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Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept

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Running title: **Deconstructing the iso/anisohydric concept**

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Abstract

In this review we address the relationship between stomatal behaviour, water potential regulation and hydraulic transport in plants, focusing on the implications for the iso/anisohidric classification of plant drought responses at seasonal timescales. We first revise the history of the isohydry concept and its possible definitions. Then, we use published data to answer two main questions: (1) is greater stomatal control in response to decreasing water availability associated with a tighter regulation of leaf water potential (Ψ_L) across species? And (2) is there an association between tighter Ψ_L regulation (~isohydric behaviour) and lower leaf conductance over time during a drought event? These two questions are addressed at two levels: across species growing in different sites and comparing only species coexisting at a given site. Our analyses show that, across species, a tight regulation of Ψ_L is not necessarily associated with greater stomatal control or with more constrained assimilation during drought. Therefore, iso/anisohydry defined in terms of Ψ_L regulation cannot be used as an indicator of a specific mechanism of drought-induced mortality or as a proxy for overall plant vulnerability to drought.

Keywords:

Anisohydry; Drought; Embolism; Hydraulic architecture; Isohydry; Plant strategies; Seasonal responses; Stomata; Water relations; Xylem transport

Introduction

Plants differ tremendously in the water potentials they experience, not only when comparing species along wide gradients of water availability but also within sites (Ackerly 2004; Martínez-Vilalta *et al.* 2014; Pivovaroff *et al.* 2016). The ecological implications of this variability are large, as xylem and leaf water potentials are arguably the most important variables determining the water status of plants (Kramer 1988; Bhaskar & Ackerly 2006). Low (negative) water potentials put the plant hydraulic transport system under stress, as xylem embolism and analogous processes in extraxylary tissues tend to increase hydraulic resistance along the soil-plant-atmosphere continuum (Sperry *et al.* 2002). If water potentials become too low water transport may cease altogether, ultimately leading to complete desiccation and plant death (Tyree & Sperry 1988; Tyree & Zimmermann 2002; Choat *et al.* 2012). There are several mechanisms by which plants can regulate water transport to avoid hydraulic failure, including structural and physiological adjustments (Maseda & Fernández 2006). At short time scales, however, stomatal closure is the main mechanism by which plants limit transpiration losses and, thus, maintain water potentials within tolerable limits.

The regulation of plant water potential is not only important because of its role in determining plant responses to drought and other stress factors but also because of its influence on metabolic processes, including plant growth through turgor-driven cell expansion (Slatyer & Taylor 1960; Lockhart 1965; Kramer 1983). This central role explains why, since the development of the pressure chamber by Scholander *et al.* (1965), water potential has become one of the most frequently measured variables in plant physiology and plant physiological ecology. Concurrently, there has been an effort to characterize different modes of water potential regulation (*cf*. next section). The iso/anisohidric classification, based on the ability to regulate leaf water potential (Ψ_L), has been among the most successful of these attempts (Stocker 1956; Jones 1998; Tardieu & Simonneau 1998). Isohydric species are those that maintain midday Ψ_L relatively stable as environmental conditions change, whereas anisohydric species track environmental fluctuations in water availability, with no discernible threshold of minimum Ψ_L. Because of the role of stomata in regulating leaf conductance to water vapour (g_L) and, therefore, transpiration and plant water status, the iso/anisohydric classification is usually interpreted in terms of stomatal behaviour: isohydric species maintain relatively stable Ψ_L precisely because of their more strict stomatal control, whereas anisohydric species would show a looser regulation of transpiration (Jones 1998; Tardieu & Simonneau 1998).

Interpreted in this way, the iso/anisohydric categorization has strong implications for the maintenance of assimilation under varying environmental conditions and, in general, for the carbon economy of plants. This notion was used by McDowell *et al.* (2008) to distinguish between two interrelated physiological mechanisms leading to plant mortality under drought. Isohydric species would close stomata earlier during drought and, therefore, would depend more heavily on carbohydrate reserves to meet continued carbon demands for respiration, osmoregulation or defense. As a result, they would be more prone to die from carbon starvation. At the other extreme, anisohydric species would close stomata later at the expense of suffering lower water potentials; which would make them more vulnerable to hydraulic failure. There is no doubt that this framework is appealing and has been hugely influential in shaping the research agenda on drought-induced mortality in the last decade (Adams *et al.* 2009; Mitchell *et al.* 2012; Hartmann *et al.* 2013; Sevanto *et al.* 2014); however, is it correct?

A case in point can be found in Figures 1 and 2 of the seminal paper by Tardieu & Simonneau (1998), in which a diurnal course of stomatal conductance and Ψ_L is shown for sunflower (a paradigmatic anisohydric species) and maize (isohydric) under different levels of drought stress. These figures show that the reduction in stomatal conductance under severe water deficit relative to full irrigation is even more dramatic for sunflower (~100% reduction) than for maize (~66% reduction), despite a much tighter leaf water potential regulation in the latter species. A similar pattern emerges when comparing cultivars with isohydric and anisohydric behaviour in *Vitis vinifera*, one of the model systems in the study of the relationship between stomatal regulation and drought resistance in plants (Schultz 2003; Lovisolo *et al.* 2010). In their literature synthesis Lovisolo *et al.* (2010) show similar stomatal behaviour for cultivars with contrasted Ψ_L (*cf*. Figure 1 in their paper). These results seem hard to reconcile with the view that tighter water potential regulation is necessarily associated with stronger stomatal control across species or cultivars. More recent studies also challenge the existence of a direct, unambiguous association between water potential regulation and seasonal gas exchange when comparing coexisting species (Quero *et al.* 2011), including the piñon-juniper system in SW USA, the model system behind the hydraulic framework described in the previous paragraph (Garcia-Forner *et al.* 2016a; but see also Limousin *et al.* 2013; Woodruff *et al.* 2014).

There are at least three issues that complicate the link between stomatal control and water potential regulation across species. The first one has to do with definitions: although the general concepts of isohydry and anisohydry are quite intuitive, how do we exactly define them in practice considering that most species are likely to lie somewhere in between these two extreme theoretical behaviours? Secondly, there is the conceptual difficulty that the changes of the water potential gradient through the plant do not depend only on stomatal responses, but on the relative sensitivity of transpiration and plant hydraulic conductance to declining water availability (Martínez-Vilalta *et al.* 2014). This is further complicated by the fact that gas exchange and plant hydraulics are tightly coordinated across species (Meinzer 2002; Sperry *et al.* 2002; Mencuccini 2003; Brodribb *et al.* 2014). Finally, when considering the implications of different stomatal behaviours on the carbon economy of plants what matters is for how long stomata are closed under a given drought and, thus, we need to focus on the temporal dynamics. Precisely because isohydric and anisohydric species operate at different water potentials, the fact that stomata are more sensitive to declining Ψ_L in isohydric species does not imply that they will close earlier during drought. The relevant question becomes whether the stomata of isohydric species are more sensitive relative to the water potentials at which they operate, and therefore constrain assimilation further in these species.

In this review we aim at disentangling the relationship between stomatal behaviour, water potential regulation and hydraulic transport in plants, focusing on seasonal timescales. We first revise the history of the isohydry concept and its possible definitions. Then, we use data retrieved from the literature to address two main questions: (1) is greater stomatal control in response to decreasing water availability associated with a tighter regulation of leaf water potential across species? (2) is there an association between tighter water potential regulation (~isohydric behaviour) and lower leaf conductance (g_L) over time during a drought event? These two questions are addressed at two levels: across species growing in different sites and comparing only species coexisting at a given site. We finish by discussing the mechanisms behind the observed patterns and the ecological implications in terms of characterizing plant responses to drought.

A brief history of the isohydry concept

The classification of plants based on their capacity to maintain a favorable water balance is a classic theme in environmental plant physiology (*e.g.*, Larcher 2003), which has led to a very rich, and not always consistent terminology. Terrestrial vascular plants are able to maintain their water content relatively stable despite fluctuations in water availability, thanks to a cuticle that minimizes evaporative water losses and large central vacuoles that stabilize the water content in the protoplasm (homoiohydric *sensu* Walter 1931). It was early realized, however, that vascular plants differ substantially in the degree to which they regulate transpiration to maintain an adequate water balance over diurnal and seasonal timescales. This variability led to the distinction between the hydrostable and hydrolabile types (Stälfelt 1939) and between the isohydric and anisohydric behaviours (Berger-Landefeldt 1936; Stocker 1956). There is a close correspondence between these two classifications, with hydrostable/isohydric species having sensitive stomata and relatively constant water status, and hydrolabile/anisohydric plants having opposite characteristics. Importantly, these two classifications originated before the water potential concept became widely used in plant physiology (Slatyer & Taylor 1960; Scholander *et al.* 1965), and therefore focused more (initially) on the ability to regulate transpiration than on the capacity to maintain relatively stable Ψ_L per se. The iso-/anisohydry dichotomy also predated the work on xylem water transport showing that hydraulic conductivity was also a function of water potential (Milburn 1966; Zimmermann 1983; Tyree & Sperry 1989). All this might explain why a formal definition of the iso-/anisohydric behaviours has remained somewhat elusive and current definitions usually mix the cause (stomatal control) with its expected consequence (water potential regulation) (*e.g.*, Jones 1998; Klein 2014; Meinzer *et al.* 2014; Skelton *et al.* 2015).

The current use of the iso-/anisohydry dychotomy stresses the physiological responses, but it is tightly connected to a plethora of more ecological classifications of plant water use under drought (Schultz 2003). These latter classifications have also a long tradition and oppose plants that tend to reduce transpiration early on during drought development to save water (water savers, pessimistic or drought avoiders) with plants that maintain transpiration rates for longer under drought (water spenders, optimistic or drought tolerant) (Shantz 1927; Turner 1979; Jones 1980; Ludlow 1989). These classifications have obvious implications in terms of competition for limited soil water resources and the coexistence of different plant functional types in water-limited systems (Bunce *et al.* 1977). More broadly, they reflect the general distinction between conservative/stress tolerant and acquisitive/competitive strategies of plant resource use (Grime 1974; Díaz *et al.* 2016).

Towards a definition of iso- and anisohydry

It is our view that the iso-/anisohydry concepts will only be operational and useful in advancing our understanding of plant water relations if we are able to define them precisely in terms of measurable quantities. Despite the original focus on the regulation of transpiration (Berger-Landefeldt 1936;

Stocker 1956), it seems more consistent with the current use of the terms and their etymology to emphasize the maintenance of relatively constant leaf water potential (Tardieu & Simonneau 1998; Sperry *et al.* 2002). However, even in this more restrictive meaning isohydry can be defined in several ways and at different temporal scales. We focus here on seasonal patterns and advocate for a continuous measure of the degree of isohydry instead of distinguishing only between two idealized extreme behaviours, which would always be somewhat arbitrary (Klein 2014; Martínez-Vilalta *et al.* 2014).

Figure 1 presents a hydraulic framework where alternative definitions of the degree of isohydry can be mapped. Reduced soil water availability (lower, more negative Ψ_{soil}) may affect plant conductance in two ways, by lowering its hydraulic conductance (K_H) and/or its leaf conductance (g_L). These reductions, however, have opposite effects on the water potential difference through the plant ($\Delta\Psi = |\Psi_L - \Psi_{soil}|$): whereas lower K_H increases $\Delta\Psi$, lower g_L decreases $\Delta\Psi$ (everything else being equal). The net change in $\Delta\Psi$ will thus depend on the balance between these two processes (*i.e.*, the relative sensitivity of transpiration vs. hydraulic transport to declining Ψ_{soil}) (Martínez-Vilalta *et al.* 2014), with the complication that any change in Ψ_L through changes in $\Delta\Psi$ will feedback onto K_H and g_L. These feedbacks underlie the tight coordination between hydraulic and water vapor transport at the plant level (Sperry & Love 2015). The dual control of g_L by Ψ_{soil} and Ψ_L reflects the response of g_L to both soil water availability and leaf water status (Tardieu & Simonneau 1998), albeit in a very simplified way (Damour *et al.* 2010; Buckley & Mott 2013). On the other hand, the response of K_H to Ψ_{soil} and Ψ_L reflects hydraulic losses in different parts of the plant experiencing different water potentials (*e.g.*, rhizosphere and roots vs. stems or leaves). An important aspect here is the temporal reversibility of these relationships. No hysteresis is normally assumed for g_L responses (but see Martorell *et al.* 2014; Tombesi *et al.* 2015), whereas K_H recovery following increases in soil water availability (higher Ψ_{soil} and Ψ_L) remains highly controversial, particularly with regards to rapid refilling of previously embolized xylem conduits under negative water potentials (Sperry 2013; Delzon & Cochard 2014; Trifilò *et al.* 2014).

We examine here three possible definitions of the degree of isohydry, assuming that predawn leaf water potential (Ψ_{PD}) reflects soil water availability as perceived by the plant (but see Donovan *et al.* 2003), and that midday leaf water potential (Ψ_{MD}) measures Ψ_L under maximum daily water demand. Firstly, one could define isohydry simply in terms of the minimum seasonal Ψ_{MD} experienced by a given species or population, with relatively high (close to zero) values implying a more isohydric behaviour. This is the definition adopted in practice by many studies, but it has important limitations, as it is greatly affected by the severity of the drought conditions for which Ψ_{MD} values are reported. In practice, most of the variability in minimum Ψ_{MD} across species is explained by minimum Ψ_{PD} (Figure 2), and within a site (constant climate) Ψ_{PD} is largely affected by rooting extension and depth (Davis *et al.* 1998; West *et al.* 2012; Nardini *et al.* 2016) (see also section 'What determines minimum operating leaf water potentials across species?' below). A second definition that stresses more the regulation of water potential inside the plant would be the seasonal variability of Ψ_{MD}, with more isohydric species showing less variability. This variability could be measured as the range or standard deviation of seasonal Ψ_{MD} values. This definition reduces, to some extent, the effect of differences in rooting systems across species, but it is still heavily affected by the range of water availability conditions under which water potential measurements are taken. Finally, a third alternative would be to define isohydry from the relationship between Ψ_{MD} and Ψ_{PD}, with flatter slopes defining more isohydric behaviours. One such measures was proposed by Martínez-Vilalta *et al.* (2014) (σ parameter). Note, however, that other definitions are possible, for instance assuming more complex relationships between Ψ_{MD} and Ψ_{PD} or regressing $\Delta\Psi$ against Ψ_{PD} (instead of Ψ_{MD} against Ψ_{PD}), which may result in significantly different species rankings (Meinzer et al. 2016).

Do isohydric species close stomata earlier than anisohydric species?

We claim here that the relationship between stomatal control and the regulation of leaf water potential, as well as its implications for plant survival under drought, have not been assessed as thoroughly as one might expect for what we feel is one of the foundations of our current understanding of plant drought responses. In order to test whether tighter water potential regulation (~isohydric behaviour) is associated with lower g_L under drought, we collected two databases, one across species growing in different sites and a second one focusing on species coexisting at the same sites. These databases, as well as the results of the corresponding analyses, are presented as case studies in the following sub-sections. Note that we use the more general term leaf conductance to water vapour (g_L) throughout the manuscript, but the values we take from the literature are frequently reported as stomatal conductances. These two conductances are considered equivalent unless stomata are nearly or completely closed, in which case other elements (*e.g.*, cuticular conductance) become relevant.

1. The relationship between stomatal control and water potential regulation across species

For this analysis we used the global water potentials database from Martínez-Vilalta *et al.* (2014). This database contains Ψ_{PD} and Ψ_{MD} data from 83 articles and includes 102 species growing under Temperate (*n* = 44, including one Boreal species), Mediterranean (*n* = 33), Tropical (*n* = 15) and Dry (*n* = 10) climates. We first asked whether using different measures of isohydry (*cf*. above) resulted in different rankings of species. Our results show that this is very clearly the case, particularly when comparing the σ parameter with the two definitions directly based on Ψ_{MD} (Figure 3). The correlation between the minimum Ψ_{MD} and the seasonal range of Ψ_{MD} values across species was high (r = -0.94), whereas it was very low and not even statistically significant when relating σ with minimum Ψ_{MD} values (r = -0.04) or with the Ψ_{MD} range (r = 0.18). As an example, *Tamarix chinensis*, the second most isohydric species in terms of σ (lowest decline in Ψ_{MD} per unit of reduction in Ψ_{PD}) reached rather low minimum Ψ_{MD} (-4.6 MPa) and showed considerable seasonal range of Ψ_{MD} values (2.9 MPa). These results highlight how important it is to agree on a precise definition of isohydry and stick to it if we are to make any progress on that matter.

In a second step, we retrieved seasonal g_L data from the papers included in the Martínez-Vilalta *et al.* (2014) database. A total of 33 papers reported this variable as well as water potentials, including 44 species (Table S1). In some species, g_L measurements were very abundant at high Ψ_{PD} (well-watered conditions), effectively providing several g_L values at a given Ψ_{PD}. To avoid putting excessive weight to these measurements data was previously summarized by calculating the maximum g_L by 0.1 MPa intervals whenever more than one measurement was available per bin, and these values were used in all further analyses. Using average instead of maximum g_L per bin provided essentially identical results. Mixed linear models were used to fit the (seasonal) relationship between $\log_{10}(g_L)$ and Ψ_{PD} within and across species. Species and the combination of study by treatment (when present) nested within species were included as random effects on the intercept (both) and slope of the model (only species). 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 (see Martínez-Vilalta *et al.* (2014) for details). A logarithmic transformation of g_L (exponential relationship between g_L and Ψ_L) is commonly used (*e.g.*, Hoffmann *et al.* 2011) and in our case it improved the distribution of residuals and model fit in terms of the Akaike information criterion (AIC) relative to other functional relationships between g_L and Ψ_{PD} (linear, power). The resulting model provided an overall good fit to the data (conditional $R^2 = 0.95$, marginal $R^2 = 0.50$), and the species-level random slopes (γ) were used as an estimate of stomatal sensitivity to Ψ_{PD} for each species. Similarly, the intercept of the relationship was used to calculate maximum g_L at $\Psi_{PD} = 0$ (g_{L0}). An exponential relationship between g_L and Ψ_{PD}, as assumed here, implies that the relative change in g_L per unit change in Ψ_{PD}, determined by the slope, is constant. Consequently, the γ values estimated for each species were used to calculate the water potential decline required to reduce g_L by 50% ($\Psi_{gL50} = \ln(0.5)/\gamma$). These values ranged between -0.62 MPa (*Vicia fava*) and -8.7 MPa (*Larrea* *tridentata*). The value of Ψ_{gL50} could not be estimated for *Tamarix ramosissima* because its almost flat relationship between $\log_{10}(g_L)$ and Ψ_{PD} resulted in an unrealistically low value (~-71 MPa).

Species' estimates of stomatal sensitivity (γ values) were used to test whether stomatal behaviour is associated with different measures of isohydry in terms of water potential regulation. The results of these analyses showed that, as expected, species with less sensitive stomata experience lower minimum Ψ_{MD} values ($R^2 = 0.20$, $P = 0.003$) and higher seasonal changes in Ψ_{MD} ($R^2 = 0.21$, $P = 0.002$) (Figure 4a,b). However, stomatal sensitivity was unrelated to σ ($R^2 = 0.00$, $P = 0.94$) (Figure 4c), implying that higher stomatal sensitivity did not result in stronger Ψ_{MD} regulation as Ψ_{PD} declined under drought. Very similar relationships were obtained if Ψ_{gL50} was used instead of γ to characterize stomatal responses (excluding *T. ramosissima*). Maximum leaf conductance (g_{L0}) was unrelated to the three isohydry measures we employed ($P > 0.3$ in all cases). Overall, these results indicate that species operating at higher (less negative) water potentials tend to close stomata faster with declining Ψ_{PD} than species experiencing lower water potentials, but this does not imply an association between stomatal control and water potential regulation inside the plant (as measured by the σ parameter) across species.

We can further ask whether different degrees of water potential regulation are associated to greater constrains to gas exchange through stomatal regulation (over time). Leaf conductance (g_L) values were obtained from the same studies from which water potential data had been retrieved (Table S1) and were pre-processed in exactly the same way as explained above (*e.g.*, 0.1 MPa binning). We analyzed the relationship between our three measures of isohydry and two measures of seasonal stomatal behaviour: average g_L over the whole study period covered by each study ($g_{L,mean}$), as a measure of absolute gas exchange during a drought event; and the ratio of $g_{L,mean}$ to maximum measured g_L ($g_{L,ratio}$) over the same period, as a relative measure of gas exchange. Our results show that none of our three measures of isohydry was related to $g_{L,mean}$ across species ($P > 0.36$ in all cases)

(Figure 5d,e,f). Similarly, the σ parameter and $g_{L,ratio}$ were unrelated ($P = 0.58$) (Figure 5c). However, species experiencing lower minimum Ψ_{MD} or wider seasonal ranges of Ψ_{MD} presented lower values of $g_{L,ratio}$ ($R^2 = 0.16$, $P = 0.007$; and $R^2 = 0.12$, $P = 0.022$; respectively) (Figure 5a,b). This result implies that species operating at lower water potentials or experiencing wider water potential fluctuations closed stomata more strongly during the period covered by each study than those species operating at less negative water potentials, contrary to the notion that lower g_L is associated with maintaining less negative water potentials across species. Interestingly, stomatal sensitivity (measured as γ or Ψ_{gL50}) was unrelated to $g_{L,mean}$ or $g_{L,ratio}$ ($P > 0.25$ in all cases), due to the fact that species with more sensitive stomata tended to operate at higher Ψ_L (Figure 4) and, thus, closed stomata to a similar extent than species with less sensitive stomata but operating at lower Ψ_L.

2. Stomatal control vs. water potential regulation among coexisting species

Assessing the relationship between stomatal control and water potential regulation across species occupying different environments, as done in the previous section, may be problematic because it mixes plants growing under very different conditions, including exposure to drought stress. To overcome this limitation we conducted a similar analysis focusing on the comparison of coexisting species measured concurrently in the same sites, and thus having similar exposure to drought. A new global database was compiled using mostly published sources. We searched the literature for studies fulfilling the following criteria: (1) they compared different species (or cultivars with contrasted stomatal behaviour in the case of *Vitis vinifera*) growing at the same site under the same environmental conditions; (2) focused on the study of drought effects (including experimental and naturally occurring droughts) over a period of weeks to months; (3) reported multiple measures of Ψ_{PD}, Ψ_{MD} and g_L; (4) these three variables were measured concurrently and could be linked to each other (directly or through third variables such as time); and (5) the range of measured Ψ_{PD} values was > 1 MPa for at least one of the species included in the study, to ensure drought severity was substantial. We also added an unpublished dataset including measures on *Phillyrea latifolia* and

Quercus ilex planted on the ground in a tunnel greenhouse and subjected to a drought-simulation experiment (N. Garcia-Forner *et al.*, unpublished). Altogether, we compiled data from 15 datasets, covering mostly Mediterranean (n = 9) and Dry climates (n = 3) (Table S2). Each study compared between two and seven species growing under the same environmental conditions (33 species in total), except two studies on *Vitis vinifera* that compared two different cultivars each.

Using this database we calculated the species-level slopes of the relationships between Ψ_{MD} and Ψ_{PD} (parameter σ) and between $\log_{10}(g_L)$ and Ψ_{PD} (parameter γ) as explained in the previous section. We fitted a different mixed model for each study, with species as a random effect on the intercept and slope. Model fits were generally good, with conditional R^2 = 0.3 – 0.98 for the regressions between Ψ_{MD} and Ψ_{PD} and conditional R^2 = 0.32 – 0.93 for the regressions between $\log_{10}(g_L)$ and Ψ_{PD}. As before, species' estimates of stomatal sensitivity (γ and Ψ_{gL50} values) were used to test whether stomatal behaviour is associated with different measures of isohydry (minimum Ψ_{MD}, range of Ψ_{MD} values and σ) using mixed models with site as a random factor. In all cases, model fit in terms of AIC was best when random effects were included on the intercept only. The overall relationships were similar to those obtained in the previous section using the global database (compare Figure 6a,c,e with Figure 4), with γ being positively related to minimum Ψ_{MD} (P = 0.001), negatively related to Ψ_{MD} range (P = 0.002), and unrelated to σ (P = 0.17). However, since we were interested in the comparison within sites and the previous analysis mixes the effect of stomatal sensitivity within and between sites, we also used mixed linear models to fit the relationships between the three measures of isohydry and centered γ (or Ψ_{gL50}) values. Centering was achieved by subtracting the average γ (or Ψ_{gL50}) for the corresponding site to each species' γ (or Ψ_{gL50}) value, and ensured that fixed effects were evaluated only within sites. The relationship between centered stomatal sensitivity and the three isohydry measures was not significant (P > 0.05 in all cases) (Figure 6b,d,f), although the (negative) effect of γ on σ was close to significant (P = 0.06). Overall, these results indicate that stomatal sensitivity and water potential regulation are largely unrelated when comparing coexisting species within a site.

We used a similar approach to explore whether different degrees of water potential regulation are associated to greater constrains on gas exchange through stomatal regulation when comparing coexisting species. As before, mixed linear models with site as random factor were used to fit the relationships between $g_{L,mean}$ and $g_{L,ratio}$ (response variables) and the three measures of isohydry (centered minimum Ψ_{MD}, centered Ψ_{MD} range and centered σ). In all cases, the best fitting model in terms of AIC included the random effect of site on the intercept but no effect of the fixed explanatory variable ($P > 0.35$ for all model comparisons), indicating that our three measures of isohydry were unrelated to stomatal behaviour when comparing different species measured within a site (Figure 7). The corresponding plots using non-centered explanatory variables instead of centered values are provided in Figure S1.

Why are water potential regulation and stomatal behaviour decoupled across species?

The results reported in the previous sections have to be considered with caution, as they come from a synthesis of different data sources, each covering different time periods and using potentially different experimental protocols. However, our analyses at two different levels (across species and within sites) suggest that water potential regulation and stomatal control are largely unrelated across species. Of course, this is not to mean that these variables are not related in general. It is very well established both theoretically and empirically that, for a given plant, stomatal closure reduces transpiration and hence limits the water potential difference between the soil and the leaves and the risk of hydraulic failure (Tyree & Sperry 1988; Jones & Sutherland 1991; Sperry *et al.* 2002; Cochard *et al.* 2002). However, the situation becomes more complex when we compare different species, even if they grow at the same site. This is because water potential dynamics are affected by several plant attributes that are coordinated across species, including stomatal behaviour but also hydraulic architecture and root properties. At a given point in time, rooting extension and depth will determine how a certain soil water availability is translated into a plant Ψ_{PD} (Jackson *et al.* 2000; Sperry & Hacke 2002; Martínez-Vilalta *et al.* 2002). The water potential drop in the plant ($\Delta\Psi$), and hence Ψ_{MD}, depends on the ratio between transpiration rate and hydraulic transport capacity (Martínez-Vilalta *et al.* 2014); which are largely determined by the ratio between stomatal and hydraulic conductance. Dynamic aspects are important here, as both stomatal and hydraulic conductances are affected by Ψ_{MD} (Figure 1). At longer time scales, stomatal conductance and transpiration (including the effects of vapour pressure deficit and leaf area dynamics) will determine the rate of water extraction from the soil and, therefore, will feed back into Ψ_{PD}: species showing higher transpiration rates will deplete soil water faster and hence experience also faster reductions in Ψ_{PD} over time. In addition, once the hydraulic system of the plant is disconnected from the soil (complete loss of hydraulic conductivity somewhere in the hydraulic pathway) Ψ_{PD} will cease to track fluctuations in soil water potential.

Arguably, the ultimate minimum water potential a plant can withstand is determined by the vulnerability of its hydraulic system (Brodribb & Cochard 2009; Nardini *et al.* 2013; Urli *et al.* 2013; Brodribb *et al.* 2014). The high degree of phylogenetic conservatism in vulnerability to xylem embolism (Maherali *et al.* 2004) supports the notion that hydraulic vulnerability may have driven differences in water potential regulation over evolutionary time scales. Relatively high hydraulic vulnerability (*e.g.*, low resistance to xylem embolism) tends to be associated with tight stomatal control across species (Brodribb *et al.* 2003; Arango-Velez *et al.* 2011; Klein 2014) and also within species (*e.g.*, when comparing *Vitis vinifera* cultivars with contrasted stomatal behaviour, Tombesi *et al.* 2014). This association is also supported by the positive relationship between the water potential causing 50% loss of hydraulic conductivity in stem xylem (Ψ_{PLC50}), obtained from the Choat *et al.* (2012) database, and stomatal sensitivity as obtained from our analysis (*cf.* ‘1. The relationship between stomatal control and water potential regulation across species’ section above) (Figure S2).

The proximal mechanism underlying the coordination between vapour and liquid phase water transport in plants is provided by the response of stomata to hydraulic signals (Meinzer 2002; Sperry *et al.* 2002; Buckley 2005; Meinzer *et al.* 2009). This response is complex and has several potentially problematic aspects, including the fact that Ψ_L, the obvious integrator of leaf water status to which stomata may respond through its effect on guard cell turgor (Figure 1), is also the same variable that is maintained relatively constant as a result of stomatal control. There is indeed plenty of evidence showing stomatal responses to hydraulic signals without significant changes in bulk Ψ_L (Sperry & Pockman 1993; Saliendra *et al.* 1995; Salleo *et al.* 2000; Hubbard *et al.* 2001). However, this is still consistent with a regulation of stomatal conductance through a negative feedback with Ψ_L if we consider that embolism itself may provide the amplification required to achieve nearly homeostatic regulation of leaf water potential (Buckley 2005) (Figure 1). Hormonal signals play also a prominent role in modulating stomatal responses, particularly through abscisic acid (ABA) synthesis in roots and leaves and its subsequent accumulation in leaves (Mittelheuser & Van Steveninck 1969; Zhang & Davies 1989; Bauer *et al.* 2013; Tombesi *et al.* 2015). Recent evidence suggests that stomatal closure under drought stress evolved from a passive, purely hydraulic process, to the more complex mechanism involving hormonal signalling and active ion exchange between guard and epidermal cells currently characterizing most angiosperms, with stomatal regulation in conifers being intermediate between these two modes (Brodribb & McAdam 2010; McAdam & Brodribb 2014, 2015).

Even within conifers, stomatal closure seems to be induced by two contrasted mechanisms. Whereas some species show fast stomatal closure under drought in response to sustained high levels of ABA in leaves, a second group of species show slower stomatal responses at lower Ψ_L (Brodribb *et al.* 2014). Importantly, these two contrasting modes of stomatal regulation are associated to differences in vulnerability to xylem embolism, with the first mode of stomatal regulation described above being characteristic of species with more vulnerable xylem (Brodribb *et al.* 2014). This association has been interpreted to imply that the first group of species shows a more active stomatal regulation, whereas the second group responds directly to Ψ_L (Brodribb *et al.* 2014). These results, however, are also compatible with the view that hydraulic signals are important drivers of stomatal movements in both groups, as the amplifying effect of xylem embolism on hydraulic signaling would occur at higher water potentials in more vulnerable species. Although it is unclear to what extent this framework may extend to angiosperms, it is intriguing to speculate that divergent pathways of stomatal regulation may underlie the large differences in hydraulic safety margins between angiosperms and gymnosperms (Choat *et al.* 2012).

What determines minimum operating leaf water potentials across species?

If stomatal control is largely unrelated to water potential regulation across species, what drives the large differences in minimum leaf water potential among coexisting species? Or, in other words, what plant attributes are associated with maintaining relatively high and stable (as opposed to low and declining) leaf water potentials under drought? The first one is obviously deep rooting, particularly considering the tight relationship between Ψ_{MD} (~Ψ_L) and Ψ_{PD} (~Ψ_{soil}) reported in Figure 2. Species with more extensive and deeper root systems are able to access more stable water sources, thus buffering changes in hydrological conditions (Jackson *et al.* 2000; Oliveira *et al.* 2005). Accordingly, deep-rooted species should be able to maintain less negative and more stable water potentials (particularly Ψ_{PD}), everything else being equal. Although there are many cases in which this is the case (Bucci *et al.* 2009; West *et al.* 2012), there appear to be also counterexamples in which species known to be relatively shallow-rooted operate at higher water potentials than coexisting deep-rooted species (West *et al.* 2007; Plaut *et al.* 2012; Aguadé *et al.* 2015).

Minimum Ψ_L is also associated to the vulnerability to xylem embolism, both at the local and global scales (Pockman & Sperry 2000; Choat *et al.* 2012), with more resistant species being able to operate at lower Ψ_L. Globally, a positive relationship between resistance to xylem embolism and rooting depth is to be expected, as these two characteristics tend to occur under similar environmental conditions (Schenk & Jackson 2002; Maherali *et al.* 2004; Choat *et al.* 2012). Within a given site, however, species that are hydraulically more vulnerable and cannot sustain very low water potentials may require deeper root systems. Accordingly, a number of studies report negative relationships between resistance to xylem embolism and rooting depth (Hacke *et al.* 2000; Sperry & Hacke 2002; Lopez *et al.* 2005), although exceptions occur (Pivovaroff *et al.* 2016; Nardini *et al.* 2016). Species that are shallow-rooted and relatively vulnerable to xylem embolism may disconnect their hydraulic system from the soil early during drought development. This disconnection may be purely hydraulic or physical, involving fine root mortality (Bauerle *et al.* 2008; Espeleta *et al.* 2009), and it is frequently associated to drought deciduousness (Kolb & Davis 1994; Miranda *et al.* 2010; Hoffmann *et al.* 2011). Dynamic aspects related to vertical water redistribution in the soil may also be important in explaining differences in Ψ_L dynamics between coexisting species with different root distributions (Meinzer *et al.* 2004; Neumann & Cardon 2012).

If the hydraulic system of the plant remains connected to the soil, the rate of transpiration and water uptake will affect Ψ_{soil} dynamics in the rooting zone and, hence, will contribute to explain differences in Ψ_L dynamics across species (Mitchell *et al.* 2012) (Figure 1). Maintenance of relatively high transpiration rates under drought (high g_L) will deplete soil water resources faster and will result in steeper declines in Ψ_{soil} and Ψ_{PD} over time. An important additional aspect is that in general this effect will be driven not only by the water uptake of the target plant but also by all individuals with roots within its belowground neighborhood (Casper & Jackson 1997; Zavala & Bravo de la Parra 2005). Our results suggest that the positive relationship between stomatal sensitivity and minimum Ψ_{MD} across species (Figures 4 and 6a) may be more associated to the effect of water uptake (or to the covariation with rooting depth) than to the role of stomatal control on water potential regulation inside the plant (as measured by the σ parameter).

Assuming steady state, the water potential gradient within the plant will be determined by the maximum transpiration rate per unit of hydraulic transport capacity, which defines the leaf water potential at $\Psi_{soil} \approx 0$; and by the relative sensitivity of transpiration and the plant hydraulic system to declining Ψ_{PD} (σ; *cf.* Figure 1) (Martínez-Vilalta *et al.* 2014). An important result of recent data syntheses is that the vulnerability of stem xylem to embolism (measured as Ψ_{PLC50}) appears to be more variable than stomatal sensitivity across species, with Ψ_{gL50} rarely falling below -4 MPa (Klein 2014; Manzoni 2014; Skelton *et al.* 2015; Mencuccini *et al.* 2015). This result is difficult to reconcile with the fact that species with lower stem Ψ_{PLC50} (Ψ_{PLC50} << -4 MPa) have higher σ values (σ ~ 1) (Martínez-Vilalta *et al.* 2014), unless we consider that hydraulic bottlenecks are more likely to be in the roots (Hacke *et al.* 2000; Jackson *et al.* 2000; Martínez-Vilalta *et al.* 2002) or leaves than in the stem (Tyree & Ewers 1991; Pivovaroff *et al.* 2014; Bouche *et al.* 2016; Hochberg *et al.* 2016). Since stomata typically close around the leaf turgor loss point (Ψ_{tlp}), the fact that stomata rarely close at very low Ψ_L likely reflects the limits of osmoregulation and the inability of plant leaves to maintain turgor at very low Ψ_L (Brodribb *et al.* 2003; Brodribb & Holbrook 2003; Hao *et al.* 2010; Bartlett *et al.* 2012). Although a recent global synthesis shows relatively low plasticity in Ψ_{tlp} in most species (Bartlett *et al.* 2014), high plasticity in Ψ_{tlp} has been reported in some 'anisohydric' species and likely represents an adaptation for coping with low and fluctuating water potentials (Meinzer *et al.* 1986, 2014). Substantial reductions in Ψ_{tlp} with declining Ψ_L (together with high hydraulic compartmentalization in the leaf, Buckley *et al.* 2015) may help explain the puzzling result that many species from dry habitats appear to operate largely below their Ψ_{tlp} as determined on fully rehydrated samples (Meinzer *et al.* 2014).

In most field situations non-steady state conditions prevail, implying that the water content in the plant is not constant. Hydraulic capacitance, the water content change per unit of variation in water potential, allows the plant to (partially) uncouple the changes in transpiration from water potential dynamics, effectively dampening the temporal fluctuations in Ψ_L (Meinzer *et al.* 2003, 2009; Sperry *et* *al.* 2007). Species differ widely in their sapwood capacitance, and this variability is associated with other hydraulic traits. In particular, higher sapwood capacitance seems to be associated with higher water potentials, lower resistance to xylem embolism and narrower hydraulic safety margins (Pratt *et al.* 2007; Sperry *et al.* 2007; Meinzer *et al.* 2009; Mcculloh *et al.* 2014). Clearly, capacitance and water storage need to be considered as additional elements, together with changes in stomatal and hydraulic conductance, determining the water potential regulation inside the plant, and hence Ψ_L at a given Ψ_{soil} (Matheny *et al.* 2015). At very low water potentials stomata close completely and plant water losses are driven by leaf cuticular conductance. If severe embolism has not yet developed, cuticular conductance will determine the time needed to reach hydraulic failure and thus low cuticular conductance can confer substantial drought tolerance (Scoffoni *et al*. 2011; Blackman *et al.* 2016). However, our knowledge on the determinants and implications of the variability of cuticular conductance across species is limited (the last review we are aware of was written 20 years ago by Kerstiens (1996)) and requires further research.

Implications for drought-induced mortality mechanisms

An important implication of our results is that isohydric species in terms of water potential regulation are not necessarily more carbon limited than anisohydric species. When comparing species coexisting within a given site there is no relationship between any of the three measures of isohydry used in this study and average g_L (either in absolute terms or relative to the seasonal maximum g_L; Figure 7). When this relationship is assessed across species growing at different sites, species experiencing lower minimum Ψ_{MD} or wider seasonal Ψ_{MD} range tend to have lower average g_L (relative to its maximum) (Figure 5), despite also having lower stomatal sensitivity (Figure 4). These results simply reflect that the range of minimum Ψ_{MD} across species and sites is wider than the range of stomatal sensitivities, which appears to be relatively constrained across species (Klein 2014; Manzoni 2014; Skelton *et al.* 2015; Mencuccini *et al.* 2015). Dynamic aspects may also contribute to this pattern, as relatively open stomata will result in higher rates of water use, faster declines of Ψ_{soil}

(~Ψ_{PD}) and, possibly, lower minimum Ψ_L. Of course, these considerations do not invalidate the argument that species closing stomata earlier during drought are likely to be more carbon constrained (everything else being equal) (McDowell *et al*. 2008; McDowell 2011). However, it places the emphasis on the temporal dynamics of gas exchange and drought responses (*cf*. Mitchell *et al.* 2012; McDowell *et al.* 2013) instead of focusing only on the responses to Ψ_L, which cannot be interpreted by itself precisely because isohydric and anisohydric species differ in the water potentials at which they operate (by definition). This view favours measures of stomatal control in which stomatal sensitivity is defined relative to hydraulic vulnerability (Martínez-Vilalta *et al.* 2014; Skelton *et al.* 2015).

There is convincing evidence that the plant carbon economy plays a relevant role during drought-induced mortality, at least in some cases (particularly for conifer species) (Galiano *et al.* 2011; O'Brien *et al.* 2014; Dickman *et al.* 2015; Garcia-Forner *et al.* 2016b). However, the carbon starvation mechanism as such remains controversial (McDowell & Sevanto 2010; Sala *et al.* 2010; Hartmann 2015) due to the difficulty in determining the precise timing of tree death and resolving the complex interactions between water and carbon relations under extreme drought (McDowell *et al.* 2011; Sevanto *et al.* 2014; Mencuccini *et al.* 2015). Arguably, however, one of the most important outcomes of the recent boost in drought-induced mortality research is the realization that hydraulic deterioration is ubiquitous under lethal drought, whereas reductions in carbon reserves are not (Hartmann *et al.* 2013; Rowland *et al.* 2015; Adams *et al.*, submitted). Although in some ways this result does not take us much further than the original hydraulic framework presented by McDowell *et al.* in 2008, it has important implications, as it emphasizes the importance of plant hydraulics and paves the ground for models of canopy conductance and drought responses based on relatively simple hydraulic principles (Sperry & Love 2015; Sperry *et al*. 2016). It also suggests hydraulic safety margins as a reasonable proxy for vulnerability to drought (Choat *et al.* 2012; Delzon & Cochard 2014). Of course assessing hydraulic safety margins is not free of complications (see next section) and does not give a complete account of drought resistance strategies in plants (Klein *et al.* 2013), but in our opinion it remains the best single predictor for drought responses we have currently at hand.

Conclusions and future directions

In this review we have shown that, contrary to what is usually assumed, a tight regulation of Ψ_L is not necessarily associated with greater stomatal control across species. Therefore, we advocate for a clear and quantitative definition of iso/anisohydry that separates these two concepts. This distinction is important, as iso/anisohydry defined in terms of Ψ_L regulation tells us little by itself about leaf gas exchange dynamics or the degree of hydraulic or carbon limitations under drought. Therefore, it cannot be used as an indicator of a specific mechanism of drought-induced mortality (*sensu* McDowell *et al*. 2008) or as a proxy for overall vulnerability to drought. The way we understand and define the iso/anisohydryc behaviours has important implications for the modelling of drought responses at scales that range from the individual to the ecosystem and the Biosphere (Roman *et al.* 2015; Combe *et al.* 2016).

Several issues remain that limit our understanding of plant water relations and our capacity to predict vegetation responses under ongoing climate change. Among other aspects, significant advances could be achieved by:

- Improving our understanding of how relevant traits scale up from the tissue to the whole-plant levels (Sperry *et al*. 2007; Meinzer *et al*. 2010; Petit & Anfodillo 2011) and, in particular, resolving where the hydraulic bottleneck is in the soil-plant-atmosphere continuum and how this bottleneck might change during drought. Candidates include the rhizosphere, the xylem of different organs and extraxylary tissues. This is a long-standing issue in plant hydraulics (Tyree & Ewers 1991; Sperry *et al.* 2002; Tyree & Zimmermann 2002; Sack & Holbrook 2006) and important contributions continue to be published (*e.g.*, Bouche *et al.* 2016; Hochberg *et al.* 2016).

- Accounting explicitly for the temporal (dynamic) dimension of drought responses, including: (i) the role of cuticular conductance, capacitance and water storage under extreme drought (Blackman *et al*. 2016); (ii) the temporal covariation between soil water deficit and atmospheric water demand and their interactive effects on plant water status; and (iii) the reversibility of stomatal and hydraulic conductance losses. Although there are strong arguments for hydraulic failure and repair not being routine in trees (Cochard & Delzon 2013; Delzon & Cochard 2014), evidence for novel refilling continues to accumulate (Brodersen & McElrone 2013; Trifilò *et al.* 2014; McCulloh & Meinzer 2015; Rolland *et al.* 2015). Whatever the outcome of this debate, it needs to be consistent with the evidence showing that vulnerability to xylem embolism plays a central role in plant water relations and drought responses, and therefore needs to address the limits of refilling and its associated costs. New imaging techniques (Brodersen *et al.* 2010; Cochard *et al.* 2015; Choat *et al.* 2016; Knipfer *et al.* 2016) hold promise for resolving this burning issue.
- Improving our understanding of the covariation between key water relations traits, including: rooting depth, maximum hydraulic conductance, maximum leaf conductance to water vapor, hydraulic vulnerability, stomatal sensitivity to plant water status, hydraulic capacitance, osmotic adjustment capacity and leaf habit. Community level assessments of these traits are becoming more frequent and complete (Hoffmann *et al.* 2011; West *et al.* 2012; Skelton *et al.* 2015; Pivovaroff *et al.* 2016). However, we still do not know enough about their relationships to define clear trait syndromes that would characterize general water-use and drought resistance strategies of plants. An additional important research question is establishing how these water relations traits map into the spectrum of variability defined by more standard plant functional traits (Markesteijn *et al.* 2011; Reich 2014; Díaz *et al.* 2016). The fact that the acquisitive versus conservative resource economies identified in global

assessments of plant form and function correspond well with ecological classifications of plant water-use strategies (*cf.* ‘A brief history of the isohydry concept’ above) is encouraging. Global functional trait databases (Kattge *et al.* 2011; Choat *et al.* 2012) will be instrumental to make progress in this area.

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Figure legends

Figure 1. Diagram illustrating some of the variables and relationships controlling leaf water potential (Ψ_L). Solid lines indicate positive relationships between variables, whereas broken lines indicate negative relationships. The dotted broken line indicates an effect on a control valve. Reduced soil water availability (higher absolute value of Ψ_{soil}) may affect plant conductance in two ways, by lowering its hydraulic conductance (K_H) and its leaf conductance (g_L). These reductions have opposite effects on the water potential gradient through the plant ($\Delta\Psi$), so that the net change in $\Delta\Psi$ will depend on the balance between these two processes, with the complication that changes in leaf water potential (Ψ_L) will feedback onto K_H and g_L. High transpiration rates (through high g_L) cause faster reductions in Ψ_{soil}, unless the hydraulic system of the plant becomes disconnected from the soil. Important plant attributes and processes (rooting depth, capacitance, osmoregulation) have been omitted for simplicity. See text for further details.

Figure 2. Relationship between seasonal minimum predawn (Ψ_{PD}) and midday leaf water potentials (Ψ_{PD}, both in MPa) across 102 plant species from the global database from Martínez-Vilalta *et al.* (2014). The overall regression line is depicted (solid line, with grey shadow indicating 95% confidence intervals). The 1:1 relationship is indicated by a broken line.

Figure 3. Three different measures of isohydry measured on 102 plant species (data from Martínez-Vilalta *et al.* 2014): minimum midday water potential (minimum Ψ_{MD}, MPa), seasonal range of Ψ_{MD} (MPa), and the slope of the relationship between Ψ_{MD} and Ψ_{PD} (σ, MPa MPa^{-1}). Species are ordered according to the former measure (left panel). Left and right y-axis provide species names for alternate bars to improve readability (odd ranks in the left axis and even ones in the right axis).

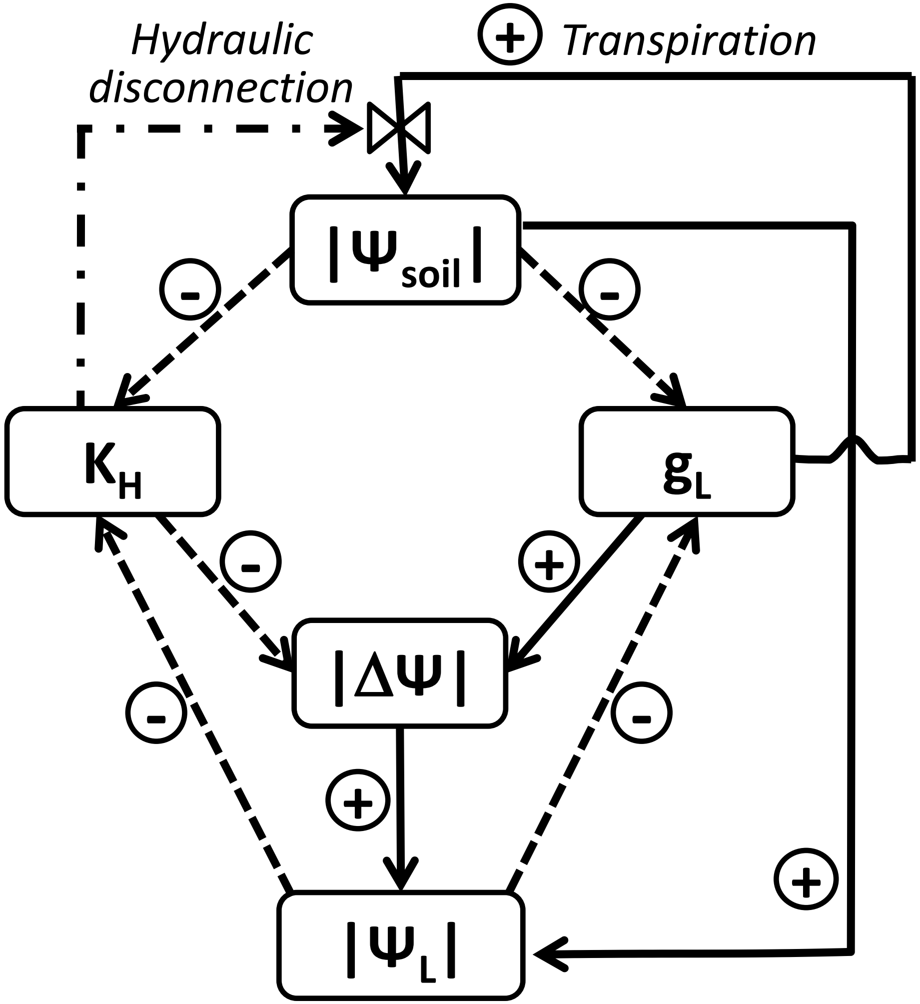
Figure 4. Relationship between stomatal sensitivity to decreasing predawn leaf water potential (γ, in log(mmol m^{-2} s^{-1}) MPa^{-1}) and three different measures of isohydry: (a) minimum midday water

potential (minimum Ψ_{MD}, MPa), (b) seasonal range of Ψ_{MD} (MPa) and (c) the slope of the relationship between Ψ_{MD} and Ψ_{PD} (σ, MPa MPa^{-1}) for 44 species (see text for details). Stomatal sensitivity was estimated as the slope of the (seasonal) relationship between $\log_{10}(g_L)$ and Ψ_{PD} (see text for further details). Solid and dashed lines indicate significant and non-significant relationships between variables. Grey-vertical lines show the equivalence of γ in terms of the water potential required to reduce leaf conductance to water vapour by 50 % (Ψ_{gL50}, in MPa). Species abbreviations are given in Table S1.

Figure 5. Relationship between stomatal behaviour over time and three different measures of isohydry: minimum midday water potential (minimum Ψ_{MD}, MPa), seasonal range of Ψ_{MD} (MPa), and the slope of the relationship between Ψ_{MD} and Ψ_{PD} (σ, MPa MPa^{-1}). Stomatal behaviour over time is characterized using two variables: the ratio of average g_L to maximum measured g_L ($g_{L,ratio}$; panels a, b, c) and average g_L ($g_{L,mean}$; panels d, e, f). Solid and dashed lines indicate significant and non-significant relationships between variables. Species abbreviations are given in Table S1.

Figure 6. Relationship between stomatal sensitivity to decreasing predawn leaf water potential and three different measures of isohydry for species coexisting at a given site (see text for details). Isohydry measures include minimum midday water potential (minimum Ψ_{MD}, MPa), seasonal range of Ψ_{MD} (MPa), and the slope of the relationship between Ψ_{MD} and Ψ_{PD} (σ, MPa MPa^{-1}). Stomatal sensitivity was estimated as the slope of the (seasonal) relationship between $\log_{10}(g_L)$ and Ψ_{PD} (see text for further details), and it is expressed in two different ways: as absolute γ values (log(mmol m^{-2} s^{-1}) MPa^{-1}; panels a, c, e) and as centered γ values (log(mmol m^{-2} s^{-1}) MPa^{-1}; panels b, d, f). Each dot indicates a species and colors designate studies. Species measured in the same study are linked by colored lines, to facilitate assessing the relationships within sites. Grey-vertical lines show the equivalence of γ in terms of the water potential required to reduce leaf conductance to water vapor by 50 % (Ψ_{gL50}, in MPa). Study codes are given in Table S2.

Figure 7. Relationship between stomatal behaviour over time and three different measures of isohydry for species coexisting at a given site (centered values; see text for details). Isohydry measures include minimum midday water potential (minimum Ψ_{MD}, MPa), seasonal range of Ψ_{MD} (MPa) and the slope of the relationship between Ψ_{MD} and Ψ_{PD} (σ, MPa MPa^{-1}). Stomatal behaviour over time is characterized using two variables: the ratio of average g_L to maximum measured g_L ($g_{L,ratio}$; panels a, b, c) and average g_L ($g_{L,mean}$; panels d, e, f). Each dot indicates a species and colours designate studies. Species measured in the same study are linked by coloured lines, to facilitate assessing the relationships within sites. Study codes are given in Table S2.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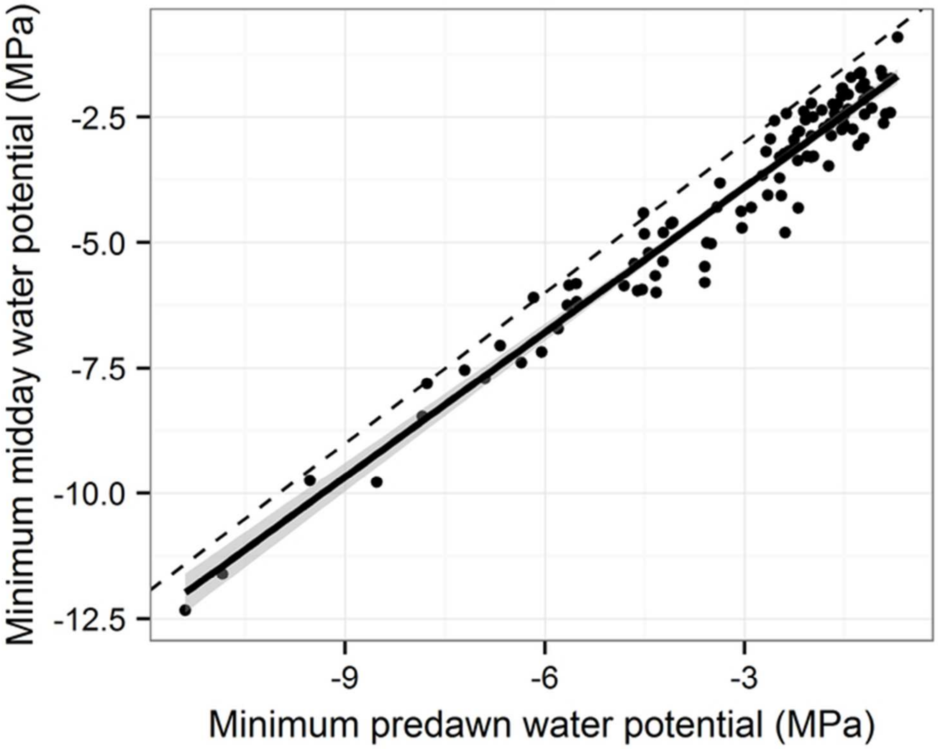

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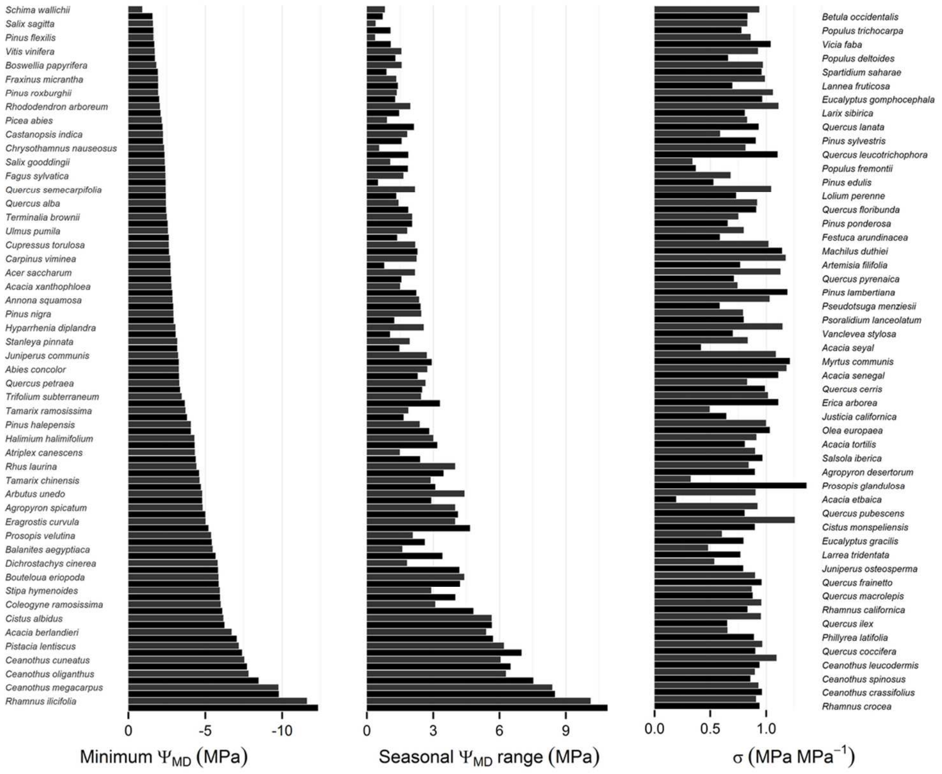

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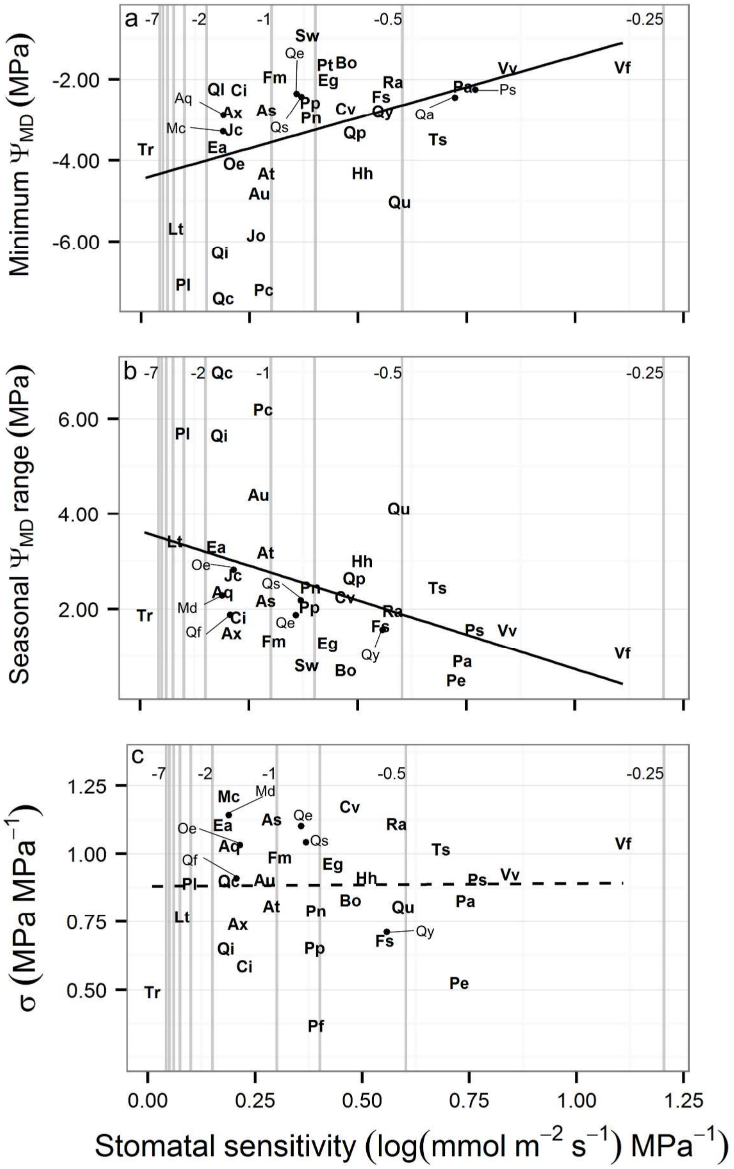

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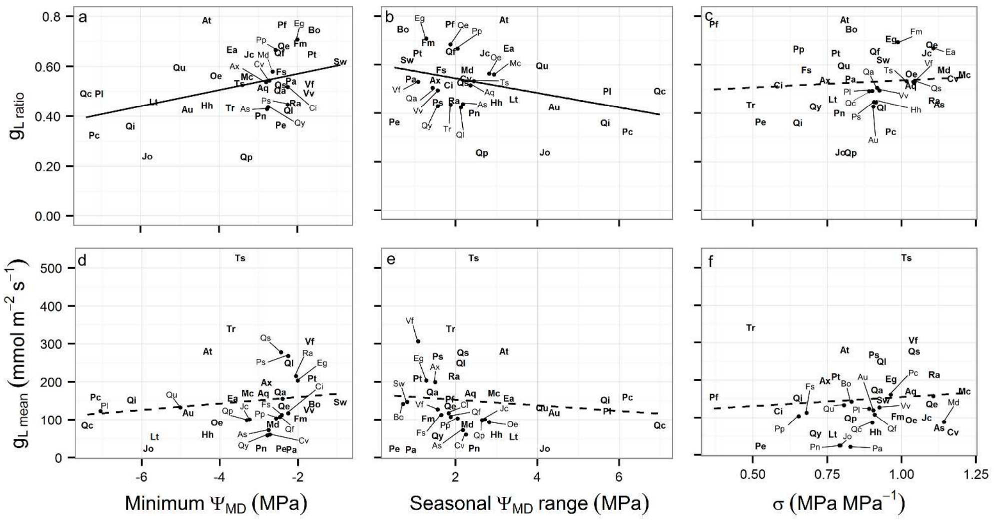

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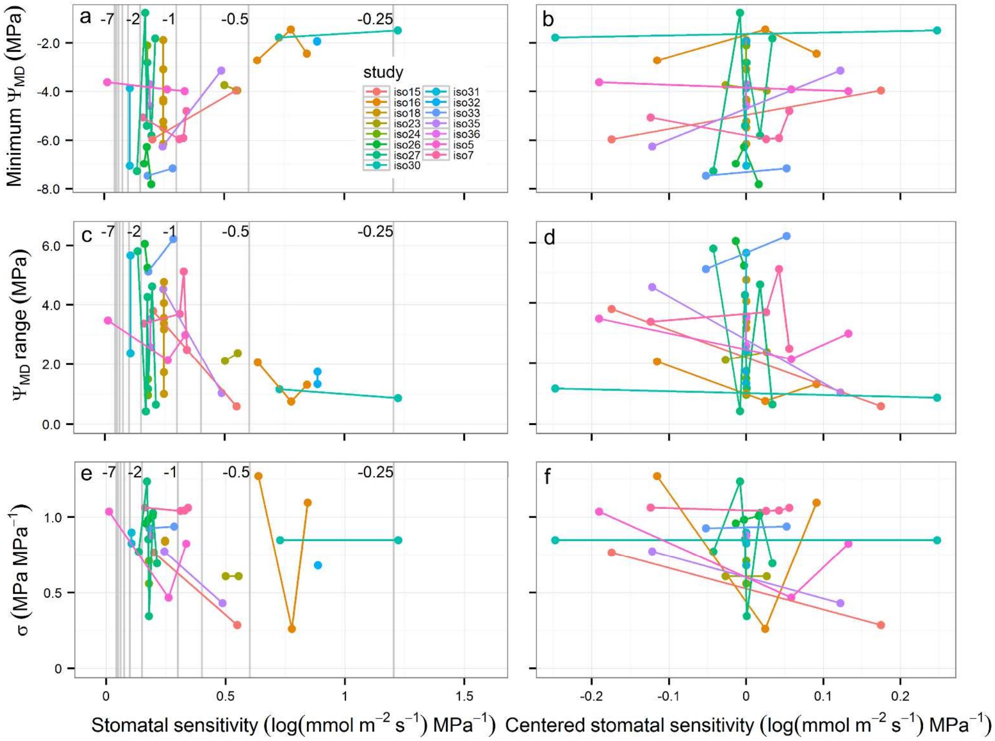

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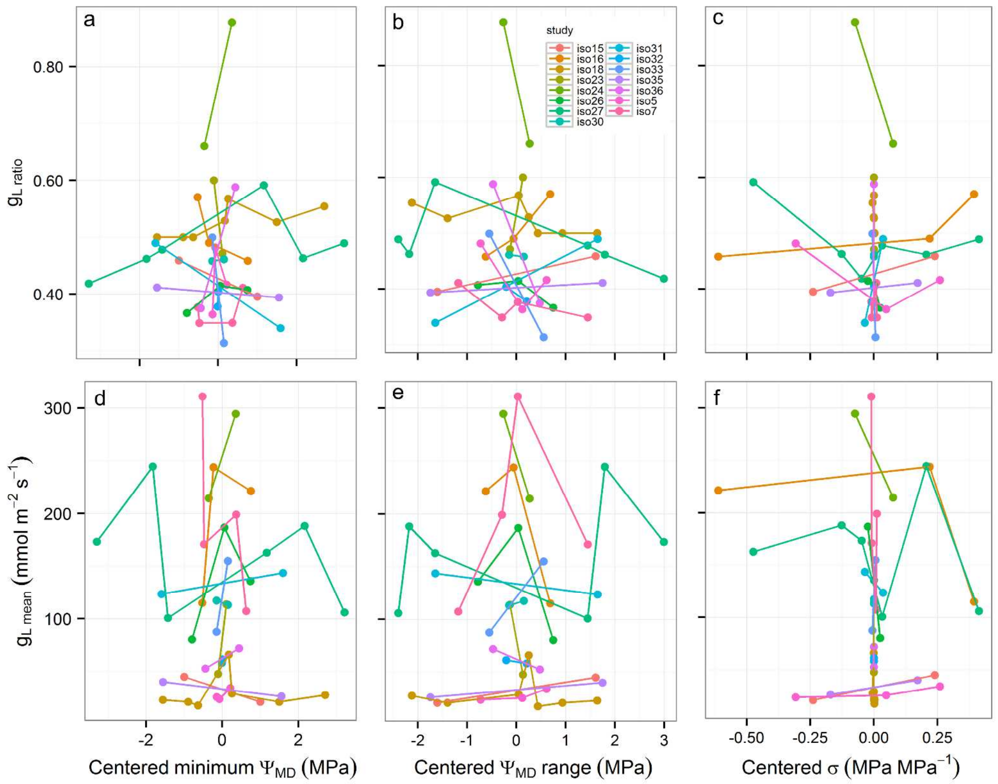

1041x822mm (96 x 96 DPI)

Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept

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Figure S1. Relationship between stomatal behaviour over time and three different measures of isohydry for species coexisting at a given site (see text for details). Isohydry measures include minimum midday water potential (minimum Ψ_{MD}, MPa), seasonal range of Ψ_{MD} (MPa) and the slope of the relationship between Ψ_{MD} and Ψ_{PD} (σ, MPa MPa^{-1}). Stomatal behaviour over time is characterized using two variables: the ratio of average g_L to maximum measured g_L ($g_{L,ratio}$; panels a, b, c) and average g_L ($g_{L,mean}$; panels d, e, f). Each dot indicates a species and colours designate studies. Species measured in the same study are linked by coloured lines. Study codes are given in Table S2.

Figure S2. Relationship between stomatal sensitivity to decreasing predawn leaf water potential (γ, in log(mmol m^{-2} s^{-1}) MPa^{-1}) and the water potential at 50 % loss of hydraulic conductivity in the stem (Ψ_{50PLC}) across species. Separate linear regressions are depicted for angiosperms and gymnosperms in red and blue, respectively. Grey shadows around lines indicating 95% confidence intervals. A linear model accounting for the differences between angiosperms and gymnosperms in the intercept of the relationship resulted in a highly significant Ψ_{PLC50} effect ($R^2 = 0.33$, $P = 0.007$). Overall model fit increased substantially if *Tamarix ramosissima*, a clear outlier of the relationship, was excluded from the analysis ($R^2 = 0.44$, $P < 0.001$). Species abbreviations are given in Table S1. Ψ_{50PLC} data was obtained from Choat *et al*. (2012).

Figure S1

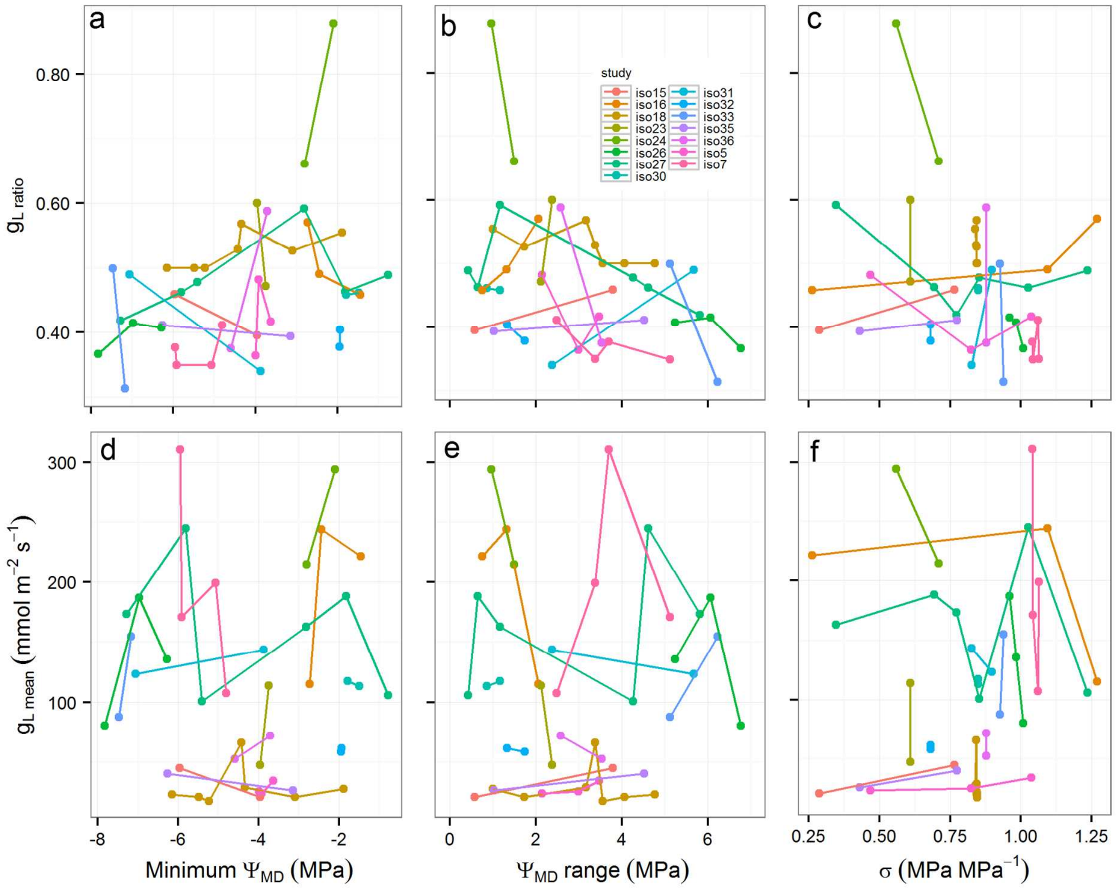
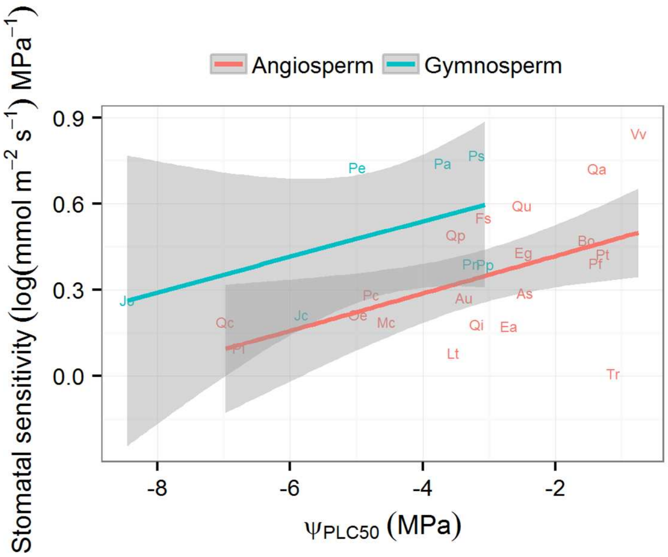

a
b
c
d
e
f
study
iso15
iso16
iso18
iso23
iso24
iso26
iso27
iso30
iso31
iso32
iso33
iso35
iso36
iso5
iso7
$g_{L\ ratio}$
0.80
0.60
0.40
$g_{L\ mean}$ (mmol m^{-2} s^{-1})
300
200
100
-8
-6
-4
-2
Minimum Ψ_{MD} (MPa)
0
2
4
6
Ψ_{MD} range (MPa)
0.25
0.50
0.75
1.00
1.25
σ (MPa MPa^{-1})

Figure S2


Angiosperm
Gymnosperm
Stomatal sensitivity (log(mmol m^{-2} s^{-1}) MPa^{-1})
0.9
0.6
0.3
0.0
-8
-6
-4
-2
ψPLC50 (MPa)
Vv
Pe
Pa
Ps
Qa
Qu
Fs
Qp
Bo
Eg
Pt
Pf
PrPp
Pc
Au
As
Jo
Qc
Jc
Oe
Mc
Qi
Ea
Pl
Lt
Tr

Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept

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Table S1. Characteristics of the species considered in this study and list of data sources used to build the database of leaf water potentials and stomatal responses (*cf*. '1. The relationship between stomatal control and water potential regulation across species' section in main text).

Species name	Abbreviation	Family	Group	Functional Type	Climate	References
Acacia tortilis	At	Fabaceae	Angiosperm	Broadleaf	Tropical	Otieno *et al.* 2005; Gebrekirstos *et al.* 2006
Acacia xanthophloea	Ax	Fabaceae	Angiosperm	Broadleaf	Tropical	Otieno *et al.* 2005
Acer saccharum	As	Sapindaceae	Angiosperm	Broadleaf	Temperate	Ellsworth & Reich 1992; Loewenstein & Pallardy 1998
Annona squamosa	Aq	Annonaceae	Angiosperm	Broadleaf	Tropical	Endres 2007
Arbutus unedo	Au	Ericaceae	Angiosperm	Broadleaf	Mediterranean	Castell *et al.* 1994; Werner *et al.* 1999; Martínez-Vilalta *et al.* 2002; Martínez-Vilalta *et al.* 2003; Clemente *et al.* 2005; Ripullone *et al.* 2009
Betula occidentalis	Bo	Betulaceae	Angiosperm	Broadleaf	Temperate	Smith *et al.* 1991
Carpinus viminea	Cv	Betulaceae	Angiosperm	Broadleaf	Temperate	Singh *et al.* 2006
Castanopsis indica	Ci	Fagaceae	Angiosperm	Broadleaf	Temperate	Poudyal *et al.* 2004
Erica arborea	Ea	Ericaceae	Angiosperm	Shrub	Mediterranean	Tognetti *et al.* 2000
Eucalyptus gomphocephala	Eg	Myrtaceae	Angiosperm	Broadleaf	Tropical	Franks *et al.* 2007
Fagus sylvatica	Fs	Fagaceae	Angiosperm	Broadleaf	Temperate	Aranda *et al.* 2000, 2005
Fraxinus micrantha	Fm	Oleaceae	Angiosperm	Broadleaf	Temperate	Singh *et al.* 2006
Halimium halimifolium	Hh	Cistaceae	Angiosperm	Shrub	Mediterranean	Zunzunegui *et al.* 2000, 2009

Juniperus communis	Jc	Cupressaceae	Gymnosperm	Conifer	Temperate	Tognetti *et al.* 2000
Juniperus osteosperma	Jo	Cupressaceae	Gymnosperm	Conifer	Temperate	Leffler *et al.* 2002; West *et al.* 2007
Larrea tridentata	Lt	Zygophyllaceae	Angiosperm	Shrub	Tropical	Pavlik 1980; Meinzer *et al.* 1988; Pockman & Small 2010
Machilus duthiei	Md	Lauraceae	Angiosperm	Broadleaf	Temperate	Singh *et al.* 2006
Myrtus communis	Mc	Myrtaceae	Angiosperm	Shrub	Mediterranean	Tognetti *et al.* 2000; Mendes *et al.* 2001
Olea europaea	Oe	Oleaceae	Angiosperm	Broadleaf	Mediterranean	Ben Ahmed *et al.* 2009
Phillyrea latifolia	Pl	Oleaceae	Angiosperm	Broadleaf	Mediterranean	Martínez-Vilalta *et al.* 2002; Martínez-Vilalta *et al.* 2003; Serrano & Penuelas 2005
Picea abies	Pa	Pinaceae	Gymnosperm	Conifer	Temperate	Lu *et al.* 1995
Pinus edulis	Pe	Pinaceae	Gymnosperm	Conifer	Temperate	West *et al.* 2007
Pinus nigra	Pn	Pinaceae	Gymnosperm	Conifer	Mediterranean	Lebourgeois *et al.* 1998
Pinus ponderosa	Pp	Pinaceae	Gymnosperm	Conifer	Temperate	Lanini & Radosevich 1986; Feeney *et al.* 1998; Kolb *et al.* 1998; Stone *et al.* 1999; Fischer *et al.* 2002; Eggemeyer *et al.* 2006; Simonin *et al.* 2006; Gaylord *et al.* 2007
Pinus sylvestris	Ps	Pinaceae	Gymnosperm	Conifer	Temperate	Irvine *et al.* 1998; Poyatos *et al.* 2008, 2013
Pistacia lentiscus	Pc	Anacardiaceae	Angiosperm	Shrub	Mediterranean	Vilagrosa *et al.* 2003
Populus fremontii	Pf	Salicaceae	Angiosperm	Broadleaf	Mediterranean	Smith *et al.* 1991; Horton *et al.* 2001
Populus trichocarpa	Pt	Salicaceae	Angiosperm	Broadleaf	Temperate	Smith *et al.* 1991; Johnson *et al.* 2002
Quercus alba	Qa	Fagaceae	Angiosperm	Broadleaf	Temperate	Loewenstein & Pallardy 1998
Quercus coccifera	Qc	Fagaceae	Angiosperm	Shrub	Mediterranean	Werner *et al.* 1999; Vilagrosa *et al.* 2003
Quercus floribunda	Qf	Fagaceae	Angiosperm	Broadleaf	Temperate	Singh *et al.* 2006; Joshi *et al.* 2009
Quercus ilex	Qi	Fagaceae	Angiosperm	Broadleaf	Mediterranean	Castell *et al.* 1994; Sala & Tenhunen 1994; Tognetti *et al.*

						1998; Fotelli *et al.* 2000; Martínez-Vilalta *et al.* 2002; Martínez-Vilalta *et al.* 2003; Serrano & Penuelas 2005; Cubera & Moreno 2007a, 2007b; Poyatos *et al.* 2013
Quercus lanata	Ql	Fagaceae	Angiosperm	Broadleaf	Temperate	Poudyal *et al.* 2004; Singh *et al.* 2006
Quercus leucotrichophora	Qe	Fagaceae	Angiosperm	Broadleaf	Temperate	Singh *et al.* 2006
Quercus petraea	Qp	Fagaceae	Angiosperm	Broadleaf	Temperate	Bréda *et al.* 1993; Aranda *et al.* 2000, 2005; Rodríguez-Calcerrada *et al.* 2006
Quercus pubescens	Qu	Fagaceae	Angiosperm	Broadleaf	Mediterranean	Tognetti *et al.* 1998; Fotelli *et al.* 2000; Poyatos *et al.* 2008
Quercus pyrenaica	Qy	Fagaceae	Angiosperm	Broadleaf	Mediterranean	Rodríguez-Calcerrada *et al.* 2006
Quercus semecarpifolia	Qs	Fagaceae	Angiosperm	Broadleaf	Temperate	Poudyal *et al.* 2004
Rhododendron arboreum	Ra	Ericaceae	Angiosperm	Broadleaf	Temperate	Poudyal *et al.* 2004; Singh *et al.* 2006
Schima wallichii	Sw	Sesiidae	Angiosperm	Broadleaf	Temperate	Poudyal *et al.* 2004
Tamarix ramosissima	Tr	Tamaricaceae	Angiosperm	Shrub	Temperate	Xu *et al.* 2009; Nippert *et al.* 2010
Trifolium subterraneum	Ts	Fabaceae	Angiosperm	Herb	Mediterranean	Socias *et al.* 1997
Vicia faba	Vf	Fabaceae	Angiosperm	Herb	Mediterranean	Sau & Inés Mínguez 2000
Vitis vinifera	Vv	Vitaceae	Angiosperm	Shrub	Mediterranean	Williams & Araujo 2002; Schultz 2003; Patakas *et al.* 2005

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Table S2. List of the studies, species and data sources included in the comparison of coexisting species within sites (case study 2 in main text).

Study code	**Site**	**Species**	**Life stage**	**Climate**	**Study system**	**Experimental treatment**	**Reference**
iso5	Paracou, French Guiana	*Eperua falcata, Diplotropis purpurea, Virola surinamensis*	Juvenile	Tropical	Greenhouse (potted)	Yes	Bonal & Guehl 2001
iso7	Thessaloniki Forest Research Institute, Greece	*Quercus frainetto, Quercus macrolepis, Quercus pubescens, Quercus ilex*	Juvenile (2-year-old)	Mediterranean	Greenhouse (potted)	Yes	Fotelli *et al*. 1999
iso15	Sevilleta LTER, NM, USA	*Pinus edulis, Juniperus monosperma*	Mature	Dry	Field	Yes	Limousin *et al*. 2013
iso16	Baskett Research & Education area, Missouri, USA	*Juglans nigra, Acer saccharum, Quercus alba*	Mature	Temperate	Field	No	Loewenstein & Pallardy 1998
iso18	Prades Mountains, Spain	*Acer monspessulanum, Arbutus unedo, Cistus albidus, C. laurifolius, Ilex aquifolium, Juniperus oxycedrus, Sorbus torminalis*	Mature	Mediterranean	Field	No	Martínez-Vilalta *et al*. 2002
iso23	Univ. Queensland, Australia	*Eucalyptus cloeziana, Eucalyptus argophloia*	Juvenile (6-months)	Tropical	Greenhouse (potted)	Yes	Ngugi *et al*. 2004
iso24	Kibwezi, Kenya	*Acacia xanthophloea, Acacia tortilis*	Mature	Dry	Field	No	Otieno *et al*. 2005
iso26	Zaragoza, Spain	*Quercus coccifera, Quercus ilex* ssp. *Ballota, Quercus suber*	Juvenile (5-years-old)	Mediterranean	Greenhouse (potted)	Yes	Peguero-Pina *et al*. 2009
iso27	Sierra de Cardeña y Montoro, Spain	*Cistus ladanifer , Daphne gnidium, Pistacia lentiscus, Myrtus*	Mature	Mediterranean	Field	No	Quero *et al*. 2010

		communis, Quercus ilex ssp. *Ballota, Olea europaea*					
iso30	Montpellier, France	*Vitis vinifera* 'Semillon', *Vitis vinifera* 'Grenache'	Juvenile (8 year-old)	Mediterranean	Field	Yes	Schultz 2003
iso31	Prades Mountains, Spain	*Quercus ilex, Phillyrea latifolia*	Mature	Mediterranean	Field	No	Serrano & Peñuelas 2005
iso32	University of Perugia, Italy	*Vitis vinifera* 'Sangiovese', *Vitis vinifera* 'Montepulciano'	Mature (8 years-old)	Mediterranean	Greenhouse (potted)	Yes	Tombesi *et al*. 2015
iso33	Valencia, Spain	*Quercus coccifera, Pistacia lentiscus*	Juvenile (2 years-old)	Mediterranean	Greenhouse (potted)	Yes	Vilagrosa *et al*. 2003
iso35	Los Alamos, NM, USA	*Pinus edulis, Juniperus monosperma*	Mature	Dry	Field	Yes	Garcia-Forner *et al*. 2016a
iso36	Caldes de Montbui, Spain	*Quercus ilex, Phillyrea latifolia*	Juvenile (7 years-old)	Mediterranean	Greenhouse (not potted)	Yes	Garcia-Forner (unpublished data)

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