

## IDEA AND PERSPECTIVE

# The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis

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### Abstract

Increasing drought is one of the most critical challenges facing species and ecosystems worldwide, and improved theory and practices are needed for quantification of species tolerances. Leaf water potential at turgor loss, or wilting ( $\pi_{\text{tlp}}$ ), is classically recognised as a major physiological determinant of plant water stress response. However, the cellular basis of  $\pi_{\text{tlp}}$  and its importance for predicting ecological drought tolerance have been controversial. A meta-analysis of 317 species from 72 studies showed that  $\pi_{\text{tlp}}$  was strongly correlated with water availability within and across biomes, indicating power for anticipating drought responses. We derived new equations giving both  $\pi_{\text{tlp}}$  and relative water content at turgor loss point ( $RWC_{\text{tlp}}$ ) as explicit functions of osmotic potential at full turgor ( $\pi_{\text{o}}$ ) and bulk modulus of elasticity ( $\epsilon$ ). Sensitivity analyses and meta-analyses showed that  $\pi_{\text{o}}$  is the major driver of  $\pi_{\text{tlp}}$ . In contrast,  $\epsilon$  plays no direct role in driving drought tolerance within or across species, but sclerophylly and elastic adjustments act to maintain  $RWC_{\text{tlp}}$ , preventing cell dehydration, and additionally protect against nutrient, mechanical and herbivory stresses independent of drought tolerance. These findings clarify biogeographic trends and the underlying basis of drought tolerance parameters with applications in comparative assessments of species and ecosystems worldwide.

### Keywords

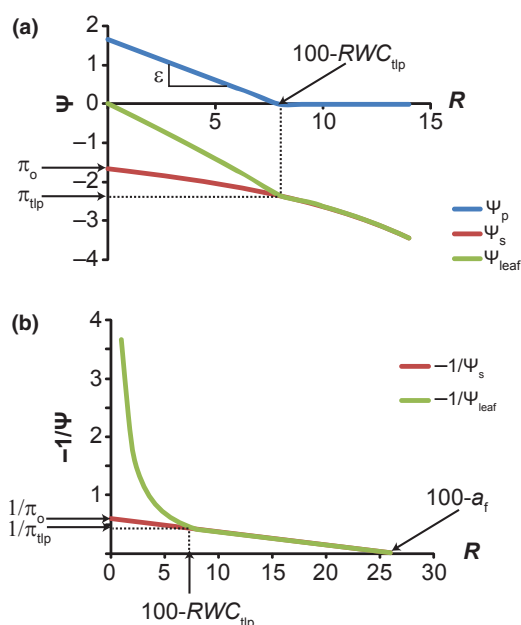
Biogeography, biomes, climate, plant hydraulics, plant traits.

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## INTRODUCTION

Climate change is predicted to increase the incidence and severity of droughts in ecosystems worldwide (Sheffield & Wood 2008). Species differences in drought tolerance are integral determinants not only of present distributions but also of future scenarios, including the probability of extinctions (Engelbrecht *et al.* 2007; Bonan 2008; Feeley *et al.* 2011). Predicting the impact of climate change on plant performance and survival is a major challenge facing plant science and ecology (Grierson *et al.* 2011). However, there remain fundamental gaps in our knowledge of which traits can be used to assess ecological drought tolerance. Cell turgor loss is arguably the best recognised classical indicator of plant water stress, having impacts on cellular structural integrity, metabolism and whole-plant performance (Kramer & Boyer 1995; McDowell 2011). Consequently, the leaf water potential at turgor loss, or bulk turgor loss point ( $\pi_{\text{tlp}}$ , units MPa) has been used to assess physiological drought tolerance for decades. Despite its potential use for quantifying ecological drought tolerance (Niinemets 2001; Brodribb & Holbrook 2003; Lenz *et al.* 2006; Blackman *et al.* 2010), no study to our knowledge has tested the relationship between  $\pi_{\text{tlp}}$  and water supply within or across biomes, or its performance as an indicator of drought tolerance relative to other plant traits. In addition, significant ambiguities concerning the underlying physiological and anatomical determinants of  $\pi_{\text{tlp}}$  are featured prominently in textbooks of physiological and whole plant ecology (e.g. Jones 1992; Larcher 2003; Nobel 2009). We undertook new analyses to clarify this topic and its importance, given the critical need for physiological measures that can be used to assess species' drought tolerances and thus their likely sensitivity to ongoing climate change.

The  $\pi_{\text{tlp}}$  is classically measured in assessments of drought tolerance, as one of six key bulk leaf parameters relating to cellular composition and structural properties typically calculated from a plot of leaf water potential ( $\Psi_{\text{leaf}}$ ) against water volume in drying leaves, known as the pressure-volume (p-v) curve (see primer in Fig. 1 and Table 1). The  $\pi_{\text{tlp}}$  is often recognised as the 'higher-level' trait that quantifies leaf and plant drought tolerance most directly, because a more negative  $\pi_{\text{tlp}}$  extends the range of  $\Psi_{\text{leaf}}$  at which the leaf remains turgid and maintains function (Sack *et al.* 2003; Lenz *et al.* 2006). Plants with low  $\pi_{\text{tlp}}$  tend to maintain stomatal conductance, hydraulic conductance, photosynthetic gas exchange and growth at lower soil water potential ( $\Psi_{\text{soil}}$ ), which is especially important when droughts occur during the growing season (Abrams & Kubiske 1990; Sack *et al.* 2003; Baltzer *et al.* 2008; Mitchell *et al.* 2008; Blackman *et al.* 2010). The  $\pi_{\text{tlp}}$  is thus a trait quantifying the ability to 'tolerate' drought, rather than to 'avoid' drought by ceasing gas exchange and surviving on stored water, shedding leaves or dying back to below-ground parts or to seeds (e.g. as done by annuals, deep-rooted perennials, or phreatophytes, CAM succulents or drought-dormant species; Chaves *et al.* 2002; Brodribb & Holbrook 2005; Ogburn & Edwards 2010). The  $\pi_{\text{tlp}}$  also defines the  $\Psi_{\text{soil}}$  below which the plant cannot take up sufficient water to recover from wilting. Known as the 'permanent wilting point', this was previously thought to correspond to a  $\Psi_{\text{soil}}$  of  $-1.5$  MPa (Veihmeyer & Hendrickson 1928), but the  $\pi_{\text{tlp}}$  is now known to vary across species, and thus may influence ecological distributions with respect to water availability. Some have focused on a second p-v curve parameter as a possible determinant of drought tolerance, the relative water content at  $\pi_{\text{tlp}}$  ( $RWC_{\text{tlp}}$ ). The other four parameters, i.e. the apoplastic water fraction ( $a_i$ ), modulus of elasticity ( $\epsilon$ ), osmotic potential at full



**Figure 1** A primer of pressure-volume curve construction. (a) plot of water potential vs.  $100 - \text{total relative water content}$  ( $100 - RWC = R$ , units %); the leaf water potential ( $\Psi_{\text{leaf}}$ , units MPa) is the sum of the pressure potential ( $\Psi_p$ ) and solute potential ( $\Psi_s$ ). The slope of  $\Psi_p$  between  $R = 0$  and turgor loss point ( $R = 100 - RWC_{\text{tlp}}$ ) is the modulus of elasticity ( $\epsilon$ , units MPa). The y-intercept of the  $\Psi_s$  curve is the osmotic potential at full turgor ( $\pi_o$ ), and when  $\Psi_p = 0$ ,  $\Psi_{\text{leaf}} = \pi_{\text{tlp}}$  is the water potential at turgor loss point ( $\pi_{\text{tlp}}$ ). The apoplastic fraction ( $a_f$ ) is the  $R$  at which  $\Psi_{\text{leaf}}$  trends towards  $-\infty$ . (b) A plot of  $-1/\Psi$  vs.  $R$  facilitates parameter estimation.

hydration ( $\pi_o$ ) and the tissue capacitance ( $C$ ; see Table 1 and Fig. 1 for their derivation and significance) have also been correlated with various aspects of drought tolerance (Niinemets 2001; Brodribb & Holbrook 2003; Lenz *et al.* 2006; Baltzer *et al.* 2008). Indeed, numerous studies have evaluated  $\epsilon$ ,  $\pi_o$  and  $a_f$  as functional determinants, or 'drivers' of  $\pi_{\text{tlp}}$  within species, as plants adjust  $\pi_{\text{tlp}}$  in response to drought, and across species according to habitat water supply (e.g. Joly & Zaerr 1987; Niinemets 2001; Lenz *et al.* 2006).

Decades of research have improved p-v curve analysis and clarified its biological meaning (Höfler 1920; Tyree & Hammel 1972; Richter 1978; Tyree 1981; Abrams & Kubiske 1990; Niinemets 2001; Brodribb & Holbrook 2003; Lenz *et al.* 2006; Baltzer *et al.* 2008). However, in our view the application of  $\pi_{\text{tlp}}$  as an ecological drought tolerance trait has been slowed by four inter-related major controversies concerning its mechanistic basis and interpretation that have confused generations of students in physiology and ecology. By 'controversy' we mean problems that engendered debate among two or more scientific points of view and that remain unresolved because of a lack of a theoretical framework or information for decision. We developed new theory and meta-analyses to resolve these controversies:

(1) *How are p-v parameters related to water supply within and across biomes?*

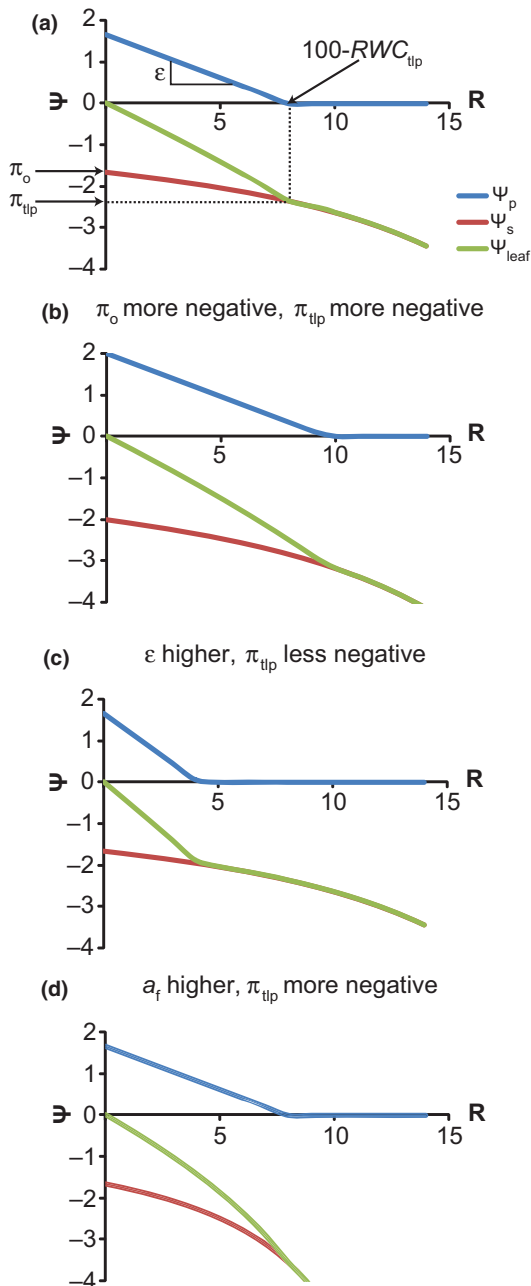
We need improved means for rapidly assessing species' drought tolerances. The  $\pi_{\text{tlp}}$  and other p-v parameters, especially  $RWC_{\text{tlp}}$ ,  $\epsilon$  and  $\pi_o$ , have been alternatively proposed as predictors of physiological drought tolerance, and possibly of realised ecological drought tolerance, although without a global test to our knowledge. Meanwhile, leaf mass per area (LMA) has been proposed as a correlate or predictor of drought tolerance in many species sets, partly because it is associated with  $\epsilon$  (e.g. Niinemets 2001; Wright *et al.* 2005;

**Table 1** A primer of terms and symbols used in leaf pressure-volume (p-v) analysis of water relations and drought tolerance, including measured metrics and parameters derived from p-v analysis, units, derivation and biological significance. See Fig. 1 for graphical depiction of parameters. For the derivation and significance of capacitance, see Supplementary Information, 'The role of capacitance and elasticity in drought survival'

| Symbol  | Variables  | Unit | Derivation   | Significance  |
|---|--|------|--|---|
| <b>Measured metrics</b>                                       |  |      |  |   |
| $\Psi_w$  | Bulk leaf water potential                              | MPa  | Volume-weighted average of water potentials (forces acting on water) in the leaf     | Index of leaf hydration and demand for water                              |
| $\Psi_s$  | Osmotic potential                                      | MPa  | The portion of the p-v curve following turgor loss point gives $\Psi_w = \Psi_s$     | A lower water potential caused by concentration of cell solute            |
| $\Psi_p$  | Pressure potential                                     | MPa  | In the portion of the p-v curve before turgor loss point, $\Psi_w - \Psi_s = \Psi_p$ | A higher water potential caused by turgor pressure against the cell walls |
| $RWC$   | Relative water content                                 | %    | Fraction of saturated water mass present in leaf                                     | Leaf hydration  |
| $R$   | $100 - RWC$  | %    | —  | —   |
| <b>Parameters derived from pressure-volume curve analysis</b> |  |      |  |   |
| $\pi_{\text{tlp}}$  | Turgor loss point                                      | MPa  | Point at which $\Psi_p = 0$ and $\Psi_w = \Psi_s$                                    | Point at which leaf cells become flaccid, on average                      |
| $\epsilon$  | Modulus of elasticity                                  | MPa  | $\frac{\Delta \Psi_p}{\Delta RWC_{\text{symplastic}}}$                               | Wall stiffness, calculated from symplastic water content                  |
| $\epsilon^*$  | Modulus of elasticity                                  | MPa  | $\frac{\Delta \Psi_p}{\Delta RWC_{\text{total}}}$                                    | Wall stiffness, calculated from total water content                       |
| $\pi_o$   | Osmotic potential at full rehydration                  | MPa  | $\Psi_s$ at full hydration ( $R = 0$ )   | Solute concentration in cells   |
| $a_f$   | Apoplastic fraction                                    | %    | $RWC$ at $\Psi_s = -\infty$  | Extracellular water content   |
| Total $RWC_{\text{tlp}}$                                      | Relative water content at turgor loss point            | %    | Total $RWC$ at which $\Psi_w = \pi_{\text{tlp}}$                                     | Leaf hydration at which cells become flaccid                              |
| Symplastic $RWC_{\text{tlp}}$                                 | Relative symplastic water content at turgor loss point | %    | Symplastic $RWC$ at which $\Psi_w = \pi_{\text{tlp}}$                                | Symplastic leaf hydration at which cells become flaccid                   |

Poorter *et al.* 2009; Markesteijn *et al.* 2011a), but to our knowledge there have been no comparative tests of the correlation of p-v parameters and LMA with water availability within or across biomes.

(2) *What traits underlie  $\pi_{\text{tlp}}$  adjustment during drought within species and  $\pi_{\text{tlp}}$  differences across species?* The importance of other p-v traits in determining the higher-level trait  $\pi_{\text{tlp}}$  within and across species has remained controversial. Plants of given species improve their drought tolerance by making their  $\pi_{\text{tlp}}$  more negative, and this might be done in three possible ways (Fig. 2): accumulating solutes (decreasing  $\pi_o$ ),



**Figure 2** Graphical illustration of the impacts on  $\pi_{\text{tlp}}$  of changing given pressure-volume (p-v) curve parameters in a plot of  $\Psi_{\text{leaf}}$ ,  $\Psi_p$  and  $\Psi_s$  against  $R$  (symbols as in Fig 1 and Table 1) (a) The p-v curve of Fig. 1a. (b) When  $\pi_o$  is more negative but  $\epsilon$  and  $a_f$  are fixed, the  $RWC_{\text{tlp}}$  decreases, and the  $\pi_{\text{tlp}}$  is shifted to more negative. (c) When  $\epsilon$  is higher, but  $\pi_o$  and  $a_f$  are fixed, the  $RWC_{\text{tlp}}$  increases, the  $\pi_{\text{tlp}}$  is shifted to less negative. (d) When  $a_f$  is higher, the  $\pi_{\text{tlp}}$  is shifted to more negative, via a reduction of  $\epsilon$  (see formula for  $\epsilon$  in Table 1).

reducing symplastic water content by redistributing more water outside of the cell walls (increasing  $a_f$ ), and/or increasing cell wall flexibility (decreasing  $\epsilon$ ), known as osmotic, apoplastic and elastic ‘adjustments’, respectively. Osmotic adjustment has been observed in numerous species to enable the maintenance of growth and yield during drought (e.g. Gonzalez *et al.* 1999; Merchant *et al.* 2007) but strong arguments have also been made for the importance of elastic and apoplastic adjustments (Joly & Zaerr 1987; Iraki *et al.* 1989; Chimenti & Hall 1994; Kozłowski & Pallardy 2002; Moore *et al.* 2008), although  $\epsilon$  and  $a_f$  have been observed to increase (Bowman & Roberts 1985; Joly & Zaerr 1987; Chimenti & Hall 1994; Kozłowski & Pallardy 2002) or decrease (Kubiske & Abrams 1991; Kozłowski & Pallardy 2002) during drought. As these parameters are typically adjusted simultaneously, their relative importance in influencing  $\pi_{\text{tlp}}$  has remained unclear.

An analogous controversy has surrounded the role of  $\pi_o$ ,  $a_f$  and  $\epsilon$  in determining *interspecific* differences in  $\pi_{\text{tlp}}$ . A study of compiled data for 51 shrub and tree species reported a 10-fold variation across species in  $\epsilon$  but only fourfold in  $\pi_o$ , and concluded that  $\epsilon$  has greater potential to influence  $\pi_{\text{tlp}}$  and drought tolerance (Niinemets 2001). In contrast, three studies that examined fewer species found  $\pi_o$  but not  $\epsilon$  to predict differences in  $\pi_{\text{tlp}}$  (Lenz *et al.* 2006; Baltzer *et al.* 2008; Mitchell *et al.* 2008).

(3) *How exactly are  $\pi_{\text{tlp}}$  and/or  $RWC_{\text{tlp}}$  important in plant water relations?* Although most have considered that a more negative  $\pi_{\text{tlp}}$  benefits drought tolerance, as described above, a counter-argument has been made that a *less* negative  $\pi_{\text{tlp}}$  may be beneficial. According to this view, a *less* negative  $\pi_{\text{tlp}}$  enables leaves to quickly lose turgor and close their stomata as  $\Psi_{\text{leaf}}$  declines and thereby maintain a high  $RWC_{\text{tlp}}$  (Walter & Stadelmann 1968; Read *et al.* 2006). Indeed, some have argued that maintaining cell hydration is more important than turgor, as dehydration can induce shrinkage, wall structural damage and potentially osmotic stress due to very strong ion concentration, all of which could disrupt metabolic processes. Indeed, a total cell relative water content below 75% severely inhibits ATP, RuBP and protein production (Lawlor & Cornic 2002). The importance of  $\pi_{\text{tlp}}$  and  $RWC_{\text{tlp}}$  as drought tolerance predictors has been frequently debated without resolution (e.g. Sinclair & Ludlow 1985; Kramer 1988; Schulte 1992).

(4) *What are the roles of the modulus of elasticity, sclerophylly and malacophylly in drought tolerance?* The convergent evolution of sclerophyllous plants, with mechanically tough leaves and stiff cell walls, in mediterranean and semi-desert systems was classically interpreted as indicating an importance in drought tolerance, although a number of other conditions may select for tough evergreen leaves, such as low nutrients or evergreen shade (Grubb 1986; Sack 2004; Markesteijn *et al.* 2011b). Although sclerophylly can be defined in several ways, such as a high leaf mass per area (LMA) or lignin concentration (Read & Sanson 2003), the feature most closely related to water relations is  $\epsilon$ , and several have hypothesised that a high  $\epsilon$  contributes critically to species-differences in drought tolerance (Salleo & Nardini 2000; Niinemets 2001; Read *et al.* 2006). The relationship between high  $\epsilon$ ,  $\pi_{\text{tlp}}$ , and drought tolerance has been termed ‘one of the oldest controversies in ecology’ (Lamont *et al.* 2002) and has given rise to numerous hypotheses. A first hypothesis is that a high  $\epsilon$  causes  $\Psi_{\text{leaf}}$  to decline rapidly as leaves dehydrate, allowing sustained water uptake from drying soil (Bowman & Roberts 1985). A second hypothesis considers high  $\epsilon$  to actually *lower*  $\pi_{\text{tlp}}$ , contrary to the mechanisms in Fig. 2 (Larcher 2003; Lenz *et al.* 2006). A third hypothesis is that, consistent with Fig. 2, a high  $\epsilon$  contributes to a less negative  $\pi_{\text{tlp}}$  and

this would enable stomata to close quickly with turgor loss and maintain a high  $RWC_{\text{tbp}}$ , which would benefit drought tolerance as described for controversy (2) (Walter & Stadelmann 1968; Read *et al.* 2006). A fourth hypothesis considers a high  $\varepsilon$  to provide mechanical support for cells with very negative  $\pi_o$  and  $\pi_{\text{tbp}}$ , to prevent bursting due to excessive turgor pressure when they are fully hydrated (Jones 1992). A fifth hypothesis is that a higher  $\varepsilon$  could mechanically constrain shrinkage in cells with very negative  $\pi_o$  and  $\pi_{\text{tbp}}$ , allowing  $RWC_{\text{tbp}}$  to remain high, the so-called 'cell water conservation hypothesis' (Cheung *et al.* 1975; Jones 1992). Finally, a sixth hypothesis is that a high  $\varepsilon$  and sclerophyll might in fact play no direct role in drought tolerance, and instead improve carbon and/or nutrient balance by contributing to longer leaf lifespans (Grubb 1986; Sack 2004; Markesteijn *et al.* 2011b). Indeed, a number of species persist in arid-zones despite having relatively low  $\varepsilon$ , i.e. the malacophylls, or soft-leaved species of dry areas (Walter 1985). No study to our knowledge has investigated these hypotheses in detail, although the contradictions have slowed interpretation of p-v parameters and sclerophyll.

Here, we provide a unique perspective to resolve controversies 1–4 from the fundamental cellular relationships to the biome scale. We first determined new mathematical relationships among p-v parameters. We then applied these relationships in sensitivity analyses and meta-analyses of a new global database, and related p-v parameters to aridity within and across biomes. We compiled data for  $\pi_{\text{tbp}}$ ,  $\pi_o$ ,  $\varepsilon$ ,  $a_f$ ,  $RWC_{\text{tbp}}$  and LMA for species of a wide range of growth forms and habitat preferences in the global literature. These p-v data were originally generated using the bench-drying method (using a pressure chamber; Koide *et al.* 2000) ( $n = 317$  species from 72 studies; Supplementary Information). For species from studies that did not include LMA, we compiled mean values from the Global Plant Network (GLOPNET) dataset (Wright *et al.* 2004).

#### DERIVATION OF NEW EQUATIONS FOR $\pi_{\text{tbp}}$ and $RWC_{\text{tbp}}$ as functions of other p-v parameters

We present two equations summarising the p-v curve as a function of its parameters, given classical assumptions based on the structure and physiology of the leaf (e.g. Tyree & Hammel 1972; Baltzer *et al.* 2008; Mitchell *et al.* 2008; see Supplementary Information, 'Derivation and verification of new fundamental equations'). Solving these equations for  $\pi_{\text{tbp}}$  and  $RWC_{\text{tbp}}$  gave the following novel relationships:

$$\pi_{\text{tbp}} = \frac{\pi_o \varepsilon}{\pi_o + \varepsilon} \quad (1)$$

$$RWC_{\text{tbp}} = \frac{\pi_o + \varepsilon}{\varepsilon} \quad (2)$$

Previous studies have used statistical regression to relate  $\pi_{\text{tbp}}$  differences to the other p-v parameters (e.g. Schulte & Hinckley 1985), but there has been little basis for favouring any particular model, without knowledge of the underlying relationships among parameters. Despite their elegance and usefulness, this is the first presentation to our knowledge of eqns 1 and 2.

Notably, in eqn 2 and elsewhere,  $RWC_{\text{tbp}}$  refers to the symplastic relative water content at turgor loss point, i.e. that within the leaf cells, unless specified otherwise as the total  $RWC_{\text{tbp}}$ , which includes the water in the apoplast. The two are inter-related as:

$$\text{total } RWC_{\text{tbp}} = (100 - a_f) \times RWC_{\text{tbp}} + a_f \quad (3)$$

Furthermore, in deriving eqns 1 and 2, we followed the classical method (e.g. Tyree & Hammel 1972; Koide *et al.* 2000) of defining  $\varepsilon$  as the slope of  $\Psi_p$  against symplastic rather than total relative water content, correcting for  $a_f$  (Fig. 1; Table 1). Thus, the  $a_f$  was considered implicitly in the calculation of  $\varepsilon$  (Fig. 1; Table 1). However in some studies, when the data did not allow clear estimation of the  $a_f$ , modulus of elasticity was calculated as the slope of  $\Psi_p$  against *total* rather than symplastic relative water content ( $\varepsilon^*$ ; e.g. Sack *et al.* 2003; Lenz *et al.* 2006; Baltzer *et al.* 2008); the two measures are inter-related as  $\varepsilon^* = \frac{\varepsilon}{(100 - a_f)}$ . If using  $\varepsilon^*$ , the analogous equations for  $\pi_{\text{tbp}}$  and  $RWC_{\text{tbp}}$  are:

$$\pi_{\text{tbp}} = \frac{\pi_o \varepsilon^*}{\frac{\pi_o}{100 - a_f} + \varepsilon^*} \quad (1a)$$

$$RWC_{\text{tbp}} = \frac{\frac{\pi_o}{100 - a_f} + \varepsilon^*}{\varepsilon^*} \quad (2a)$$

We additionally considered each of the analyses described below using eqns 1a and 2a, as this allowed the separate consideration of  $a_f$ . Those analyses confirmed the findings below, and are presented in the Supplementary Information, 'Alternative formulation of  $\varepsilon$  and the impact of apoplastic fraction'.

The application of eqns 1 and 2 in combination with global meta-analyses enabled the resolution of all four major controversies.

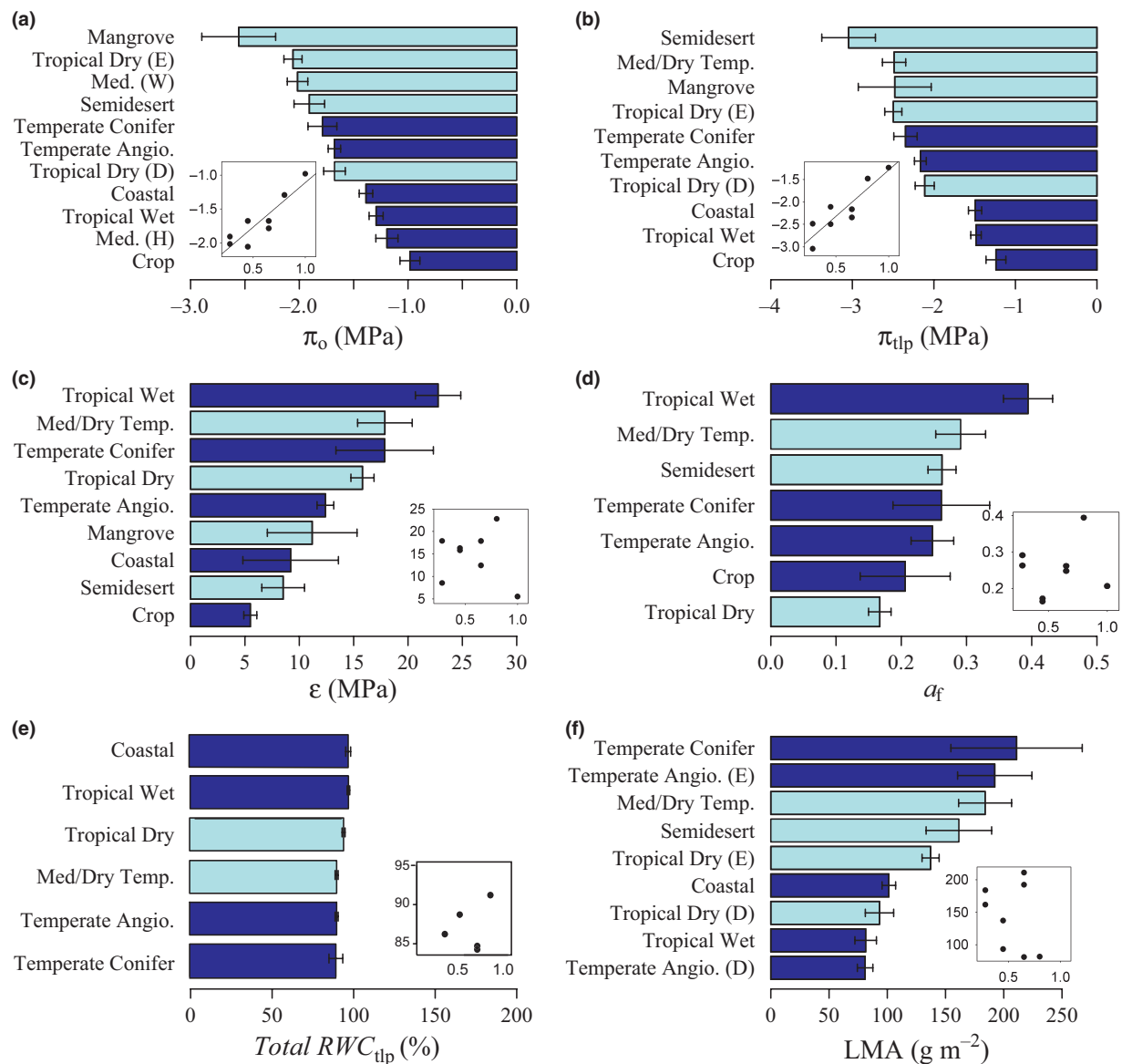
#### Resolution of controversy (1): The $\pi_{\text{tbp}}$ and $\pi_o$ correspond with ecological drought tolerance

Given the inter-relationship among p-v parameters in eqns 1 and 2, multiple parameters may be predictive of ecological drought tolerance. Thus, we tested the relationship of each p-v parameter with moisture gradients within and across biomes, and additionally tested leaf mass per area (LMA), a functional trait commonly measured as an indicator of drought tolerance. These analyses showed that  $\pi_{\text{tbp}}$  and  $\pi_o$  are excellent indicators of drought tolerance, and much more powerful than LMA.

First, we compared species among biome categories: semi-desert, mediterranean-climate/dry temperate, temperate forest (conifers and angiosperms), coastal vegetation, mangrove, crop herb and wet and dry tropical forest ( $n = 20$ – $30$  species per biome), using ANOVAs. We first tested differences within each biome in each p-v parameter and in LMA between woody and herbaceous species, and between evergreen and deciduous species and when no differences were found, those categories were pooled for an overall biome mean (Sokal & Rohlf 1995). In addition, for each variable, we tested biome means for correlation with Priestley–Taylor coefficients of biome water availability ( $\alpha$ ; Prentice *et al.* 1992).

Although all traits varied significantly among biomes (ANOVA; Table S1), only  $\pi_{\text{tbp}}$  and  $\pi_o$  showed separation of moist from dry biomes (Fig. 3, dark and light blue bars). In addition, the biome means for  $\pi_{\text{tbp}}$  and  $\pi_o$  correlated tightly with biome water availability as quantified by Priestley–Taylor coefficients ( $\alpha$ ;  $r = 0.90$ ,  $P = 0.03$ – $0.006$ ; Fig. 3, inset panels). No other traits correlated with water availability.





**Figure 3** Global data for pressure-volume parameters (symbols as in Table 1) and leaf mass per area (LMA), with mean  $\pm$  standard error across biome categories, with inset plots of biome category means against the Priestley–Taylor coefficient of annual moisture availability ( $\alpha$ ). Biome categories: semi-desert, mediterranean-type vegetation/dry temperate woodland, tropical dry and wet forest, temperate forest angiosperm and conifer, coastal vegetation, mangrove and crop herb. Data within biomes were separated into herb (H) vs. woody (W), or evergreen (E) vs. deciduous (D) when significantly different (Table S1). Only  $\pi_o$  and  $\pi_{tlp}$  showed separation of moist and dry biomes (light and dark blue bars respectively), and correlated with  $\alpha$  across biomes (both  $r^2 = 0.81$ ,  $P = 0.03$  to  $0.006$ ).

Second, to test traits in their ability to predict drought tolerance *within* biomes, we conducted two analyses. First, we compared LMA and  $\pi_{tlp}$  values of wet- and dry-forest species compiled from studies of temperate and tropical systems (Baltzer *et al.* 2008, 2009; Blackman *et al.* 2010), using *t*-tests. The LMA did not reflect differences in forest water availability (Fig. S1a–c), whereas  $\pi_{tlp}$  shifted strongly to more negative values from wet to dry forests ( $P \leq 0.05$ , Fig. S1d). Next, we used stepwise regression to test the relationship of LMA to a published drought tolerance index for tropical woody species (Sokal & Rohlf 1995; Engelbrecht & Kursar 2003; Poorter & Markesteijn 2008), and the relationship of both LMA and p-v parameters to a drought tolerance index for temperate woody species (Niinemets & Valladares 2006). LMA was poorly correlated with the drought tolerance index for tropical woody species ( $r^2 < 0.001$ ; Fig. S1a, b). However, for the

temperate forest species, LMA as well as p-v parameters correlated with the drought tolerance index. The  $\pi_{tlp}$  and  $\pi_o$  were negatively correlated with species' drought tolerance index ( $r = -0.51$  and  $-0.42$ ,  $P < 0.01$ ) whereas  $\epsilon$  and log-transformed LMA were positively correlated with the index ( $r = 0.24$  and  $r = 0.63$ , respectively,  $P < 0.001$ ); neither total  $RWC_{tlp}$  nor  $a_f$  related to drought tolerance. Using both LMA and  $\pi_{tlp}$  improved prediction of drought tolerance in this species set (Fig. S2;  $r^2$  increased from 0.40 and 0.26, respectively, for the traits individually, to 0.47), as these traits were uncorrelated ( $r^2 < 0.1$ ).

We conclude that  $\pi_{tlp}$  and  $\pi_o$  are reliable indicators of species drought tolerance within and across biomes, in contrast with other p-v parameters. The evidence did not support a mechanistic linkage of LMA with drought tolerance; high LMA values were found in

moist as well as dry biomes and tropical forests. Notably, LMA can be related to drought tolerance in given species sets, especially when drought stress coincides with other environmental conditions for which high LMA confers a benefit. For example, among deciduous species LMA tends to be higher for species adapted to more exposed areas, whereas among evergreen species LMA tends to be higher for species adapted to deep shade, nutrient shortage and/or herbivore pressure (Walters & Reich 1999; Sack 2004; Lusk *et al.* 2008; Markesteijn *et al.* 2011b). In contrast, as expected from their more direct physiological role, the  $\pi_{\text{tip}}$  and  $\pi_o$  showed far stronger correspondence with ecological distribution with respect to water supply.

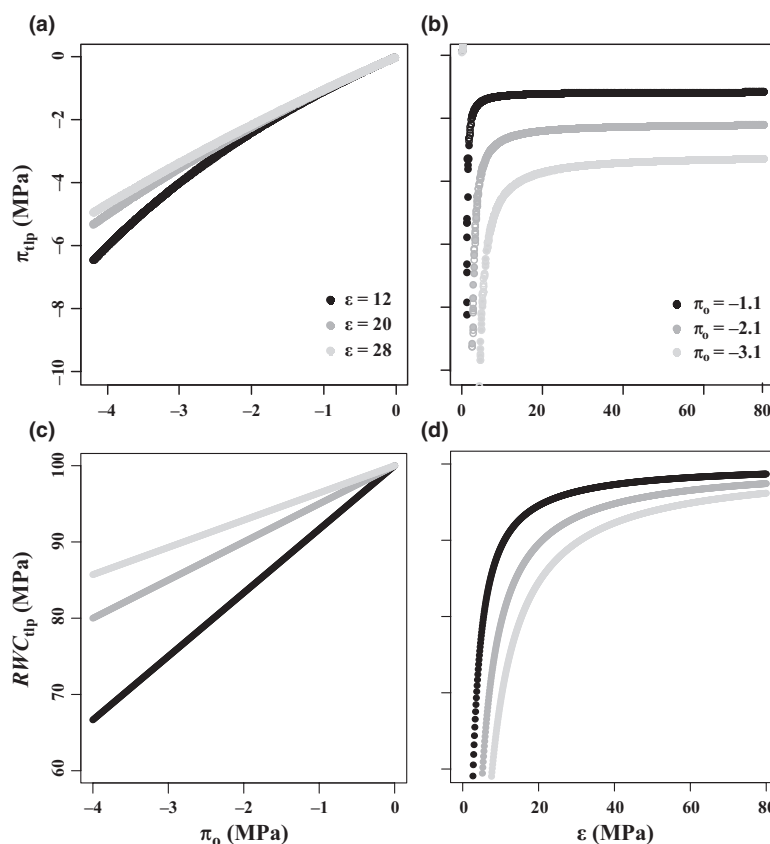
### Resolution of controversy (2): $\pi_o$ determines differences in $\pi_{\text{tip}}$ within- and across-species

Given the importance of  $\pi_{\text{tip}}$ , clarifying its underlying basis is critical. Using eqn 1, which showed that the  $\pi_{\text{tip}}$  is a function of  $\pi_o$  and  $\varepsilon$ , we tested the theoretical sensitivity of  $\pi_{\text{tip}}$  to other p-v parameters, and then applied the equation and meta-analyses of the global dataset to determine which parameters drove actual differences in  $\pi_{\text{tip}}$  within and among species. These analyses all indicated that  $\pi_{\text{tip}}$  is influenced by  $\pi_o$  with a negligible direct role for  $\varepsilon$ .

The structure of eqn 1 indicated that the sensitivity of  $\pi_{\text{tip}}$  to a given parameter may vary widely depending on the value of the other

parameter. We used simulations to characterise the relationship of  $\pi_{\text{tip}}$  to changes in  $\pi_o$  and  $\varepsilon$  values across ranges of realistic parameter values (Fig. 4a and b; we performed analogous analyses for  $RWC_{\text{tip}}$  using eqn 2 in 'Resolution of Controversy (4)'; Fig. 4c and d). Simulations that held one parameter constant demonstrated the effect of shifts in the other parameter on  $\pi_{\text{tip}}$ . Several new principles emerged. The decline of  $\pi_{\text{tip}}$  as  $\pi_o$  becomes more negative is very strong at all values of  $\pi_o$  and any value of  $\varepsilon$ , although increasingly rapid at low  $\varepsilon$  (Fig. 4a). However, the  $\pi_{\text{tip}}$  is not sensitive to  $\varepsilon$  in the same way; reducing  $\varepsilon$  can in principle make  $\pi_{\text{tip}}$  values more negative, but only within a narrow range of low  $\varepsilon$  values, and depends on  $\pi_o$  (Fig. 4b). The  $\pi_o$  defines the possible range of covariation in  $\pi_{\text{tip}}$  and  $\varepsilon$ : the  $\pi_o$  sets not only the highest  $\pi_{\text{tip}}$  attainable, but also the lowest  $\varepsilon$  attainable, because the relationship of  $\pi_{\text{tip}}$  to  $\varepsilon$  is asymptotic, and biologically infeasible values of  $\pi_{\text{tip}}$  occur when  $\varepsilon \leq -\pi_o$  (Fig. 4b). Thus, the range of  $\varepsilon$  that impacts  $\pi_{\text{tip}}$  depends on  $\pi_o$ : a more negative value of  $\pi_o$  results in sensitivity of  $\pi_{\text{tip}}$  to  $\varepsilon$  over a greater range of  $\varepsilon$  values (Fig. 4b). Indeed, variation in  $\varepsilon$  has little influence on  $\pi_{\text{tip}}$  under most local conditions, but theoretically, in extreme parameter spaces (i.e. low  $\varepsilon$ , low  $\pi_o$ ),  $\varepsilon$  might be strongly influential.

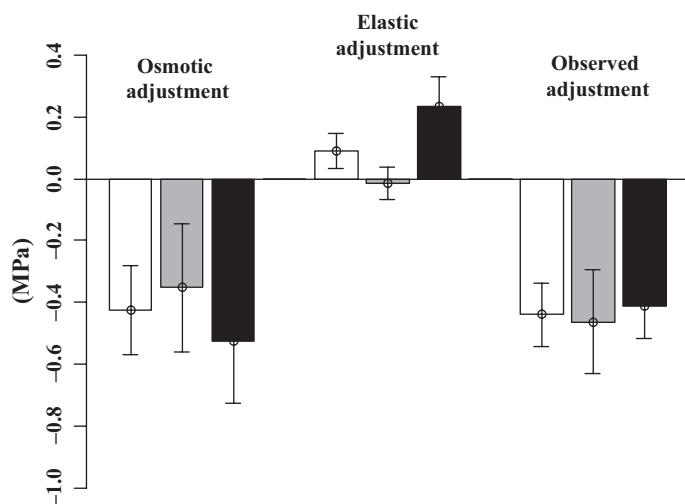
The strong sensitivity of  $\pi_{\text{tip}}$  to  $\pi_o$  was borne out in the global dataset for changes in given species during drought. Drought treatments led to a reduction of  $\pi_{\text{tip}}$  for plants of given species by  $0.44 \pm 0.10$  MPa on average (paired *t*-test,  $P < 0.001$ ,  $n = 25$ ). To determine the importance of  $\pi_o$  and  $\varepsilon$  in driving shifts of  $\pi_{\text{tip}}$  within



**Figure 4** Simulations based on equations 1 and 2, demonstrating the implications of the relationships among the pressure-volume parameters (symbols as in Fig 1 and Table 1). Each point would represent parameters determined in a single p-v curve. (a) The  $\pi_{\text{tip}}$  is sensitive to  $\pi_o$  across the full range of values, and especially at lower  $\varepsilon$ . (b) Decreases in  $\varepsilon$  result in more negative  $\pi_{\text{tip}}$  only within a narrow range of low  $\varepsilon$  values. The range of values of  $\varepsilon$  values with an influence on  $\pi_{\text{tip}}$  increases as  $\pi_o$  becomes more negative. (c & d) The  $RWC_{\text{tip}}$  is also sensitive to  $\pi_o$  and shows a considerably stronger response to  $\varepsilon$ .

species during drought (Fig. 2), we used eqn 1 to partition the role of the different parameters. We determined the 'post-drought  $\pi_{\text{tLP}}$ ' that would be attained with the shift of each parameter singly, by applying eqn 1 using the post-drought value for that parameter while fixing the other parameter at its pre-drought value. The post-drought  $\pi_{\text{tLP}}$  achieved by shifting each parameter was tested for significance by comparing with the pre-drought  $\pi_{\text{tLP}}$  ( $t$ -tests across all taxa,  $n = 25$  species or varieties), and considering separately the taxa that increased ( $n = 14$ ) and decreased in  $\varepsilon$  ( $n = 11$ ) during drought (Fig. 5). We found that shifts to more negative  $\pi_o$ , i.e. osmotic adjustment, accounted almost entirely for the observed decreases in  $\pi_{\text{tLP}}$ . In contrast, shifts in  $\varepsilon$  had negligible impact on  $\pi_{\text{tLP}}$ . In fact, on average,  $\varepsilon$  shifted upward, which would have made  $\pi_{\text{tLP}}$  less negative by 0.1 MPa considering all taxa, and by 0.2 MPa considering only taxa that increased  $\varepsilon$  (all  $P < 0.05$ ). In those taxa that did decrease  $\varepsilon$ , however, this did not occur in the range of parameter values in which  $\pi_{\text{tLP}}$  was sensitive to  $\varepsilon$ , and this shift accounted for a decrease in  $\pi_{\text{tLP}}$  of on average 0.01 MPa. Thus, osmotic adjustment was the only mechanism employed by plants to render  $\pi_{\text{tLP}}$  more negative during drought.

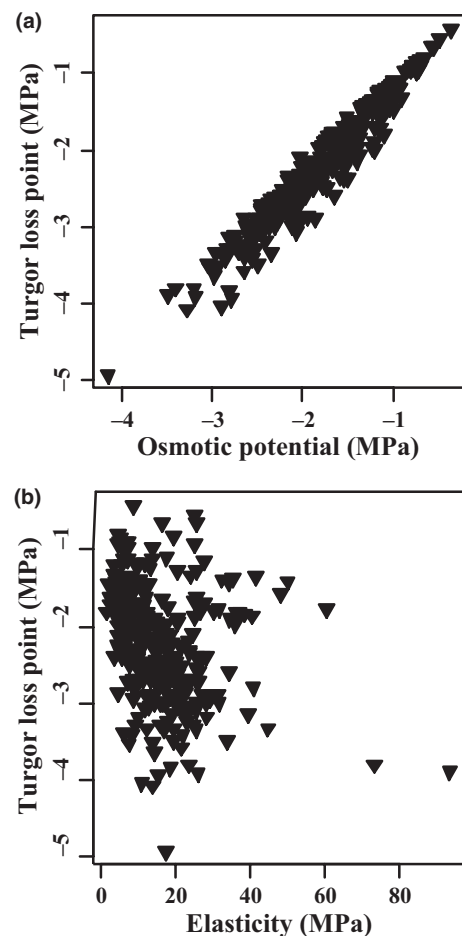
To comprehensively determine the importance of  $\pi_o$  and  $\varepsilon$  to interspecific differences in  $\pi_{\text{tLP}}$ , we conducted three analyses. All analyses showed that, despite the mathematical sensitivity of  $\pi_{\text{tLP}}$  to  $\varepsilon$  at certain values, there is no evidence that variation in  $\varepsilon$  drives functional variation in  $\pi_{\text{tLP}}$ . First, we used correlations to determine pairwise relationships between variables. Consistent with the previous analysis, across the global dataset,  $\pi_{\text{tLP}}$  was strongly correlated with  $\pi_o$  (Fig. 6a). There was a notable inverse correlation of  $\pi_{\text{tLP}}$  and  $\varepsilon$  (Fig. 6b), contrary to the mechanistic relationship expected from eqn 1 (i.e., in Fig. 4b). A partial correlation analysis of  $\pi_{\text{tLP}}$ ,  $\varepsilon$  and  $\pi_o$  allowed considering the correlations among parameters while holding another 'fixed', i.e. testing the correlation between two variables after removing the influence of a third variable (Sokal & Rohlf 1995).



**Figure 5** The impact of shifts in pressure-volume parameters on turgor loss point ( $\pi_{\text{tLP}}$ ) for given species during drought. Mean values  $\pm$  standard error for the adjustment of  $\pi_{\text{tLP}}$  observed in response to drought for given species from a global dataset, and that driven by the change in each component pressure-volume parameter alone, using eqn 1. White bars represent all taxa ( $n = 25$  species and varieties), grey bars those taxa that decreased in the modulus of elasticity ( $\varepsilon$ ) in response to drought ( $n = 14$ ), and black bars those taxa that increased in  $\varepsilon$  in response to drought ( $n = 11$ ).

Although controlling for the variation in  $\varepsilon$  did not affect the partial correlation of  $\pi_{\text{tLP}}$  and  $\pi_o$  across the global dataset (log-transformed data;  $r_{\text{partial}} = 0.95$ ;  $P < 0.001$ ), controlling for the variation in  $\pi_o$  changed the direction of the correlation of  $\pi_{\text{tLP}}$  and  $\varepsilon$  to positive ( $r_{\text{partial}} = 0.40$ ;  $P < 0.001$ ), as expected from eqn 1. Thus, any ability of a lower  $\varepsilon$  to directly drive a more negative  $\pi_{\text{tLP}}$  (Fig. 2) was reversed by a strong negative correlation of  $\pi_o$  and  $\varepsilon$ , a relationship previously reported in smaller species sets (Niinemets 2001; Sack *et al.* 2003; Lenz *et al.* 2006). The  $\pi_{\text{tLP}}$  is actually insensitive to  $\varepsilon$ , and the apparent association of low  $\pi_{\text{tLP}}$  with high  $\varepsilon$  across species arises secondarily from the negative correlation of  $\varepsilon$  with  $\pi_o$ , a general relationship further discussed in 'Resolution of controversy (4)'. This analysis indicated no direct role for  $\varepsilon$  in directly driving species-differences in  $\pi_{\text{tLP}}$ .

Second, to determine the degree that species'  $\pi_{\text{tLP}}$  values individually would be sensitive to changes in  $\pi_o$  and  $\varepsilon$ , we calculated partial derivatives  $\partial\pi_{\text{tLP}}/\partial\varepsilon$  and  $\partial\pi_{\text{tLP}}/\partial\pi_o$  from eqn 1 using the parameter values for each species in the global dataset. The partial derivatives, i.e. the slope of the relationship between  $\pi_{\text{tLP}}$  and each parameter at each observed parameter value, indicated how  $\pi_{\text{tLP}}$  would change with



**Figure 6** The relationship of turgor loss point ( $\pi_{\text{tLP}}$ ) to other pressure volume parameters (osmotic potential at full turgor,  $\pi_o$ ; modulus of elasticity,  $\varepsilon$ ) across species in a global dataset. Note that this analysis alone, although previously the most comprehensive analysis of variation in pressure-volume curve parameters across species (Niinemets 2001; Lenz *et al.* 2006) cannot elucidate the nonlinear relationships among parameters or partition the sensitivity of  $\pi_{\text{tLP}}$  to individual parameters, but this analysis still indicates a strong, direct impact of  $\pi_o$  in determining variation in  $\pi_{\text{tLP}}$  and no direct determining role for  $\varepsilon$ . (a)  $\pi_{\text{tLP}}$  vs.  $\pi_o$ ;  $r = 0.96$ ,  $P < 1 \times 10^{-15}$  (b)  $\pi_{\text{tLP}}$  vs.  $\varepsilon$ ;  $r = 0.28$ ,  $P < 0.001$ .

actual shifts in each variable. To test the importance of shifts in the two parameters, we compared mean partial derivative values across species using paired *t*-tests ( $n = 89$ ) and the equations:

$$\frac{\partial \pi_{\text{dip}}}{\partial \varepsilon} = \frac{\pi_o^2}{(\varepsilon + \pi_o)^2} \quad (4)$$

$$\frac{\partial \pi_{\text{dip}}}{\partial \pi_o} = \frac{\varepsilon^2}{(\varepsilon + \pi_o)^2} \quad (5)$$

Consistent with the previous analyses, the  $\pi_{\text{dip}}$  was far more responsive to changes in  $\pi_o$  than  $\varepsilon$ : across all species, the mean value for  $\partial \pi_{\text{dip}} / \partial \pi_o$  was 30-fold greater than  $\partial \pi_{\text{dip}} / \partial \varepsilon$  ( $t = 41.1$ ,  $P < 2.2 \times 10^{-16}$ ; paired *t*-test; Fig. 7). We also graphically compared the observed partial derivative values to those calculated from randomly generated p-v parameters, to determine whether plants preferentially occupied parameter spaces that made them more sensitive to a given parameter, or evenly occupied all the theoretically feasible parameter combinations and would thus be sensitive to changes in both  $\pi_o$  and  $\varepsilon$ . The observed values of  $\partial \pi_{\text{dip}} / \partial \varepsilon$  did not enter the theoretically plausible parameter space wherein  $\pi_{\text{dip}}$  is more sensitive to  $\varepsilon$  than  $\pi_o$  (Fig. 7).

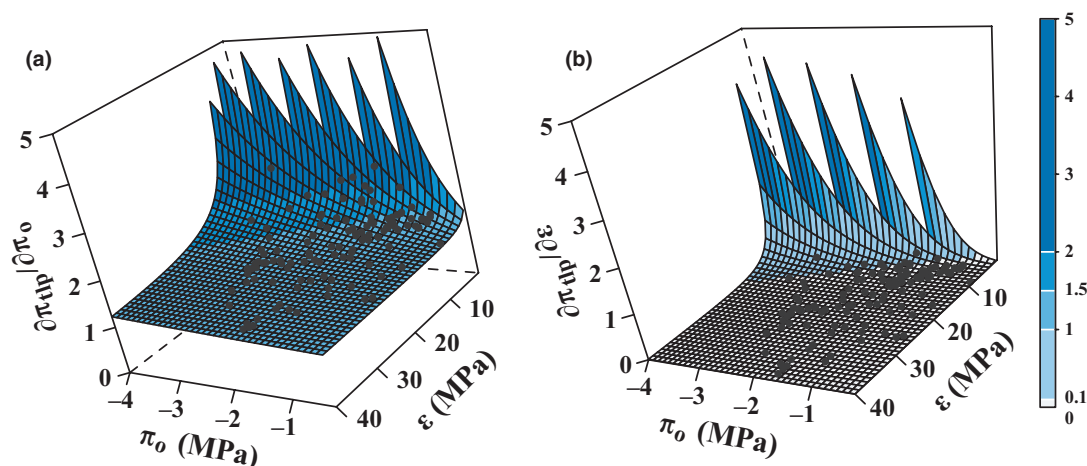
Finally, given that eqn 1 showed a stronger sensitivity of  $\pi_{\text{dip}}$  to  $\varepsilon$  at low values of  $\varepsilon$ , we determined the sensitivity of  $\pi_{\text{dip}}$  in those species with lowest  $\varepsilon$  values, and even here found stronger sensitivity to  $\pi_o$ . We identified the 25 observations in the global dataset with lowest  $\varepsilon$ , and, using eqn 1, tested the amount that  $\pi_{\text{dip}}$  was made more negative by reducing  $\varepsilon$  or by increasing  $\pi_o$  by 20%. For these species with low  $\varepsilon$ , the  $\pi_o$  and  $\pi_{\text{dip}}$  values were relatively high; mean values  $\pm$  SE (MPa) were, respectively,  $3.5 \pm 0.15$ ,  $-1.0 \pm 0.07$  and  $-1.4 \pm 0.07$ . Reducing  $\varepsilon$  by 20%, without change in  $\pi_o$ , led to an average decrease of  $\pi_{\text{dip}}$  by  $0.28 \text{ MPa} \pm 0.07$  whereas making  $\pi_o$  more negative by 20% had twice the effect, decreasing  $\pi_{\text{dip}}$  by  $0.56 \text{ MPa} \pm 0.098$  (paired *t*-test;  $P < 0.001$ ).

Our analyses demonstrated that shifts in  $\pi_{\text{dip}}$  for plants of given species are driven by osmotic and not elastic adjustment, and that

differences within and across species in  $\pi_{\text{dip}}$  are attributable entirely to  $\pi_o$ . Although lower values of  $\varepsilon$  may in principle result in lower  $\pi_{\text{dip}}$ , that only can occur in a limited range of parameter values (i.e. when  $\varepsilon$  is very low, especially when  $\pi_o$  is high). Within species on average, and across species, this effect was completely overcome by the general inverse correlation of  $\varepsilon$  and  $\pi_o$ , such that higher  $\varepsilon$  was associated with a more negative  $\pi_{\text{dip}}$ . Although numerous studies had concluded that  $\varepsilon$  had a strong role in driving  $\pi_{\text{dip}}$  reductions within species or differences in  $\pi_{\text{dip}}$  across species, based on finding substantial variation in  $\varepsilon$  values,  $\pi_o$  was the important factor due to the far greater sensitivity of  $\pi_{\text{dip}}$  to  $\pi_o$ .

### Resolution of controversy (3): a low $\pi_{\text{dip}}$ but not low $RWC_{\text{dip}}$ is associated with drought tolerance

Previous researchers have debated the importance of  $\pi_{\text{dip}}$  vs.  $RWC_{\text{dip}}$  as traits predictive of drought tolerance (Sinclair & Ludlow 1985; Kramer 1988; Schulte 1992). The analysis of species across biomes indicated that  $\pi_{\text{dip}}$  and not total  $RWC_{\text{dip}}$  was correlated with habitat moisture (Fig. 3). This conclusion was supported by additional analyses within and across species. Whereas within species  $\pi_{\text{dip}}$  declined strongly during drought (see ‘Controversy (2)’ above),  $RWC_{\text{dip}}$  showed a non-significant decline of only 1.2% (paired *t*-test,  $P = 0.08$ ;  $n = 13$ ). Indeed, across species globally,  $RWC_{\text{dip}}$  showed relatively narrow variation, with a coefficient of variation (cv) of 11%, much lower than the cv of 34% for  $\pi_{\text{dip}}$ . There was only an empirically weak and statistically non-significant tendency for  $RWC_{\text{dip}}$  to decline as  $\pi_{\text{dip}}$  become more negative ( $P = 0.06$ ;  $r^2 = 0.03$ ; slope = 1.9;  $n = 76$ , indicating a decline of  $\sim 2\%$  in  $RWC_{\text{dip}}$  per MPa of  $\pi_{\text{dip}}$ ). The  $RWC_{\text{dip}}$  appears to be conserved above 60% in all species, corresponding to a total  $RWC_{\text{dip}}$  of 75%, consistent with previous demonstrations that dehydration below this level severely inhibits metabolism (Lawlor & Cornic 2002). These data indicate that variation in  $\pi_{\text{dip}}$  is considerably more significant to physiological and ecological drought tolerance.



**Figure 7** The sensitivity of  $\pi_{\text{dip}}$  to shifts in  $\pi_o$  and  $\varepsilon$  in a global dataset, characterised as the partial derivative of  $\pi_{\text{dip}}$  with respect to each parameter. The contour surface shows the partial derivatives calculated from 1000 randomly generated parameter values across the range of parameter space, indicating the theoretically possible  $\pi_{\text{dip}}$  responsiveness. The black points are partial derivatives of the observed values (89 total; excluding the 2% of partial derivative values  $> 10$ , for visual clarity). Although it was theoretically possible for  $\partial \pi_{\text{dip}} / \partial \varepsilon > \partial \pi_{\text{dip}} / \partial \pi_o$ , as seen in the rapid contour rise at low  $\varepsilon$  and  $\pi_o$ , all species occupied the spaces where  $\partial \pi_{\text{dip}} / \partial \pi_o > \partial \pi_{\text{dip}} / \partial \varepsilon$  (the points were higher in the left than the right panel as emphasised by the darker colour of the plane and its higher position, made clearer by the rotation of the plots).



#### Resolution of controversy (4): Sclerophylly (high $\epsilon$ ) has no direct role in drought tolerance, but plays supporting roles

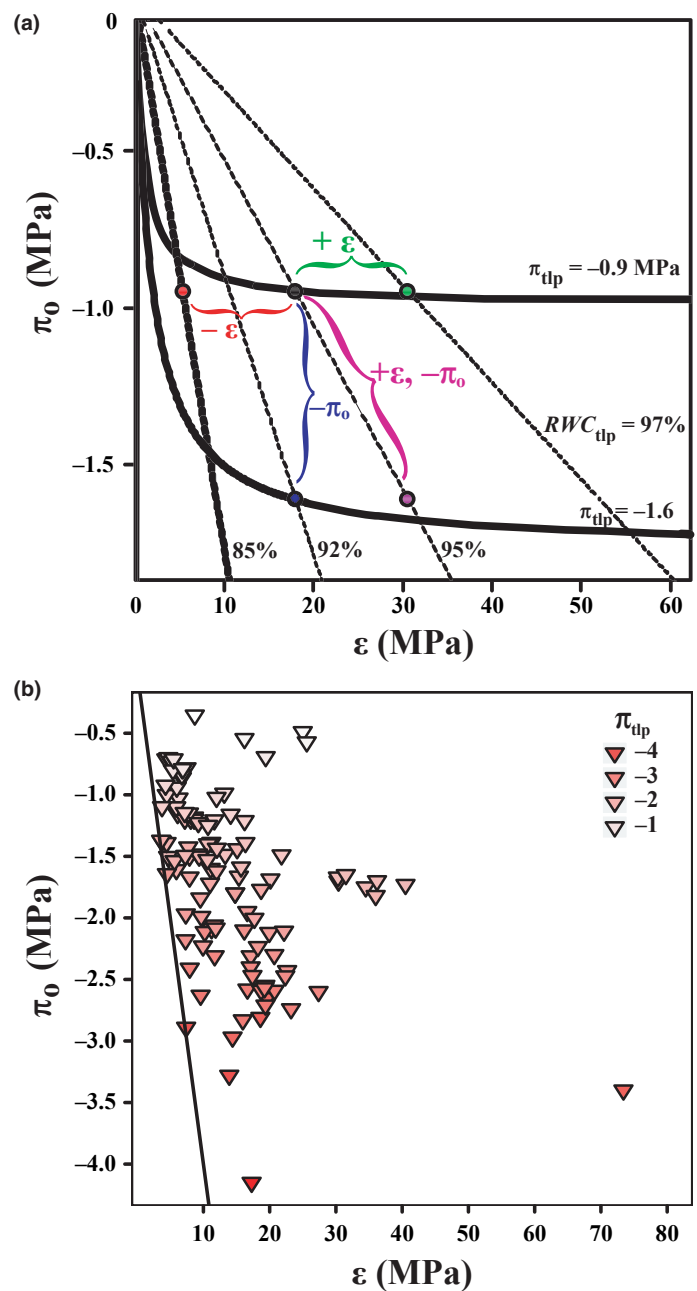
The above analyses indicated no primary contribution of  $\epsilon$  to drought tolerance through lowering  $\pi_{\text{tlp}}$ . However, we considered six additional hypotheses for a role of  $\epsilon$  in drought tolerance. We found strong evidence to support an indirect role in cell water conservation, and further benefits for tolerance of other resource shortages and/or mechanical and herbivory stresses.

The first and second hypotheses could be rejected based on theory. The idea that a higher  $\epsilon$  confers drought tolerance by inducing steep declines in  $\Psi_{\text{leaf}}$  below  $\Psi_{\text{soil}}$  as leaves lose water, enabling water to be taken up from the roots (Bowman & Roberts 1985; Niinemets 2001) can be rejected according to the Ohm's Law analogy for the soil-plant-atmosphere continuum. Although for excised leaves a higher  $\epsilon$  will lead to a more rapid decline of  $\Psi_{\text{leaf}}$  with loss of a given volume of water, in intact plants it is not  $\epsilon$ , but plant hydraulic conductance ( $K_{\text{plant}}$ ) that will determine  $\Psi_{\text{leaf}}$  and its difference from  $\Psi_{\text{soil}}$  for a given transpiration rate ( $E$ ): ( $\Psi_{\text{leaf}} = \Psi_{\text{soil}} - E/K_{\text{plant}}$ ; Tyree & Zimmermann 2002). The  $\Psi_{\text{leaf}}$  will thus always be below  $\Psi_{\text{soil}}$  regardless of  $\epsilon$ . Likewise, the second hypothesis, that a high  $\epsilon$  actually *lowers*  $\pi_{\text{tlp}}$ , contrary to the mechanisms in Fig. 2, can be rejected from the graphical analysis of the p-v curve (Fig. 2) and the analysis of eqn 1 (Fig. 4a and 4b). Proposed a number of times, including in textbooks (e.g. Larcher 2003), this fallacy apparently arises from a misleading plot of the p-v relationship (Supplementary Information, Fig. S3).

The third and fourth hypotheses could be rejected based on our analyses. The idea that a high  $\epsilon$  would confer drought tolerance by driving a less negative  $\pi_{\text{tlp}}$  and thereby rapid turgor loss such that stomata could close quickly to conserve leaf water at a high  $RWC_{\text{tlp}}$  (Walter & Stadelmann 1968; Read *et al.* 2006) was not supported because  $\pi_{\text{tlp}}$  is very insensitive to  $\epsilon$  at high values of  $\epsilon$  (Figs 4 and 7). In addition, a more negative, rather than less negative  $\pi_{\text{tlp}}$  was related to greater drought tolerance, within and across species and biomes (Figs 3, 5). Finally, early stomatal closure can be developed independently of  $\pi_{\text{tlp}}$ . While across species the  $\Psi_{\text{leaf}}$  at stomatal closure correlates with  $\pi_{\text{tlp}}$ , the stomata close in response to a low water potential in or near the guard cells and/or to chemical signals, which can be decoupled from bulk leaf  $\pi_{\text{tlp}}$  (Davies & Zhang 1991; Brodribb & Holbrook 2003). Thus, several species close their stomata at  $\Psi_{\text{leaf}}$  values less negative than their  $\pi_{\text{tlp}}$ , enabling survival on stored water which is lost slowly given low minimum epidermal conductance after stomatal closure (Guyot *et al.* 2012).

The fourth hypothesis was the idea that a high  $\epsilon$  and stiffer cell walls might be required mechanically for cells with very negative  $\pi_o$ , to withstand high turgor pressures at full hydration (Jones 1992). However, cell walls do not apparently need such high  $\epsilon$  to sustain turgor pressure. Cell walls can withstand experimental pressures many times higher than their turgor pressure before rupture (Carpita 1985; Blewett *et al.* 2000).

Indeed, the data strongly supported the fifth hypothesis, a role of high  $\epsilon$  allowing cells to prevent dehydration below a dangerous threshold  $RWC_{\text{tlp}}$ . The idea that a high  $\epsilon$  allows cells to maintain a higher  $RWC_{\text{tlp}}$  despite very negative  $\pi_o$  and  $\pi_{\text{tlp}}$ —the 'cell water conservation hypothesis' (Cheung *et al.* 1975; Jones 1992) is depicted in Fig. 8, wherein illustrative values of  $\epsilon = 18$  MPa and  $\pi_o = -0.95$  MPa were shifted by 50% to increase or decrease  $\epsilon$ , or to decrease  $\pi_o$ , or to simultaneously increase  $\epsilon$  and decrease  $\pi_o$ . As



**Figure 8** The mechanism for cell water conservation (symbols as in Table 1). (a) From initial values (black point), decreasing  $\pi_o$  strongly reduced  $\pi_{\text{tlp}}$  and  $RWC_{\text{tlp}}$  (blue), whereas increases or decreases in  $\epsilon$  raised or lowered  $RWC_{\text{tlp}}$  with slight impact on  $\pi_{\text{tlp}}$  (green and red respectively). Coordinated  $\pi_o$  and  $\epsilon$  adjustments reduced  $\pi_{\text{tlp}}$  and maintained  $RWC_{\text{tlp}}$  (purple). (b) In the global dataset,  $\pi_o$  and  $\epsilon$  were inversely correlated ( $r = 0.42$ ;  $P < 1 \times 10^{-14}$ ), and  $\pi_{\text{tlp}}$  decreased with  $\pi_o$  but not  $\epsilon$  (darker red = more negative values), consistent with cell water conservation. All values of symplastic  $RWC_{\text{tlp}}$  were above 60%, corresponding to total  $RWC_{\text{tlp}}$  of 75%, a threshold for metabolic inhibition (solid line).

expected,  $\epsilon$  reduction only slightly decreased  $\pi_{\text{tlp}}$ , whereas  $\pi_o$  reduction was considerably more effective in lowering  $\pi_{\text{tlp}}$ . However, reductions of  $\epsilon$  and  $\pi_o$  both resulted in  $RWC_{\text{tlp}}$  declines (Fig. 4c and d). In contrast, a coordinated reduction of  $\pi_o$  and *increase* of  $\epsilon$  lowered  $\pi_{\text{tlp}}$  while maintaining a constant  $RWC_{\text{tlp}}$ , which would achieve both tolerance of lower  $\Psi_{\text{soil}}$  and prevention of dangerous cell dehydration and shrinkage.

Given the potential importance of this mechanism, to test its theoretical effectiveness we used eqn 2 to apply the sensitivity analyses previously applied for  $\pi_{\text{dp}}$  to determine the sensitivity of  $RWC_{\text{dp}}$  to its underlying parameters within and among species. First, we used eqn 2 to calculate how shifts in  $\varepsilon$  and  $\pi_o$  in response to drought affected  $RWC_{\text{dp}}$  for given species from the global database (for  $n = 13$  taxa overall, and for the 5 taxa that decreased  $\varepsilon$ , and for the 8 taxa that increased  $\varepsilon$ ). As discussed above, droughted plants exhibited no significant decrease in their  $RWC_{\text{dp}}$  values (paired  $t$ -test,  $P = 0.09$ ). However, for the eight taxa that increased  $\varepsilon$  during drought, the adjustments made in  $\pi_o$  alone, to reduce  $\pi_{\text{dp}}$ , would have caused a 6.1% decline in post-drought  $RWC_{\text{dp}}$  to  $79.4 \pm 3.5\%$ , whereas adjustments in  $\varepsilon$  alone would have caused a 4.6% increase in post-drought  $RWC_{\text{dp}}$  to  $88.4 \pm 2.2\%$ , and these coordinated adjustments allowed  $RWC_{\text{dp}}$  to be maintained even when  $\pi_{\text{dp}}$  was lowered. For the five taxa that decreased  $\varepsilon$  during drought, adjustments in  $\pi_o$  alone would have caused only a non-significant 0.04% decline in post-drought  $RWC_{\text{dp}}$ , whereas adjustments in  $\varepsilon$  would have caused a 0.4% increase. Overall, these data indicate that plants in both groups reduced  $\pi_o$  to drive a lower  $\pi_{\text{dp}}$  (both  $P < 0.01$ ), and species underwent elastic adjustment if needed to maintain a high  $RWC_{\text{dp}}$ .

We also tested whether differences across species in  $\varepsilon$  likewise counteracted low  $\pi_o$  to maintain  $RWC_{\text{dp}}$ . We conducted a partial derivative sensitivity analysis for  $RWC_{\text{dp}}$  as for  $\pi_{\text{dp}}$  above. The partial derivatives  $\partial RWC_{\text{dp}}/\partial \varepsilon$  and  $\partial RWC_{\text{dp}}/\partial \pi_o$  were calculated for species in the global database and compared with paired  $t$ -tests ( $n = 76$  species) using the equations:

$$\frac{\partial RWC_{\text{dp}}}{\partial \varepsilon} = \frac{-\pi_o}{\varepsilon^2} \quad (7)$$

$$\frac{\partial RWC_{\text{dp}}}{\partial \pi_o} = \frac{1}{\varepsilon} \quad (8)$$

Across species,  $RWC_{\text{dp}}$  was significantly more sensitive to adjustments in  $\pi_o$  than  $\varepsilon$ , with  $\partial RWC_{\text{dp}}/\partial \pi_o$  nearly sevenfold greater than  $\partial RWC_{\text{dp}}/\partial \varepsilon$  (paired  $t$ -test,  $P < 2 \times 10^{-16}$ ,  $n = 76$ ). These analyses indicated that the decreases in  $\pi_o$  necessary to generate a very negative  $\pi_{\text{dp}}$  also would drive a strong decline in  $RWC_{\text{dp}}$ , requiring a higher  $\varepsilon$  to prevent decreases in  $RWC_{\text{dp}}$ . As previously discussed, none of the species in our global database had a total  $RWC_{\text{dp}}$  of less than 75% (corresponding here to a 60% symplastic  $RWC_{\text{dp}}$ ), which would significantly impair metabolic function, suggesting that plants favour the adjustment of  $\varepsilon$  to maintain sufficient  $RWC_{\text{dp}}$  (Lawlor & Cornic 2002).

The mechanistic feasibility of cell water conservation explains very well the correspondence of  $\varepsilon$  with drought adaptation which had been at first sight ambiguous, i.e. its negative correlation across species with  $\pi_{\text{dp}}$ , with high values in many plants of dry biomes, and its frequent increase in droughted plants. We note that other effects may also contribute to these trends. For example, a higher  $\varepsilon$  may be directly linked with the reduction of  $\pi_o$ , if carbon is redirected from cell wall extension for osmotic adjustment, resulting in a less flexible cell wall (Iraki *et al.* 1989; Moore *et al.* 2008). Furthermore, a low  $\pi_o$  and high  $\varepsilon$  may be coordinated with other structural features that benefit drought tolerance, such as the general trend for dry-adapted plants to exhibit a higher density of smaller cells, which increases wall investment and makes solute accumulation more efficient in lower cell volumes (Cutler *et al.* 1977). Some have proposed that a high  $\varepsilon$  may also enable

more rapid refilling of embolisms in leaf xylem (Salleo *et al.* 1997), but recent studies reported that even species with low  $\varepsilon$  can have strong refilling capacity (Trifilo *et al.* 2003; Scoffoni *et al.* 2012).

The sixth hypothesis for a role of  $\varepsilon$  was also supported by our review of the literature and meta-analyses. Sclerophylly and high  $\varepsilon$  would have additional benefits for plants in arid areas that are not directly linked to water relations, e.g. via a high LMA and high leaf density, conferring leaf longevity (Loveless 1961; Groom & Lamont 1999; Chaves *et al.* 2002; Wright & Westoby 2002). Higher leaf longevity provides an economic advantage, especially given low nutrient supply and/or a short dry season or winter, allowing greater total photosynthetic returns when leaf replacement costs exceed maintenance costs (Orians & Solbrig 1977; Mooney *et al.* 1983; Salleo *et al.* 1997). Indeed, many sclerophyllous species in semi-arid climates evolved tough leaves in response to nutrient or mechanical stress under moister climate regimes, and numerous species that currently inhabit wet zones exhibit sclerophylly (Buckley *et al.* 1980; Ackerly 2004). Conversely, sclerophylly is not necessary for drought adaptation; numerous species of dry areas exhibit soft leaves (malacophylly), including succulent and semi-deciduous species (Walter 1985). As these species have high  $\pi_o$ , their  $\pi_{\text{dp}}$  would be unresponsive to  $\varepsilon$  (Fig. 4). Thus, these species would not gain any direct advantage from having a low  $\varepsilon$  with respect to lowering their  $\pi_{\text{dp}}$ . The low  $\varepsilon$  of deciduous malacophylls may simply reflect low cell wall investment in short-lived leaves (Fig. 4; Goldstein & Nobel 1991; Loik & Nobel 1991). In addition, in dry-habitat plants with soft leaves and water storage tissues, flexible cell walls may further contribute to greater water storage capacitance after stomatal closure, given an impermeable cuticle (Ogburn & Edwards 2010). Such a low  $\varepsilon$  in water storage tissues would contribute to succulence, a drought avoidance mechanism independent of low  $\pi_{\text{dp}}$ , which contributes to an ability to survive water shortage, although not enabling sustained gas exchange and growth in drying soil for  $C_3$  or  $C_4$  plants (see Supplementary Information, 'The role of capacitance and elasticity in drought survival').

### Scales of drought tolerance

Our analyses supported a strong association of  $\pi_{\text{dp}}$  with not only physiological but also ecological drought tolerance. The  $\pi_{\text{dp}}$  reflects the ability of the bulk leaf tissue to maintain function during drought, and is also correlated with other leaf drought tolerance traits, including the  $\Psi_{\text{leaf}}$  values at which growth ceases, stomatal conductance and leaf hydraulic conductance decline by 50%, and leaves desiccate irreversibly (Abrams & Kubiske 1990; Sack *et al.* 2003; Baltzer *et al.* 2008; Mitchell *et al.* 2008; Blackman *et al.* 2010). However, as for other functional traits,  $\pi_{\text{dp}}$  must be understood as one factor in leaf and whole-plant water relations and species distributions with respect to water supply, as there are cases where other factors would become equally or more significant. At the leaf level, the  $\pi_{\text{dp}}$  is an average characteristic of all cells, and it is usually robust to heterogeneity in cell anatomy, structure and function across the leaf (Tyree & Hammel 1972; Tyree 1981). However, for some taxa this average may not well represent the turgor loss point of the photosynthetic mesophyll cells, or bundle sheath cells that contribute to hydraulic conductance, or the epidermal pavement cells or guard cells that control stomata, and thus in cases may not be a good predictor of these cells' loss of function.

Furthermore, although leaf and whole-plant drought tolerance are generally coordinated, as shown by the relationships between  $\pi_{\text{dp}}$  and water availability in this study, some plants with relatively tolerant

leaves may be drought-sensitive at the whole-plant level (e.g. *Magnolia grandiflora* due to its shallow roots; Scoffoni *et al.* 2011). Conversely, as mentioned above, species with sensitive leaves may have excellent drought survival—especially succulent species, when  $\pi_{\text{tp}}$  is likely to be less important than the capacitance of water storage cells (Chaves *et al.* 2002; Ogburn & Edwards 2010). Other species may go dormant, shed leaves or whole shoots during the drought season, or persist in episodically very dry habitats through desiccation tolerance of vegetative parts and/or seeds (Jenks & Wood 2007).

Even beyond whole-plant drought tolerance, at ecological scales, the interplay between drought tolerance and competitive and trophic interactions will contribute to species distributions and population dynamics (Chesson *et al.* 2004). Even so, recent work has provided strong evidence that drought tolerance indeed scales up in many cases to determine communities and their dynamics in dry as well as typically wet systems (e.g. Engelbrecht *et al.* 2007; McDowell 2011; Blackman *et al.* 2012).

## CONCLUSIONS

We are in strong need of traits for rapidly assessing species' drought tolerances. Prediction of drought tolerance and distributions for diverse species and vegetation types based on traits is increasingly necessary given climate change. A direct role of  $\pi_{\text{tp}}$  and  $\pi_o$  in determining physiological drought tolerance is well established, but the lack of demonstration of a role in realised ecological drought tolerance and the controversies of their interpretation slowed their application in comparative and community level trait studies. In our global meta-analysis,  $\pi_{\text{tp}}$  showed a strong association with water availability within and across biomes, and was typically more effective than LMA and other p-v parameters as a functional trait representing drought tolerance.

Beyond establishing the importance of  $\pi_{\text{tp}}$  as a functional trait at species and biome levels, we resolved long-standing controversies surrounding its interpretation. The derivation and application of eqns 1 and 2 clarified the mechanistic bases for  $\pi_{\text{tp}}$  and  $RWC_{\text{tp}}$  and provided a novel mathematical framework to resolve questions of their significance and their determination. These analyses showed that variation in  $\pi_{\text{tp}}$  within and across species is due virtually entirely to shifts in  $\pi_o$ , with coordinated adjustments in  $\epsilon$  having no direct impact on  $\pi_{\text{tp}}$ , but acting to compensate for variation in  $\pi_o$  and allowing maintenance of a high  $RWC_{\text{tp}}$ , thereby preventing dangerous levels of cell dehydration and shrinkage.

These findings also clarified species-level drought responses and biogeographic trends in sclerophylly. A high  $\epsilon$  has an indirect role in drought adaptation, including maintaining  $RWC_{\text{tp}}$  and thereby, cell hydration, when  $\pi_o$  is low. This provides a benefit for sclerophylly in many arid-adapted species, given the strong relationship of  $\pi_o$  with drought tolerance. However, not all arid-zone species have high  $\epsilon$ ; malacophyllous species that avoid drought with water storing or deciduous leaves and low solute concentrations can have low  $\epsilon$ . Furthermore, sclerophylly would also be selected in leaves without a low  $\pi_o$  and thus poor drought tolerance, to extend leaf lifespan during other resource shortages or stresses.

The new perspective presented here points to a renewed value of  $\pi_{\text{tp}}$ , and a clear framework for the importance of its underlying parameters. Given the remarkable degree that  $\pi_o$  and  $\pi_{\text{tp}}$  scale up, cell water relations has previously unappreciated predictive power at the levels of leaf, whole plant and even the biome. Given additional

consideration of other factors that can contribute to tolerance or avoidance of drought, the  $\pi_{\text{tp}}$  has strong value as a functional trait for species and ecosystems, to allow increasing assessment of their comparative drought tolerance and their potential sensitivity to climate change.

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MB, CS and LS all contributed to the design, research and writing of this article.

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