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LETTER

Mapping 'hydroscapes' along the iso- to anisohydric continuum of stomatal regulation of plant water status

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Abstract

The concept of iso- vs. anisohydry has been used to describe the stringency of stomatal regulation of plant water potential (ψ). However, metrics that accurately and consistently quantify species' operating ranges along a continuum of iso- to anisohydry have been elusive. Additionally, most approaches to quantifying iso/anisohydry require labour-intensive measurements during prolonged drought. We evaluated new and previously developed metrics of stringency of stomatal regulation of ψ during soil drying in eight woody species and determined whether easily-determined leaf pressure–volume traits could serve as proxies for their degree of iso- vs. anisohydry. Two metrics of stringency of stomatal control of ψ, (1) a 'hydroscape' incorporating the landscape of ψ over which stomata control ψ, and (2) the slope of the daily range of ψ as pre-dawn ψ declined, were strongly correlated with each other and with the leaf osmotic potential at full and zero turgor derived from pressure–volume curves.

Keywords

Anisohydry, drought resistance, isohydry, leaf osmotic potential, leaf turgor, plant traits, plant water potential, stomata.

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INTRODUCTION

The uptake, transport and loss of water by plants are governed by suites of coordinated structural and functional traits. Greater expression of certain traits related to maintenance of water transport and hydration under dry conditions typically involves trade-offs against other traits. This makes prediction of species' performance and survival under different drought scenarios potentially complex (McDowell *et al*. 2011; Mitchell *et al*. 2013; Anderegg *et al*. 2015; Mencuccini *et al*. 2015; Skelton *et al*. 2015). For instance, shallow-rooted species with strong expression of drought avoidance traits typically show more stringent stomatal control of transpiration than similarly rooted drought tolerators. Drought avoiders with relatively shallow roots thus may experience strong constraints on carbon assimilation when soil water availability is low, whereas drought tolerators sustain photosynthetic gas exchange through enhanced investment of resources in structural reinforcement of xylem to resist embolism, and in osmoregulation to maintain leaf turgor. Although liquid phase transport properties determine the root-to-leaf gradient in xylem tension at a given rate of flow, failure of stomata to coordinate vapour phase water loss with decreases in soil-to-leaf hydraulic conductance would result in rapid desiccation of plant tissues and catastrophic hydraulic dysfunction. The ability of stomata to rapidly balance transpiration with dynamic variation in plant hydraulic properties is well documented (e.g. Meinzer & Grantz 1990; Cochard *et al*. 2000; Hubbard *et al*. 2001) as is the general coordination of stomatal behaviour and photosynthetic gas exchange with steady state plant hydraulic properties (Santiago *et al*. 2004; Brodribb *et al*. 2005). However, this behaviour appears to be mostly mediated by variation in leaf and stomatal guard cell turgor rather than directly by hydraulics (Cowan 1977; Brodribb & Holbrook 2003; Brodribb *et al*. 2003).

Species-specific differences in the stringency with which stomata limit transpiration in drying soil and the resulting degree of homoeostasis of maximum plant water deficits have been described in terms of a continuum of iso- to anisohydric behaviour (Tardieu & Simonneau 1998; Domec & Johnson 2012; Klein 2014; Martínez-Vilalta *et al*. 2014). Broadly, isohydric species tend to maintain a relatively constant midday minimum leaf water potential (ψ_{min}) as soil and pre-dawn leaf water potential (ψ_{pd}) decline, whereas stomatal behaviour in anisohydric species allows ψ_{min} to decline nearly in parallel with ψ_{pd}. Although iso- and anisohydric species co-occur in drought-prone ecosystems, it appears that anisohydric species may experience lower mortality during extreme droughts (McDowell *et al*. 2008). The possibility that species' survival during extreme drought is related to the stringency of their stomatal control has prompted intense interest in developing means of quantifying species-specific operating ranges along the iso- to anisohydric continuum (Klein 2014; Martínez-Vilalta *et al*. 2014; Garcia-Forner *et al*. 2016).

Although considerable progress has been made in characterising the relative degree of iso- vs. anisohydric regulation of

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plant water status, most approaches require intensive monitoring of plant water potential and stomatal behaviour during soil drying (Ewers *et al.* 2007; West *et al.* 2007; Klein 2014; Skelton *et al.* 2015; Garcia-Forner *et al.* 2016). Further progress in defining species' operating ranges along this continuum would be facilitated by identifying suitable proxies that can be derived from relatively rapid, short-term measurements rather than repeated, intensive monitoring. Such proxies may prove useful in modelling changes in vegetation composition in response to anticipated future increases in the frequency of extreme droughts.

Stomatal behaviour is determined, in large part, by leaf turgor, making it reasonable to postulate that leaf pressure–volume (P-V) traits, such as the water potential at the turgor loss point (ψ_{TLP}) and the osmotic potential at full turgor ($\psi_{\pi 100}$), could serve as proxies for both drought tolerance and the degree of iso- vs. anisohydry exhibited by a species. In a meta-analysis conducted on 62 Australian species, Lenz *et al.* (2006) found that ψ_{TLP} was more negative in species from lower rainfall sites and that diurnal fluctuations in ψ were larger in species with more negative ψ_{TLP}. In a more recent meta-analysis across different vegetation types, Bartlett *et al.* (2012a) found that both ψ_{TLP} and $\psi_{\pi\ 100}$ became more negative with declining annual soil moisture availability. This suggests that both these P-V traits could serve as useful proxies of drought tolerance. However, the extent to which the patterns identified by Lenz *et al.* (2006) and Bartlett *et al.* (2012a) are related to species-specific differences in the degree if iso- vs. anisohydry as defined above is unknown.

To address this, we characterised leaf P-V relationships in eight evergreen and deciduous woody angiosperm species selected to represent different operating ranges along the iso- to anisohydric continuum based on literature describing their ecophysiological behaviour, traits and geographical distribution. To standardise environmental conditions, the plants were grown in containers in a greenhouse where we characterised their trajectories of daily maximum and minimum ψ after withholding irrigation. We then used these data to develop, assess and compare different metrics of iso- vs. anisohydry and evaluate the degree to which these metrics were correlated with key leaf P-V characteristics such as ψ_{TLP} and $\psi_{\pi\ 100}$. We hypothesised that leaf P-V traits would serve as robust proxies for different metrics of iso- vs. anisohydric behaviour because of coordination of stomatal behaviour with leaf water status, particularly turgor as determined by $\psi_{\pi\ 100}$ and ψ_{TLP}.

MATERIALS AND METHODS

Plant material and growing conditions

Eight woody species were selected for comparison (Table S1) based on literature describing their ecophysiology and their availability from a local nursery (Sevenoaks Native Nursery, Albany, OR). The species: *Betula occidentalis* (BEOC), *Ceanothus cuneatus* (CECU), *Cercocarpus ledifolius* (CELE), *Heteromeles arbutifolia* (HEAR), *Quercus douglasii* (QUDO), *Quercus garryana* (QUGA), *Rhamnus ilicifolia* (RHIL), *Salix scouleriana* (SASC) are representative of a range of vegetation types that include riparian, forest, oak woodland, chaparral and sagebrush scrub. In March 2015, ten 1–2-yr-old container-grown plants of each species were transported to an air-conditioned greenhouse on the Oregon State University campus in Corvallis, Oregon and transplanted to 9.8-L pots containing a 1:1 mixture of sandy loam soil and commercial growth medium (Metro-mix 840, Sun Gro Horticulture, Agawam, MA). All pots were irrigated daily until late August when irrigation was completely stopped or gradually reduced for five individuals of each species. It was necessary to gradually reduce irrigation for *Betula occidentalis* and *Salix scouleriana* to obtain sufficient data for their trajectories of ψ_{pd} and ψ_{min} during soil drying (see below). Time courses of air temperature, relative humidity and photosynthetically active radiation inside the greenhouse during the experiment are shown in Fig. S1. Plant size characteristics at the time irrigation regimes were changed are summarised in Table S2.

Plant water potential and pressure–volume curves

Pre-dawn and afternoon minimum leaf water potential (ψ_{pd} and ψ_{min} respectively) were measured with a pressure chamber (Model 1505D-EXP, PMS Instrument Company, Albany, OR) on control and treatment plants on one occasion prior to imposition of the drought treatment (25 August 2015) and on four to six occasions in four to five treatment and control plants after the drought treatment was imposed. The number of occasions depended on the time required for a given treatment plant to reach the point at which $\psi_{min} = \psi_{pd}$, indicative of the limits of stomatal control of plant water status. The duration of the drought treatment ranged from 38 days in *B. occidentalis* and *S. scouleriana*, which had the largest amounts of leaf area, to 59 days in *C. ledifolius* and *R. ilicifolia*, which had the smallest leaf areas. The remaining species were subjected to 42 days of drought. For measurements of ψ_{pd}, one leaf or small leafy shoot per individual was wrapped in aluminium foil and enclosed in a sealable plastic bag in the late afternoon. The following morning, the leaves or shoots were excised with a razor blade and the bags sealed and placed in a cooler for transport to the laboratory where measurements began within 15 min. This procedure minimised, but did not exclude the potential impact of nocturnal transpiration on ψ_{pd} because the rest of the plant was not enclosed to prevent transpiration. Samples for determination of ψ_{min} were excised between 1300 and 1400 h local time. Once ψ_{min} and ψ_{pd} of treated plants became equal or if ψ became more negative than about −9 MPa, that individual's ψ was no longer measured.

For pressure–volume curve (Tyree & Hammel 1972) determination, leaves or small leafy shoots were excised from well-irrigated plants at dawn or shortly thereafter, immediately sealed in plastic bags containing moist paper towelling, and transported to the laboratory where P-V curves were initiated within 30 min of sample excision with samples at their native state of hydration. Curves and their parameters were determined according to the procedures described in Meinzer *et al.* (2014). Sample saturated weights were determined from the *y*-intercept of a linear regression fitted to the initial portion of a plot of sample weight vs. balance pressure (Meinzer *et al.* 2014). Values of ψ_{TLP} and $\psi_{\pi\ 100}$ reported here are means

from P-V curves determined on four to seven individuals. Representative P-V curves for each species are shown in Fig. S2.

Analyses

Different measures of the stringency of stomatal control of plant water status during soil drying were obtained by applying four approaches to the analysis of trajectories of ψ_{pd} vs. ψ_{min}. In the first approach, the slope of a linear regression fitted to the entire trajectory of ψ_{min} vs. ψ_{pd} during soil drying was determined ('Slope (all)'; dotted lines Fig. 1a and b) following the procedure of Martínez-Vilalta *et al.* (2014). According to this procedure, slopes would be zero and one, respectively, for perfectly isohydric and anisohydric species, indicative of constant vs. steadily declining ψ_{min}. The second procedure aimed to isolate the data where stomata have most control over ψ_{min} ('Slope (to $\psi_{min} = \psi_{pd}$)'; Fig. 1c and d). This involved, first, removing data more negative than the point where $\psi_{min} = \psi_{pd}$ (i.e. beyond where stomatal closure

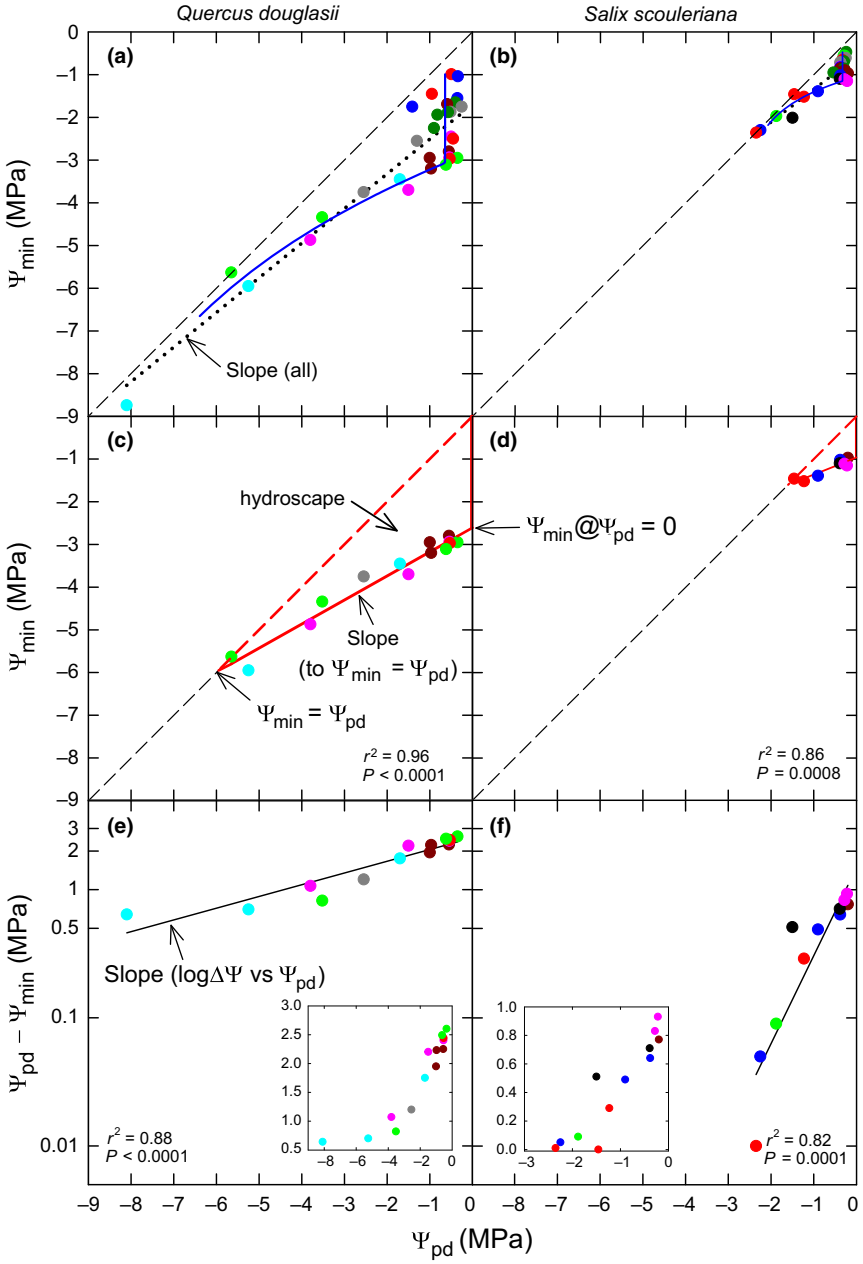

Figure 1 Trajectories of plant water potential during soil drying for *Quercus douglasii* and *Salix scouleriana*: (a-d) Relationships between pre-dawn water potential (ψ_{pd}) and afternoon minimum water potential (ψ_{min}) and (e-f) ψ_{pd} vs. the difference between ψ_{pd} and ψ_{min}. Dashed lines in (a-d) represent $\psi_{min} = \psi_{pd}$. Note log *y*-axis scale in (e) and (f) and linear *y*-axis scale on insets. Curved blue lines in (a) and (b) represent approximate fits to the trajectories of ψ_{min} vs. ψ_{pd}. See text and Table 1 for definitions of variables identified by arrows and criteria applied in fitting dotted and solid regression lines in (a–f) and in determining hydroscape areas bounded by red lines in (c–d). Symbol colours represent different individual plants.

ameliorates ψ_{min} with further declines in ψ_{pd}). Then, starting where $\psi_{min} = \psi_{pd}$, points with less negative ψ_{pd} were added until the r^2 for a linear fit reached its maximum value. This second step excluded very mild values where ψ_{min} likely varied with irradiance only, independently of ψ_{pd} (vertical portions of trajectories Fig. 1a and b). The *y*-intercept of this regression (ψ_{min}@ $\psi_{pd} = 0$) is the predicted ψ_{min} in fully saturated soil and its intercept with the 1:1 line ($\psi_{min} = \psi_{pd}$) is the threshold at which stomatal closure is no longer effective in limiting ψ_{min} as ψ_{pd} becomes more negative. The third approach consisted of determining the area of a triangle bounded by the regression line fitted using the second procedure described above, the *y*-axis, and the 1:1 line in a plot of ψ_{min} vs. ψ_{pd} (red lines; Fig. 1c and d). We have named this area a 'hydroscape' because it incorporates the ranges of ψ_{pd} and ψ_{min}, or water potential landscape, over which stomata are effective in controlling leaf ψ as the soil dries. Thus, species with larger hydroscapes are expected to be more anisohydric. Because this two-dimensional metric also defines an area over which a plant is able to sustain net CO_2 assimilation, it may provide more information about overall drought tolerance and mechanisms of drought-induced mortality than metrics involving the rate of change in ψ_{min} as ψ_{pd} declines. The hydroscape is analogous to the water use envelope described by Sperry *et al*. (2002) in which the ability of a plant to extract soil water is constrained by the soil water potential and stomatal responses to changes in soil-to-leaf hydraulic conductance as the soil dries. The fourth approach involved determining the slope of a linear regression fitted to a plot of $\log(\psi_{pd} - \psi_{min})$ vs. ψ_{pd} ('Slope ($\log\Delta\psi$ vs. ψ_{pd})'; Fig. 1e and f). This is similar to the approach described by Garcia-Forner *et al*. (2016), except that we have linearised the often nonlinear relationship between $\psi_{pd} - \psi_{min}$ and ψ_{pd} (insets Fig. 1e and f) to facilitate quantitative comparisons of species-specific slopes. These metrics of the degree of iso- vs. anisohydry and their abbreviations are summarised in Table 1. A Pearson correlation matrix was developed to evaluate relationships among P-V curve traits, the above metrics of iso- vs. anisohydric behaviour, and other features of the trajectories of ψ_{min} vs. ψ_{pd} during soil drying. Ordinary least squares linear regressions were fitted to plots of variables showing significant relationships. Linear regressions of ψ_{pd} against ψ_{min} or $\psi_{pd} - \psi_{min}$ (Figs 1, S3 and S4 only) mix within and between individual sources of variation in the analysis due to logistical constraints associated with obtaining larger data sets. However, the lack of idiosyncratic patterns between individuals suggested the interpretations from these regressions were biologically meaningful and good estimates of species characteristics.

Table 1 Abbreviations and descriptions of variables used to characterise leaf water relations components and trajectories of leaf water potential during soil drying

Variable	Description
$\psi_{\pi\ 100}$	Bulk leaf osmotic potential at full turgor (MPa)
ψ_{TLP}	Bulk leaf water potential at the turgor loss point (MPa)
RWD@TLP	Bulk leaf relative water deficit at the turgor loss point
ψ_{min}@$\psi_{pd} = 0$	Estimate of daily minimum leaf water potential when pre-dawn water potential is zero, i.e. *y*-intercepts of regressions in Fig. 1c and d (MPa)
$\psi_{min} = \psi_{pd}$	Estimate of the water potential at which pre-dawn and daily minimum leaf water potential become equal during soil drying (MPa)
Slope (all)	Slope of the linear regression fitted to the entire trajectory of ψ_{min} vs. ψ_{pd} during soil drying (MPa MPa^{-1})
Slope (to $\psi_{min} = \psi_{pd}$)	Slope of regression line fitted to relationship between ψ_{min} and ψ_{pd} from the point at which $\psi_{min} = \psi_{pd}$ to the point at which the r^2 was maximised by adding less negative values of ψ_{pd} as in Fig. 1c and d (MPa MPa^{-1})
Slope ($\log\Delta\psi$ vs. ψ_{pd})	Slope of linear regression fitted to plot of $\log(\psi_{pd} - \psi_{min})$ vs. ψ_{pd} as in Fig. 1e and f ($\log$(MPa) MPa^{-1})
Hydroscape area	Area of triangle bounded by the regression line, *y*-axis and 1:1 line in a plot of ψ_{min} vs. ψ_{pd} as in Fig. 1c and d (MPa^2)

See text and Fig. 1 for additional information and details concerning derivation of variables.

RESULTS

Leaf water potential trajectories

The data for *Quercus douglasii* and *Salix scouleriana* shown in Fig. 1 are representative of the extremes among the study species in terms of stringency of stomatal control of plant water status in drying soil, with *S. scouleriana* being highly isohydric and *Q. douglasii* being highly anisohydric. Data for the remaining six species are shown in Figs. S3 and S4. Trajectories of ψ_{min} vs. ψ_{pd} during soil drying generally exhibited three phases (Fig. 1a and b; Figs. S3 and S4, top row). At values of $\psi_{pd} > \sim -0.6$ MPa, variation in ψ_{min} was much greater than that of ψ_{pd} resulting in a nearly vertical trajectory of ψ_{pd} vs. ψ_{min}. In the second phase, a threshold was reached beyond which ψ_{min} abruptly began to decline more gradually with declining ψ_{pd}. This phase continued to the point at which $\psi_{min} = \psi_{pd}$, indicative of complete stomatal closure and the loss of stomatal control of ψ_{min}. The third phase consisted of an essentially 1:1 relationship between ψ_{min} and ψ_{pd} as the soil continued to dry. The nearly vertical trajectories in phase 1 were likely associated with the impact of variations in irradiance rather than ψ_{pd} on stomatal conductance and therefore ψ_{min} among the days on which ψ was measured.

Metrics of iso- vs. anisohydry

Linear regressions fitted to relationships between ψ_{min} and ψ_{pd} over a constrained range of ψ_{min} and ψ_{pd} as described above (Slope (to $\psi_{min} = \psi_{pd}$); Table 1) (Fig. 1c and d; Figs. S3 and S4, middle row) provided estimates of two relevant metrics of stringency of stomatal control of plant water status in drying soil; ψ_{min} at $\psi_{pd} = 0$ (i.e. the *y*-intercepts in Fig. 1c and d) and the point of complete stomatal closure (i.e. $\psi_{min} = \psi_{pd}$). The slopes of these regressions were significantly different from zero and one ($P < 0.05$) for all species and ranged from 0.4 MPa MPa^{-1} in *B. occidentalis* and *S. scouleriana* to 0.8 MPa MPa^{-1} in *Ceanothus cuneatus*, indicating that according to this criterion, none of them

Table 2 Pearson correlation matrix for leaf water relations components and characteristics of leaf water potential trajectories during soil drying

	ψ_{TLP} (MPa)	$\psi_{\pi\ 100}$ (MPa)	RWD @TLP	$\psi_{min}@\psi_{pd} = 0$ (MPa)	$\psi_{min} = \psi_{pd}$ (MPa)	Slope (all) (MPa MPa^{-1})	Slope (to $\psi_{min} = \psi_{pd}$) (MPa MPa^{-1})	Hydroscape area (MPa^2)
$\psi_{\pi\ 100}$	**1.00*****							
RWD@TLP	−0.50	−0.54						
$\psi_{min}@\psi_{pd} = 0$	**0.87****	**0.86****	0.42					
$\psi_{min} = \psi_{pd}$	**0.76***	**0.76***	−0.51	0.50				
Slope (all)	−0.21	−0.23	0.32	−0.10	−0.59			
Slope (to $\psi_{min} = \psi_{pd}$)	−0.41	−0.41	0.31	−0.10	**−0.89****	0.70		
Hydroscape area	**−0.96*****	**−0.96*****	0.58	**−0.92****	**−0.79***	0.28	0.44	
Slope ($\log\Delta\psi$ vs. ψ_{pd})	**0.89****	**0.88****	−0.35	**0.74***	**0.92****	−0.48	−0.70	**−0.91****

Significant correlations are in bold type. Significance levels: *$P \leq 0.035$; **$P \leq 0.006$; ***$P < 0.0002$. Abbreviations are defined in Table 1.

exhibited near perfect isohydric or anisohydric behaviour under the conditions imposed. The slopes obtained with this method were less steep than, and not significantly correlated with, slopes of regressions fitted to the entire range of ψ observed for each species (dotted lines Fig. 1a and b; Figs. S3 and S4; Table 2).

Hydroscape areas (Table 1; bounded by red lines, Fig. 1c and d) ranged from about 0.7 MPa^2 in *S. scouleriana* to 8.4 MPa^2 in *Q. garryana*. The relative range of hydroscape areas among species was about six to eight times greater than that of the slopes of the two types of linear regressions fitted to the relationships between ψ_{min} and ψ_{pd} (data not shown). Hydroscape areas were not significantly correlated with slopes of either type of linear regression (Table 2). Slopes of regressions fitted to plots of the daily range of ψ (i.e., $\psi_{pd} - \psi_{min}$) vs. ψ_{pd}, another measure of the stringency of stomatal control of plant water status in drying soil (Table 1), were also highly significant for all species (Fig. 1e and f; Figs. S3 and S4), showing a 7.5-fold relative range, somewhat less than that of hydroscape areas. Species-specific values of Slope ($\log\Delta\psi$ vs. ψ_{pd}) were strongly correlated ($P = 0.002$) with their hydroscape areas (Fig. 2).

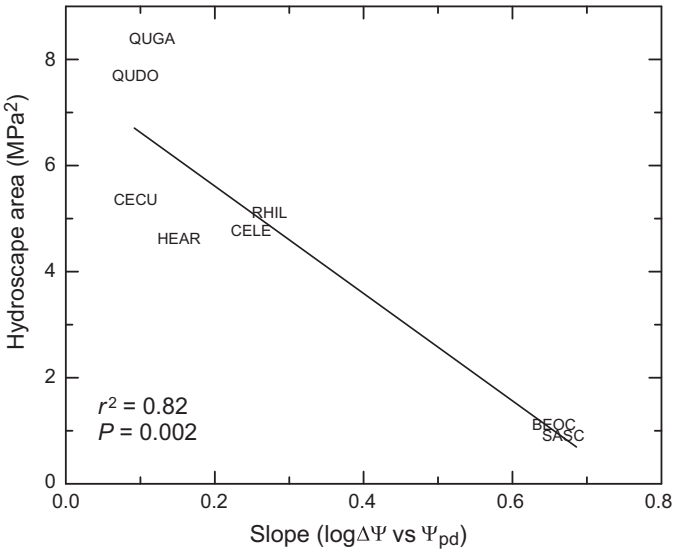

Figure 2 Relationship between hydroscape area on a plot of pre-dawn plant water potential (ψ_{pd}) vs. daily minimum water potential (ψ_{min}) and the log-transformed slope of the relationship between ψ_{pd} and $\psi_{pd} - \psi_{min}$. See Table 1, Fig. 1 and text for details. Species codes are BEOC: *Betula occidentalis*, CECU: *Ceanothus cuneatus*, CELE: *Cercocarpus ledifolius*, HEAR: *Heteromeles arbutifolia*, QUDO: *Quercus douglasii*, QUGA: *Quercus garryana*, RHIL: *Rhamnus ilicifolia*, SASC: *Salix scouleriana*.

Leaf pressure–volume traits as proxies for the degree of iso- vs. anisohydry

Neither ψ_{TLP} nor $\psi_{\pi\ 100}$ was significantly correlated with the slopes of the two types of regressions fitted to the trajectories of ψ_{min} vs. ψ_{pd} (Slope (all), Slope (to $\psi_{min} = \psi_{pd}$); Table 2). However, both ψ_{TLP} and $\psi_{\pi\ 100}$ were strongly correlated with $\psi_{min}@\psi_{pd} = 0$, hydroscape area, and Slope ($\log\Delta\psi$ vs. ψ_{pd}) (Fig. 3; Table 2). Over a range of species-specific ψ_{TLP} from −1.60 to −3.44 MPa, $\psi_{min}@\psi_{pd} = 0$ (see Table 1 for definition) declined linearly from about −1.0 to −2.9 MPa (Fig. 3a). The slope of the regression line (0.96) did not differ significantly from 1.0, resulting in a relatively consistent offset of about 0.76 ± 0.12 MPa between $\psi_{min}@\psi_{pd} = 0$ and ψ_{TLP} across the eight species. Hydroscape areas increased linearly with declining species-specific ψ_{TLP} ($P = 0.0001$) at a rate of about 4 MPa^2 per MPa decline in ψ_{TLP} (Fig. 3b). The stringency of stomatal control of diurnal variation in ψ in drying soil (Slope ($\log\Delta\psi$ vs. ψ_{pd}); see Table 1 for definition) increased with increasing (less negative) species-specific ψ_{TLP} ($P = 0.003$; Fig. 3c). Relationships between $\psi_{\pi\ 100}$ and the three metrics of stomatal control of plant water status described above were nearly as strong as those observed for ψ_{TLP} (Fig. 3b, d and f; Table 2). Interestingly, the 95% confidence interval of the regression fitted to the relationship between $\psi_{min}@\ \psi_{pd} = 0$ and $\psi_{\pi\ 100}$ included the 1:1 line (Fig. 3b), suggesting that these two variables were roughly equal within species. The bulk leaf relative water deficit at the turgor loss point (RWD@TLP) was not significantly correlated with any of the metrics of stomatal control of leaf water status (Table 2). Comparison of Figure 3a and b suggested that differences between ψ_{TLP} and $\psi_{\pi\ 100}$ may have been similar among species. When species-specific averages of ψ_{TLP} were plotted against $\psi_{\pi\ 100}$, a highly significant linear relationship was obtained (Fig. 4). However, the slope of the relationship (1.21 MPa MPa^{-1}) was significantly different from one, resulting in an increase in $\psi_{\pi\ 100} - \psi_{TLP}$ from 0.43 MPa in *B. occidentalis* to 0.74 MPa in *Q. garryana*.

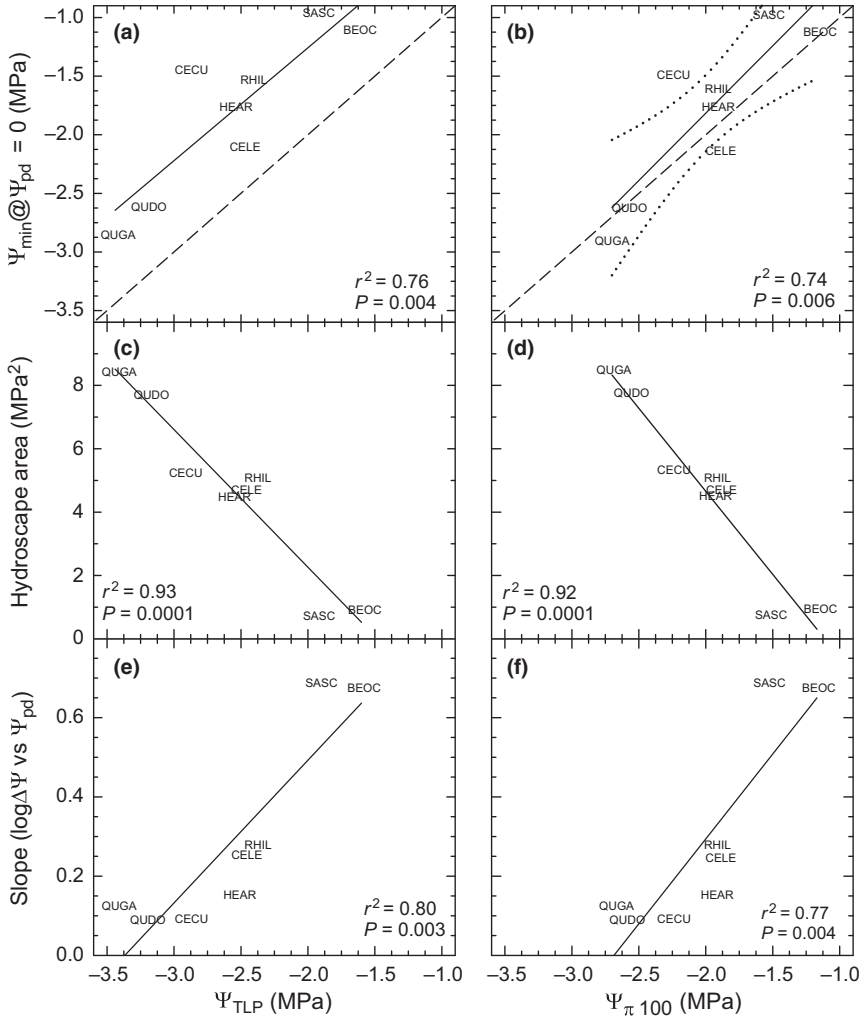

Figure 3 Bulk leaf water potential at the turgor loss point (ψ_{TLP}) and osmotic potential at full turgor ($\psi_{\pi\ 100}$) in relation to (a, b) daily minimum water potential (ψ_{min}) when pre-dawn water potential (ψ_{pd}) = 0, (c, d) hydroscape area, and (e, f) the log-transformed slope of the relationship between $\psi_{pd} - \psi_{min}$ and ψ_{pd}. Dotted lines in (b) represent the 95% confidence interval for the regression. See Table 1, Fig. 1 and text for details and Fig. 2 for species codes.

DISCUSSION

Identifying suitable proxies for quantifying modes of stomatal control of plant water status in drying soil has been somewhat elusive. Here, we assessed trajectories of ψ_{pd} and ψ_{min} during soil drying in eight diverse woody species selected to represent different degrees of iso- vs. anisohydric behaviour and native habitats with a range of moisture regimes. We found that two key leaf water relations traits, ψ_{TLP} and $\psi_{\pi\ 100}$ determined on well-irrigated individuals, served as robust proxies for two measures of the stringency of stomatal control of plant water status in drying soil: (1) the so-called hydroscape area (Tables 1 and 2; Fig. 1c and d) and (2) the log-transformed slope of the diurnal variation in ψ vs. ψ_{pd} (Tables 1 and 2; Fig. 1e and f). Both metrics define quantifiable positions along a continuum of iso- to anisohydry on which no species would exhibit perfect iso- or anisohydric behaviour. Additionally, both ψ_{TLP} and $\psi_{\pi\ 100}$ were strongly correlated with ψ_{min} in moist soil (ψ_{min}@ ψ_{pd} = 0) and the point at which $\psi_{min} = \psi_{pd}$, the limit of effective stomatal control of plant water status (Table 2). As such, the results of our survey of eight woody angiosperm species grown under similar conditions represent a proof of concept. Although the approach employed here has mechanistic underpinnings involving stomatal regulation in relation to leaf turgor and osmotic properties (see below), it awaits further testing with additional species, including conifers, under field conditions.

Comparison of metrics of stomatal control of plant ψ in drying soil

It is now apparent that perfect iso- vs. anisohydric behaviour as originally defined is rare if it exists at all (Martínez-Vilalta *et al.* 2014). Moreover, it appears that within species, individuals can switch between iso- and anisohydric behaviour depending on environmental conditions and developmental stage (Schultz 2003; Domec & Johnson 2012; Rogiers *et al.* 2012). Nevertheless, describing stomatal control of plant water status in terms of a continuum of behaviours seems useful if operating ranges along this continuum can be quantified. In principle, the slope of a plot of ψ_{min} vs. ψ_{pd} could yield a

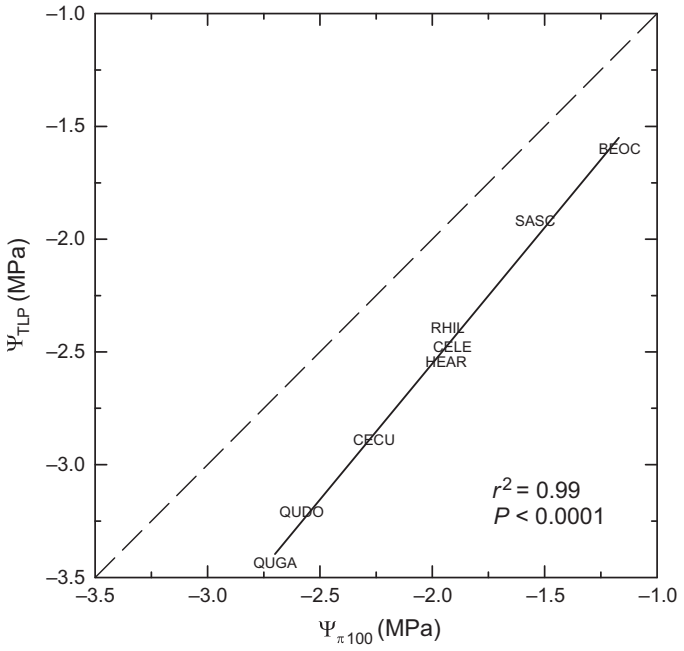

Figure 4 Relationship between the bulk leaf water potential at the turgor loss point (ψ_{TLP}) and bulk leaf osmotic potential at full turgor ($\psi_{\pi\ 100}$) for the eight study species. See Fig. 2 for species codes.

quantitative estimate of the stringency of stomatal control of ψ_{min} in drying soil. We used different criteria to estimate two types of slopes of linear regressions fitted to the trajectory of ψ_{min} vs. ψ_{pd} of each species studied here. One slope was obtained from a regression fitted to the entire trajectory of ψ_{min} vs. ψ_{pd} (Martínez-Vilalta *et al.* 2014) and a second slope from a regression over a constrained range of ψ_{min} vs. ψ_{pd} (Table 1; Fig. 1c and d) to account for the impact of variable light conditions on ψ_{min} in moist soil at one extreme, and to exclude the impact of data obtained beyond the point at which stomatal closure is no longer effective in limiting ψ_{min} at the other extreme. However, these slopes were not significantly correlated with each other or with any of the leaf pressure–volume traits characterised or with any of the other metrics of iso- vs. anisohydric behaviour applied here, except $\psi_{min} = \psi_{pd}$ (Table 2).

The limitations of using slopes of trajectories of ψ_{min} vs. ψ_{pd} to quantify species' positions along the isohydric/anisohydric continuum are exemplified by a comparison of the behaviour of the highly drought resistant species *Q. douglasii* and the drought avoiding riparian species *S. scouleriana* (Fig. 1). Slopes of regressions fitted to their entire trajectories of plant ψ data (Fig. 1a and b) were 0.81 and 0.77 MPa MPa^{-1} for *Q. douglasii* and *S. scouleriana*, respectively, a difference of only 5%. This ambiguity occurs because slopes of regressions fitted to the entire trajectories of ψ_{min} vs. ψ_{pd} are sensitive to the range of ψ_{min} in moist soil as well as the degree to which the data extend beyond the point at which stomatal closure no longer affects ψ_{min}.

Applying more stringent filtering of data to fit regressions (Fig. 1c and d) resulted in a slope that was 43% greater for *Q. douglasii*, consistent with its apparent greater degree of anisohydry. Yet, as indicated above, this latter type of slope was also not significantly correlated with any of the other metrics of iso- vs. anisohydric behaviour applied here. This ambiguity occurs because species with identical values of $\psi_{min} = \psi_{pd}$ (point of complete stomatal closure) may operate over substantially different ψ landscapes if their values of $\psi_{min}@\ \psi_{pd} = 0$ differ (Fig. S5a). Similarly, species with identical values of Slope (to $\psi_{min} = \psi_{pd}$), but different values of $\psi_{min} = \psi_{pd}$ and $\psi_{min}@\ \psi_{pd} = 0$ would operate over different hydroscape areas and therefore differ in stringency of stomatal regulation of gas exchange in relation to plant ψ (Fig. S5b). Thus, the hydroscape integrates more information and more accurately quantifies the degree of iso-anisohydry than simpler metrics such as the slope of ψ_{min} vs. ψ_{pd} over the entire or constrained range, thereby decreasing ambiguity in positioning species along the iso- to anisohydric continuum.

In contrast to slopes of trajectories of ψ_{min} vs. ψ_{pd} in drying soil, the hydroscape, the area on a plot of ψ_{min} vs. ψ_{pd} over which stomata were able to exert effective control of plant water status, was strongly correlated with the degree to which stomatal behaviour dampened diurnal fluctuations in ψ (i.e. $\Delta\psi$) with increasing plant water deficit (Fig. 2). Because $\Delta\psi$ often declines exponentially with declining ψ_{pd} (insets Fig. 1e and f; Garcia-Forner *et al.* 2016), linearising the relationship as done here allows for more straightforward quantification of the degree to which stomata constrain $\Delta\psi$ as ψ_{pd} declines. Unlike the two slopes discussed above, 'Slope (log$\Delta\psi$ vs. ψ_{pd})' (Table 1) is not directly affected by the highest values of ψ_{min} in moist soil and is therefore a more direct measure of the sensitivity of stomatal control of plant water status in drying soil. The hydroscape integrates the influence of this slope along with that of the value of $\psi_{min}@\ \psi_{pd} = 0$, which appears to be determined largely by ψ_{TLP} (Fig. 3).

Leaf pressure–volume traits as proxies for operating ranges along the isohydric/anisohydric continuum

Prior analyses of leaf pressure–volume traits in multiple species across gradients of aridity have pointed to strong associations between ψ_{TLP}, $\psi_{\pi\ 100}$ and overall drought tolerance (Nilsen *et al.* 1984; Lenz *et al.* 2006; Mitchell *et al.* 2008; Bartlett *et al.* 2012a) as well as components of drought tolerance such as xylem vulnerability to embolism (Choat *et al.* 2007; Blackman *et al.* 2010). Our results suggest that ψ_{TLP} and $\psi_{\pi\ 100}$ are also robust proxies for an important component of drought tolerance: the ranges of ψ_{pd} and ψ_{min} that define a hydroscape over which species can operate before complete stomatal closure prevents CO_2 uptake. Among the eight species studied here, a 1.8 MPa range of ψ_{TLP} was associated with a 10-fold difference in hydroscape area and a sevenfold relative range of the rate at which stomata constrained $\Delta\ \psi$ as ψ_{pd} declined (Fig. 3). Thus, ψ_{TLP} and $\psi_{\pi\ 100}$ served as proxies for quantitative indices of iso- vs. anisohydric behaviour within a diverse group of woody species studied under similar conditions. Interestingly, our results implied that the difference between ψ_{min} in moist soil and ψ_{TLP} was similar among species (0.76 MPa, Fig. 3a) regardless of their operating positions along a continuum of iso- to anisohydry. Consistent with this, the offset between ψ_{TLP} and mean daily ψ_{min} for mature, field-grown individuals of 13 tropical forest and

savanna tree species (Bucci *et al*. 2006; Meinzer *et al*. 2008) was 0.75 ± 0.05 MPa over a range of ψ_{TLP} and ψ_{min} similar to that observed in this study (Fig. S6). These results suggest that when soil water availability is adequate, the relationship between ψ_{TLP} and ψ_{min} may be highly conserved among woody species native to different biomes and growing under different conditions. Differences in ψ_{TLP} among species were largely driven by differences in $\psi_{\pi\ 100}$ (Bartlett *et al*. 2012a), but the slope of the relationship between the two variables was significantly different from one, suggesting the potential for a minor role of increased tissue elasticity in amplifying the impact of $\psi_{\pi\ 100}$ on ψ_{TLP} at lower values of $\psi_{\pi\ 100}$ (Fig. 4).

Leaf pressure–volume traits and stomatal regulation of plant ψ under natural conditions

Although leaf P-V traits and diurnal variation in leaf ψ of the species studied here would likely differ in individuals growing in their native habitats, ψ_{TLP} and $\psi_{\pi\ 100}$ should still be robust proxies for key measures of stringency of stomatal control of plant water status as they have been shown to be for overall drought tolerance across species and vegetation types (Bartlett *et al*. 2012a, 2014; Maréchaux *et al*. 2016). Despite different rates of soil drying related to differences in leaf area and stomatal control of transpiration in container-grown plants in this study, leaf P-V traits determined on a subset of well-irrigated control plants were strongly correlated with two quantitative measures of stringency of stomatal control of water status in plants from which water was withheld. Stomatal pores are turgor-operated valves that integrate a wide range of stimuli through complex signal transduction pathways into an opening and closing response governed by solute exchange and mechanical relationships between stomatal guard cells and surrounding epidermal cells (Raschke 1975). Although guard cell osmotic potential and turgor are likely to differ from those in the bulk leaf, the robustness of the relationships observed here is consistent with direct functional relationships between bulk leaf water status, osmotic properties and stomatal behaviour (Fuchs & Livingston 1996; Comstock & Mencuccini 1998; Buckley 2005). Thus, under natural conditions, species' operating ranges along a continuum of iso- to anisohydry would be expected to scale with species-specific variation in leaf osmotic properties across environmental gradients of aridity. Species with more plastic leaf osmotic properties (phenotypes or ecotypes) should occupy broader operating ranges along the isohydric/anisohydric continuum, resulting in overlapping operating ranges among species. Moreover, a high degree of plasticity of leaf osmotic properties, and therefore in ψ_{min} in wet and dry soil, may contribute to apparent transitions between iso- and anisohydric behaviour within species (Schultz 2003; Domec & Johnson 2012; Rogiers *et al*. 2012). Consistent with this, a number of species have been reported to undergo rapid, rehydration-induced changes in symplast solute content and therefore in ψ_{TLP} (Meinzer *et al*. 1986, 2014; Evans *et al*. 1990; Kubiske & Abrams 1991). However, despite widespread but moderate drought-induced plasticity in ψ_{TLP}, pre-drought ψ_{TLP} appears to be a reliable predictor of drought tolerance and species distributions along gradients of water availability (Bartlett *et al*. 2014).

In view of the available literature on the ecological distribution and ecophysiological traits and behaviour of the species and genera studied here, it was not surprising that *B. occidentalis* and *S. scouleriana* exhibited the least negative values of ψ_{TLP} and $\psi_{\pi\ 100}$ and greatest degree of isohydry according to the criteria we employed. Nevertheless, given that species such as *Ceanothus cuneatus* and *Rhamnus ilicifolia* develop highly negative leaf water potentials in chaparral vegetation prone to extreme drought (Jacobsen *et al*. 2007; Pratt *et al*. 2007), it was somewhat surprising that according to their hydroscape areas (Fig. 3) they were ranked as being less anisohydric than the two *Quercus* species. It is likely that *C. cuneatus* and *R. ilicifolia* would have substantially more negative values of ψ_{TLP} and $\psi_{\pi\ 100}$ under field conditions than observed here and that their hydroscape areas would scale accordingly. However, *Q. douglasii* has been reported to experience seasonal minimum values of ψ_{pd} as low as −5 to −7 MPa, consistent with extreme anisohydric behaviour (Xu & Baldocchi 2003; Osuna *et al*. 2015).

CONCLUSIONS

Leaf P-V traits such as ψ_{TLP} and $\psi_{\pi\ 100}$ may facilitate relatively rapid *a priori* assessment of the stringency of stomatal control of gas exchange and plant water status in drying soil, whereas determining trajectories of ψ_{pd} and ψ_{min} during soil drying requires intensive measurements for an *a posteriori* assessment of iso- vs. anisohydric behaviour. Estimates of ψ_{TLP} and $\psi_{\pi\ 100}$ derived from osmometer measurements on freeze-thawed leaf discs may further streamline rankings of species according to stomatal sensitivity to soil drying (Bartlett *et al*. 2012b; Maréchaux *et al*. 2015, 2016). However, when sufficiently complete trajectories of ψ_{pd} and ψ_{min} are available, determining hydroscapes or slopes of the diurnal range of ψ_{min} in relation to ψ_{pd} as described here may provide more robust estimates of species' operating ranges along a continuum of iso- to anisohydry than leaf P-V traits alone. The preceding features and others such as the ψ at which stomata are no longer effective in maintaining plant water status seem likely to be coordinated with xylem water transport properties such as xylem vulnerability to drought-induced embolism and may provide insights into the relative contributions of potential mechanisms of drought-induced mortality such as hydraulic failure in anisohydric species vs. depletion of carbohydrate reserves following stomatal closure in isohydric species.

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AUTHORSHIP

FCM, DRW, KAM and ARH conceived and designed the study; ALM, DDS, DEM, DRW and FCM collected data; FCM analysed the data and wrote the first draft of the manuscript. All authors contributed to manuscript editing and revision.

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