A new look at water transport regulation in plants

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Summary

- Plant function requires effective mechanisms to regulate water transport at a variety of scales. Here, we develop a new theoretical framework describing plant responses to drying soil, based on the relationship between midday and predawn leaf water potentials. The intercept of the relationship (Λ) characterizes the maximum transpiration rate per unit of hydraulic transport capacity, whereas the slope (σ) measures the relative sensitivity of the transpiration rate and plant hydraulic conductance to declining water availability.
- This framework was applied to a newly compiled global database of leaf water potentials to estimate the values of Λ and σ for 102 plant species.
- Our results show that our characterization of drought responses is largely consistent within species, and that the parameters Λ and σ show meaningful associations with climate across species. Parameter σ was ≤1 in most species, indicating a tight coordination between the gas and liquid phases of water transport, in which canopy transpiration tended to decline faster than hydraulic conductance during drought, thus reducing the pressure drop through the plant.
- The quantitative framework presented here offers a new way of characterizing water transport regulation in plants that can be used to assess their vulnerability to drought under current and future climatic conditions.

Introduction

Plants are able to survive and function under extremely variable environmental conditions, including dramatic changes in soil water availability and atmospheric evaporative demand. This could not be achieved without powerful regulatory mechanisms allowing plants to modulate water transport in response to these changes. At relatively short time scales (i.e. less than seasonal), this regulation is primarily physiological rather than structural, and takes place mostly at two sites within the soil–plant–atmosphere continuum (SPAC): stomata and the hydraulic transport system connecting the soil with the leaves (Sperry *et al.*, 2002). Several components of the hydraulic system of plants are known to vary as a function of water availability, including the hydraulic conductivity of roots and the root–soil interface (Maurel *et al.*, 2010), as well as the xylem and extraxylary tissues (Hacke, 2014). The xylem is one of the plant tissues in which the largest hydraulic conductivity losses occur during drought, and is certainly the tissue in which these losses have been most thoroughly studied (Tyree & Zimmermann, 2002).

The xylem of plants offers a low resistance pathway for water movement from roots to the evaporation sites in leaves. Certain stress factors, such as drought and freezing, induce the formation of emboli in xylem conduits, resulting in an overall loss of plant hydraulic conductance, and therefore increasing the pressure drop required to sustain a certain transpiration rate (Tyree &

Sperry, 1988; Tyree & Zimmermann, 2002). Under severe water deficit, hydraulic failure may result in the complete loss of plant hydraulic conductance and the consequent desiccation of aboveground tissues (Choat *et al.*, 2012), eventually leading to drought-induced mortality (McDowell *et al.*, 2008). Although there seems to be a certain level of reversibility in xylem embolism, even at relatively short time scales (Zwieniecki & Holbrook, 2009; Brodersen *et al.*, 2010), most of the evidence for novel refilling is controversial and should be treated with caution, as it could be affected by measurement artifacts (Sperry, 2013; Rockwell *et al.*, 2014).

Stomata provide the most obvious mechanism allowing plants to control water transport and loss under drought conditions. The aperture of stomata responds to both atmospheric water demand and soil water content through a complex array of processes that eventually result in changes in the turgor pressure of the guard cells that form the pores or that of adjacent epidermal cells (Buckley, 2005). Plants have been classified into two broad categories based on the ability of stomata to regulate the leaf water potential (Ψ_L) (Stocker, 1956; Jones, 1998; Tardieu & Simonneau, 1998). Isohydric species adjust their stomatal opening in such a way as to maintain midday Ψ_L relatively stable as environmental conditions change. On the contrary, anisohydric species have a less strict stomatal control, with no discernible threshold of minimum Ψ_L. As a result, Ψ_L of anisohydric species tracks environmental fluctuations, whereas, in isohydric species,

Ψ_L is highly buffered against these fluctuations. The fact that most plants are likely to lie somewhere in between these two extreme theoretical behaviours is problematic if the iso/anisohydric dichotomy is to be used to characterize drought response strategies, and has led to inconsistent classifications of the same species across or even within studies (Domec & Johnson, 2012). However, the iso/anisohydric categorization has been used as a central tenet to describe different strategies by which plants cope with drought stress, and also to characterize the mechanisms underlying drought-induced mortality in plants (McDowell et al., 2008).

Overall, plant responses to limited water availability are complex and include adjustments at a variety of organizational (stomata, leaf, whole plant,…) and time scales (Chaves et al., 2003; Maseda & Fernández, 2006). This variety of behaviours and the multiplicity of exceptions to any tentative general rule probably explain why a definitive classification of plant water-use strategies and responses to drought has remained somewhat elusive, despite the huge research effort that has been devoted to this topic. Our first objective here is to develop a new theoretical framework to describe plant responses to drying soil conditions based on the relationship between two commonly measured ecophysiological parameters: midday and predawn leaf water potentials. Second, we apply this scheme to a newly compiled global database of leaf water potentials.

Theoretical framework

Under steady-state conditions, water transport through the xylem (J) must balance transpiration losses from leaves (E). This equality can be expressed as (Whitehead et al., 1984):

$$E = g_L \cdot A_L \cdot D = -k_S \cdot A_S \cdot (\Psi_L - \Psi_s) = J \qquad \text{Eqn 1}$$

(g_L, leaf conductance for water vapour; D, vapour pressure deficit of the atmosphere; k_S, whole-plant hydraulic conductance per unit of basal sapwood cross-sectional area; A_L and A_S, leaf area and basal sapwood area, respectively; Ψ_L and Ψ_s, water potential in leaves and soil, respectively). The gravitational component of the water potential gradient is omitted for simplicity and for consistency with the nomenclature used below. We also assume here that steady-state conditions are a reasonable approximation at seasonal or longer time scales, whereas capacitance needs to be considered when studying shorter term responses (Meinzer et al., 2009).

Equation 1 can be rearranged to obtain:

$$\Psi_L - \Psi_s = -\frac{E}{k_S \cdot A_S} = -\frac{g_L \cdot A_L \cdot D}{k_S \cdot A_S} \qquad \text{Eqn 2}$$

As soil drought develops (i.e. Ψ_s declines, becoming more negative), some of the plant parameters in Eqn 2 remain constant or vary typically over relatively long time scales (A_S, A_L), whereas others can vary in the short term. In particular, k_S is likely to decline because of the occurrence of xylem embolism (among other processes) and g_L will be reduced by stomatal closure. The

changes in k_S and g_L with drought have been described using many different equations (Pammenter & der Willigen (1998) for k_S; Oren et al. (1999) and Granier et al. (2000) for g_L). Here, we describe both relationships as a function of Ψ_s. These functions are not intended to model a direct mechanistic link, but to capture a strong empirical pattern that arises from potentially complex mechanisms. Our argument only requires that an overall response of k_S and g_L to Ψ_s (f_k and f_g, respectively) can be defined. Equation 2 can thus be rewritten as:

$$\Psi_L = \Psi_s - \frac{A_L \cdot D \cdot g_{L,max} \cdot f_g(\Psi_s)}{A_S \cdot k_{S,max} \cdot f_k(\Psi_s)} = \Psi_s + \Lambda \cdot \frac{f_g(\Psi_s)}{f_k(\Psi_s)}$$
$$= \Psi_s + \Lambda \cdot \Theta(\Psi_s), \qquad \text{Eqn 3}$$

(Λ, measure of the (maximum) transpiration rate per unit of water transport capacity or, equivalently, the leaf water potential at $\Psi_s \approx 0$ (the pressure drop or 'pulling' capacity of the plant when there is plenty of water available in the soil); f_g and f_k, functions describing the reductions in g_L and k_S, respectively, with declining Ψ_s; Θ, ratio of these functions). Several types of f_g and f_k functions have been used in the literature, with two-parameter sigmoid or Weibull functions being among the most frequent (Neufeld et al., 1992; Pammenter & der Willigen, 1998; Sperry et al., 1998; Hoffmann et al., 2011).

It should be noted that, when $\Psi_s = 0$, by definition $f_g = f_k = 1$, so that Λ is the intercept of the relationship in Eqn 3. Interestingly, if Λ is assumed to be relatively constant, at the temporal scales of interest, compared with g_L and k_S, it follows that the relative sensitivity of stomata and plant hydraulic conductance to declining soil water potentials (f_g/f_k) determines whether the water potential gradient in the plant declines, increases or stays approximately constant as drought progresses (Fig. 1). In more general terms (i.e. without making any assumptions on how or where water transport is regulated), Eqns 2 and 3 imply that the pressure drop in the plant will increase if the hydraulic conductance declines more rapidly than the transpiration rate as drought progresses, whereas it will be reduced if the transpiration rate declines more rapidly than the plant hydraulic conductance.

If it is assumed that the Θ function in Eqn 3 is approximately linear within biologically reasonable ranges of water potentials, the relationship between Ψ_L and Ψ_s also becomes a linear function, and its slope (σ) determines the magnitude of the reduction in Ψ_L as Ψ_s declines (see Supporting Information, Notes S1):

$$\Psi_L = \Psi_s + \Lambda \cdot \Theta(\Psi_s) \approx \Lambda + \sigma \cdot \Psi_s \qquad \text{Eqn 4}$$

In this equation, the value of σ determines the behaviour of plants according to the classical iso/anisohydry paradigm. $\sigma = 0$ implies strict isohydry (constant Ψ_L as Ψ_s declines), whereas $\sigma = 1$ implies strict anisohydry (the difference between Ψ_L and Ψ_s stays constant). It should be noted that two other behaviours are possible (cf. Fig. 1): for $\sigma > 1$, there is extreme anisohydry, implying an increase in the pressure drop through the plant as Ψ_s declines, whereas $0 < \sigma < 1$ implies a sort of partial isohydry, by which the difference between Ψ_L and Ψ_s is reduced as Ψ_s declines
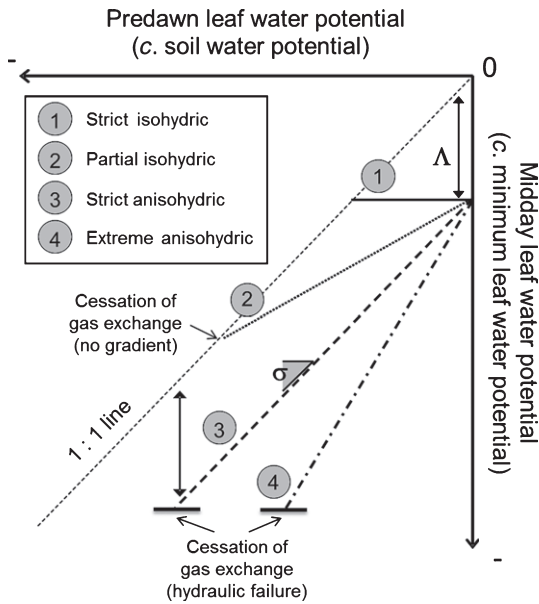
Fig. 1 Relationship between predawn and midday leaf water potentials according to our theoretical model, which assumes a linear relationship. Four different behaviours are depicted, all sharing the same intercept (Λ): strict isohydric ($\sigma = 0$), partial isohydric ($0 < \sigma < 1$), strict anisohydric ($\sigma = 1$) and extreme anisohydric ($\sigma > 1$). The point of cessation of gas exchange is also represented: for isohydric behaviours; it occurs when $\Psi_{PD} = \Psi_{MD}$; for anisohydric relationships, it occurs when Ψ_{MD} reaches the water potential inducing complete loss of plant hydraulic conductance. The 1 : 1 line is also depicted.

(please note that our nomenclature differs from that proposed by Franks *et al.* (2007) to describe similar forms to control water status).

We aimed to use this theoretical framework and a newly developed global database of leaf water potentials to test the following hypotheses: (1) the relationship described in Eqn 3 can be approximated by a linear function, as expressed in Eqn 4, and its parameters are mostly consistent within species (within limits of statistical detectability); (2) the slope (σ) of the relationship between Ψ_L and Ψ_s is close to one in most species, reflecting a close coordination between stomatal and hydraulic responses to drought; and (3) variation in σ across species will reflect differences in environmental conditions and plant hydraulic traits, so that low values of σ (transpiration reduced much more rapidly than plant hydraulic conductance during drought) will occur in environments characterized by high evaporative demands or in species showing large pressure drops under well-watered conditions (low Λ) and high vulnerability to xylem embolism (overcompensation).

Materials and Methods

Literature searches

A literature search was conducted in July 2010 using Google Scholar and the terms: (leaf OR needle) + midday + 'predawn water potential'. All the > 400 references in the initial list were checked for measured values of leaf water potential. The software

TechDig (version 2.0, Ronald B. Jones) was used to retrieve individual data points from published figures. The following criteria were used to include individual papers in the final database: (1) they had to be published in the primary scientific literature; (2) no direct modifications of water potentials in the plant were conducted, whereas experimental treatments, such as irrigation or drought simulation, were allowed; (3) leaf water potentials had been monitored over a period of > 1 month (i.e. short-term studies focusing on diurnal changes were excluded); and (4) predawn and midday water potentials were measured concurrently over time and the number of data pairs was > 5. One study (Hamerlynck *et al.*, 2000) was not considered because it was unique in showing consistently lower (more negative) predawn than midday leaf water potentials, suggesting that the measurements were conducted under very particular conditions that may not be representative. In all the analyses, midday (Ψ_{MD}) and predawn (Ψ_{PD}) leaf water potentials were used as proxies of Ψ_L and Ψ_s, respectively. In doing so, we assumed that plant and soil water potentials equilibrate overnight, which is not always the case (Donovan *et al.*, 2003).

A total of 83 articles fulfilled the previous criteria and were included in our database (see Table S1). In most cases, the studies were conducted in natural conditions in the field (83%), although some studies carried out on crop fields, potted plants or experimental containers were included. An additional filtering was carried out at the species level, so that only species for which the overall range of predawn leaf water potential was > 0.6 MPa were retained. The final database contained data for 102 species sampled in five continents (see Fig. S1), including representatives from the Temperate ($n = 44$, including one Boreal species), Mediterranean ($n = 33$), Tropical ($n = 15$) and Desert ($n = 10$) biomes. All Tropical species, except one, corresponded to the Dry Tropical biome (Table S1). The predominance of species from relatively dry regions corresponds to the fact that leaf water potentials have been widely used to study plant responses to drought, whereas they have been measured less frequently in wet environments (e.g. Tropical rainforests). With regard to growth habits and functional types, 15 species were conifers, 46 were angiosperm trees (broadleaves), 28 were angiosperm shrubs and 13 were herbaceous (see Table S1).

Additional datasets

Average climatic variables for the reference period 1961–1990 for each study location were obtained from the CRU CL 2.0 gridded dataset, with a spatial resolution of 10′ (New *et al.*, 2002). The following variables were considered: mean annual temperature (MAT), mean summer temperature (June–August in the Northern Hemisphere and December–February in the Southern Hemisphere, MST), mean diurnal temperature range (MDTR), mean annual precipitation (MAP), coefficient of variation of monthly precipitation (CVMP), mean summer precipitation (June–August, MSP), mean annual vapour pressure difference (MAD) and mean summer vapour pressure difference (June–August, MSD). These values were averaged across all the locations in which each species had been sampled to obtain a mean value for

each species. A preliminary analysis including correlation and principal component analyses showed that some of the previous climatic variables contained highly redundant information, and we selected MAT, CVMP, MSP and MSD as four relatively independent ($r < 0.5$ in all cases) climate descriptors. These four variables were used in all further analyses.

Wood hydraulic traits were obtained from the Xylem Functional Traits dataset compiled by Choat *et al.* (2012). The following variables were extracted from the database (the number of common species with our dataset, which determined the sample size of the corresponding analyses, is also given for each variable): the water potential at which 50% of hydraulic conductivity is lost as a result of xylem embolism (P_{50}) ($n = 49$), the water potential at which 88% of the hydraulic conductivity is lost as a result of xylem embolism (P_{88}) ($n = 47$), the slope of the vulnerability curve ($n = 48$), maximum specific hydraulic conductivity (K_S) ($n = 41$), maximum leaf-specific hydraulic conductivity (K_L) ($n = 27$) and leaf-to-sapwood area ratio ($A_L : A_S$) ($n = 25$). All variables correspond to measurements taken on branches. When more than one value was available for a given species, the values were averaged to obtain a single, representative value per species.

Data analysis

Mixed linear models were used to fit the (seasonal) relationship between predawn and midday leaf water potential within and across species (Figs 1, 2). Species and the combination of study by treatment nested within species were included as random effects, and the species-specific slopes and intercepts of the model were allowed to co-vary. By treatment here we refer to sets of plants of a given species that were measured under different environmental conditions in a particular study, regardless of the nature of the treatment (e.g. different experimental drought treatments, but also different measured populations in observational studies). In a preliminary analysis, different one-parameter functions were used to fit the relationship between Ψ_{MD} and Ψ_{PD}, including linear, logarithmic and exponential relationships. However, a linear function gave a much better fit in terms of the Akaike information criterion (AIC) and explained variance (see also Fig. 2), and was finally selected. Higher order functions (e.g. quadratic) were also tried, but were abandoned because the resulting model coefficients were highly correlated between each other ($r = 0.99$), indicating that they could not be resolved with the empirical data available. The species-level random coefficients of the fitted model were used to estimate the value of the intercept (Λ) and slope (σ) of the relationship between Ψ_{MD} and Ψ_{PD} for each species in our dataset.

In order to test for the consistency of the estimated parameter values within species, we selected all those species for which we had data for at least two study by treatment combinations. For this subset of species ($n = 61$), we ran a variance components analysis based on the same mixed model described above. In addition, for each of these species (separately), we ran a linear model (without random effects) of the relationship between predawn and midday leaf water potentials in which study by treatment combinations (ranging between 2 and 21, depending on
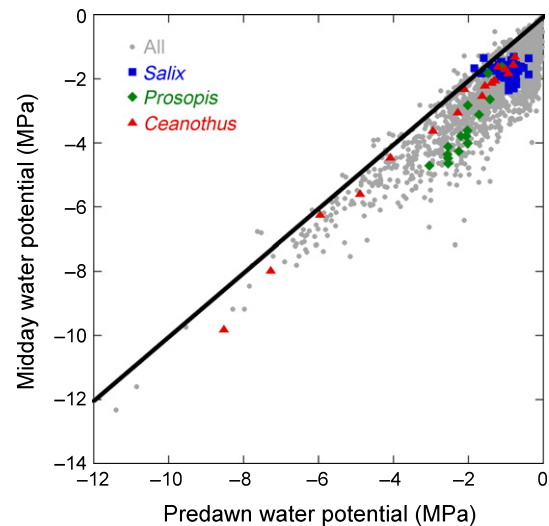
Fig. 2 Relationship between predawn and midday leaf water potentials for all the species included in this study. Three species representative of contrasting responses are highlighted in colour: *Salix gooddingii*, with rapidly shrinking water potential difference as predawn water potential declines ($\approx$ isohydric); *Ceanothus crassifolius*, with nearly constant difference between predawn and midday water potentials ($\approx$ anisohydric); and *Prosopis glandulosa*, with increasing water potential difference as predawn water potential declines ($\approx$ extreme anisohydric).

the species) were introduced as a fixed factor affecting the intercept and the slope of the relationship. This model was compared with the base model assuming a homogeneous intercept and slope across study by treatment combinations.

We used linear models to study the relationships between the species-level parameters Λ and σ and the climatic variables described above. For hydraulic traits, we used correlation analysis to study their association with parameters Λ and σ. Factors coding for functional type (conifer, angiosperm tree, angiosperm shrub, herbaceous) and biome (Temperate, Mediterranean, Tropical, Desert) were also included in the models. In all cases, we compared these base models with the equivalent model including phylogenetic effects by means of phylogenetic generalized least squares (PGLS; Paradis, 2006). In addition, phylogenetic effects on response variables were explored using the coefficient λ as a measure of phylogenetic correlation (Freckleton *et al.*, 2002), based on a Brownian motion evolutionary model ($\lambda = 0$ indicates the evolution of traits independent of phylogeny, whereas $\lambda = 1$ indicates that traits have evolved according to a Brownian motion model) and Moran's autocorrelation index *I* applied to different taxonomic levels (Paradis, 2006).

A phylogenetic tree for the study species was constructed in Phylomatic 2 (Webb & Donoghue, 2005; http://phylodiversity.net/phylomatic/html/pm2_form.html), starting from an updated megatree based on a recent classification by the Angiosperm Phylogeny Group (APGIII; https://github.com/camwebb/tree-of-trees/blob/master/megatrees/R20100701.new). Phylogenetic relationships within families were resolved using published phylogenies for Anacardiaceae (Pell, 2004; Wannan, 2006), Asteraceae (Bayer & Starr, 1998; Roberts & Urbatsch, 2004), Betulaceae (Chen *et al.*, 1999), Ericaceae (Kron *et al.*, 2002), Oleaceae (Wallander & Albert, 2000), Pinaceae (Wang *et al.*, 2000;

Gernandt *et al.*, 2005), Poaceae (Hsiao *et al.*, 1995; Hilu *et al.*, 1999; Catalán *et al.*, 2004) and Rosaceae (Potter *et al.*, 2007). Additional phylogenetic trees were used to solve other polytomies at the genus level for *Acacia* (Miller & Bayer, 2001; Bouchenak-Khelladi *et al.*, 2010), *Populus* (Hamzeh & Dayanandan, 2004) and *Quercus* (Manos *et al.*, 1999; Bellarosa *et al.*, 2005). Family-level polytomies only remained unresolved for Poaceae; some polytomies within genera for *Acacia*, *Quercus*, *Ceanothus* and *Rhamnus* were also left unresolved.

We fitted each PGLS model assuming an Ornstein–Uhlenbeck (OU) model of character evolution. All analyses were conducted with the software R (v. 2.12, the R Foundation for Statistical Computing, Vienna, Austria) using the packages *nlme*, *lme4*, *ape*, *ade4* and *pglm3.2*. The residuals of all reported models showed no obvious pattern. Significance for all statistical analyses was accepted at $\alpha = 0.05$. The R^2 (explained variance) of the mixed models was estimated including both fixed and random factors using a likelihood ratio statistic (Magee, 1990).

Results

The study species covered a wide range of minimum predawn leaf water potentials ($\Psi_{PD,min}$), ranging from -0.7 MPa in the Tropical tree *Schima wallichii* to -11.4 MPa in the Mediterranean shrub *Rhamnus crocea*. $\Psi_{PD,min}$ was affected by functional type, with significantly lower values in shrubs (2.3–3.0 MPa more negative than the other functional types, on average), and also by biome, with Mediterranean species showing the lowest $\Psi_{PD,min}$, followed by Desert species and by Temperate and Tropical species, the last two having similar values (Fig. S2).

Our linear model of the relationship between predawn and midday leaf water potentials (Eqn 4) provided a good fit to the data. Overall, the model explained 90% of the variability in Ψ_{MD}. The overall slope of the relationship between midday and predawn water potential was 0.86 MPa MPa^{-1} (i.e. a value < 1 implies that the pressure drop through the plant diminishes with drought), and varied across species between 0.19 MPa MPa^{-1} for *Acacia etbaica* (a Tropical tree) and 1.36 MPa MPa^{-1} for *Prosopis glandulosa* (a Desert tree). The value of the intercept (Λ) varied substantially across species, ranging between -3.2 MPa (*Balanites aegyptiaca*, a Tropical tree) and -0.2 MPa (*Eragrostis curvula*, a Tropical grass).

Overall, the estimated σ values for most species were consistent with either strict anisohydry ($\sigma \approx 1$, $n = 55$) or partial isohydry ($0 < \sigma < 1$, $n = 42$). Only five species showed σ values significantly > 1 (extreme anisohydry) and none showed strict isohydry as defined in this study ($\sigma \approx 0$) (Figs 3, S3). In 80 of 102 species (78%), the estimated $\sigma < 1$, implying a faster decline in canopy transpiration than in plant hydraulic conductance (hence a decline in the plant pressure drop) in response to drying soil, although the value of σ was not significantly different from one in all cases (Fig. S3). The slope parameter σ was unaffected by functional type or biome ($P > 0.05$ in all cases). The intercept Λ was also similar across functional types ($P > 0.05$), but its value was c. 0.4 MPa lower (i.e. more negative) in Desert species compared with either Temperate or Mediterranean ones ($P < 0.05$),
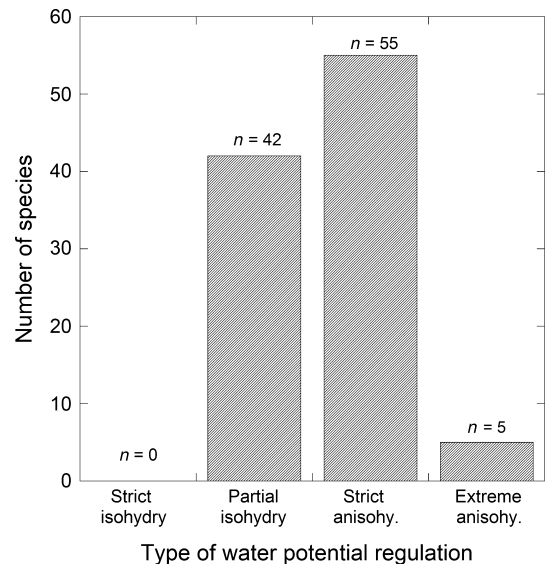
Fig. 3 Frequency histogram of the type of water potential regulation according to the observed σ values (cf. Fig. 1). Species are classified as strict isohydric if the confidence interval of σ (CI, defined as the estimated σ value $\pm$ 1SE) includes zero, as strict anisohydric if the CI includes one, as partial isohydric if $0 < \sigma < 1$ and the CI does not include zero or one, and as extreme anisohydric if $\sigma > 1$ and the CI does not include one.

whereas Tropical species showed intermediate values (see Fig. S2).

There were 61 species for which measurements were available for different studies or treatments. Parameter Λ differed significantly across study by treatment combinations (i.e. within species) in 20 of the corresponding linear models, whereas σ was different across study by treatment combinations in only eight of 61 cases. A variance components analysis on the mixed model fitting the relationship between predawn and midday leaf water potentials, but using only the data for these 61 species, showed that the species level accounted for 56% of the total variance in midday leaf water potential, compared with 10% explained by different study by treatment combinations within species. Finally, an error analysis to assess the error in the estimation of σ and Λ as a function of sample size (number of Ψ_{PD}, Ψ_{MD} data pairs) revealed that these values could be estimated relatively well using sample sizes of eight data pairs or more (Notes S2). Taken as a whole, and considering the large variability in the measured water potentials, these results indicate that the parameter values obtained were largely consistent within species and can be used to characterize species responses to varying soil water availability.

With regard to phylogenetic effects, similar results were obtained for the two fitted parameters, indicating no evidence of phylogenetic correlation (see also Fig. S4). For σ, $\lambda = 0.16$, which was significantly different from one ($\chi^2 = 46.7$, $P < 0.001$) but not from zero ($\chi^2 = 1.1$, $P = 0.29$); for Λ, the estimated λ was 0.04, which was again significantly different from one ($\chi^2 = 50.9$, $P < 0.001$) but not from zero ($\chi^2 = 0.2$, $P = 0.67$). The same test applied to $\Psi_{PD,min}$ showed a higher value of $\lambda = 0.84$, which was significantly different from zero ($\chi^2 = 5.8$, $P = 0.02$) but not from one ($\chi^2 = 1.0$, $P = 0.33$). These results were largely consistent with those of autocorrelative models showing significant Moran's

I indices at the genus and family levels for $\Psi_{L,min}$ (*I c.* 0.6 in both cases), non-significant values for the slope parameter (σ) and significant values only at the family level for Λ ($I = 0.18$).

The parameters Λ and σ were significantly related to each other (slope = 0.29, $r = 0.74$, $P < 0.001$; Fig. 4), regardless of whether or not phylogenetic effects were taken into account. The result was also very similar (slope = 0.28, $r = 0.68$, $P < 0.001$) if the relationship between midday and predawn leaf water potentials (Eqn 4) was fitted using centred instead of raw Ψ_{PD} values (results not shown). The parameters Λ and σ were both unrelated to the minimum predawn leaf water potential measured on each species ($\Psi_{PD,min}$), regardless of whether or not phylogenetic effects were included ($P > 0.05$ in all cases). However, Λ and σ were clearly associated with climate and these relationships were robust to phylogenetic effects (Fig. 5; Table 1). Parameter Λ declined with annual temperature and summer vapour pressure deficit and increased as a function of annual precipitation variability and summer precipitation (Table 1b), whereas σ was negatively related to mean summer vapour pressure deficit (Table 1a). Adding functional type into the relationships between parameters Λ and σ and climate resulted in non-significant coefficients for functional types and nearly identical coefficients for climate variables (results not shown).

Parameter σ was negatively related to the vulnerability to xylem embolism at the species level, with lower σ values occurring in more vulnerable species (i.e. more vulnerable species showed a greater reduction in their pressure drop than more resistant ones as Ψ_{PD} declined; Fig. 6). There was also a strong relationship with the slope of the vulnerability curve, so that the relationship was tighter between σ and the water potential causing 88% embolism (P_{88}) than with the water potential causing 50% loss of hydraulic conductivity (P_{50}). These relationships remained significant and very similar if phylogenetic effects were included in the models (results not shown). In all cases, the fit of the models worsened if functional type or biome
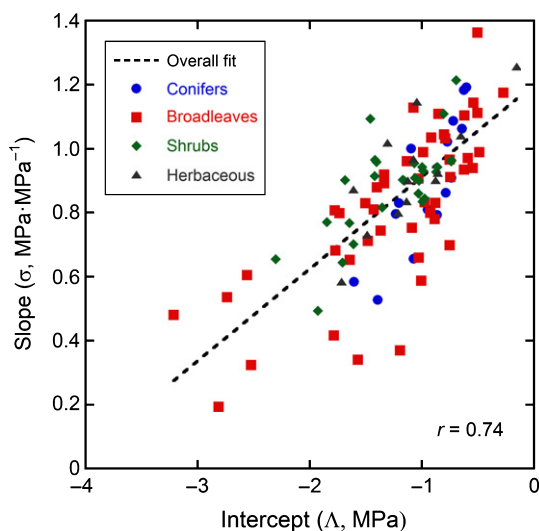
was included as an additional factor (with effects on the intercept and slope). There was no significant association between σ and specific hydraulic conductivity (K_S), leaf-specific hydraulic conductivity (K_L) or leaf-to-sapwood area ratio ($A_L : A_S$) ($P > 0.4$ in all cases). Finally, parameter Λ was unrelated to any of the hydraulic traits considered in this study ($P > 0.1$ in all cases).
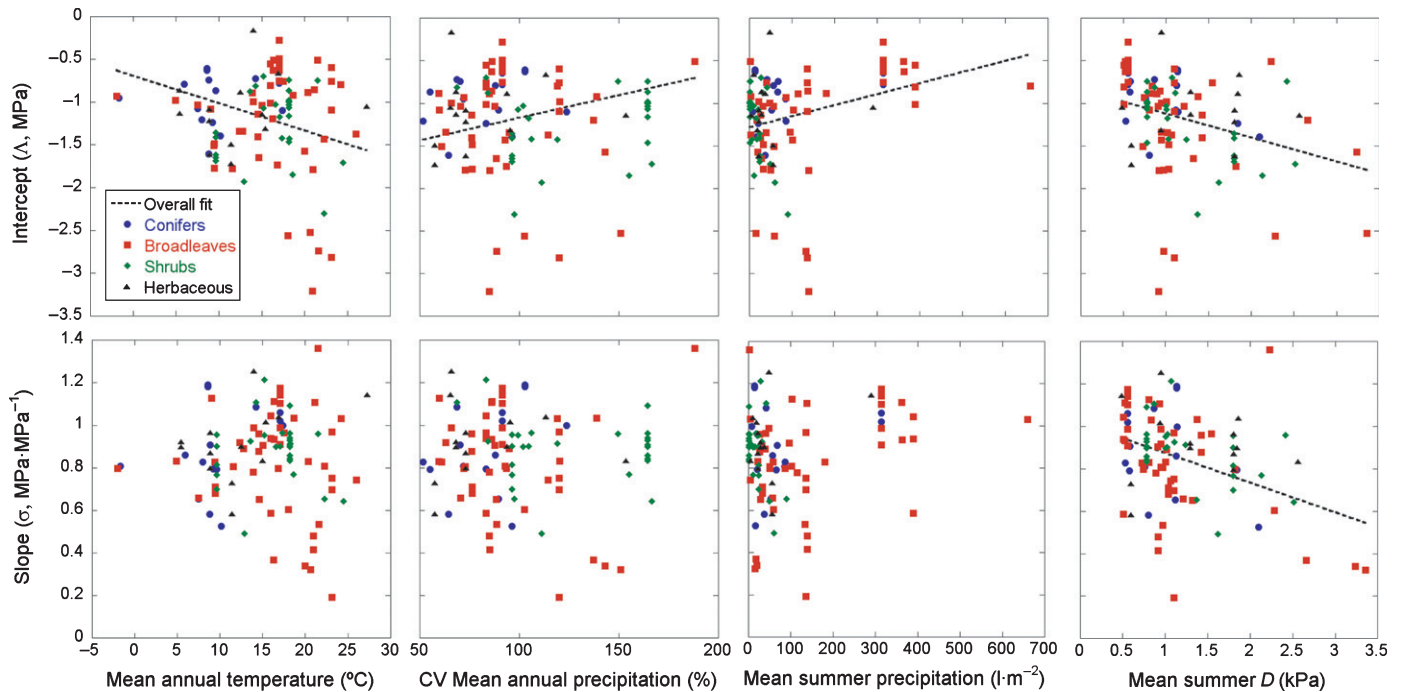
Discussion

In this paper, we propose a simple and novel approach to study water transport regulation in plants, based on the relationship between midday and predawn leaf water potentials. This relationship is characterized using two parameters: an intercept (Λ), measuring the transpiration stream relative to the plant hydraulic capacity under well-watered conditions (or, equivalently, the leaf water potential at $\Psi_s \approx 0$,), and a slope (σ) characterizing the relative sensitivity of the transpiration rate and plant hydraulic conductance to declining soil water potential (Eqn 4). We postulate that this latter parameter is more relevant than either stomatal sensitivity to drought or vulnerability to xylem embolism in determining the temporal dynamics of leaf water potentials, and that our analysis provides synthetic, quantitative measures that can be used to characterize water-use strategies and drought responses in plants.

The link between stomatal function and plant hydraulics

At the core of our approach is the realization that the response of the plant's water potential gradient to declining soil water availability is not determined directly by stomatal sensitivity to drought or, more generally, by the sensitivity of transpiration rate to drought, but by the ratio between this sensitivity and the vulnerability of the plant hydraulic system (as a result, for example, of xylem embolism). This fact has several important implications, as it makes the link between stomatal function and the dynamics of leaf water potential (in terms, for instance, of the isohydric vs anisohydric strategies) less straightforward than implied in previous reports (Jones, 1998; Tardieu & Simonneau, 1998). In our view, a plant with highly sensitive stomata closing at relatively high water potentials could still show a strict anisohydric behaviour (i.e. ever declining leaf water potentials until the minimum tolerable value is reached at any given point in the plant's hydraulic continuum) provided that its hydraulic transport system is even more sensitive than stomata to declining water availability (Fig. 1).

The notion that leaf gas exchange and plant hydraulics are tightly linked is not new (Meinzer, 2002; Sperry *et al.*, 2002; Mencuccini, 2003). Previous studies have shown that xylem hydraulic conductivity is positively related to leaf photosynthetic capacity (Brodribb & Feild, 2000) and stomatal conductance (e.g. Nardini & Salleo, 2000; Zhang & Cao, 2009; Héroult *et al.*, 2013) across species, and also that tree species with higher vulnerability to xylem embolism tend to have higher stomatal conductance (Maherali *et al.*, 2006). What is less abundant in the literature is the direct comparison between stomatal and plant

Fig. 4 Relationship between the estimated values of parameters σ and Λ. Each data point corresponds to a different species ($n = 102$). See Fig. 1 for information on the meaning of parameters σ and Λ.

Fig. 5 Relationship between σ and Λ and climate variables, including mean annual temperature (MAT), the coefficient of variation of monthly precipitation (CVMP), mean summer precipitation (June–August, MSP) and mean summer vapour pressure difference (D) (June–August, MSD). Each data point corresponds to a different species ($n = 102$). The fitted functions correspond to significant coefficients as estimated in the corresponding linear models of σ or Λ as a function of all four climate variables (see text). See Fig. 1 for information on the meaning of parameters σ and Λ.

Table 1 Models of parameters σ (a) and Λ (b) of the relationship between predawn and midday leaf water potentials as a function of climatic variables

Explanatory variables	Without phylogenetic effects		Including phylogenetic effects	
	Coefficient ± SE	t-value	Coefficient ± SE	t-value
(a) Response variable: σ				
Intercept	0.96 ± 0.08	12.12***	0.96 ± 0.08	12.12***
MAT	−0.0036 ± 0.0046	−0.78[ns]	−0.0036 ± 0.0046	−0.78[ns]
CVMP	0.0010 ± 0.0008	1.30[ns]	0.0010 ± 0.0008	1.30[ns]
MSP	0.0001 ± 0.0002	0.44[ns]	0.0001 ± 0.0002	0.44[ns]
MSD	−0.14 ± 0.04	−3.60***	−0.14 ± 0.04	−3.60***
	Model AIC = 15.3; R^2 = 0.15		Model AIC = 17.3	
(b) Response variable: Λ				
Intercept	−1.01 ± 0.19	−5.24***	−1.01 ± 0.19	−5.24***
MAT	−0.032 ± 0.011	−2.86**	−0.032 ± 0.011	−2.88**
CVMP	0.0053 ± 0.0019	2.76**	0.0053 ± 0.0019	2.77**
MSP	0.0013 ± 0.0005	2.54*	0.0013 ± 0.0005	2.55*
MSD	−0.28 ± 0.09	−3.06**	−0.28 ± 0.09	−3.05**
	Model AIC = 188.3; R^2 = 0.22		Model AIC = 190.2	

CVMP, coefficient of variation of monthly precipitation; MAT, mean annual temperature; MSD, mean summer vapour pressure difference; MSP, mean summer precipitation. Significant relationships: *, $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$; ns, not significant.

hydraulic responses to declining water potential. In one of the few articles taking this approach, Brodribb *et al.* (2003) found that the water potential inducing 50% stomatal closure was linearly correlated with the water potential inducing a 20% loss of xylem hydraulic conductivity in the stem, and that the slope of the corresponding relationship was close to one, implying that stomatal conductance was more sensitive than stem hydraulic conductivity to declining water potential. Similar results were obtained by Arango-Velez *et al.* (2011) across poplar clones.

We believe that Eqns 3 and 4 above provide a quantitative framework to interpret the close coordination between stomatal regulation and plant hydraulics. Our results show that stomatal closure tends to occur somewhat more rapidly than hydraulic conductivity loss in response to declining soil water potential, as implied by the slope (σ) values, which were slightly below one in most species (Figs 3, S3). Very shallow slope values (σ ≈ 0) would imply a safe strategy in terms of embolism avoidance, as stomata would close completely before substantial loss of
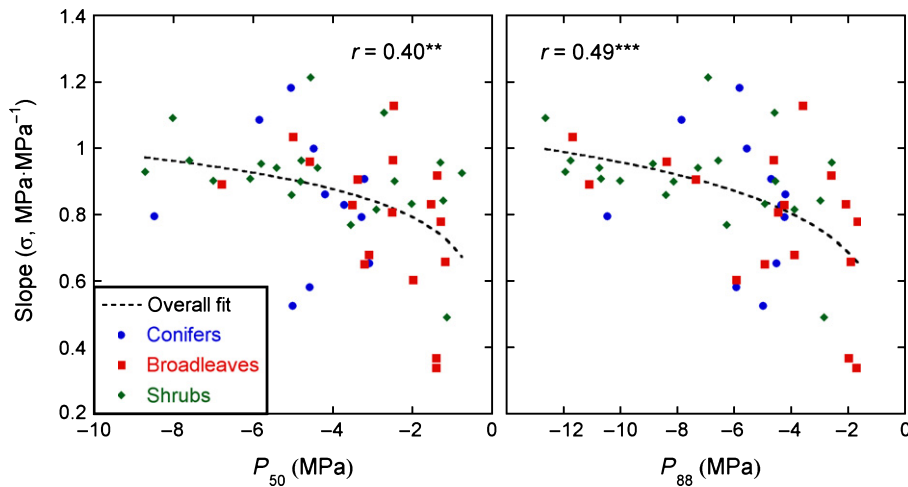
Fig. 6 Relationship between σ and two different measures of vulnerability to xylem embolism: P_{50}, the water potential causing a 50% loss in xylem hydraulic conductivity; and P_{88}, the water potential causing an 88% loss in xylem hydraulic conductivity. Each data point corresponds to a different species ($n = 102$). Simple regression curves are fitted to the data in each panel. Statistical significance of relationships is indicated as: **, $0.01 > P > 0.001$; ***, $P < 0.001$. See Fig. 1 for information on the meaning of parameter σ.

hydraulic conductivity is observed. However, such a strategy would incur a high cost in terms of reduced carbon gain, and it is thus likely to be selected against. At the opposite extreme, $\sigma > 1$ implies that hydraulic transport limitations (e.g. xylem embolism) occur more rapidly than stomatal closure in response to declining water potential. This result was observed in nearly one-quarter of the species in our database (although the standard error of the estimated slopes included one for 17 of these 22 species; Figs 3, S3). Such a strategy might seem disadvantageous because stomatal conductance loss is more easily reversed than hydraulic conductivity loss in the xylem, which may eventually lead to whole-plant mortality through hydraulic failure (Tyree & Sperry, 1988; McDowell *et al.*, 2008). However, some level of xylem embolism may be advantageous in terms of maximizing transpiration and assimilation rates (Jones & Sutherland, 1991; Manzoni *et al.*, 2013a), and may occur in phreatophytes with direct access to ground water (e.g. *Prosopis glandulosa*; Fig. S3) or in other cases in which extremely low Ψ_s values are unlikely to occur. A value of $\sigma > 1$ could also occur during the process of disconnecting hydraulically from the soil in drought-deciduous species.

Climatic and hydraulic correlates of the regulation of leaf water potential

Our two descriptors of the relationship between predawn and midday leaf water potentials (parameters Λ and σ) were associated with climate at the species level (Fig. 5). Overall, species living in drier areas (high temperature and evaporative demand, consistently low rainfall) tended to have higher pressure drops under well-watered conditions (more negative values of Λ). This result implies a greater gradient of water potential within the plant at drier sites, even under well-watered conditions. This pattern is probably related to the fact that plant species growing in drier environments are generally able to sustain more negative water potentials (Maherali *et al.*, 2004; Choat *et al.*, 2012; Manzoni *et al.*, 2013b) and tend to have lower hydraulic conductivity (Manzoni *et al.*, 2013b; but see Maherali *et al.*, 2004). The fact that σ responded only to D, with lower values (more sensitive stomata relative to the hydraulic transport system) at sites with

higher evaporative demands, is consistent with the well-known negative relationship between stomatal conductance and D (Oren *et al.*, 1999). Similarly, the tight and positive relationship between Λ and σ (Fig. 4) may be interpreted analogously to the association between the reference stomatal conductance (at $D = 1$ kPa) and the (absolute) sensitivity of stomatal conductance to D (Oren *et al.*, 1999); that is, species with higher transpiration per unit of hydraulic transport capacity (more negative values of Λ) require a stricter regulation of water loss to limit the decline in Ψ_L as soil water availability declines (lower σ).

The negative relationship between σ and the vulnerability to xylem embolism (Fig. 6) implies that species with more vulnerable xylem tend to compensate by having even more sensitive stomata. Regardless of the mechanism behind this relationship (Nardini & Salleo, 2000), its end result is the prevention of catastrophic levels of hydraulic conductivity loss. The fact that the parameters σ and Λ did not show a significant phylogenetic signal contrasts with previous results showing that there is some level of phylogenetic conservatism in the vulnerability to xylem embolism (Maherali *et al.*, 2004). It should be noted, however, that this result might be confounded, to some degree, by the tight association between σ and Λ (Fig. 4). When we tried to isolate the effect of the relative sensitivity of stomata and the hydraulic system using Eqn S5 (cf. Notes S1), the corresponding parameter (c_{gk} in Notes S1) showed phylogenetic conservatism ($\lambda = 0.32$, significantly different from zero and from one). A tight phylogenetic coordination between σ and Λ would also tend to blur the phylogenetic signal in either parameter.

Limitations and potentialities

The approach proposed here, as any similar attempt, is based on several assumptions. Perhaps the most important is that a response to soil (or predawn) water potential can be defined for both plant hydraulic conductance and stomatal conductance or, more generally, transpiration rate. This seems to be the case for the xylem, as embolism is believed to be a direct response to water potential (Tyree & Zimmermann, 2002). It should also be noted that we do not make any particular assumption as to where hydraulic conductivity losses start to develop and, as long as an

overall relationship between whole-plant hydraulic conductance and predawn water potential can be defined, our approach should be robust regardless of the location of the main hydraulic bottleneck (cf. Jackson *et al.*, 2000; Johnson *et al.*, 2011; Nardini *et al.*, 2012). A similar reasoning can be applied to stomatal conductance, although the situation is even more complex here, as stomata respond to soil water availability and leaf water status through a complex set of chemical and hydromechanical signals (Buckley, 2005; Damour *et al.*, 2010).

In addition, the fact that leaf area may vary at the temporal scale of our analysis and that the vapour pressure deficit (*D*) frequently co-varies with soil water potential implies that we are not necessarily characterizing a stomatal response to soil water potential, but an overall response of transpiration rate to water availability (cf. Eqns 2 and 3). It is also well known that soil and plant water potentials may not be in equilibrium, particularly in dry soils (Donovan *et al.*, 2003). However, this decoupling is likely to affect similarly the responses for both transpiration and plant hydraulic conductance. The previous considerations do not invalidate our analysis, but altogether they imply that the $\sigma < 1$ values observed in most species should be interpreted to mean that water loss regulation begins before hydraulic transport limitations start to occur, not necessarily that stomata are more sensitive than the hydraulic system to water potential measured at one particular point within the SPAC.

Another important assumption is that a linear function is a reasonable approximation of the relationship between predawn and midday leaf water potential within species (Eqn 4). Although this is largely consistent with the empirical data used in this study (Fig. 2) and statistically robust towards alternative assumptions, it seems clear that highly non-linear relationships are possible, particularly when the hydraulic system is much more sensitive than stomata or when stomatal conductance is close to zero (Notes S1). Our results show that a linear function is a good descriptor of the relationship in Eqn 3, and the best one using only two parameters. More complex functions (e.g. with three parameters) can be used in further studies focusing on drought responses in one or a few intensively studied species. Importantly, the interpretation of the resulting functions, even if more complex than those assumed here, would still be consistent with our general framework, as their shape would always reflect the relative sensitivity of transpiration rate and hydraulic conductance to declining water potentials. It should be noted, however, that the use of more complex functions is at the expense of simplicity and generality, and could not be applied to our global water potentials database because most individual datasets lacked the required level of detail.

Finally, our framework is based on the assumption that steady-state conditions are a reasonable approximation of water flow through plants at seasonal time scales. The importance of hydraulic capacitance is undeniable (Meinzer *et al.*, 2009), particularly at relatively short time scales, and could influence the shape of the relationship between Ψ_{MD} and Ψ_{PD}, even at the longer time scales considered here. However, our approach seems to be a reasonable first approximation and is consistent with previous synthesis efforts at comparable temporal scales (Oren *et al.*, 1999; Manzoni *et al.*, 2013a).

An important advantage of our approach is that it is based on predawn and midday leaf water potentials, perhaps the most commonly measured variables in ecophysiological studies (Bhaskar & Ackerly, 2006). In addition, our analyses demonstrate that the estimated parameters (Λ and σ) show meaningful associations with climate variables and are largely consistent within species, implying that they can be used to characterize species behaviour (see also Notes S2). In this regard, the slope parameter σ provides a quantitative index to locate species along the continuum between isohydric ($\sigma \approx 0$) and anisohydric ($\sigma \approx 1$) behaviour, and a more precise characterization of drought responses than the qualitative approaches used thus far. The fact that σ explicitly relates stomatal sensitivity to plant hydraulic vulnerability to drought provides a useful descriptor in the context of studying the mechanism of drought-induced mortality in plants, as it directly relates to the carbon starvation and hydraulic failure hypotheses and their interaction (McDowell *et al.*, 2008; McDowell, 2011). It helps to explain, for instance, why species with a stronger stomatal control are not necessarily less prone to hydraulic failure (cf. Martínez-Vilalta *et al.*, 2003; Meinzer *et al.*, 2009; Nardini *et al.*, 2013), as they may reach very high levels of xylem embolism (measured, for example, as P_{88}) before their stomata are completely closed (measured as the point at which $\Psi_{PD} = \Psi_{MD}$, Fig. 1). In this regard, our analysis could be expanded to include a third parameter to characterize species: Ψ_{PD} at the cessation of gas exchange. This value could be estimated as $\Lambda/(1 - \sigma)$ for relatively isohydric species ($\sigma < 1$) and as the water potential causing 100% loss of hydraulic conductivity for anisohydric species ($\sigma \geq 1$).

We are still far from a complete understanding of plant strategies to cope with drought, and clearly there is no single metric that is able to synthesize the plethora of responses observed across species. At the same time, however, climate change is increasing drought stress over many regions of the Earth, and reports of drought (and heat)-related forest die-off are becoming widespread (Allen *et al.*, 2010). In this context, we need to identify the species and populations that are more likely to be vulnerable to increased drought, and there is an urgent need to develop simple but reliable metrics that could be used in the context of dynamic global vegetation models (Bartlett *et al.*, 2012; Choat *et al.*, 2012; Klein *et al.*, 2014). We are convinced that the analyses presented here will facilitate the characterization of drought responses of plants and will therefore provide a valuable and fruitful addition to these efforts.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Mean annual temperature map of the World showing the sampling locations of all the studies included in our final water potentials database.

Fig. S2 Species-level values of minimum measured predawn leaf water potential, the slope parameter σ and the intercept Λ as a function of biome.

Fig. S3 Species-level values of the intercept (parameter Λ) and the slope (parameter σ).

Fig. S4 Phylogenetic tree of the studied species.

Table S1 Characteristics of the species considered in this study and list of references used to build the database of leaf water potentials

Notes S1 The theoretical framework expanded, including an error analysis.

Notes S2 Error analysis of the estimation of the slope and intercept of the relationship between predawn and midday leaf water potentials.

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