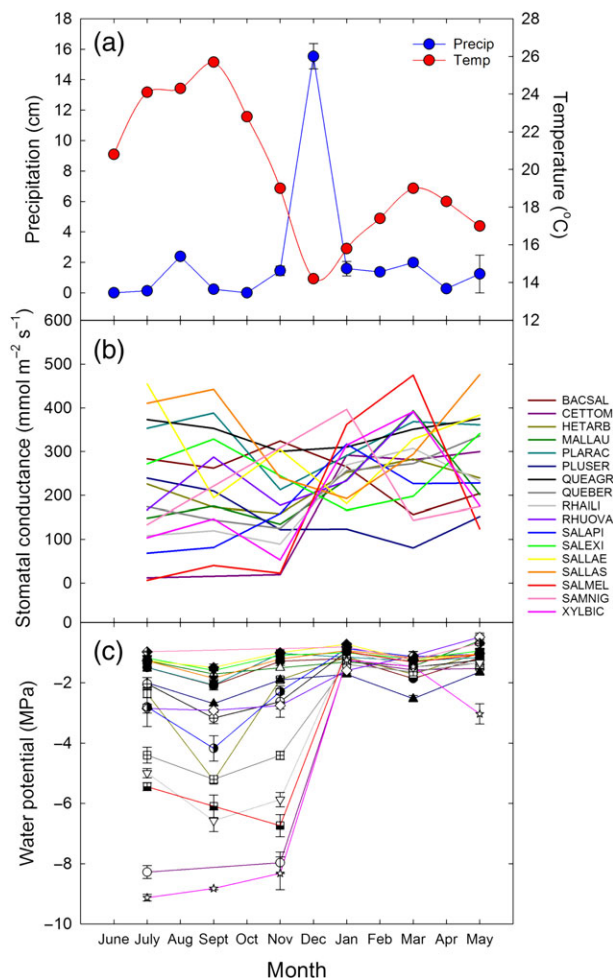


FIGURE 1 Mean total monthly precipitation (cm; blue symbols and line) and monthly mean temperature ($^{\circ}\text{C}$; red symbols and line) at the Santa Margarita Ecological Reserve for June 2014–May 2015 (a). Precipitation data is from the National Oceanic and Atmospheric National Centers for Environmental Information stations Fallbrook 5 NE, CA, USA, and Fallbrook 6.5 NE, CA, USA, and temperature data from Fallbrook 5 NE, CA, USA (Peterson & Vose, 1997). Maximum daily stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$), taken as the mean of the three highest daily stomatal conductance measurements, recorded seasonally on 17 woody species (b). Minimum daily water potential (MPa), taken as the mean of the three lowest daily water potential measurements, recorded seasonally (c). Errors bars represent SE. Corresponding species and symbols can be found in Table 1

in a day. Maximum overall stomatal conductance (g_{max}) represents the single highest stomatal conductance measurement for a species experienced during the course of a year.

2.3 | Water potentials

Water potentials were measured every 2 months, concurrently with stomatal conductance, using a pressure chamber (Model 1005; Plant Moisture Stress Instruments, Albany, OR, USA). Immediately following measurement of stomatal conductance, the same shoot was collected, double-bagged, and placed in a dark cooler before measuring bulk shoot water potential. We defined maximum water potential (P_{max}) as the single highest seasonal water potential value for each species

during the course of a year, and minimum water potential (P_{min}) as the single most negative seasonal water potential value for each species during the course of a year. Minimum seasonal water potential is the greatest xylem tension experienced by a species during the course of a year (Bhaskar & Ackerly, 2006) and is an important metric for understanding plant water status as it indicates the greatest water deficit a species must tolerate. The variability of midday water potential was defined as $P_{\text{max}} - P_{\text{min}}$.


















2.4 | Leaf cuticular conductance and leaf mass per area

Leaf cuticular conductance (g_{min}) and leaf mass per area (LMA) were determined for five leaves of each species every 4 months (July 2014, November 2014, and March 2015) to determine how much water is lost even after stomata are closed and to characterize a standard leaf functional trait, respectively. The most recently mature leaves were always selected for sample collection. Samples were collected at the end of the field day (~15:00) and double-bagged for transport to the lab. Cuticular conductance samples were immediately hydrated overnight (~12 hr); riparian species were hydrated the following morning (~4 hr) to prevent leaf burn from overhydration. After hydration, cut ends were sealed with wax. Samples were dried on the lab bench with a fan for 30 min at photosynthetically active radiation of $<10 \mu\text{mol m}^{-2} \text{s}^{-1}$ to induce stomatal closure, then weighed every 30 min for eight intervals. The g_{min} was calculated by plotting water loss versus time, for which the slope was highly linear and therefore taken to represent transpiration after stomata had closed. Transpiration rate was divided by the mole fraction vapour pressure deficit calculated from a thermohygrometer (Cole-Parmer, Vernon Hills, IL, USA). For LMA, fresh leaf area was measured for individual leaves with a leaf area meter (LI-3100, Li-Cor Biosciences, Lincoln, NE, USA). Leaves were then dried in a drying oven for 48 hr at 65°C before measuring dry mass. Leaf mass per area was calculated as dry mass (g) divided by fresh leaf area (cm^2).

2.5 | Literature survey

Species rooting depth classifications (Canadell et al., 1996; Davis, 1989; DeSouza, Silka, & Davis, 1986; Hellmers, Horton, Juhren, & O'Keefe, 1955; Miller & Ng, 1977; Thomas & Davis, 1989; USDA, 2017) and the water potential at which 50% of stem hydraulic conductivity is lost due to cavitation (P_{50} ; Jacobsen, Pratt, Ewers, & Davis, 2007; Litvak, McCarthy, & Pataki, 2012; Pivovarov et al., 2014; Pockman & Sperry, 2000; Pratt et al., 2007; Wheeler, Sperry, Hacke, & Hoang, 2005) were compiled from data from the literature. When different P_{50} values from multiple sources for a single species were found, we chose the more resistant value as highly vulnerable values for long-vesselled species may be a methodological artefact (Pivovarov Burlett, et al., 2016). We did not find P_{50} values for *Salvia apiana*, *Pluchea sericea*, *Salix lasiolepis*, or *Salix laevigata*, so we measured vulnerability curves for these species ourselves (Supplemental Materials). We did not find rooting depth values for *P. sericea*, *Rhamnus ilicifolia*, *S. laevigata*, or *Xylococcus bicolor*.

TABLE 1 Study species, including family, vegetation type, leaf mass per area (LMA), and cuticular conductance (g_{\min}), at the Santa Margarita Ecological Reserve, California, USA

Species	Family	Vegetation type	Symbol	Leaf mass per area (g cm^{-2})	Cuticular conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	Rooting depth (m)
<i>Salvia apiana</i>	Lamiaceae	Coastal sage scrub		0.0178 ± 0.0013	3.39 ± 0.27	2.7^a ; 1.5^f
<i>Salvia mellifera</i>	Lamiaceae	Coastal sage scrub		0.0163 ± 0.0020	6.32 ± 1.21	2.1^a
<i>Sambucus nigra</i>	Adoxaceae	Coastal sage scrub		0.0109 ± 0.0005	10.87 ± 2.14	0.41 (min)^g
<i>Ceanothus tomentosus</i>	Rhamnaceae	Chaparral		0.0173 ± 0.0012	14.37 ± 1.69	$1.8\text{--}3.7^{a,e,f}$
<i>Heteromeles arbutifolia</i>	Rosaceae	Chaparral		0.0144 ± 0.0015	1.91 ± 0.24	0.3^b
<i>Malosma laurina</i>	Anacardiaceae	Chaparral		0.0245 ± 0.0010	5.05 ± 0.74	$>13.2^c$; $>5.4^{e,f}$
<i>Quercus agrifolia</i>	Fagaceae	Chaparral		0.0186 ± 0.0015	2.59 ± 0.47	10.7^f
<i>Quercus berberidifolia</i>	Fagaceae	Chaparral		0.0157 ± 0.0016	4.54 ± 0.56	8.5^f
<i>Rhamnus ilicifolia</i>	Rhamnaceae	Chaparral		0.0142 ± 0.0013	2.86 ± 0.40	--
<i>Rhus ovata</i>	Anacardiaceae	Chaparral		0.0250 ± 0.0010	1.69 ± 0.19	"deep" ^d ; 0.46 (min)^g
<i>Xylococcus bicolor</i>	Ericaceae	Chaparral		0.0367 ± 0.0035	6.99 ± 0.78	--
<i>Baccharis salicifolia</i>	Asteraceae	Riparian		0.0120 ± 0.0005	6.83 ± 0.96	0.3 (min)^g
<i>Platanus racemosa</i>	Platanaceae	Riparian		0.0088 ± 0.0003	5.38 ± 1.02	0.91 (min)^g
<i>Pluchea sericea</i>	Asteraceae	Riparian		0.0164 ± 0.0008	5.51 ± 0.46	--
<i>Salix exigua</i>	Salicaceae	Riparian		0.0135 ± 0.0005	8.25 ± 1.12	0.51 (min)^g
<i>Salix laevigata</i>	Salicaceae	Riparian		0.0147 ± 0.0012	7.08 ± 0.94	--
<i>Salix lasiolepis</i>	Salicaceae	Riparian		0.0159 ± 0.0008	9.45 ± 1.46	0.66 (min)^g

Note. Species are represented by the corresponding symbols in figures. Mean LMA and g_{\min} values are reported \pm SE. When indicated by (min), rooting depth values are for minimum rooting depth. *Ceanothus* rooting depth represents the range of rooting depth among the *Ceanothus* genus. References for rooting depth:

^aHellmers et al. (1955);

^bMiller and Ng (1977);

^cDeSouza et al. (1986);

^dDavis (1989);

^eThomas and Davis (1989);

^fCanadell et al. (1996);

^gUSDA (2017).

2.6 | Statistical analysis

To calculate the water potential at stomatal closure (P_{close}), Weibull curves were fitted to the stomatal conductance versus water potential response curves using the "fitplc" package in R (Duursma & Choat, 2017). Specifically, we used the "fitcond" function because data were organized as water potential versus conductance (in this case, stomatal conductance). With this function, we set "x" to solve for the water potential at which 50% of stomatal conductance is lost ($x = 50$; P_{g50}) and the water potential at which 88% of stomatal

conductance is lost ($x = 88$; P_{close}). Our P_{close} is analogous to the Skelton et al. (2015) standardized critical point, P_{g12} , or the water potential at which 12% of stomatal conductance remains. For *Rhus ovata*, the model was successfully fit to the data, but the calculated P_{close} was determined to be unrealistic and was excluded from further analyses.

The hydraulic safety margin was calculated as minimum seasonal water potential minus stem xylem vulnerability to cavitation ($P_{\min} - P_{50}$; Delzon & Cochard, 2014; Meinzer, Johnson, Lachenbruch, McCulloh, & Woodruff, 2009). Although multiple metrics for the

degree of isohydry versus anisohydry exist, we used the Skelton definition ($P_{\text{close}}-P_{50}$; Skelton et al., 2015).

Correlations between traits were analysed in R using "rccorr" to calculate the Pearson correlation coefficient (r) and P value (Hollander & Wolfe, 1973; Press, Flannery, Teukolsky, & Vetterling, 1988). Before running Pearson correlations, data were checked for normal distribution using the Shapiro-Wilk test. If traits were not normally distributed, they were log-transformed and checked for normal distribution again. For P_{close} , log transformation did not result in normal distribution.

3 | RESULTS

3.1 | Stomatal conductance and water potentials

Coupled stomatal conductance and water potential measurements revealed differing seasonal patterns for these two traits (Figure 1b,c and Figure S1). Stomatal conductance patterns varied among species and across seasons. Water potentials, on the other hand, showed a different pattern. There was a wide range of water potentials among species during the warm, dry period (June–November), whereas all species converged on high (less negative) water potentials in the cool and wet winter and spring months (January–May).

The wide range of minimum seasonal water potentials among study species demonstrated different functional groups as related to water acquisition and regulation (Figure 1c). For example, *Malosma laurina*, *Sambucus nigra*, *Salix exigua*, *S. laevigata*, and *S. lasiolepis* all displayed high minimum seasonal water potentials (>-2 MPa). These species were also deeply rooted (*M. laurina*), drought-deciduous (*S. nigra*), or riparian (*Salix* species). Other species displayed intermediate minimum seasonal water potentials, including *Baccharis salicifolia*, *Platanus racemosa*, *P. sericea*, *R. ovata*, and *Quercus agrifolia*. These species never exhibited a water potential more negative than -4 MPa. These species were also riparian (*B. salicifolia* and *P. sericea*) or deeply rooted (*R. ovata* and *Q. agrifolia*). Some species had very negative minimum seasonal water potentials. For example, *Quercus berberidifolia*, *Heteromeles arbutifolia*, and *Salvia apiana* had minimum seasonal water potentials around -6 MPa, and *R. ilicifolia*, *Salvia mellifera*, *Ceanothus tomentosus*, and *X. bicolor* continued to maintain stomatal conductance until -7 to -9 MPa. In fact, *Q. berberidifolia*, *R. ilicifolia*, and *X. bicolor* maintained high levels of stomatal conductance (>100 mmol m $^{-2}$ s $^{-1}$) even beyond -4 MPa, whereas *H. arbutifolia*, *S. apiana*, *S. mellifera*, and *C. tomentosus* only maintained low levels of stomatal conductance (<100 mmol m $^{-2}$ s $^{-1}$) beyond -4 MPa. In general, all riparian species had minimum seasonal water potentials >-3 MPa. Deeply rooted chaparral species had minimum seasonal water potentials ≥-3.5 MPa. Shallow rooted chaparral species continued to operate until -8 MPa. Coastal sage scrub species were mixed in that their minimum seasonal water potentials varied from -2 to -8 MPa.

3.2 | Water potential at stomatal closure

Eight out of the 17 study species closed their stomata before reaching a water potential of -2 MPa. These included the riparian species *B. salicifolia*, *P. racemosa*, *P. sericea*, *S. exigua*, *S. laevigata*, *S. lasiolepis*,

the chaparral species *Q. agrifolia*, and the coastal sage scrub species *S. nigra*. Three species, including *S. apiana*, *M. laurina*, and *S. mellifera*, all closed stomata before reaching a water potential of -4 MPa. *C. tomentosus* closed stomata before reaching -6 MPa. Four species, including *R. ilicifolia*, *Q. berberidifolia*, *H. arbutifolia*, and *X. bicolor*, kept stomata open until about -10 MPa.

The water potential at which plants closed stomata (P_{close}) and minimum seasonal water potential (P_{min}) were positively correlated ($P = 0.0004$, $r = 0.77$; Figure 2a); species with a lower minimum seasonal water potential also had a lower water potential at which they closed stomata. In addition, minimum seasonal water potential was also positively correlated with stem xylem vulnerability to cavitation (P_{50} ; $P = 0.01$, $r = 0.62$; Figure 2b); species with more cavitation resistant xylem had more negative minimum seasonal water potentials. Minimum seasonal water potentials were negatively correlated with leaf mass per area ($P = 0.0004$, $r = -0.76$); leaf mass per area increased with decreasing minimum seasonal water potential. The variability of midday water potential ($P_{\text{max}}-P_{\text{min}}$) was positively correlated with leaf

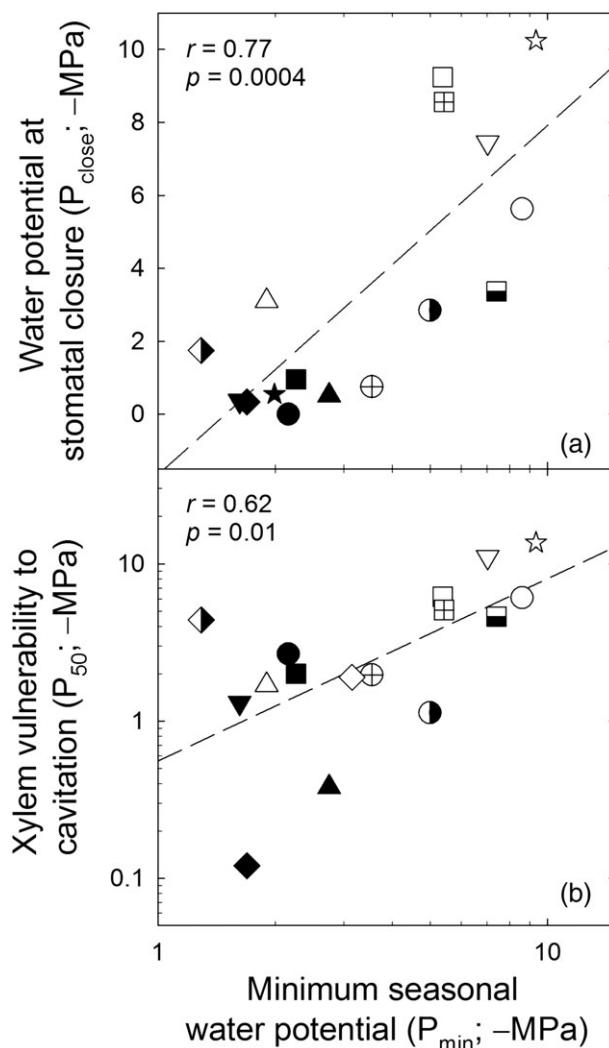


FIGURE 2 Minimum seasonal water potential (P_{min}) versus the water potential at stomatal closure (P_{close} ; a) and stem xylem vulnerability to cavitation (P_{50} ; b) for woody species at the Santa Margarita Ecological Reserve. The dashed lines represent the linear regression. Each symbol represents a different species mean; corresponding species and symbols can be found in Table 1

mass per area ($P = 0.0002$, $r = 0.78$), xylem vulnerability to cavitation ($P = 0.01$, $r = 0.62$), and water potential at stomatal closure ($P = 0.0004$, $r = 0.78$); species with a greater variability of midday water potential had higher leaf mass per area, greater resistance to xylem cavitation, and more negative water potential at stomatal closure.

3.3 | Isohydry versus anisohdry

The degree of isohdry versus anisohdry, defined as $P_{\text{close}} - P_{50}$ by Skelton et al., 2015, revealed a continuum among the study species (Figure 3). *R. ilicifolia*, *X. bicolor*, *B. salicifolia*, and *S. nigra* had $P_{\text{close}} - P_{50}$ values greater than 2 MPa, towards the isohdryic end of the spectrum. *S. mellifera*, *Q. agrifolia*, *P. racemosa*, *S. exigua*, and *C. tomentosus* had $P_{\text{close}} - P_{50}$ values between 2 and 0 MPa. *P. sericea*, *S. laevigata*, *M. laurina*, and *S. apiana* had $P_{\text{close}} - P_{50}$ values between 0 and -2 MPa. *H. arbutifolia* and *Q. berberidifolia* had $P_{\text{close}} - P_{50}$ values more negative than -2 MPa, towards the anisohdryic end of the spectrum.

3.4 | Hydraulic safety margins

Stem xylem vulnerability to cavitation (P_{50}) was positively correlated with the water potential at which stomata closed (P_{close} ; $P = 0.002$, $r = 0.72$; Figure 4); more vulnerable species closed their stomata at less negative water potentials and more resistant species closed their stomata at more negative water potentials. The hydraulic safety margin ($P_{\text{min}} - P_{50}$) was positively correlated with the degree of isohdry-anisohdry ($P_{\text{close}} - P_{50}$; $P = 0.04$, $r = 0.52$; Figure 5); more isohdryic species had a greater hydraulic safety margin.

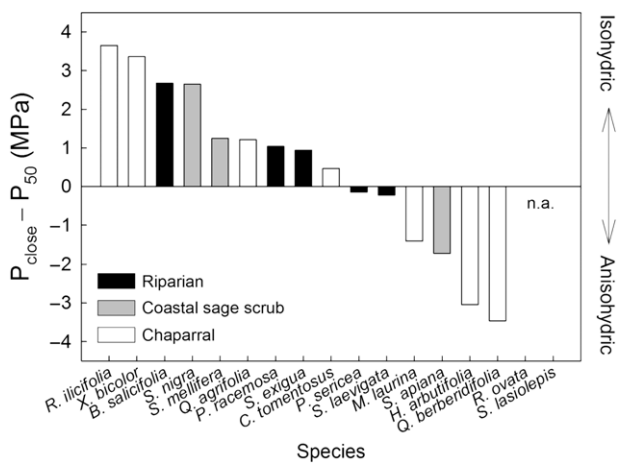


FIGURE 3 Degree of isohdry versus anisohdry measured as the difference between water potential at stomatal closure and stem xylem vulnerability to cavitation ($P_{\text{close}} - P_{50}$; $n = 15$) for woody species at the Santa Margarita Ecological Reserve. Black bars represent riparian species, grey bars represent coastal sage scrub species, and white bars represent chaparral species. There are no data for *Rhus ovata*; whereas a model was successfully fit to the stomatal response curve, the calculated P_{close} was determined to be unrealistic and so was excluded from further analyses. In addition, there is no data for *Salix lasiolepis* as a model could not be successfully fitted to the vulnerability curve to determine P_{50}

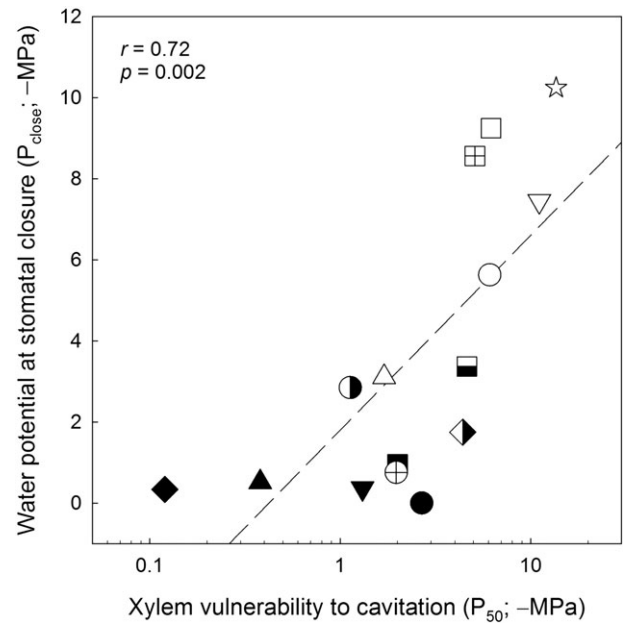


FIGURE 4 Stem xylem vulnerability to cavitation (P_{50}) versus the water potential at stomatal closure (P_{close}) and for woody species at the Santa Monica Ecological Reserve. The dashed lines represent the linear regression. Each symbol represents a different species mean; corresponding species and symbols can be found in Table 1

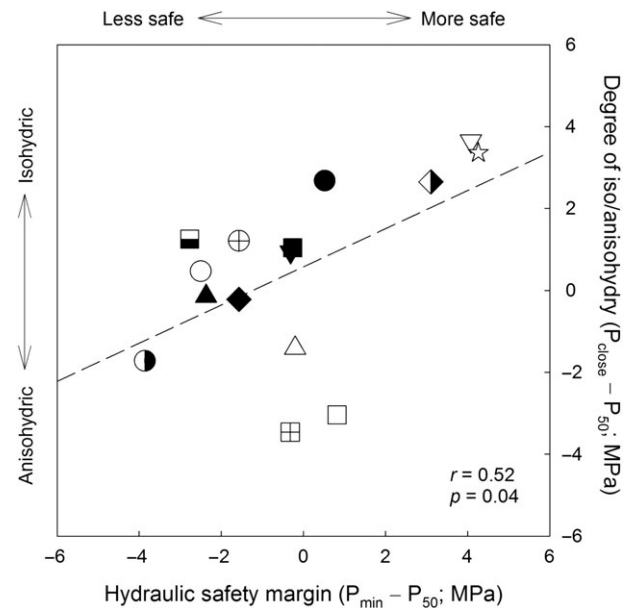


FIGURE 5 The hydraulic safety margin ($P_{\text{min}} - P_{50}$) versus the degree of isohdry-anisohdry ($P_{\text{close}} - P_{50}$) for woody species at the Santa Margarita Ecological Reserve. Each symbol represents a different species mean; corresponding species and symbols can be found in Table 1

3.5 | Cuticular conductance and leaf mass per area

Cuticular conductance (g_{min}) was not correlated with any other measured trait (Table 1). Leaf mass per area (Table 1) was negatively correlated with water potential at stomatal closure (P_{close} ; $P = 0.004$, $r = -0.68$; Figure S2); leaf mass per area increased with decreasing water potential at stomatal closure.

4 | DISCUSSION

The goal of this study was to characterize the coordination of stomatal and stem xylem traits in the biodiverse MTE region of California using *in situ* field-based measurements. By merging stomatal regulation with xylem hydraulic strategies across a range of species types and life history strategies, we paint a complete drought response framework picture. In doing so, we had three hypotheses. The first was that the stomatal strategies of species would be grouped by vegetation type, with riparian species being strongly isohydric as they would maintain high water potentials across seasons, coastal sage scrub being intermediate as the deciduous nature of these species would allow them to shut down during the summer drought to avoid a detrimental water status and hydraulic failure, and chaparral species being strongly anisohydric as they continue to function despite seasonal conditions. Surprisingly, this was not the case. At all points along the isohydric–anisohydric continuum investigated in this study, there was a mix of riparian, coastal sage scrub, and chaparral species. Second, we tested for coordination among measured leaf and stem traits; we hypothesized that stomatal and xylem traits were coordinated in this diverse plant community, and that the degree of isohydric–anisohydric behaviour would be associated with the hydraulic safety margin of a species. Indeed, $P_{\text{close}} - P_{50}$ was correlated with the hydraulic safety margin ($P_{\text{min}} - P_{50}$); more isohydric species had a larger hydraulic safety margin. In addition, minimum seasonal water potentials (P_{min}) were positively correlated with stem xylem vulnerability to cavitation (P_{50}) and the water potential at stomatal closure (P_{close}); P_{min} decreased as P_{50} decreased and P_{close} decreased. We also report for the first time P_{close} values below -10 MPa, which contradicts a recent review on stomatal closure that posits all species close their stomata by -4 MPa (Martin-StPaul, Delzon, & Cochard, 2017). Also, the water potential at stomatal closure and stem xylem vulnerability to cavitation were positively correlated with each other. Overall, these results point to coordination among individual stomatal, hydraulic, and functional traits. Finally, we evaluated the coordination of additional standard leaf functional traits, including leaf mass per area and cuticular conductance, with isohydric and anisohydric strategies and found that leaf mass per area was negatively correlated with minimum seasonal water potential and the water potential at stomatal closure, but cuticular conductance was not correlated with any other measured traits.

4.1 | Coordination of hydraulic safety margin with degree of isohydry–anisohydry

Confirmation of the coordination of the hydraulic safety margin and degree of isohydry versus anisohydry supports a new framework for predicting plant responses to drought (Skelton et al., 2015). The hydraulic safety margin is closely associated with drought mortality risk on a global scale (Anderegg et al., 2016; Choat et al., 2012; Nardini, Battistuzzo, & Savi, 2013), so incorporating stomatal strategies with drought mortality via isohydry–anisohydry adds another avenue for determining drought responses. This understanding is crucial especially during severe drought conditions as it is not a question of *if* stomata close, but a matter of *when*. Therefore, if species that operate within a wider hydraulic safety margin also tend to be more

isohydric, this stomatal strategy would be more successful under extreme drought conditions. Another interpretation of this made by Martin-StPaul et al., (2017) is “... greater safety margin between stomatal closure and embolism formation would have allowed plants to adapt to extreme drought conditions and to colonize xeric environments.” In addition, the coordination of hydraulic safety margins and degree of isohydry versus anisohydry merges the roles that leaves and stems play in drought response strategies. Many studies only focus on one organ or the other, but leaves and stems represent a coordinated pathway along the soil–plant–atmosphere continuum. Studying the coordination of hydraulic supply (stem xylem) with evaporative demand (leaf stomata) deserves further attention to make progress in understanding plant responses to a changing climate.

4.2 | The role of minimum seasonal water potentials

Minimum seasonal water potentials are an integrated measure of variables affecting plant water status. For example, they are related to (a) access to water, whether via rooting depth or establishment in a moist microsite; (b) regulation of water use as related to hydraulic conductivity, xylem resistance to cavitation, stomatal conductance, and even cuticular conductance; (c) storage of water through sapwood capacitance and leaf water storage tissue; and (d) water demand, as related to photosynthesis, hydraulic architecture, and phenology. In addition, environmental conditions, such as soil water availability and atmospheric conditions, affect water potentials as well. For example, when water was available and atmospheric demand was low, such as during cool and wet winter climatic conditions, midday water potentials converged among study species (Figure 1). However, when temperatures warmed and water became limiting, the diversity of drought response mechanisms associated with these traits led to a wide range of minimum seasonal water potentials (Pivovarov, Pasquini, et al., 2016). For example, deeply rooted, drought deciduous, and riparian species had high (less negative) minimum seasonal water potentials and less resistant xylem. By establishing in a moist microsite, guaranteeing access to water through deep roots, or shutting down during unfavourable conditions, these species avoided drought and maintained high water status regardless of seasonal precipitation patterns. However, if the water table dropped, the river dried up, or drought conditions became extended, these species would not have the cavitation resistance to survive. The species that operated over a broad range of water potentials and had lower (more negative) minimum seasonal water potentials were all known to be shallow rooted (Table 1). The species that experienced more negative minimum seasonal water potentials (P_{min}) also had lower water potentials at which stomata closed (P_{close}) and greater xylem resistance to cavitation (more negative P_{50} ; Figure 2). The coordination of minimum seasonal water potentials with both xylem traits (P_{50}) and stomatal traits (P_{close}) points to the significance of P_{min} . However, the role that access to water and rooting depth have in maintaining high (less negative) water potentials leads to questions of whether P_{min} or $P_{\text{max}} - P_{\text{min}}$ are the best proxies for stomatal control strategies such as isohydry (Martínez-Vilalta & García-Fórner, 2017). For example, a plant can have less stringent stomatal regulation, but as long as it has access to water via deep roots, moist microsite establishment, or during favourable conditions, its

water potential will stay high. Indeed, this is the case with many cavitation vulnerable species that are also deeply rooted (Venturas et al., 2016). Disentangling stomatal regulation from rooting depth or access to water may be a technical challenge in field studies, but will contribute to our understanding of the coordination and regulation of hydraulic supply and evaporative demand. For example, in the MTE of South Africa, West et al. (2012) found that anisohydric but shallow rooted shrubs were negatively affected by an imposed experimental drought, whereas both deep and shallow rooted isohydric species were unaffected.

4.3 | Xylem vulnerability and stomatal control

The positive relationship between stem xylem vulnerability to cavitation (P_{50}) and the water potential at stomatal closure (P_{close}) among a diverse sampling of species demonstrates that carbon uptake through stomata is a trade-off of hydraulic risk (Sperry et al., 2016, 2017; Wolf, Anderegg, & Pacala, 2016; Figure 4). Although stomatal closure has been found to be correlated with other physiological traits, including the decline in leaf hydraulic conductance and loss of turgor (Brodribb & Holbrook, 2003), in many previous studies no correlation between P_{50} and P_{close} has been found (Brodribb & Holbrook, 2003; Brodribb, Holbrook, Edwards, & Gutierrez, 2003). This is surprising because xylem and stomatal function are tightly coordinated (Brodribb, McAdam, & Carins Murphy, 2017). However, this relationship may be dependent on vegetation type. In our study, species more resistant to xylem cavitation closed their stomata at more negative water potentials. Species with more resistant xylem can safely operate at more negative water potentials and so do not need to close their stomata with the immediate onset of drought. Theoretically, they can continue to photosynthesize and gain carbon for longer time periods or during less favourable conditions than less drought resistant species, though this is debated (Martinez-Vilalta & Garcia-Forner, 2017). However, a recent study also conducted in the California chaparral during the exceptional 2014 drought found that chaparral species with high cavitation resistance experienced high mortality rates, whereas deeply rooted species had high survival rates (Venturas et al., 2016). Obviously, although coordination between the P_{50} and P_{close} may vary depending on the vegetation-type, disentangling rooting depth and stomatal behaviour is of value (Feng, Dawson, Ackerly, Santiago, & Thompson, 2017).

4.4 | Cuticular conductance: A last resort?

Standard leaf functional traits, such as leaf mass per area and cuticular conductance, are also critical components for understanding plant drought survival. For example, in a study of 10 Mediterranean species exposed to simulated drought, functional traits associated with conservative behaviour, including low transpiring area, deep roots, and low water use, resulted in high drought survival rates, whereas fast-growing, high resource use species were vulnerable to drought (Lopez-Iglesias, Villar, & Poorter, 2014). Likewise, in a study of species' ecology, leaf traits, and water relations at a desert-chaparral ecotone under drought, in addition to rooting depth and xylem cavitation resistance, high leaf mass per area was associated with high mortality

(Paddock et al., 2013). Cuticular conductance (g_{min}) is a measure of how much water is lost from a leaf once the stomata are closed (Kerstiens, 1996). This water can be lost through the cuticle or even leaky stomata. Cuticular conductance acts as the final barrier to water loss for a plant. In our study, cuticular conductance was not correlated with any other measured trait. This means that in extreme drought, when all transpiration through open stomata has ceased, the water lost through the cuticle is orthogonal to the axis of strategy variation described by the isohydric–anisohydric spectrum.

5 | CONCLUSION

Studying plant responses to drought allows us to assess plant mortality risk (Pivovarovoff, Pasquini, et al., 2016), which appears to be independent of mean annual precipitation (Choat et al., 2012). The movement of water through plants via the soil–plant–atmosphere continuum involves both xylem hydraulic and stomatal components, so understanding the coordinated function of leaves and stems in woody plant drought survival strategies is imperative (Pivovarovoff et al., 2014). Furthermore, measuring traits in the field under exceptional drought conditions at the community level in a biodiverse region significantly contributes to our understanding of plant responses to drought.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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