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Title

Does the turgor loss point characterise drought response in dryland plants?

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Abstract

The water potential at turgor loss point (Ψ_{tlp}) has been suggested as a key functional trait for determining plant drought tolerance, due to its close relationship with stomatal closure. Ψ_{tlp} may indicate drought tolerance as plants which maintain gas exchange at lower midday water potentials as soil water availability declines also have lower Ψ_{tlp}. We evaluated 17 species from seasonally dry habitats, representing a range of life-forms, under well-watered and drought conditions, to determine how Ψ_{tlp} relates to stomatal sensitivity (pre-dawn water potential at stomatal closure: Ψg_{s0}) and drought strategy (degree of isohydry or anisohydry; $\Delta\Psi_{\mathrm{MD}}$ between WW conditions and stomatal closure). Although Ψg_{s0} was related to Ψ_{tlp}, Ψg_{s0} was better related to drought strategy ($\Delta\Psi_{\mathrm{MD}}$). Drought avoiders (isohydric) closed stomata at water potentials higher than their Ψ_{tlp}; whereas, drought tolerant (anisohydric) species maintained stomatal conductance at lower water potentials than their Ψ_{tlp} and were more dehydration tolerant. There was no significant relationship between Ψ_{tlp} and $\Delta\Psi_{\mathrm{MD}}$. While Ψ_{tlp} has been related to biome water availability, we found that Ψ_{tlp} did not relate strongly to stomatal closure or drought strategy; for either drought avoiders or tolerators. We therefore suggest caution in using Ψ_{tlp} to predict vulnerability to drought.

Keywords: drought; water relations; anisohydry; isohydry; P-V curve; TLP

Introduction

Drought-related mortality and die-back are increasingly being reported from vegetation communities globally (Allen, Macalady, Chenchouni, Bachelet, McDowell, Vennetier, Kitzberger, Rigling, Breshears & Hogg, 2010). Consequently there is growing interest in being able to predicting plant species vulnerability to water limitations from physiological traits such as leaf turgor loss point, to understand potential changes in plant distributions and ecosystem structure (Bartlett, Zhang, Kreidler, Sun, Ardy, Cao & Sack, 2014, Maréchaux, Bartlett, Sack, Baraloto, Engel, Joetzjer & Chave, 2015, Mitchell & O'Grady, 2015).

Drought is a meteorological term; but the majority of plant physiology papers use it to describe how plants respond to the stress of increasing water deficits. There are many definitions and theories about how plants adapt to drought, but ultimately the survival of a plant is determined by its ability to avoid or tolerate drought; i.e., it’s drought strategy. The two contrasting drought strategies can also be broadly classified as isohydry and anisohydry, where isohydry is generally associated with dehydration avoidance and anisohydry with dehydration tolerance (Stocker, 1956; Tardieu & Simonneau, 1998). Both strategies can co-occur in dryland ecosystems (Blum, 2005) and are not necessarily related to life-form or regeneration strategy (Galmés, Flexas, Savé & Medrano, 2007, Vilagrosa, Hernández, Luis, Cochard & Pausas, 2013, West, Dawson, February, Midgley, Bond & Aston, 2012). Isohydric plants are thought to have a have high stomatal sensitivity and avoid water stress by closing their stomata to maintain stable midday water potentials (Ψ_{MD}) regardless of environmental conditions (Tardieu & Simonneau, 1998). Whereas anisohydric species have lower stomatal sensitivity and maintain gas exchange by lowering their midday water potentials as water availability declines (Blum, 2005, Sperry, Hacke, Oren & Comstock, 2002, Stocker, 1956, Tardieu & Simonneau, 1998). As such, the degree to which plants drop their midday water potentials under drought conditions ($\Delta\Psi_{\mathrm{MD}}$) before stomatal closure can be used to describe a plant's drought strategy (Delzon, 2015, Farrell, Szota, Williams & Arndt, 2013b, Franks, Drake & Froend, 2007).

The leaf water potential at zero turgor, or the turgor loss point (Ψ_{tlp}), is increasingly being used as a functional trait for determining drought tolerance (Bartlett, Scoffoni & Sack, 2012b, Bartlett *et al.*, 2014, Sack, Cowan, Jaikumar & Holbrook, 2003). Ecologically, Ψ_{tlp} has been shown to be strongly correlated with water availability both within and across biomes (Bartlett *et al.*, 2012b) and species from drier sites typically have lower Ψ_{tlp} (Lenz, Wright & Westoby, 2006, Merchant, Callister, Arndt, Tausz & Adams, 2007, Mitchell & O'Grady, 2015). Physiologically, plants with lower Ψ_{tlp} generally show greater tolerances to lower water potentials (Blackman, Brodribb & Jordan, 2010) as plants with a lower Ψ_{tlp} can maintain leaf turgor and therefore maintain metabolic function, stomatal conductance and growth at lower soil water contents (Kramer & Boyer, 1995). Therefore, Ψ_{tlp} might be a key trait to characterise the ability to maintain leaf function under moderate drought, as plants which maintain gas exchange at lower midday water potentials, should also have lower Ψ_{tlp} (Delzon, 2015). However, most of the ecological and physiological relationships of Ψ_{tlp} have been observed in broad scale meta-analyses. While these have been very useful in discovering general patterns and the link between Ψ_{tlp} and environmental aridity, they have not elucidated the role of Ψ_{tlp} in the more immediate responses of plants to water deficit. In other words, it is unclear if Ψ_{tlp} can be used as an indicator or trait to evaluate how plants adjust to a developing soil water deficit. The relationships of Ψ_{tlp} with stomatal sensitivity or water potential adjustment during drought have not been investigated in detail and these are important to better understand how useful Ψ_{tlp} is as a physiological indicator during drought.

Stomatal sensitivity to drought stress has been correlated with Ψ_{tlp}, with stomatal closure occurring before Ψ_{tlp} to maintain leaf turgor (Brodribb, Holbrook, Edwards & Gutierrez, 2003, Hinckley, Duhme, Hinckley & Richter, 1983, Mitchell, O'Grady, Tissue, White, Ottenschlaeger & Pinkard, 2013, Morgan, 1984). However, this relationship has been shown for very few species and life-forms: four tropical (Brodribb *et al.*, 2003) and three temperate tree species (Mitchell *et al.*, 2013). Others have also suggested that the link between turgor maintenance and gas exchange is ambiguous (Sperry, 2000) and that stomatal closure is primarily coordinated with loss in leaf hydraulic conductance through cavitation (Brodribb & Holbrook, 2003). As Ψ_{tlp} reflects bulk leaf turgor, stomatal closure may also occur earlier than the Ψ_{tlp} in some species due to chemical signalling or where bulk leaf water potential differs from guard cell turgor (Blum, 2011). Therefore, there is a need to confirm the relationship between Ψ_{tlp} and water potential at stomatal closure for a broader range of species and life-forms to determine whether Ψ_{tlp} can be used as a trait to characterise stomatal sensitivity to drought stress.

The relationship between the adjustment of water potential and Ψ_{tlp} also requires more detailed investigation. Although recent studies have compared Ψ_{tlp} of many species and related these to climatic water availability (Bartlett *et al.*, 2012b, Lenz *et al.*, 2006, Mitchell & O'Grady, 2015, Mitchell, Veneklaas, Lambers & Burgess, 2008), these comparative studies have not considered differences in plant drought strategies. Due to differences in stomatal regulation and maintenance of leaf water potential, Meinzer *et al.* (2014) hypothesised that isohydric species that maintain a stable Ψ_{MD} under drought conditions (drought avoiders) would have a higher Ψ_{tlp} than species that anisohydric species, which adjust the Ψ_{MD} to more negative values under drought (drought tolerators). While there is evidence in some species for this hypothesis in two tree species (Meinzer et al., 2014), there is a need to determine whether Ψ_{tlp} is associated with drought strategy across a broader range of species and life-forms.

We evaluated 17 species with a range of life-forms from seasonally dry habitats under glasshouse conditions to determine: 1) whether Ψ_{tlp}, relates to stomatal sensitivity to drought stress; and 2) whether Ψ_{tlp} is related to drought strategy; i.e, the ability of plants to lower midday water potentials in response to drought.

Materials and Methods

Species selection

To determine differences in drought response between life-forms which grow in seasonally dry habitats, we selected 20 species with five different life-forms from four different habitats with restricted water availability due to shallow or very free-draining soils with low water holding capacity. These habitats included rock outcrop, inland woodland, coastal dune and rocky grassland plant communities from across south-eastern Australia (Victoria; Table 1). Following establishment, the 20 species were reduced to 17 on account of obligate dormancy in two of the geophytes and loss of one herb species due to poor establishment. These 17 species were then unevenly distributed between the life-forms with: two geophytes; four grass-like monocots; three herbs; four prostrate shrubs; and four small shrubs (<1 m tall). Table 1 describes the habitat, life-form and mean annual aridity index for natural range (Atlas of Living Australia) of each species. Based on their minimum aridity indices (Table 1), all species were distributed in areas considered semi-arid (0.2-0.5) or arid (0.03-0.2) (UNEP, 1997).

Plants were obtained as six-month old seedlings from commercial nurseries. In early spring (September 2011), 15 plants of uniform size of each species were planted into 4 l black plastic containers (200 mm diameter, 190 mm height) containing 3.5 kg of a scoria-based substrate (60% aerolite black scoria 8 mm minus blockmix; 20% 7 mm red scoria aggregate and 20% coir) with a water holding capacity of 46% and a bulk density of 1.26 g cm^{-3} (Farrell, Ang & Rayner, 2013a). Twelve grams of low phosphorous slow release fertiliser (Osmocote® plus, Scotts Australia Pty Ltd.; 16 nitrogen (N):1.3 phosphorus (P):9.1 potassium (K)) was added to the surface of each pot one week post-planting. Plants were grown in a glasshouse at the Burnley Campus, University of Melbourne at an average temperature of 23 °C during the experiment. All plants were watered to pot capacity twice a week before the start of the experiment in mid-summer (January, 2012).

Experimental design

Five plants of each species were randomly allocated to two treatments: well-watered (WW) and drought (D). The remaining five plants were harvested at the start of the experiment to obtain initial fresh weights. An additional 10 substrate-only pots were used to determine evaporation rates from WW and D treatments during the experiment (five pots per treatment). Pots were arranged in a complete randomised block design (five blocks). The experiment ran for 73 days, between late-

January (mid-summer) and mid-April (mid-autumn). Well-watered plants were watered twice weekly (Monday and Thursday) to pot capacity (2 l per pot) while each pot in the drought treatment was dried out to approximately 10% SWC before being rewatered to pot capacity (see below) and again left to dry out. This cycle of drying and rewatering was repeated for 6 weeks before plants were left to dry out without further rewatering. All physiological measures were collected during the final dry down period for each species.

Soil water content over time

Soil water content (SWC) was determined from pot weights pre- and post-watering. SWC was calculated by first correcting pot weight for estimated plant weight at each weighing; estimated as: initial mean fresh weight + daily biomass gain; where daily biomass gain = (final fresh weight – initial fresh weight) / number of days in experiment. Initial and final fresh weights were obtained from harvests of five plants of each species at the start and end of the experiment. SWC was then calculated as: (corrected pot weight – substrate dry weight) / substrate dry weight. Substrate dry weight was determined for substrate from the 10 bare pots (five per treatment) at the end of the experiment after drying in a 105ºC oven to a constant weight.

Stomatal conductance

Stomatal conductance (g_s) was measured with a LI-6400 gas exchange system (LI-COR Inc. Lincoln, NE) between 800 and 1130h every 1-2 days during the final dry down period to determine the point of stomatal closure ($g_s < 0.05$ mol H_2O m^{-2} s^{-1}) in drought plants. Well-watered plants were also measured regularly during this period. Photosynthetic photon flux density (red–blue light source) was set at 1500 µmol m^{-2} s^{-1} and CO_2 concentration in the chamber at 400 µmol mol^{-1}. Temperature in the chamber was set at 25 °C. Relative humidity was adjusted to maintain chamber values between 55 and 60 %. Leaves were allowed to reach equilibrium for 3-4 minutes prior to measurement. Photographs of measured leaves were taken and leaf area determined using ImageJ (Rasband, 1997-2012).

Plant water potentials

Pre-dawn (Ψ_{PD}; 0400-0500 h) and midday (Ψ_{MD}; 1230-1300 h) leaf water potentials were measured regularly to determine water potential at effective stomatal closure ($g_s < 0.05$ mol H_2O m^{-2} s^{-1}). Midday water potentials were also used to determine species drought strategy, (degree of isohydry and anisohydry) described as the drop in midday water potential between well-watered plants and in drought plants when stomatal closure had occurred ($\Delta\Psi_{MD}$ = mean D Ψ_{MD} – mean WW Ψ_{MD}); where greater $\Delta\Psi_{MD}$ indicates greater anisohydry. Tissue samples were excised, sealed immediately into zip-lock bags and stored in an insulated box until measurement. Leaf water potential was measured using a Scholander-type pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA). Due to short petiole lengths, the majority of water potentials were measured on apical branches with two to three leaves; while the remainder (mostly monocots) were determined on individual leaves.

Pressure-volume curves

Pressure-volume (PV) curves were used to determine tissue or cellular water relations of the 17 species. We measured PV curve parameters during the final dry down period of drought plants when plants reached a SWC of approximately 15%. Well-watered plants were measured in one week at the end of the experiment. This meant that sampling could be staggered across species with three to five species per measurement day. Five replicates of each species were measured using the bench drying method (Turner, 1988). As per the pre-dawn and midday water potential measurements, either whole leaves or apical branches were sampled for PV curve measurements at pre-dawn (0400-0500 h) and recut under deionised water before weighing to determine fresh weight and then rehydrating in a dark room at 21 to 24 ºC for 4-6 hours. Sections were rehydrated in 50 ml centrifuge tubes filled with deionised water, with only the cut end of the leaf or apical branch submerged. Following rehydration, samples were blotted dry, recut and weighed before measuring leaf water potential (Ψ_L; as above), to determine saturated weights. Only samples which had re-hydrated to a water potential of more than -0.2 MPa were used to develop PV curves. Samples were then left on the bench to dry and leaf water potential and weight were regularly measured as leaf *RWC* decreased. Tissue water relations parameters including: turgor loss point (Ψ_{tlp}), relative water content at full turgor (RWC_{tlp}), bulk modulus of elasticity (ε_{max}) and osmotic pressure at full turgor (π_o) were calculated using the PV curve fitting routine (Microsoft Excel 2000; K.Tu, University of California Berkeley v5.6, http://landflux.org/Tools.php) based on the approach of Schulte and Hinckley (1985). *RWC* was determined as: *RWC* = (fresh weight – dry weight)/(saturated weight – dry weight) x 100. At the end of the PV curve measurement period, samples were oven dried at 70 °C to a constant weight to determine dry weight.

Data analysis

Stomatal conductance variables including pre-dawn leaf water potential at stomatal closure (Ψg_{s0}) and at 50% stomatal closure (Ψg_{s50}), as well as stomatal conductance at Ψ_{tlp} as a percentage of maximum stomatal conductance under well-watered conditions ($g_s\Psi_{\mathrm{tlp}}$ (% of $g_{s\ max}$)); were derived from models predicting g_{s} from Ψ_{PD} (Table S1 and Figures S1-4). Linear and curvilinear models were fitted to relationships between Ψ_{PD} (actually the inverse of, given that Ψ_{PD} values are negative) and g_{s} in R, version 3.0.3 (R Core Team, 2013). Residual plots from linear regression indicated that nonlinear relationships existed for all species, therefore power, logarithmic, exponential and sigmoidal (logistic and Weibull) models were fitted using the 'stats' package. The 'best' model for each species was selected as having the lowest residual standard error. Models were fitted using all measured replicates from both WW and D treatments. Prediction intervals (95%) were determined for each model and adjusted R^2 (with associated *P-values*) were determined by plotting observed versus predicted values of g_s (log-transformed where necessary to achieve linearity). Models were rejected where (i) residual values were not normally distributed and/or (ii) where heterogeneity of variance was observed. The same procedure was followed to describe the relationship between g_s and SWC (see Table S2), such that relative stomatal closure could be compared among species at a standard soil matric potential, in this case permanent wilting point (-1.5 MPa; derived for a similar substrate from Farrell et al. (2013a); stomatal conductance at the permanent wilting point (g_sPWP (% of $g_{s\ max}$)) .

For plant water potential and PV curve-derived traits, differences between species within drought or well-watered treatments were analysed using one-way ANOVA. Significant differences between species means were determined by Tukey's *post hoc* test ($P < 0.05$). Differences between drought and well-watered treatments within species were analysed with Mann Whitney U tests as data were not normally distributed. Relationships between Ψ_{tlp} or drought strategy (; $\Delta\Psi_{\mathrm{MD}}$ = mean D Ψ_{MD} – mean WW Ψ_{MD}) and stomatal conductance variables obtained from stomatal response curves: predawn water potential at stomatal closure (Ψg_{s0}) and Ψ_{PD} at 50% stomatal closure (Ψg_{s50}), stomatal conductance at Ψ_{tlp} as a percentage of WW stomatal conductance ($g_s\Psi_{\mathrm{tlp}}$ (% of $g_{s\,max}$)) and stomatal conductance at the permanent wilting point (g_sPWP (% of $g_{s\,max}$) were analysed using simple linear regression. Relationships between drought strategy ($\Delta\Psi_{\mathrm{MD}}$) and Ψ_{tlp} or dehydration tolerance (Ψg_{s0} (% of Ψ_{tlp})) were also analysed using simple linear regression. All data presented in figures and tables are non-transformed. These data analyses used GenStat 15 (VSN International Ltd, Hemel Hempstead, UK.).

Results

Leaf water potential and drought strategy

Midday water potential (Table 2) at stomatal closure was not significantly different from well-watered conditions in eight of the 17 species: the two geophytes (*Arthropodium milleflorum* and *Pelargonium rodneyanum*), all monocots except *Dianella revoluta*, two of the herbs (*Isotoma axillaris* and *Stylidium graminifolium*) and one shrub (*Platylobium obtusangulum*). In contrast, the remaining species all had significantly lower water potentials (Ψ_{MD}) at stomatal closure than under well-watered conditions. By using the difference between mean Ψ_{MD} at stomatal closure (drought) and when well-watered ($\Delta\Psi_{\mathrm{MD}}$) to describe drought strategy (Delzon, 2015, Farrell *et al.*, 2013b, Franks *et al.*, 2007) along a continuum of isohydry and anisohydry, all herbs and shrubs were more anisohydric than the geophytes and monocots (Table 2). The lowest midday water potentials were measured in the shrubs: *Enchylaena tomentosa* (-5.40 MPa), *Eutaxia microphylla* (-4.75 MPa) and *Senna artemisiodes* (-4.43 MPa).

PV curve-derived traits under drought and well-watered conditions

Tissue water relations parameters including: turgor loss point (Ψ_{tlp}), relative water content at full turgor (RWC_{tlp}), bulk modulus of elasticity ($\varepsilon_{\mathrm{max}}$) and osmotic pressure at full turgor (π_{o}) for the 17 species are shown in Table 3. These traits were not related to life-form; however, the two geophytes had higher values for Ψ_{tlp} and π_{o} under well-watered conditions. *Lomandra filiformis* (monocot) had the lowest Ψ_{tlp} (-2.96 MPa), π_{o} (-2.64 MPa), $\varepsilon_{\mathrm{max}}$ (31.3 MPa; very inelastic tissue) and the highest RWC_{tlp} (94.6%) under well-watered conditions. *E. tomentosa*, a shrub with succulent leaves, had the lowest RWC_{tlp} (73.4%).

These patterns were also evident under drought conditions. However, some species adjusted their tissue water relations under drought conditions (*P-values* in Table 4). Half of the shrub species lowered their Ψ_{tlp} under drought conditions, while *L. filiformis* (monocot) increased its Ψ_{tlp} under drought conditions. All species which lowered Ψ_{tlp} also lowered π_{o}, although these adjustments only reduced RWC_{tlp} in *Correa glabra*. Lowering of RWC_{tlp} under drought conditions was insufficient to result in lowered Ψ_{tlp} in *P. obtusangulum*, *E. microphylla* (shrubs) and *F. nodosa* (monocot).

The water potential at turgor loss (Ψ_{tlp}) was strongly influenced by osmotic pressure at full turgor (π_{o}); with species with low Ψ_{tlp} also having a low π_{o} ($P \leq 0.001$; $R^2 = 0.924$). This relationship did not differ significantly between drought and well-watered plants ($P = 0.278$) or between life-forms. The relationships between Ψ_{tlp} and RWC_{tlp} (D: $P = 0.589$; WW: $P = 0.711$) or ε_{max} (D: $P = 0.079$; WW: $P = 0.302$) were not significant, even when the outlier (*L. filiformis*) was excluded from analysis (data not shown). There was no relationship between Ψ_{tlp} and the aridity of the environments where the plants naturally occur, based on the aridity index data presented in Table 1 (regressions not shown).

Relationships between Ψ_{tlp} and stomatal sensitivity to drought

Under drought conditions, differences in pre-dawn water potential at stomatal closure (Ψg_{s0}) were related to values of Ψ_{tlp} determined on drought plants ($P = 0.01$; $R^2 = 0.33$; Fig. 1A). Species with lower Ψg_{s0} had lower Ψ_{tlp} and these were generally anisohydric shrubs (Fig. 1A; Table 2). However, four species (three shrubs and a monocot) showed stomatal closure at water potentials lower (i.e., below the 1:1 line) than their Ψ_{tlp} (Fig. 1A). Eight species, including the geophytes and most monocots, showed Ψg_{s0} values higher than their Ψ_{tlp}, while the remaining five species showed $\Psi g_{\text{s0}} \approx \Psi_{\text{tlp}}$ (Fig. 1A). Relationships between Ψ_{tlp} and water potential at 50% stomatal closure (Ψg_{s50}; Fig. 1B), stomatal conductance at Ψ_{tlp} as a percentage of stomatal conductance under WW conditions ($g_s\Psi_{\text{tlp}}$ (% of $g_{s\,max}$); Fig. 1C) and stomatal conductance at the permanent wilting point (g_sPWP (% of $g_{s\,max}$); Fig 1D) were not significant. Relationships between stomatal conductance measures and the other PV-derived traits (RWC_{tlp}, ε_{max} and π_{o}) were not significant (data not shown).

Relationships between drought strategy ($\Delta\Psi_{MD}$) and stomatal conductance

Under drought conditions, differences in pre-dawn water potential at stomatal closure (Ψg_{s0}) were also related to drought strategy, or the degree of isohydry and anisohydry, described as the difference between Ψ_{MD} at stomatal closure (drought) and when well-watered ($\Delta\Psi_{\text{MD}}$) ($P \leq 0.001$; $R^2 = 0.57$;

Fig. 2A). Species with lower Ψg_{s0} decreased $\Delta\Psi_{MD}$ and were therefore more anisohydric (three shrubs and one monocot), while species with higher Ψg_{s0} showed similar Ψ_{MD} under drought and were therefore isohydric (geophytes). Species with higher $\Delta\Psi_{MD}$ also showed higher stomatal conductance at Ψ_{PD} equivalent to their Ψ_{tlp}, ranging from 12-35% of maximum g_s ($P = 0.042$; $R^2 = 0.25$; Fig. 2C). Although, one isohydric monocot species (*Ficinia nodosa*), was also able to maintain 28% stomatal conductance at its Ψ_{tlp}. Relationships between $\Delta\Psi_{MD}$ and water potential at 50% stomatal closure (Ψg_{s50}) were not significant (P = 0.21; Fig. 2B). There was also no relationship between $\Delta\Psi_{MD}$ and g_sPWP (% of $g_{s\,max}$; P = 0.80; Fig. 2D).

Dehydration tolerance and drought strategy

The Ψ_{tlp} was not related to drought strategy ($\Delta\Psi_{MD}$; $P = 0.26$; Fig. 3A), i.e., plants that were anisohydric and were able to decrease their Ψ_{MD} did not have a lower Ψ_{tlp}. However, the ability of plants in our experiment to exceed their Ψ_{tlp} i.e. have more negative Ψ_{PD} at stomatal closure than their Ψ_{tlp}, was related to drought strategy ($R^2 = 0.33$; $P = 0.017$; Fig. 3B). We used Ψg_{s0} as a percentage of Ψ_{tlp} (Ψg_{s0} (% of Ψ_{tlp})) to describe a species' dehydration tolerance. Species with greater anisohydry (greater $\Delta\Psi_{MD}$) were more tolerant of dehydration than isohydric species as they continued to maintain stomatal conductance at pre-dawn water potentials that were greater than their Ψ_{tlp}.

Discussion

1. Relationship between Ψ_{tlp} and stomatal sensitivity to drought stress

Justification of the use of Ψ_{tlp} as a key functional trait for understanding species distributions along climate or soil water gradients in ecological studies has relied on its relationship with leaf water potential at stomatal closure; i.e., stomatal sensitivity (Bartlett *et al.*, 2012b, Maréchaux *et al.*, 2015, Meinzer *et al.*, 2014, Mitchell & O'Grady, 2015). However, as previously discussed, this relationship has only been demonstrated with very few species (Brodribb *et al.*, 2003, Mitchell *et al.*, 2013). In our study, plant water potential at stomatal closure (Ψg_{s0}) was related to Ψ_{tlp}, with higher plant water potential (less negative Ψ_{PD}) at stomatal closure in species with higher Ψ_{tlp}. This relationship is in agreement with studies on drought tolerant shrubs (Hinckley, Duhme, Hinckley & Richter, 1980, Hinckley *et al.*, 1983). However, many of the 17 species in our experiment closed their stomata at Ψ_{PD} lower or higher than their Ψ_{tlp}, regardless of life-form. Hence, while we observed a significant relationship between Ψ_{tlp} and stomatal closure across all species, plant species seem to exhibit individual sensitivities to stomatal closure that are not necessarily related to $\Psi_{\text{tlp.}}$

Lower water potential at stomatal closure than Ψ_{tlp} occurred in several of the shrub species, which indicates that these species had lost leaf turgor before ceasing stomatal conductance. Maintenance of stomatal conductance despite losing turgor has been reported elsewhere in drought tolerant shrubs, (Guyot, Scoffoni & Sack, 2012, Scholz, Bucci, Arias, Meinzer & Goldstein, 2012, Vilagrosa, Bellot, Vallejo & Gil-Pelegrín, 2003), trees (Alder, Sperry & Pockman, 1996) and grasses (Barnes, 1985). The role of Ψ_{tlp} in driving stomatal closure has been questioned by several authors (Ali, Jensen, Mogensen, Andersen & Henson, 1999, Turner, 1986) who showed that stomatal closure is more closely related to soil water potential than leaf water potential or Ψ_{tlp}. Further, guard cell turgor, rather than bulk leaf turgor is more closely related to stomatal function)(Blum, 2011, Cowan, 1978).

It has also been suggested that the association between turgor maintenance and stomatal control of water use is primarily driven by cavitation-induced losses of leaf hydraulic conductance (K_{leaf}) (Brodribb & Holbrook, 2003, Sperry, 2000) and stomatal conductance can continue despite partial cavitation (Manzoni, Vico, Katul, Palmroth, Jackson & Porporato, 2013, Vilagrosa *et al.*, 2003).

Brodribb *et al.* (2003) is often cited to demonstrate the relationship between stomatal closure and Ψ_{tlp} as they showed a relationship between leaf water potential at 50% stomatal conductance (which they considered closure) and Ψ_{tlp} in eight tree species. However, they also showed species which ceased stomatal conductance at water potentials higher than their Ψ_{tlp}, consistent with our observations. In our study, species which ceased stomatal conductance at Ψ_{PD} values higher than their Ψ_{tlp} included the geophytes (*A. milleflorum* and *P rodneyanum*), two monocots (*D. revoluta* and *S.glauca*), two herbs (*C. apiculatum* and *S. graminifolium*) and one shrub (*P. nivea*). Early stomatal closure likely indicates high stomatal sensitivity in these species, typical of non-woody species from water limited environments (Chaves, Pereira, Maroco, Rodrigues, Ricardo, Osorio, Carvalho, Faria & Pinheiro, 2002). However, early closure in the geophyte and monocot species is likely due to succulence, with tubers (geophytes) and fleshy rhizomes (monocots) (Pate & Dixon, 1981) storing water to enable metabolism to continue despite conservative water use strategies (Eggli & Nyffeler, 2009). In the case of the geophytes, both species had drought sensitive leaves (high Ψ_{tlp}); but at the whole plant level water storing tubers may have enabled them to maintain leaf water potential during drought (Farrell *et al.*, 2013b). Therefore, due to high water storage capacitance, early stomatal closure in these species is likely to provide benefits for leaf survival (Guyot *et al.*, 2012), by buffering leaf water potential (Holloway-Phillips & Brodribb, 2011).

Leaf water potential at stomatal closure (Ψg_{s0}) was significantly related to drought strategy ($R^2 = 0.57$, Fig. 2A), with anisohydric species ceasing stomatal conductance at lower water potentials than isohydric species. This makes sense because isohydric species use stomatal control to balance changes in leaf water potential as soil water potential declines and avoid further water deficit (Tardieu & Simonneau, 1998). Anisohydric species were also able to maintain higher rates of stomatal conductance at Ψ_{tlp} and in some shrub species this was up to 35% of rates under WW conditions. Similar results have been reported in shallow-rooted Patagonian desert shrubs (Scholz *et al.*, 2012) and non-hemiphytic *Ficus* spp. (Hao, Sack, Wang, Cao & Goldstein, 2010) which maintained 40-60% stomatal apertures under drought conditions despite loss of leaf turgor. This strategy has advantages in terms of carbon gain but could be negative if hydraulic failure occurs (Scholz *et al.*, 2012).

Our results suggest that the Ψ_{tlp} of a species does not give a clear indication as to whether that species is more or less likely to maintain a higher water status at stomatal closure and therefore persist/survive for a longer period under drought conditions. The $\Delta\Psi_{\mathrm{MD}}$ seems to be more closely related to stomatal sensitivity; however, it is essential that we understand under what circumstances either the Ψ_{tlp} or $\Delta\Psi_{\mathrm{MD}}$ are related to performance under drought conditions if we are to predict how species are likely to respond to shifts in climate.

2. *Relationship between* Ψ_{tlp} *and drought strategy*

It has been suggested that Ψ_{tlp} could be related to anisohydry, i.e., the decrease in Ψ_{MD} between well-watered and drought conditions (Delzon, 2015). This has been demonstrated in grape vines, with lower Ψ_{tlp} in more drought tolerant cultivars (Tombesi, Nardini, Farinelli & Palliotti, 2014). In our study Ψ_{tlp} was not related to drought strategy; however, the degree to which water potentials at stomatal closure exceeded their Ψ_{tlp} was weakly related to drought strategy ($R^2 = 0.33$; Fig. 3B). Species which exceeded their Ψ_{tlp} to the greatest extent were considered to be more tolerant of dehydration as they were able to continue functioning beyond loss of turgor in a dehydrated state (Blum, 2005). This corresponds with Turner's (1986) suggestion that dehydration tolerance will be greater in anisohydric plants with poorly developed dehydration postponement characteristics. Interestingly, many of these species did not adjust their Ψ_{tlp} in response to drought, despite suggestions that osmotic adjustment increases drought tolerance (Turner, 1986). Our results are in agreement with White, Beadle and Worledge (1996) who found no evidence of osmotic or elastic adjustment in drought tolerant eucalypt species which had undergone several drought cycles under field conditions. Many of the species which lost turgor in our study had sclerophyllous leaves (low specific leaf area) which has been suggested would make them more tolerant of negative turgor (Mitchell *et al.*, 2008, Sperry, 2000). However, we found no relationship between specific leaf area and the degree to which water potentials at stomatal closure exceeded Ψ_{tlp}. Some authors have also proposed that loss of turgor is a drought adaptive strategy, reducing growth and gas exchange, and plants can regain positive turgor potentials after favourable conditions return (Kolb & Sperry, 1999, Mishio, 1992).

Where drought avoidance through succulence, high capacitance or stomatal sensitivity are suspected, Ψ_{tlp} will provide little insight into how these species will perform under drought conditions. We therefore suggest caution in using Ψ_{tlp} to predict vulnerability to drought in species where there is no information on their drought strategy. In contrast, the change in midday water potential between well-watered and drought conditions ($\Delta\Psi_{\mathrm{MD}}$) was better related to drought response (Ψg_{s0}) and hence could be a useful measure to integrate in studies relating climate or other traits with drought vulnerability. $\Delta\Psi_{\mathrm{MD}}$ considers a species position along the continuum of drought avoidance and tolerance strategies (Rosado, Dias & de Mattos, 2013) and reflects the range of strategies which co-exist in dryland ecosystems (Vilagrosa *et al.*, 2013). Although we measured $\Delta\Psi_{\mathrm{MD}}$ under experimental conditions using stomatal closure as the threshold for drought, this measure could also be determined using field measures of Ψ_{MD} during wet and dry seasons (Drake & Franks, 2003, Nardini, Pedà & Rocca, 2012). This makes $\Delta\Psi_{\mathrm{MD}}$ a viable alternative to the proposed osmometer derived measurement of Ψ_{tlp} (Bartlett, Scoffoni, Ardy, Zhang, Sun, Cao & Sack, 2012a, Bartlett *et al.*, 2014). As minimum Ψ_{MD} values are commonly reported $\Delta\Psi_{\mathrm{MD}}$ could easily be calculated and used for meta-analyses of published data allowing greater insight into drought resistance strategies across life-forms and ecosystems.

Conclusions

Although generally water potential at stomatal closure (Ψg_{s0}) was related to Ψ_{tlp}, stomatal sensitivity of individual species was determined by their drought strategy ($\Delta\Psi_{\mathrm{MD}}$). Drought tolerant and more anisohydric species, which maintained stomatal conductance at lower water potentials than their $\Psi\pi_{\mathrm{tlp}}$, were more tolerant of dehydration, whereas drought avoiding and isohydric species closed their stomata at water potentials higher than indicated by their Ψ_{tlp}. However, there was no relationship between Ψ_{tlp} and drought strategy. As a functional trait, Ψ_{tlp} does provide some insight into how drought avoiders persist under drought conditions. However, using Ψ_{tlp} for predicting drought tolerance is likely to be misleading in cases where whole plant drought resistance differs from leaf-level resistance due to avoidance strategies such as succulence or dormancy. Therefore, although ecologically Ψ_{tlp} has been shown to reflect water availability across biomes and soil gradients, we caution the use of $\Psi\pi_{tlp}$ as a trait to predict drought tolerance and vulnerability of individual species to drought. Potentially, $\Delta\Psi_{MD}$ could be a useful measure for predicting drought response because it better reflects the range of co-existing drought strategies in dryland ecosystems.

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Figure captions

Fig. 1 Relationships between turgor loss point (Ψ_{tlp}; derived from PV curves) and stomatal conductance variables, including; pre-dawn water potential at stomatal closure ($\Psi g_{s0\,=}\,g_s < 0.05$ mol H_2O m^{-2} s^{-1}; A), pre-dawn water potential at 50% stomatal conductance (Ψg_{s50}; B), stomatal conductance at the Ψ_{tlp} as a percentage of maximum stomatal conductance under well-watered conditions ($g_s\Psi_{\mathrm{tlp}}$ (% of $g_{s\ max}$); C) and stomatal conductance at the permanent wilting point, also as a percentage of $g_{s\ max}$ (g_sPWP (% of $g_{s\ max}$); D), for 17 species under drought conditions. Stomatal conductance variables extrapolated from stomatal response curves for each species (Tables S1 and S2). Different symbols indicate life-form and bars on values represent mean standard error (SE) for Ψ_{tlp}, SE not shown for stomatal conductance variables as these were modelled values.

Fig. 2 Relationships between drought strategy (degree of isohydry and anisohydry described by the drop in midday water potential between well-watered and drought plants; $\Delta\Psi_{\mathrm{MD}}$ = mean Ψ_{MD} well-watered – mean Ψ_{MD} drought when stomatal conductance was approximately zero) and stomatal conductance variables, including; pre-dawn water potential at stomatal closure ($\Psi g_{s0\,=}\,g_{\mathrm{s}} < 0.05$ mol H_2O m^{-2} s^{-1}; A), pre-dawn water potential at 50% stomatal conductance (Ψg_{s50}; B), stomatal conditions ($g_s\Psi_{\mathrm{tlp}}$ (% of $g_{s\ max}$); C) and stomatal conductance at the permanent wilting point, also as a percentage of $g_{s\ max}$ (g_sPWP (% of $g_{s\ max}$); D), for 17 species under drought conditions. Stomatal conductance variables were extrapolated from stomatal response curves for each species (Tables S1 and S2). Different symbols indicate life-form. Mean standard errors not shown as stomatal conductance variables were modelled values and $\Delta\Psi_{\mathrm{MD}}$ was calculated from mean values.

Fig. 3 Relationships between drought strategy (degree of isohydry and anisohydry described by the drop in midday water potential between well-watered and drought plants; $\Delta\Psi_{\mathrm{MD}}$ = mean Ψ_{MD} well-watered – mean Ψ_{MD} drought; where drought was when stomatal conductance was approximately zero) and turgor loss point (Ψ_{tlp}; derived from PV curves; A) and dehydration tolerance (B) for 17 species under drought conditions. Dehydration tolerance was expressed as the pre-dawn water potential at stomatal closure for each species as a percentage of their turgor loss point (Ψg_{s0} (% of Ψ_{tlp})). Predawn water potential at stomatal closure was extrapolated from stomatal response curves for each species (Table S1). Different symbols indicate life-form and bars on Ψ_{tlp} values represent mean standard error (SE). SE not shown on percentage values or $\Delta\Psi_{\mathrm{MD},}$ as $\Delta\Psi_{\mathrm{MD}}$ was calculated from mean values.

FigS1. Relationship between (the inverse of) predawn leaf water potential (Negative Ψ_{PD}) and mid-morning stomatal conductance (g_s) for geophytes. Model equations and measures of fit are presented in Table S1.

FigS2. Relationship between (the inverse of) predawn leaf water potential (Negative Ψ_{PD}) and mid-morning stomatal conductance (g_s) for monocots. Model equations and measures of fit are presented in Table S1.

FigS3. Relationship between (the inverse of) predawn leaf water potential (Negative Ψ_{PD}) and mid-morning stomatal conductance (g_s) for herbs. Model equations and measures of fit are presented in Table S1.

FigS4. Relationship between (the inverse of) predawn leaf water potential (Negative Ψ_{PD}) and mid-morning stomatal conductance (g_s) for shrubs. Model equations and measures of fit are presented in Table S1.

Table 1 Life-forms and descriptions of the 17 species used in the drought experiment.

Family	Species	Habitat	Aridity index		
			Mean	**Min**	**Max**
Geophytes					
Asparagaceae	*Arthropodium milleflorum* (DC.) J.F.Macbr.	Rock outcrop	1.16	0.09	7.47
Geraniaceae	*Pelargonium rodneyanum* Mitch. ex Lindl.	Rocky grassland	0.84	0.35	5.27
Monocots					
Hemerocallidaceae	*Dianella revoluta* R.Br.	Inland woodland	0.73	0.07	5.44
Cyperaceae	*Ficinia nodosa* (Rottb.) Goetgh., Muasya & D.A.Simpson	Coastal dune	0.92	0.13	4.91
Asparagaceae	*Lomandra filiformis* (Thunb.) Britten	Rocky grassland	0.83	0.19	5.71
Hemerocallidaceae	*Stypandra glauca* R.Br.	Rock outcrop	0.72	0.10	2.65
Herbs					
Asteraceae	*Chrysocephalum apiculatum* (Labill.) Steetz	Inland woodland	0.55	0.04	8.35
Campanulaceae	*Isotoma. axillaris* Lindl.	Rock outcrop	0.76	0.16	5.02
Stylidiaceae	*Stylidium graminifolium* Sw. ex Willd.	Coastal dune	1.50	0.13	8.52
Shrubs					
Rutaceae	*Correa glabra* Lindl.	Rock outcrop	0.56	0.11	1.47
Fabaceae	*Eutaxia microphylla* (R.Br.) C.H.Wright & Dewar	Rocky grassland	0.45	0.10	1.59
Chenopodiaceae	*Enchylaena tomentosa* R.Br.	Inland woodland	0.25	0.04	1.39
Dillenaceae	*Hibbertia obtusifolia* DC.	Rock outcrop	0.79	0.27	4.45
Asteraceae	*Olearia axillaris* (Labill.) F.Muell. ex Benth.	Coastal dune	0.61	0.09	1.88
Lamiaceae	*Prostanthera nivea* A.Cunn. ex Benth.	Rocky grassland	0.57	0.23	1.52
Fabaceae	*Platylobium obtusangulum* Hook.	Coastal dune	0.98	0.25	2.75
Fabaceae	*Senna artemisiodes ssp. X coriacea* (Benth.) Randell	Inland woodland	0.23	0.04	4.18

Aridity index based on the annual mean of monthly precipitation to potential ET (pan) and values (mean, min and max) presented are for all known records of each species and represent the range of potential aridity (Atlas of Living Australia). The minimum aridity indices for all species are considered semi-arid (0.2-0.5) or arid (0.03-0.2) (UNEP, 1997).

Table 2 Mean leaf water potential at pre-dawn (Ψ_{PD}) and midday (Ψ_{MD}) under well-watered (WW) and drought (D) conditions once stomatal conductance (g_s) was approximately zero (<0.05 g_s mol H_2O m^{-2} s^{-1}) and drought strategy (degree of isohydry and anisohydry, described as the drop in midday water potential between well-watered and drought plants; $\Delta\Psi_{MD}$ = mean Ψ_{MD} WW – mean Ψ_{MD} D).

	Ψ_{PD} (MPa)			**Ψ_{MD} (MPa)**			**$\Delta\Psi_{MD}$**
Species	**WW**	**D**	***P-value***	**WW**	**D**	***P-value***	**(MPa)**
Geophytes							
A. milleflorum	-0.29_{cde} (0.01)	-0.48_{cde} (0.05)	0.063 (1.5)	-0.61_{bcd} (0.17)	-0.75_{d} (0.20)	0.533 (2)	-0.14
P. rodneyanum	-0.27_{cdef} (0.07)	-0.90_{cde} (0.08)	0.100 (0)	-0.85_{bcd} (0.03)	-1.13_{d} (0.07)	0.100 (0)	-0.28
Monocots							
D. revoluta	-0.21_{cdef} (0.05)	-1.05_{cde} (0.20)	0.029 (0)	-0.89_{bcd} (0.04)	-1.58_{d} (0.10)	0.029 (0)	-0.69
F. nodosa	-0.12_{ef} (0.02)	-1.53_{bcde} (0.58)	0.100 (0)	-0.50_{cd} (0.03)	-1.21_{cd} (0.53)	0.100 (0)	-0.71
L. filiformis	-0.12_{f} (0.02)	-2.74_{abcde} (0.64)	0.008 (0)	-1.79_{a} (0.20)	-2.33_{bcd} (0.66)	1.00 (0)	-0.54
S. glauca	-0.25_{cdef} (0.05)	-1.28_{cde} (0.30)	0.036 (0)	-1.15_{bc} (0.08)	-1.88_{d} (0.39)	0.057 (0)	-0.73
Herbs							
C. apiculatum	-0.50_{b} (0.03)	-2.41_{bcde} (0.68)	0.016 (0)	-0.98_{bcd} (0.15)	-3.30_{abcd} (1.11)	0.095 (0)	-2.32
I. axillaris	-0.18_{cdef} (0.03)	-1.23_{cde} (0.44)	0.200 (0)	-0.28_{d} (0.03)	-1.67_{d} (0.32)	0.200 (0)	-1.39
S. graminifolium	-0.17_{def} (0.04)	-2.90_{abc} (0.80)	0.200 (0)	-0.62_{bcd} (0.06)	-3.8_{abcd} (1.10)	0.200 (0)	-3.18
Shrubs							
C.glabra	-0.31_{cd} (0.01)	-2.86_{abcd} (0.25)	0.016 (0)	-1.05_{bc} (0.10)	-3.03_{abcd} (0.62)	0.029 (0)	-2.03
E. microphylla	-0.36_{bc} (0.02)	-3.63_{ab} (0.62)	0.016 (0)	-1.17_{b} (0.08)	-4.75_{ab} (0.44)	0.016 (0)	-3.58
E. tomentosa	-1.69_{a} (0.02)	-4.60_{a} (1.40)	0.048 (0)	-2.09_{a} (0.04)	-5.4_{a} (0.55)	0.048 (0)	-3.31
H. obtusifolia	-0.21_{def} (0.03)	-2.31_{abcde} (0.43)	0.016 (0)	-0.82_{bcd} (0.09)	-3.15_{abcd} (0.52)	0.016 (0)	-2.33
O. axillaris	-0.29_{cd} (0.03)	-2.08_{bcde} (0.69)	0.032 (2)	-0.71_{bcd} (0.12)	-2.32_{cd} (0.37)	0.024 (1)	-1.61
P. nivea	-0.25_{cdef} (0.02)	-1.90_{abcde} (0.24)	0.029 (0)	-0.90_{bcd} (0.05)	-2.25_{abcd} (0.44)	0.029 (0)	-1.35
P. obtusangulum	-0.25_{cdef} (0.05)	-2.09_{abcde} (0.70)	0.200 (0)	-0.79_{bcd} (0.17)	-2.73_{bcd} (0.65)	0.133 (0)	-1.94
S. artemisiodes	-0.18_{def} (0.02)	-4.15_{a} (0.58)	0.050 (0)	-0.78_{bcd} (0.07)	-4.43_{abc} (1.11)	0.050 (0)	-3.65

Values in parentheses show mean standard error. Different letters indicate significant differences between species within variable (one-way ANOVA all *P* <0.001; Tukey *post hoc* test). *P-values* in table show significant differences between WW and D treatments within each species (Mann-Whitney U test; *P*<0.05; u value in parentheses).

Table 3 Physiological traits derived from pressure-volume curve analysis of well-watered (WW) and drought (D) plants.

	Ψ_{tlp} (MPa)		**π_o (MPa)**		**ε (MPa)**		**RWC_{tlp} (%)**	
	WW	**D**	**WW**	**D**	**WW**	**D**	**WW**	**D**
Geophytes								
A. milleflorum	-1.17_{ab} (0.04)	-1.09_{ab} (0.21)	-0.98_{abcd} (0.07)	-0.87_{abc} (0.20)	8.26_{a} (2.54)	5.35_{abc} (1.71)	$88.3_{cdefghi}$ (0.03)	87.8_{efg} (0.01)
P. rodneyanum	-1.13_{ab} (0.03)	-1.12_{abc} (0.07)	-0.94_{abc} (0.02)	-0.96_{abcd} (0.07)	5.87_{a} (0.39)	7.77_{abc} (1.16)	89.6_{defghi} (0.01)	90.1_{fg} (0.01)
Monocots								
D. revoluta	-1.04_{ab} (0.09)	-1.46_{bcd} (0.19)	-0.78_{ab} (0.10)	-1.13_{bcde} (0.13)	3.17_{a} (0.77)	7.09_{abc} (2.02)	91.3_{efghi} (0.00)	88.3_{efg} (0.03)
F. nodosa	-0.82_{a} (0.10)	-0.69_{a} (0.05)	-0.67_{a} (0.10)	-0.53_{a} (0.05)	4.88_{a} (1.60)	2.44_{a} (0.50)	93.6_{fghi} (0.01)	87.7_{efg} (0.01)
L. filiformis	-2.96_{g} (0.06)	-2.75_{h} (0.08)	-2.64_{e} (0.07)	-2.49_{h} (0.05)	31.30_{b} (3.93)	44.94_{d} (4.43)	94.6_{i} (0.00)	94.1_{g} (0.01)
S. glauca	-1.97_{ef} (0.28)	-2.39_{gh} (0.05)	-1.46_{cd} (0.29)	-1.9_{g} (0.03)	6.61_{a} (2.54)	11.13_{bc} (1.46)	83.6_{cde} (0.01)	84.2_{cdef} (0.02)
Herbs								
C. apiculatum	-1.30_{abc} (0.05)	-1.33_{bcd} (0.07)	-0.99_{abcd} (0.04)	-0.96_{abcde} (0.06)	4.55_{a} (0.77)	3.83_{ab} (0.58)	75.6_{ab} (0.01)	69.6_{ab} (0.02)
I. axillaris	-1.26_{abc} (0.08)	-1.09_{abc}(0.16)	-0.99_{abcd} (0.09)	-0.69_{ab} (0.06)	4.68_{a} (0.94)	2.08_{a} (0.22)	85.4_{cdef} (0.01)	86.4_{defg} (0.03)
S. graminifolium	-1.80_{cdef} (0.04)	-1.79_{defg} (0.06)	-1.50_{d} (0.03)	-1.43_{defg} (0.05)	10.31_{a} (0.68)	7.70_{abc} (0.87)	84.6_{cde} (0.01)	84.0_{cdef} (0.02)
Shrubs								
C.glabra	-1.89_{def} (0.03)	-2.32_{fgh} (0.12)	-1.44_{cd} (0.03)	-1.71_{fg} (0.06)	6.35_{a} (0.42)	6.76_{abc} (0.29)	84.3_{cde} (0.01)	77.0_{abc} (0.01)
E. microphylla	-1.51_{bcde} (0.04)	-1.64_{bcde} (0.15)	-1.32_{bcd} (0.03)	-1.40_{def} (0.14)	11.29_{a} (2.02)	12.27_{c} (2.44)	86.1_{cdefg} (0.01)	80.6_{cde} (0.02)
E. tomentosa	-2.13_{f} (0.05)	-2.27_{fgh} (0.14)	-1.45_{cd} (0.06)	-1.44_{defg} (0.07)	5.39_{a} (1.52)	3.68_{ab} (0.40)	73.4_{a} (0.03)	68.3_{a} (0.03)
H. obtusifolia	-1.33_{abc} (0.06)	-1.83_{defg} (0.07)	-1.06_{abcd} (0.05)	-1.37_{cdef} (0.07)	5.64_{a} (0.59)	6.03_{abc} (0.64)	82.5_{bcd} (0.01)	84.4_{cdef} (0.01)
O. axillaris	-1.61_{bcdef} (0.13)	-1.71_{cdef} (0.15)	-1.22_{abcd} (0.06)	-1.23_{cdef} (0.16)	5.69_{a} (0.78)	4.99_{abc} (1.07)	82.0_{bc} (0.02)	78.4_{bcd} (0.02)
P. nivea	-1.29_{abc} (0.05)	-1.88_{defg} (0.14)	-0.96_{abcd} (0.01)	-1.46_{efg} (0.11)	4.17_{a} (0.52)	6.97_{abc} (0.63)	86.0_{cdefg} (0.01)	82.5_{cdef} (0.02)
P. obtusangulum	-1.39_{bcd} (0.13)	-1.49_{bcde} (0.15)	-1.21_{abcd} (0.12)	-1.26_{cdef} (0.15)	11.70_{a} (2.43)	9.65_{abc} (2.64)	84.0_{cde} (0.01)	90.4_{fg} (0.00)
S. artemisiodes	-1.56_{bcde} (0.09)	-2.12_{efgh} (0.07)	-1.28_{bcd} (0.11)	-1.72_{fg} (0.04)	8.25_{a} (1.77)	10.75_{abc} (2.11)	86.7_{cdefgh} (0.01)	88.6_{efg} (0.00)

Traits include leaf water potential at turgor loss (Ψ_{tlp}), osmotic pressure at full turgor (Π_o), bulk modulus of elasticity (ε) and relative water content at turgor loss point (RWC_{tlp}). Values in parentheses show mean standard error. Different letters indicate significant differences between species within treatment and variable (one-way ANOVA; Tukey *post hoc* test; all *P-values* <0.001). *P-values* for differences between well-watered and drought plants are shown in Table 4.

Table 4 *P-values* (Mann-Whitney U test; u value in parentheses) for differences in pressure-volume curve traits between well-watered and drought plants (values and SE in Table 3).

	P-values			
	Ψ_{tlp} (MPa)	**π_o (MPa)**	**ε (MPa)**	**RWC_{tlp} (%)**
Geophytes				
A. milleflorum	0.25 (3)	0.25 (3)	0.57 (5)	1.00 (7)
P. rodneyanum	0.42 (8)	0.42 (8)	0.10 (4)	0.55 (9)
Monocots				
D. revoluta	0.25 (3)	0.25 (3)	0.14 (2)	0.39 (4)
F. nodosa	0.22 (6)	0.22 (6)	0.41 (6)	0.03 (2)
L. filiformis	0.04 (2)	0.11 (4)	0.07 (3)	1.00 (12)
S. glauca	0.15 (5)	0.15 (5)	0.15 (5)	0.55 (9)
Herbs				
C. apiculatum	1.00 (12)	0.69 (10)	0.55 (9)	0.06 (3)
I. axillaris	0.63 (4)	0.57 (0)	0.06 (0)	1.00 (6)
S. graminifolium	0.84 (11)	0.42 (8)	0.03 (2)	0.69 (10)
Shrubs				
C.glabra	0.03 (2)	0.02 (1)	0.55 (9)	0.02 (1)
E. microphylla	0.42 (8)	0.69 (10)	1.00 (12)	0.02 (1)
E. tomentosa	0.91 (9)	0.91 (9)	0.41 (6)	0.41 (6)
H. obtusifolia	0.01 (0)	0.01 (0)	0.84 (11)	0.55 (9)
O. axillaris	0.55 (9)	0.42 (8)	0.69 (10)	0.22 (6)
P. nivea	0.01 (1)	0.01 (1)	0.02 (1)	0.42 (8)
P. obtusangulum	0.56 (7)	0.91 (9)	0.41 (6)	0.02 (0)
S. artemisiodes	0.02 (0)	0.02 (0)	0.56 (7)	0.19 (4)

Significant differences are highlighted in grey. Traits include leaf water potential at turgor loss (Ψ_{tlp}), osmotic pressure at full turgor (π_o), bulk modulus of elasticity (ε) and relative water content at turgor loss point (RWC_{tlp}).

Table S1 ‘Best’ models describing the relationship between negative pre-dawn water potential ($-\Psi_{\mathrm{PD}}$) and stomatal conductance (g_{s}) for each species.

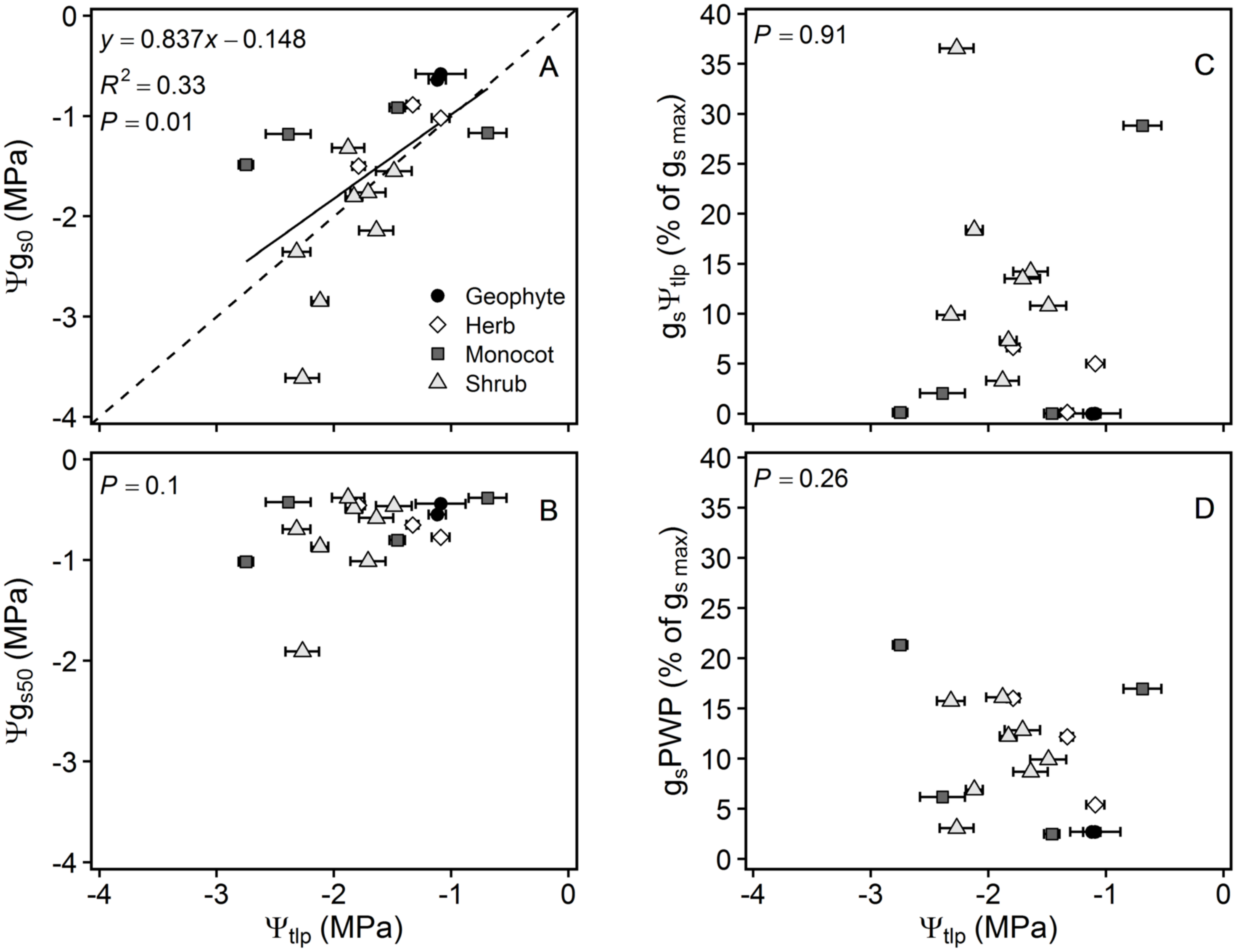
	Model equation	***Residual standard error***	R^2_{adj}
Geophytes			
A. milleflorum	$g_s = 0.454 / 1 + e^{(0.441 - (-\Psi\mathrm{PD}))/-0.066}$	0.152	0.64
P. rodneyanum	$g_s = 0.411 / 1 + e^{(0.548 - (-\Psi\mathrm{PD}))/-0.046}$	0.155	0.68
Monocots			
D. revoluta	$g_s = 0.273 / 1 + e^{(0.803 - (-\Psi\mathrm{PD}))/-0.075}$	0.059	0.80
F. nodosa	$g_s = 0.412e^{-1.803(-\Psi\mathrm{PD})}$	0.089	0.73
L. filiformis	$g_s = 0.372 / 1 + e^{(1.006 - (-\Psi\mathrm{PD}))/-0.258}$	0.146	0.58
S. glauca	$g_s = 0.341e^{-1.628(-\Psi\mathrm{PD})}$	0.064	0.76
Herbs			
C. apiculatum	$g_s = 0.562 / 1 + e^{(0.650 - (-\Psi\mathrm{PD}))/-0.102}$	0.156	0.63
I. axillaris	$g_s = 0.549 / 1 + e^{(0.774 - (-\Psi\mathrm{PD}))/-0.107}$	0.274	0.64
S. graminifolium	$g_s = 0.486e^{-1.516(-\Psi\mathrm{PD})}$	0.125	0.68
Shrubs			
C. glabra	$g_s = 0.524e^{0.998(-\Psi\mathrm{PD})}$	0.126	0.75
E. microphylla	$g_s = 0.638e^{-1.189(-\Psi\mathrm{PD})}$	0.169	0.53
E. tomentosa	$g_s = 0.725 / 1 + e^{(1.815 - (-\Psi\mathrm{PD}))/-0.690}$	0.142	0.65
H. obtusifolia	$g_s = 0.658e^{-1.429(-\Psi\mathrm{PD})}$	0.163	0.60
O. axillaris	$g_s = 0.454 / 1 + e^{(0.950 - (-\Psi\mathrm{PD}))/-0.389}$	0.132	0.53
P. nivea	$g_s = 0.548e^{-1.816(-\Psi\mathrm{PD})}$	0.138	0.77
P. obtusangulum	$g_s = 0.506e^{-1.494(-\Psi\mathrm{PD})}$	0.123	0.71
S. artemisiodes	$g_s = 0.485e^{-0.799(-\Psi\mathrm{PD})}$	0.140	0.75

Best models were identified as those with the lowest residual standard error; with functions either logistic or exponential. Both well-watered (WW) and drought (D) treatments were combined for each species (n = 53-100). Adjusted R^2 (R^2_{adj}) and *P-values* (all <0.001) were determined from linear models of observed g_{s} and predicted values of g_{s} for each species (log-transformed to achieve linearity where necessary).

Table S2 'Best' models describing the relationship between soil water content (SWC) and stomatal conductance (g_s) for each species.

	Model equation	*Residual standard error*	R^2_{adj}
Geophytes			
A. milleflorum	$g_s = 0.439 / 1 + e^{(19.0 - \mathrm{SWC}) / 2.74}$	0.131	0.70
P. rodneyanum	$g_s = 0.395 / 1 + e^{(23.1 - \mathrm{SWC}) / 3.88}$	0.148	0.52
Monocots			
D. revoluta	$g_s = 0.284 / 1 + e^{(23.7 - \mathrm{SWC}) / 3.97}$	0.063	0.81
F. nodosa	$g_s = 0.362 / 1 + e^{(17.6 - \mathrm{SWC}) / 5.26}$	0.093	0.72
L. filiformis	$g_s = 0.434 / 1 + e^{(21.7 - \mathrm{SWC}) / 9.61}$	0.131	0.54
S. glauca	$g_s = 0.210 / 1 + e^{(15.3 - \mathrm{SWC}) / 2.25}$	0.065	0.67
Herbs			
C. apiculatum	$g_s = 0.487 / 1 + e^{(21.9 - \mathrm{SWC}) / 6.42}$	0.157	0.60
I. axillaris	$g_s = 0.695 / 1 + e^{(23.0 - \mathrm{SWC}) / 4.81}$	0.220	0.67
S. graminifolium	$g_s = 0.395 / 1 + e^{(18.1 - \mathrm{SWC}) / 5.40}$	0.108	0.67
Shrubs			
C. glabra	$g_s = 0.372 / 1 + e^{(15.8 - \mathrm{SWC}) / 3.94}$	0.119	0.62
E. microphylla	$g_s = 0.481 / 1 + e^{(18.0 - \mathrm{SWC}) / 3.74}$	0.130	0.68
E. tomentosa	$g_s = 4.591 / 1 + e^{(120 - \mathrm{SWC}) / 32.19}$	0.146	0.54
H. obtusifolia	$g_s = 0.628 / 1 + e^{(28.9 - \mathrm{SWC}) / 10.01}$	0.134	0.69
O. axillaris	$g_s = 0.470 / 1 + e^{(24.2 - \mathrm{SWC}) / 7.83}$	0.080	0.82
P. nivea	$g_s = 0.462 / 1 + e^{(23.3 - \mathrm{SWC}) / 8.54}$	0.126	0.58
P. obtusangulum	$g_s = 0.395 / 1 + e^{(22.8 - \mathrm{SWC}) / 6.15}$	0.097	0.74
S. artemisiodes	$g_s = 0.438 / 1 + e^{(17.4 - \mathrm{SWC}) / 3.15}$	0.130	0.68

Best models were identified as those with the lowest residual standard error (all logistic functions). Both well-watered (WW) and drought (D) treatments were combined for each species (n = 53-100). Adjusted R^2 (R^2_{adj}) and *P-values* (all <0.001) were determined from linear models of observed g_s and predicted values of g_s for each species (log-transformed to achieve linearity where necessary).



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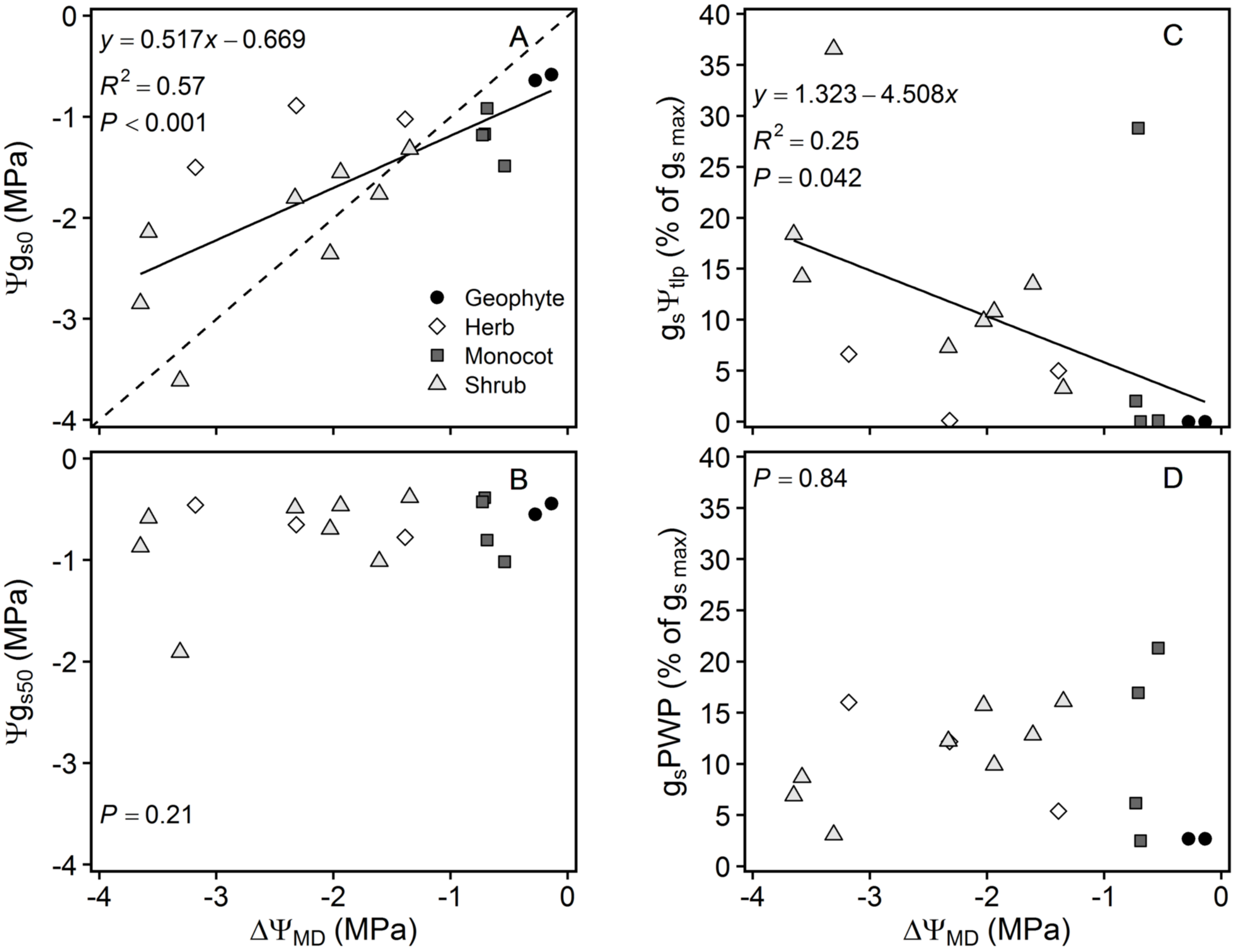

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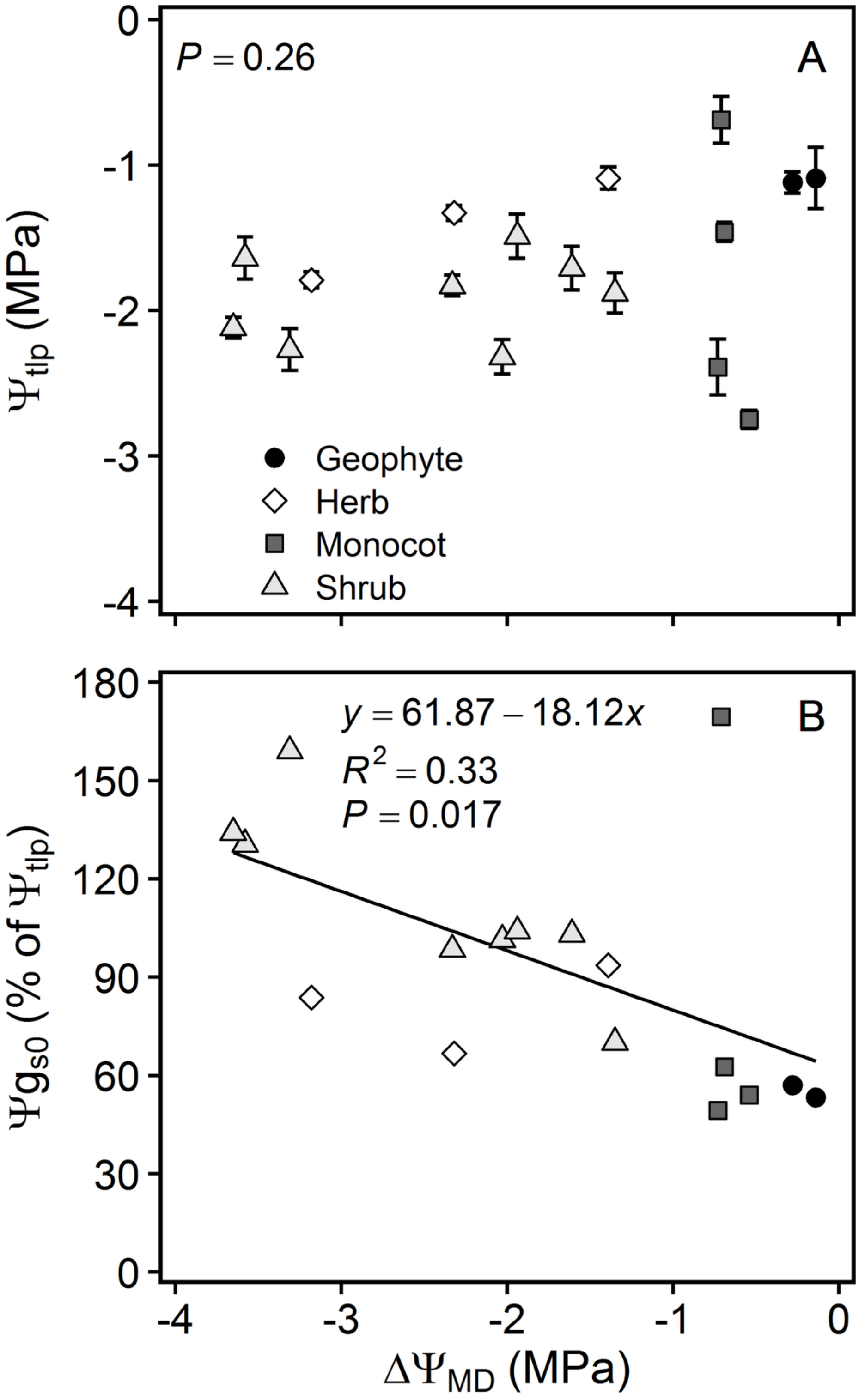

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