Leaf water storage increases with salinity and aridity in the mangrove *Avicennia marina*: integration of leaf structure, osmotic adjustment, and access to multiple water sources

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Abstract

Leaf structure and water relations were studied in a temperate population of *Avicennia marina* subsp. *australasica* along a natural salinity gradient (28 to 49 parts per thousand (ppt)) and compared with two subspecies grown naturally in similar soil salinities to those of subsp. *australasica* but under different climates: subsp. *eucalyptifolia* (salinity 30 ppt, wet tropics) and subsp. *marina* (salinity 46 ppt, arid tropics). Leaf thickness, LMA and water content increased with salinity and aridity. Turgor loss point declined with increase in soil salinity, driven mainly by differences in osmotic potential at full turgor, as the modulus of elasticity (ε) did not vary significantly with salinity. Nevertheless, high ε contributed to maintenance of high cell hydration at turgor loss points. Despite similarity among leaves in leaf water storage capacitance, total leaf water storage increased with increasing salinity and aridity. The time that stored water could alone sustain an evaporation rate of 1 mmol m^{-2} s^{-1} ranged from 77 for subspecies *eucalyptifolia* to 126 min for ssp. *marina*. Achieving full leaf hydration or turgor required water from sources other than the roots, emphasizing the importance of multiple water sources to growth and survival of *Avicennia marina* across gradients in salinity and aridity.

Key words: leaf hydration, modulus of elasticity, PV curve, turgor loss point, water relations.

Introduction

There is an urgent need to understand relationships between leaf traits and drought tolerance (Bartlett *et al*., 2012). The urgency arises because an understanding of leaf design may help to anticipate responses of trees to edaphic and atmospheric drought, and mitigate tree die-back. Such die-back is occurring globally (Allen *et al.*, 2010; McDowell & Allen, 2015) in forest systems as different as tropical rainforests (Rowland *et al.*, 2015) and mangroves (Lovelock *et al.*, 2009; Duke *et al.*, 2016). Mangroves are halophytic woody trees and shrubs that occur in tidal, saline wetlands (Feller *et al.*, 2010). These systems contribute important ecosystem services to fisheries, forestry, and the social well-being of coastal communities in the tropics and subtropics. Mangroves are also a fundamental model study system for genetic capacity in salt tolerance. The structure and function of mangrove forests varies along complex environmental gradients in salinity and climatic aridity (Duke *et al.*, 1998), factors that, respectively, affect the availability of water at the roots and the demand for water at the leaves. These factors will change in response to altered climate and sea level due to global warming. It is important to understand how mangroves cope with salinity and aridity to better manage these resources in a changing environment.

Mangroves, like other plants, must take up and store water to maintain leaf hydration. However, coping with a saline environment entails special challenges for the maintenance of favourable water and ion balances. Despite the abundance of water in mangrove habitats, salinity can limit the capacity of roots to absorb water while excluding most ions from entry into the transpiration stream. Seawater, for example, contains 35 parts per thousand (ppt) solute which includes 483 mM Na^+ and 558 mM Cl^- (Harvey, 1966) and has an osmotic potential of -2.4 MPa. For plants to absorb water, water potentials in roots must be lower than in surrounding soil. In halophytes like mangroves, turgor is maintained in tissues despite very negative water potentials through adjustment of intracellular solute concentrations, including high levels of Na^+ and Cl^-. These ions are sequestered from sensitive metabolic sites as metabolism in halophytes is as sensitive to high ion concentrations as in glycophytes (Flowers, 1972;

Ball & Anderson, 1986), and the ions contribute to osmotic adjustment in their primary storage site, the vacuole (Flowers *et al.*, 1977). Osmotic adjustment in the cytoplasmic compartment occurs mainly through the accumulation of compatible solutes (Jefferies, 1981; Flowers & Colmer, 2008). While these principles of halophytic cellular physiology are well established, questions remain about the contributions to salinity tolerance of higher levels of organization, such as leaves.

As carbon cannot be gained without the expenditure of water, acquisition of adequate water to sustain carbon gain is essential for both survival and growth. Under extreme conditions, leaves may need to close stomata and persist on stored water until conditions become favourable for water uptake. However, mangroves that cope with persistently highly saline soil must continue to spend water for carbon gain for growth. Water uptake (Ball, 1988; Bazihizina *et al.*, 2009; Reef *et al.*, 2015), transport (Sperry *et al.*, 1988; Melcher *et al.*, 2001; Ewers *et al.*, 2004; Lopez-Portillo *et al.*, 2005; Lovelock *et al.*, 2006), and use (Ball & Farquhar, 1984b; Ball & Farquhar, 1984a; Clough & Sim, 1989; Nguyen *et al.*, 2015) are typically lower in high than low salinities. These characteristics would lead to a higher requirement for leaf water storage for transient water use at high salinity. Indeed, Lechthaler *et al.* (2016) showed that leaf evaporation rates in the mangroves *Rhizophora mucronata* and *Bruguiera gymnorrhiza* depended on stored water because water transport to leaves was not sufficient to balance rates of water loss, especially when salinity was high.

Stored water can play an important role in drought tolerance. Leaf water storage depends on mass investment in structure, and thus leaf dry mass and water mass per area should tend to scale proportionally. Indeed, leaf mass per area, i.e. LMA, is a key trait that often, but not always correlates with tolerance of drought (Niinemets, 2001; Bartlett *et al.*, 2012) and salinity (Ball *et al.*, 1988). In a meta-analysis, Poorter *et al.* (2009) reported a simple linear increase in LMA with increasing salinity. However, LMA alone is not a general adaptation to drought tolerance. Bartlett *et al.* (2012) found no direct linkage between LMA and the maintenance of turgor and hydration during dehydration to the turgor loss point. They suggested that reported correlations between LMA and drought tolerance in specific plant groups probably reflected "the coincidence of drought stress and other environmental conditions for which high LMA confers a benefit" (Bartlett *et al.*, 2012).

In addition to having thick leaves for water storage, species must have sufficient solute concentrations to allow maintenance of turgor even as the water is withdrawn. Sufficient osmotica depends on the habitat occupied. Indeed, leaf water potentials reported for field-grown mangroves vary with the natural soil salinities in which they grow, which range from slightly brackish to hypersaline (Scholander *et al.*, 1964; Scholander, 1968; Naidoo, 1989; Rada *et al.*, 1989; Sternberg *et al.*, 1991; Constable, 2014; Walker, 2014). Maintenance of a minimal level of hydration is essential for survival and maintenance of turgor is required for growth. Both are achieved through osmotic adjustment. Lower (i.e. more negative) osmotic potential and turgor loss point with increasing growth salinity are common features in mangroves (Rada *et al.*, 1989; Suarez & Sobrado, 2000; Melcher *et al.*, 2001; Paliyavuth *et al.*, 2004; Sobrado, 2007). Indeed, osmotic potential at full turgor is a reliable predictor of the turgor loss point which in turn correlates with drought tolerance (Bartlett et al, 2012), and is likely also to correlate with salinity tolerance.

Rigid cell walls, which are often associated with high LMA, have consequences for leaf water relations. The bulk modulus of elasticity is defined as the change in turgor pressure per fractional change in cell volume (Cheung *et al.*, 1975). In other words, the bulk modulus of elasticity increases with the rigidity of the cell walls. Variation in bulk modulus of elasticity affects cellular water relations because the more rigid the cell wall, the greater the change in turgor pressure, and hence also water potential, for a given water loss above the turgor loss point. In this way, mechanical constraints on water loss by rigid walls conserve water content at the turgor loss point (Cheung *et al.*, 1975). It follows from this interpretation that cell wall rigidity would increase with increasing salinity. However, both increases and decreases have been reported: bulk modulus of elasticity decreased with increasing salinity in *Rhizophora mangle, Conocarpus erectus*, and *Coccoloba uvijera* (Rada *et al.*, 1989) and *Avicennia germinans* (Suarez *et al.*, 1998) but increased with increasing salinity in *Avicennia germinans* (Suarez & Sobrado, 2000), *Avicennia alba, Bruguiera gymnorrhiza, Heritiera littoralis* *and Xylocarpus granatum* (Paliyavuth et al., 2004). Thus, the role and variability in modulus of elasticity require clarification.

Pressure – volume relationships (PV curves) provide a way to examine most aspects of leaf water relations, including the modulus of elasticity, water storage capacitance, osmotic potential at full turgor and at the turgor loss point. Analysing PV curves with respect to leaf anatomy, Nguyen *et al.* (2016) revealed a cascade of water storage compartments that were operational over different ranges of leaf water potentials in one population of field-grown *A. marina*. They showed that liquid water can be absorbed from the lamina surface and stored in cells and specialized extracellular spaces (trichome lumina and cisternae) at water potentials higher than those experienced at the roots. This stored water, thus, must come from sources that are distinct from the soil. Quantification of the amount of extracellular water was problematic but it could account for as much as 10% of total leaf water based on the average size and density of trichomes. Thus, Nguyen *et al.* (2016) estimated that extracellular water together with that stored inside the cells, especially in the hypodermis, could support a sustained evaporation rate of 1 mmol m^{-2} s^{-1} for approximately 2 h without input from the roots as leaves dehydrated from full hydration to the turgor loss point. These results invite the question: how do changes in leaf anatomy relate to water relations of *A. marina* with variation in environmental conditions?

Avicennia marina is one of the most salt tolerant and widely distributed of mangrove species along complex gradients in salinity and aridity. There are three subspecies of *A. marina* whose Australian distribution varies with climatic conditions: subsp. *eucalyptifolia* in wet tropics, subsp. *marina* in arid tropics, and subsp. *australasica* in temperate areas with intermediate rainfall (Duke *et al.*, 1998; Li *et al.*, 2016). Using these subspecies as sources of variation, the leaf water relations, anatomy, and physical properties of naturally field grown leaves were measured to test the hypotheses that with increasing salinity and aridity (1) LMA increases with increases in the bulk modulus of elasticity and leaf succulence, (2) osmotic potentials at full turgor and at the turgor loss point decrease, (3) leaf water storage capacitance and total water storage increase, and (4) leaf water relations reflect increasing importance of access to multiple water sources additional to the soil.

Materials and Methods

Plant materials

All leaf samples were collected from plants growing naturally along gradients in salinity and aridity. Variation in leaf traits with salinity was studied in *A. marina* subsp. *australasica* growing at three sites along the Clyde River (Batemans Bay, New South Wales, Australia) where salinity of soil water extracted from 30 cm depth at low tide (McKee, 1993) averaged 28 ± 0.4 (35°38'50.3"S 150°08'39.5"E), 40 ± 0.4 (35°42'15.1"S 150°10'25.2"E), and 49 ± 0.6 ppt (35°42'16.2"S 150°10'18.8"E). Sea water (35 ppt) has a water potential of -2.4 MPa, and so soil water salinities at the three sites were approximately equivalent to water potentials of -1.9, -2.7, and -3.4 MPa. Differences among subspecies were based on comparison of *A. marina* subsp. *australasica* with subsp. *eucalyptifolia* from the wet tropics (Daintree, Queensland, 16°17'29.8"S 145°25'10.2"E) and subsp. *marina* from the arid tropics (Giralia Bay, Western Australia, 22°27'34.0"S 114°14'31.9"E). Soil salinity where subsp. *eucalyptifolia* grew was 30 ± 0 ppt (-2.1 MPa), and subsp. *marina* grew was 46 ± 0.7 ppt (-3.2 MPa). Hereafter, the five groups of plants are called by the first two letters of the subspecies names followed by a subscript with the soil salinity in which the plants grew, i.e. Au_{28}, Au_{40}, Au_{49}, Eu_{30}, and Ma_{46}. Note that some data for Au_{49} were reproduced from Nguyen *et al.*, 2016 and are identified in table captions where appropriate. Differences between climatic conditions at the study sites are summarized in Fig. 1.

Leaf features

One fully exposed branch bearing only sun leaves was chosen from each of five co-occurring trees in each of the five study sites for all measurements of leaf properties as previously described (Nguyen *et al.*, 2016). Care was taken to select leaves that appeared average in size for a given population under a given set of conditions, i.e. similar age, aspect, and exposure to full sunlight. Briefly, branches were rehydrated and two well-matched leaf pairs were selected for study and randomly allocated to one of two sets of measurements. One pair of leaves was used for measurement of physical properties and construction of a PV curve relationship with both sets of measurements made on the same leaf. The second leaf in the pair was a spare in case measurements needed to be repeated. The second leaf pair was used for anatomical measurements.

Leaf physical properties

Leaf area (S, cm^2, m^2), dry mass (DM, g), maximum water content (WC_{max}, g), leaf dry mass per area (LMA, g m^{-2}), maximum leaf water content per area (WCA_{max}, g m^{-2}), and per dry mass (WCD_{max}, g g^{-1}), were measured on the same set of leaves that were used for PV analyses, as described in Nguyen *et al*. (2016).

Leaf anatomy

Transverse and paradermal leaf sections were prepared, stained, and observed as previously described (Nguyen *et al.,* 2015, 2016). Lamina thickness and the fractional contribution of each tissue layer to total lamina thickness were calculated from transverse sections. The number of cells per unit area (mm^{-2}) in the hypodermis, palisade mesophyll, and spongy mesophyll was calculated from transverse sections through these tissues; the number of trichomes and upper epidermal cells per unit area were calculated from paradermal sections.

Leaf water relations

Pressure volume (PV) curves with three domains (Fig. 2) were constructed and analysed as in Nguyen *et al.* (2016) with one exception. Bulk modulus of elasticity (ε, MPa) was calculated only for domain 2 of the PV curve as: $\varepsilon_{D2} = \frac{\Delta P}{\Delta V/V}$ where ΔP is the difference in turgor pressure and ΔV/V is the corresponding fractional difference in cellular volume between the points at full turgor (ψ_{ft}, RWC_{ft}) and at turgor loss (ψ_{tlp}, RWC_{tlp}) as shown in Fig. 2. Those two points were determined by conventional methods (Scholander *et al.*, 1964; Tyree & Hammel, 1972; Cheung *et al.*, 1975; Turner, 1988) using linear regressions of $1/\psi_{leaf}$ as a function of relative water deficit, i.e. 1 – RWC, for the appropriate regions of the PV curves (Nguyen *et al.*, 2016). These calculated values of ψ_{ft} and ψ_{tlp} mark the transitions between domains 1 and 2, and domains 2 and 3, respectively (Nguyen *et al.*, 2016).

The difference in turgor pressure between ψ_{ft} and ψ_{tlp} was calculated as $\Delta P = \psi_{ft} - \psi_{\pi}^{ft}$ where ψ_{ft} is leaf water potential at full turgor, ψ_{π}^{ft} is the osmotic potential at full turgor. The corresponding fractional difference in cellular volume between ψ_{ft} and ψ_{tlp} was calculated as:

$$\Delta V/V = \frac{WC_{ft} - WC_{tlp}}{WC_{ft}} = \frac{(FM_{max} - DM)(RWC_{ft} - RWC_{tlp})}{(FM_{max} - DM)RWC_{ft}} = \frac{RWC_{ft} - RWC_{tlp}}{RWC_{ft}}$$

where WC is leaf water content, FM_{max} is leaf maximum fresh mass, DM is leaf dry mass, RWC is relative water content; ft and tlp denote the points of full turgor and turgor loss, respectively, on the PV curve as shown in Fig. 2. Substituting terms, the bulk modulus of elasticity was calculated for domain 2 of the PV curve as:

$$\varepsilon_{D2} = \frac{(\psi_{ft} - \psi_{\pi}^{ft})\, RWC_{ft}}{RWC_{ft} - RWC_{tlp}}.$$

Water storage capacitance (Q, mol m^{-2} MPa^{-1}), i.e. the amount of water released per unit leaf area per unit change in leaf water potential, was calculated for domains 1 and 2, following Brodribb and Holbrook (2003) as:

$$Q = \frac{DM}{S} \frac{WC_{max}}{DM} \frac{1}{M} \frac{\Delta RWC}{\Delta \Psi_{leaf}}$$

where M is molar mass of water (g mol^{-1}), ΔRWC is the difference between relative water contents spanning a domain as shown for ΔRWC_{D1} and ΔRWC_{D2} in Fig. 2, and $\Delta\psi_{leaf}$ is the difference between leaf water potentials spanning a domain as shown for $\Delta\Psi_{D1}$ and $\Delta\Psi_{D2}$ in Fig. 2.

Leaf water storage per unit area was calculated, respectively, for domains 1 (W_{D1}, mol m^{-2}) and 2 (W_{D2}, mol m^{-2}) of the three-domain PV curves (Nguyen *et al.*, 2016) as: W = Q($\Delta\Psi_{leaf}$). The sum of W_{D1} and W_{D2} is the total water storage (W_{tot}, mol m^{-2}).

Data analysis

Data were analysed with Genstat version 16 (Payne, 2014) through one-way ANOVA and simple linear regression. Data were normally distributed and did not require transformation before analyses. Fisher's Least Significant Difference and Tukey tests were applied *post hoc* to determine differences between treatment means whenever relationships with $P \leq 0.050$ were found.

Note that abbreviations using in the text are summarized in Table 1.

Results

Testing the four key hypotheses revealed strong differences in leaf water storage across the aridity and salinity gradients. First, increase in LMA with salinity and aridity was linked to increase in leaf water storage, which was achieved through increase in number of cell layers while maintaining fractional tissue contributions to lamina thickness. Second, turgor loss points declined with increase in soil salinity, driven primarily by differences in osmotic potential at full turgor as there were no consistent effects of salinity on the bulk modulus of elasticity. Third, there was little variation in leaf water storage capacitance (mol m^{-2} MPa^{-1}) but total leaf water storage (mol m^{-2}) increased with increase in salinity and aridity. Finally, PV curves revealed that water from sources other than roots would be required for maximum leaf hydration and turgor. These results are presented in detail below.

Physical properties of the leaves

Leaf physical properties varied both within and among subspecies (Table 2). Within *A. marina* subsp. *australasica*, leaf area, dry mass, and maximum water content were smaller at higher salinity (Fig. 3a-c). Leaf area declined more than dry mass with increasing salinity, consequently, leaf mass per area (LMA) significantly increased with salinity (P = 0.011, Fig. 3d). However, the maximum water content per unit dry mass

(WCD_{max}, g g^{-1}) decreased slightly with increasing salinity ($P = 0.02$, Fig. 3e). As maximum water content per unit leaf area (WCA_{max}, g m^{-2}) is the product of LMA and WCD_{max}, opposing variation in these two factors prevented significant ($P = 0.154$) variation in WCA_{max} with salinity within subsp. *australasica* (Fig. 3f). The tendency for WCA_{max} to increase with increasing salinity within subsp. *australasica* was mainly driven by LMA ($r^2 = 0.55$, $P = 0.001$). A similar pattern was evident with comparison of all subspecies in which LMA, and hence also WCA_{max}, increased with increasing salinity and aridity (Table 2, Fig. 3d-f).

Leaf anatomy

All three subspecies shared similar structures with five major tissue layers comprising the lamina (Fig. 4). These layers were the adaxial epidermis, hypodermis, palisade mesophyll, spongy mesophyll, and the abaxial epidermis which was covered with trichomes and contained stomata. There were no significant differences in either lamina thickness ($P= 0.991$) or the fraction each tissue contributed to lamina thickness within subsp. *australasica* grown in a range of salinities (Table 3, Fig. 5a).

In contrast, lamina thickness differed among the three subspecies ($P <0.001$) being smallest in Eu_{30} (418 ± 16 µm) and largest in Ma_{46} (761 ± 26 µm) despite these subspecies growing in salinities similar to those of Au_{28} and Au_{49}, respectively. There were no significant differences in the fraction that the photosynthetic tissues contributed to lamina thickness (Table 3), whereas significant differences occurred in the water storage tissues, i.e. the hypodermal and trichome layers. While the fraction of lamina thickness contributed by the hypodermis was lower in Eu_{30} than in Ma_{46} ($P = 0.009$), that of the trichomes followed the opposite pattern. Nevertheless, the actual thickness of each tissue layer was the greatest in Ma_{46} and the smallest in Eu_{30} (Table 3, Fig. 5a). Thus, averaging across all subspecies, lamina thickness increased with increasing salinity and aridity, consistent with the parallel increase in WCA_{max} (Fig. 5b), and LMA (Fig. 5c).

There were differences in the ways in which variation in the thicknesses of tissue layers were achieved. There was no significant difference in either the proportion or number of cells per unit area in the adaxial epidermis among subspecies, but the cuticle layer in Ma_{46}, averaging 10 µm, was twice as thick as that of other groups ($P < 0.001$). The smaller proportion of hypodermis in Eu_{30} was due to two factors: fewer hypodermal cell layers ($P < 0.001$) comprised of a greater number of smaller cells per unit area ($P = 0.01$, Fig. 4b). Variation in the thickness of photosynthetic tissues occurred mainly through a combination of the number of cell layers and the number of palisade cells per unit area. Neither the number of cells per unit area nor the number of cell layers in the spongy mesophyll differed among subspecies (Table 3). There was no significant difference in the number of trichomes per unit area between subspecies grown in similar salinities; however, the leaves of Eu_{30} and Au_{28} had significantly higher number of trichomes per unit area than those of Ma_{46} and Au_{49} ($P < 0.001$). Nevertheless, the average volume of individual trichomes was not significantly different among subspecies ($P = 0.694$).

Leaf water relations

Key leaf water relations parameters were calculated from PV curves constructed for leaves from all five sites as summarized in Table 4. All pressure volume curves had a similar shape with three domains as described in Nguyen *et al*. (2016). Domains 1, 2, and 3 were dominated respectively by loss in extracellularly stored water, decline in turgor, and decline in osmotic potential during leaf desiccation. On average, for each 0.1 MPa decrease in Ψ_{leaf}, RWC decreased by 1.5 - 2% in domain 1, 0.3 - 0.4% in domain 2, and 1% in domain 3.

There were no detectable effects of salinity on domain 1 in subsp. *australasica*. Domain 1 represented the decrease in relative water content (RWC) from 100 to approximately 87% with a corresponding decrease in leaf water potential (ψ_{leaf}) from -0.1 to the transition between domains 1 and 2 (ψ_{ft}) at -0.9 MPa. This domain accounted for an average of 13% of RWC of the leaf. There were no significant differences between these characteristics measured in subsp. *australasica* and those of the other two subspecies, except that average Ψ_{ft} was significantly less negative in Eu_{30} (-0.7 MPa) than Au_{28} (-0.9 MPa, $P = 0.042$).

Once the extracellular water was exhausted, further decline in ψ_{leaf} with decreasing RWC was driven mainly by decline in turgor over domain 2. The turgor loss point defined the transition from domain 2 to domain 3. Leaf water potential at the turgor loss point (Ψ_{tlp}) became more negative with increasing soil water salinity both within subsp. *australasica* (r^2 = 0.77, P < 0.001) and among subspecies (r^2 = 0.58, P < 0.001). Within subspecies grown in similar salinities, Ψ_{tlp} was significantly less negative in Eu_{30} (-4.1 MPa) than in Au_{28} (-4.5 MPa), and in Ma_{46} (-4.9 MPa) than in Au_{49} (-5.1 MPa, P < 0.001).

Leaf osmotic potential at full turgor (Ψ_{π}^{ft}) was about 0.8 MPa higher than Ψ_{tlp} for all leaves, and was correlated with Ψ_{tlp} both within (r^2 = 0.4, P = 0.039) and among subspecies (r^2 = 0.67, P < 0.001, Fig. 6a). Although Ψ_{tlp} varied within and among subspecies, relative water contents at turgor loss points (RWC_{tlp}) differed only between Eu_{30} and Au_{28} in which the turgor loss point occurred at significantly lower RWC_{tlp} in Eu_{30} (71%) than in Au_{28} (78%, P = 0.037).

Bulk modulus of elasticity

Bulk modulus of elasticity calculated for domain 2 (ε_{D2}) was highly variable and average values were not significantly different either within subsp. *australasica* grown at a range of salinities (P = 0.95) or among subspecies (P = 0.514). Thus, the variation in ε_{D2} did not correlate with the progressive decrease in Ψ_{tlp} with increasing salinity (Fig. 6b). There was also no correlation between ε_{D2} and increase in LMA within subsp. *australasica* (P = 0.575) or among subspecies (P = 0.928).

Water storage

Water content per unit leaf area (WCA) was plotted as a function of ψ_{leaf} to show differences in the actual water content of leaves from the five sites during dehydration (Fig. 7a). There was a correlation between WCA_{max} and that at the turgor loss point (WCA_{tlp}) within subsp. *australasica* (r^2 =0.68, P < 0.001). This correlation became stronger with the addition of data for the other two subspecies (r^2 =0.92, P < 0.001, Fig. 7b).

Despite the differences in water content between leaves, there were no significant differences in water storage capacitances calculated from either domain 1 (Q_{D1}, P = 0.26) or domain 2 (Q_{D2}, P = 0.748), between subsp. *australasica* grown in the three salinities. Similarly, neither Q_{D1} nor Q_{D2} were significantly (P = 0.301, P = 0.176, respectively) different among subspecies (Table 4).

The total of amount of water released per unit leaf area during desiccation from full hydration to the turgor loss point was related to salinity and evaporative demand. The averaged total water storage (W_{tot}) was lowest (4.63 ± 0.37 mol H_2O m^{-2}) in leaves grown in the low salinity, wet tropics site (Eu_{30}), and highest (7.56 ± 0.44 mol H_2O m^{-2}) in leaves grown in the high salinity, arid tropics site (Ma_{46}) (Table 4). Linear regression showed a significant increase in W_{tot} with salinity both within subsp. *australasica* (r^2 = 0.32, P < 0.017) and among all three subspecies (r^2 = 0.44, P < 0.001). Domains 1 and 2 contributed roughly equally to total water storage, i.e. $W_{D1} \approx W_{D2}$ (Table 4). However, the percentage contribution from domain 2 increased at the expense of domain 1 from 47.8% (Eu_{30}) to 52.2% (Ma_{46}) with increasing salinity and aridity.

These data were placed in a field context by dividing W_{D2} into two sub-components: $W_{D2\text{-}s}$ where the stored water could be sourced from the soil, i.e. $\Psi_{leaf} < \Psi_{soil}$, and $W_{D2\text{-}ns}$ where the stored water would have to be obtained from sources other than soil, i.e. $\Psi_{soil} < \Psi_{leaf} < \Psi_{ft}$. Note that soil water salinities were measured at a depth of 30 cm and so do not include lower salinities that can occur at the soil surface during tidal flooding. In this calculation, soil water contributed exclusively to water storage in domain 2. Figure 8 showed that the contribution of soil water ($W_{D2\text{-}s}$) to total leaf water storage (W_{tot}) ranged from 28% (Eu_{30}) to 35% (Ma_{46}). These data indicated that alternative water sources with salinities lower than those in the soil were required to achieve maximum water storage in all subspecies and sites.

Discussion

Variation in LMA, osmotic adjustment, water storage, and access to multiple sources of water were reflected in the structure of *A. marina* leaves grown in environments of increasing salinity and evaporative demand. Increase in LMA was a consequence of greater water storage with increasing salinity and aridity. The core feature of leaf water relations was the capacity to maintain low osmotic potential at full turgor which, when combined with high bulk modulus of elasticity, enabled maintenance of high cellular water contents with dehydration to the turgor loss point. That in itself would enhance survival, but maintenance of cell hydration during high transpiration rates would also require water storage when water loss exceeds rates of water supply. Indeed, water storage was increased by increasing lamina thickness, particularly through increasing numbers and layers of cells (Table 3, Fig. 4). Finally, linking leaf anatomy with leaf function as described by pressure-volume relationships showed that achieving either full hydration or full turgor required access to sources of water in addition to that supplied by the roots.

Increase in leaf mass per area was associated with increase in leaf water content per area.

LMA increased with increasing soil salinity and aridity of the environments in which the plants were grown (Fig. 3d), consistent with a recent meta-analysis of halophytic and glycophytic species (Poorter *et al.*, 2009). Previous studies have shown that species with higher LMA had higher cell wall concentrations of cellulose and hemicellulose per leaf dry mass, implying greater structural reinforcement than in leaves with lower LMA (Mediavilla *et al.*, 2008). Structural compounds would have contributed to the high LMA of the sclerophyllous leaves of *A. marina* (Choong *et al.*, 1992). However, in the present study, LMA was not correlated with the bulk modulus of elasticity, a measure of cell wall rigidity, consistent with the Bartlett *et al.* (2012) meta-analysis. Differences in LMA among subspecies were related to differences in lamina thickness associated with differences in numbers and sizes of cells comprising lamina tissues (Figs. 4, 5). Finally, increase in intracellular solute concentrations to maintain favourable water relations would also contribute to the increase in LMA with increasing salinity. For example, Downton (1982) reported the accumulation of NaCl for osmotic adjustment to account for approximately 10% of leaf dry weight in lab grown *Avicennia marina*. Thus, no single attribute accounted for the increase in LMA with increase in growth salinity. Instead, increase in LMA involved different combinations of more supportive structure, higher numbers of cells per unit leaf area, and higher solute concentrations that depended on the subspecies.

These results invite the question: what drives the salinity-dependent increase in LMA across subspecies? Decreasing osmotic potentials with increasing growth salinity required increasing cellular solute concentrations, which would contribute to the increase in LMA. However, such increase in the solute concentration comes at the expense of the amount of water per unit dry mass, WCD_{max} (Fig. 3e). Thus, increase in numbers or sizes of cells per unit area, thereby increasing leaf thickness and hence also LMA, would be required to maintain or increase maximum water content per unit leaf area (WCA_{max}) in increasingly saline soils. Indeed, WCA_{max} increased with increasing LMA in response to increasing salinity and evaporative demand (Fig. 5c), requiring coordination between leaf structure and leaf water relations. These effects were more pronounced among subspecies than within subspecies grown along a salinity gradient (Fig. 3f), underscoring complications in the interpretation of meta-data where species are the source of variation. Thus, the salinity-dependent increase in LMA appears driven by increasing requirements for water storage. In other environments, species from seasonally dry or xeric habitats typically have high values of LMA (Poorter *et al.*, 2009). Based on the present study, such high LMA in combination with increasing leaf thickness, as for example in Neotropical savannas (Rossatto *et al.*, 2015), may also be related to demands for water storage.

Leaf osmotic potentials at full turgor (Ψ_{π}^{ft}) and at the turgor loss point (Ψ_{tlp}) declined with increase in the growth salinity and evaporative demand of the climate in which the plants were grown.

Regardless of the sources of variation including subspecies and climate, soil water salinity was the major determinant of Ψ_{π}^{ft} and Ψ_{tlp}, consistent with the requirements to maintain a favourable water balance and the turgor essential for growth under increasingly saline edaphic conditions. Indeed, the capacity to vary osmotic potentials and thereby adjust water potentials at the turgor loss point must play critical roles in growth and survival of *A. marina* over a wide range of salinities. Specifically, *A. marina* had a low osmotic potential at full turgor, Ψ_{π}^{ft}, and it became more negative with increase in the soil water salinity in which the plants were grown. This is consistent with a study showing acclimation in osmotic potentials associated with accumulation of progressively increasing ion levels in leaves of *A. marina* (Downton, 1982). The osmotic potential at full turgor, Ψ_{π}^{ft}, was correlated with the osmotic potential at the turgor loss point, Ψ_{tlp} (Fig. 6a) as predicted by theoretical equations (Bartlett *et al.*, 2012). These results obtained from *A. marina* were consistent with those from a meta-analysis (Fig. 6c) of responses to drought where species was the source of variation (Bartlett *et al.*, 2012), and from a study of multispecies responses to imposed and natural seasonal drought in a tropical rainforest (Binks *et al.*, 2016). Thus, growth of *A. marina* in wet soil with high salinity elicited similar responses to those of plants subjected to drying soil. Bartlett *et al.* (2012) concluded from meta-analysis that leaf osmotic potentials at full turgor (Ψ_{π}^{ft}) and at the turgor loss point (Ψ_{tlp}) were important determinants of drought tolerance. The results of the present study extend that conclusion to include salt tolerance.

Leaves had a high bulk modulus of elasticity that provided mechanical strength and contributed to maintenance of high levels of cellular hydration during dehydration to the turgor loss point.

A consequence of decreasing Ψ_{π}^{ft} and Ψ_{tlp} with increasing growth salinity is the potential for turgor stress when either soil salinity is low or leaves are fully hydrated and, conversely, the potential for osmotic stress when soil salinity is high or leaves are dehydrated. The average bulk modulus of elasticity, ε_{D2} (18 to 27 MPa), in *A. marina* was highly variable with no significant difference among subspecies grown in salinities ranging from 28 to 49 ppt (Fig. 6b). Our results contrasted with the expectation that ε_{D2} would increase, i.e. that cell walls would become more rigid, with increasing growth salinity as observed in *A. germinans* grown in salinities ranging from 0 to 32 ppt under laboratory conditions (Suarez & Sobrado, 2000). In the present study, high ε_{D2} may reflect a need for mechanical strength in field-grown leaves subject to a wide range of leaf water potentials over both daily and seasonal time scales. For example, under natural field conditions, Ψ_{leaf} of *A. marina* growing in soil with pore water salinity of 40 to 49 ppt (-2.7 to -3.4 MPa) varied from -0.1 MPa at dawn following a leaf wetting event to -6 MPa in mid-afternoon without perceptible damage (Constable, 2014; Walker, 2014). In this example, if Ψ_{π}^{ft} equals -4.2 MPa, then the turgor pressure would be as high as 4.1 MPa. Conversely, cells would be subjected to extreme osmotic stress when midday or afternoon Ψ_{leaf} approaches or is more negative than a turgor loss point of, say, -5 MPa. Maintenance of a high ε_{D2} would offer protection against cell wall failure over the wide range of leaf water potentials encountered daily by leaves of *A. marina* under natural field conditions.

In the present study, there was no correlation between bulk modulus of elasticity and turgor loss points (Fig. 6b), consistent with the meta-analysis (Fig. 6d) of Bartlett *et al.* (2012). Nevertheless, in the present study, cells remained well hydrated at the turgor loss point. Indeed, in leaves of subsp. *australasica* grown in soil water salinity ranging from 28 to 49 ppt, RWC_{tlp} decreased from 78 to 75%, respectively, while ε_{D2} averaged 26 MPa (Table 4). Similarly, average RWC_{tlp} ranged from 71 - 78% across all three subspecies. However, these RWC_{tlp} values were calculated from leaf saturated water content, which included the extracellular water that dominated domain 1 (Nguyen *et al.*, 2016). If domain 1 was excluded from calculations, effectively shifting the leaf saturated water content to that at Ψ_{ft}, then RWC_{tlp} based solely on domain 2 (dominated by cellular water) ranged from 82 - 90%. These values are greater than the estimated minimum requirement of 75% RWC to sustain cell function (Lawlor & Cornic, 2002). These data agreed with the suggestion by Cheung *et al.* (1975) and meta-analysis by Bartlett *et al.* (2012) that high bulk modulus of elasticity played an important role in conserving cell hydration during leaf desiccation. Based on the PV curves, a 1% decrease in RWC was associated with a decrease in Ψ_{leaf} of 0.1 MPa with reduction in hydration below the turgor loss point (domain 3). These data suggest *A. marina* would be able to maintain cell function for a further 0.7 – 1.5 MPa decrease in Ψ_{leaf} below the turgor loss point. This is consistent with the occurrence of plasmolysis in most living cells at 1 MPa lower than Ψ_{tlp} in leaves of *A. marina* (Nguyen *et al.*, 2016).

Leaf water storage increased with increase in the growth salinity and evaporative demand of the climate in which the plants were grown.

Leaf water storage may play critical roles in drought survival and in buffering fluctuation in leaf water potentials when rates of evaporation exceed rates of water re-supply from the roots (Lechthaler *et al.*, 2016). In *A. marina*, WCA_{max} differed among subspecies and was correlated strongly with WCA_{tlp} (Fig. 7b). WCA_{max} is a component of leaf water storage capacitance (Q, mol m^{-2} MPa^{-1}), the amount of water released per unit leaf area per unit change in water potential (Fig. 2). There was a tendency, albeit not significant, for Q to increase with increasing salinity and aridity (Table 4), partly due to increase in WCA_{max} and, hence, also LMA, consistent with previous studies in other drought-affected systems (Blackman & Brodribb, 2011). The combined effects of increasing Q, driven by increasing WCA_{max}, and decrease in the turgor loss point (ψ_{tlp}) resulted in an increase in total water storage, W_{tot}, with increasing salinity and aridity.

Although salinity strongly affected leaf water storage, the ways in which water was stored differed among subspecies and appeared to be related to the evaporative demands of the environments in which the subspecies grew. For example, leaves of Eu_{30} from the wet tropics were almost half the thickness of those of Ma_{46} from the arid tropics and had correspondingly less WCA_{max}. These subspecies differed in the relative contributions of different tissues to lamina thickness. Specifically, the hypodermal layer occupied 31% of lamina thickness in Eu_{30} and 38% in Ma_{46} while the trichome layer accounted for 19% of lamina thickness in Eu_{30} and 15% in Ma_{46} (Table 4). In addition, the greater number of trichomes per unit area with similar average volumes (Table 4) would enable greater extracellular water storage in the leaf lamina of Eu_{30} than Ma_{46}. This strategy in a wet tropical environment might be favoured by two factors. First, trichomes of *A. marina* leaves rapidly absorb liquid water from wet epidermal surfaces (Nguyen *et al.*, 2016), enabling rapid replenishment of leaf water from frequent leaf-wetting events, such as showers. Second, the highly humid atmosphere would limit evaporation, enhancing the duration of extracellular water storage in the trichome layer during the day. In contrast, water absorption by the trichome layer in Ma_{46} would occur predominantly during nocturnal leaf-wetting events in its arid tropical environment. However, that water would need to be stored intracellularly to prevent its rapid loss from the trichomes upon increase in evaporative demand after sunrise. This may account for a greater allocation of lamina thickness to the hypodermal layer in the much thicker and more heavily cutinized leaves of Ma_{46} than Eu_{30} (Figs. 4, 5). Such differences among subspecies reflect coordination between leaf structure and leaf water relations under different environmental conditions, but further work is required to distinguish the relative contributions of genotypes and environments.

The PV curves showed that leaves of *A. marina* must access water from sources with salinities lower than those measured in the soil to achieve either full hydration or full turgor.

Mangroves such as *A. marina* growing in saline wetlands are subject to spatial and temporal variation in salinity, which would affect the sources of water available for uptake. Soil pore water salinity would typically be higher than that of flooding tidal water because exclusion of salt during water uptake by the roots leads to the accumulation of salt in the rhizosphere (Passioura *et al.*, 1992). The salinity of soil pore water would fluctuate less than that of surface water. Depending on conditions, the salinity of surface flood water can vary from nearly freshwater to seawater while at the same time that of underlying soil water can be hypersaline. Thus, roots of a single plant may be exposed to a wide range of salinities over a vertical gradient from flood water through the soil. Indeed, split-root experiments have shown preferential water uptake when salinity was low in soil with spatial (Bazihizina *et al.*, 2009; Reef *et al.*, 2015) or temporal variation in salinity (Lechthaler *et al.*, 2016). Meanwhile, leaves can also be rehydrated by different sources of water, such as fog, dew and rainfall (Eller *et al.*, 2013) even in hypersaline mangrove environments (Constable, 2014; Walker, 2014).

Water potentials measured during leaf desiccation ranged from -0.1 MPa at full hydration to values more negative than those at the turgor loss points. This range of potentials can be experienced in a single day (Constable, 2014; Walker, 2014). Thus, the PV relationship informs interpretation of the daily variation in leaf water potentials. Total water storage was estimated for domains 1 and 2 of the PV curves. These domains contributed almost equally to total leaf water storage, which increased with increases in the salinity and aridity in which the plants were grown. Summing the water storage from domains 1 and 2 (i.e. from full hydration to the turgor loss point), the total water storage in leaves of the present study could alone supply the water loss needed to support photosynthesis at an evaporation rate of 1 mmol H_20 m^{-2} s^{-1} for up to 77 min in the wet tropics (Eu_{30}) and 126 min in the arid tropics (Ma_{46}) (Table 5, Fig. 8). These calculations underscore the increasing importance of stored water to leaf function with increase in salinity and aridity of the environment.

The ranges of water potentials involved in domains 1 and 2 suggest contributions of water from different sources. Extraction of water from soil and its subsequent transport to leaves requires leaf water potentials to be lower than those of soil water. If ψ_{leaf} was less negative than ψ_{soil}, then water supply to leaves must be from sources other than soil water. For domain 1, water storage (W_{D1}) was exhausted with dehydration from -0.1 MPa to an average of -0.8 MPa, which is equivalent to the water potential of 34% seawater (12 ppt), much lower than the salinities measured in soil pore water at any sites in the present study. Water stored in domain 1 could be contributed by roots if salinity was lower than 12 ppt, or by leaves receiving dew or intercepting rainfall. Indeed, Lechthaler *et al.* (2016) reported rapid recharge of water storage in leaves of seedlings in the Rhizophoraceae when salinities around roots were lowered from 30 to 5 ppt. Leaves of *A. marina* can absorb liquid water through salt secretion glands (Tan *et al.*, 2013) and through the trichome layer (Nguyen *et al.*, 2016) and have the capacity for extracellular storage of such water as reflected in domain 1 (Nguyen *et al.*, 2016). Thus, leaf-wetting events could reverse the water potential gradient from the atmosphere to the plant to the soil (Goldsmith, 2013), enabling rehydration of leaves to water potentials as high as -0.1 MPa even when roots are exposed to very high soil salinities, as has been observed under natural field conditions (Constable, 2014; Walker, 2014).

Water stored in domain 2 was released from cells with dehydration from an average leaf water potential of -0.8 MPa to the turgor loss point. The cellular water storage of domain 2, W_{D2}, was divided into two components: water storage when ψ_{leaf} was less negative ($W_{D2\text{-}ns}$) or more negative ($W_{D2\text{-}s}$) than the soil water potentials measured at the time the PV curves were constructed. On this basis, water sourced from soil would most likely contribute to storage in domain 2. Furthermore, as leaf full hydration and full turgor occurred at leaf water potentials much higher than those of ψ_{soil}, leaves would be neither fully hydrated nor fully turgid if soil pore water was the only source of water unless salinity was lowered by rainfall events or roots near the soil surface accessed flood water of lower salinity. This analysis shows the importance of spatial and temporal variation in soil salinity, together with access to alternative water sources, to the water balance of these leaves.

Conclusion

Comparative analyses of pressure volume curves revealed intricate integration of leaf structure and water relations that may contribute to growth and survival of *Avicennia marina* along complex gradients in salinity and aridity. As expected, osmotic adjustment together with a high cellular modulus of elasticity enable maintenance of turgor and hydration over progressively lower leaf water potentials with increase in soil water salinity, consistent with analyses of leaf properties in relation to drought tolerance (Bartlett *et al*., 2012). The high LMA values of the sclerophyllous leaves of *A. marina* played no direct role in leaf water relations, again consistent with meta-analysis of drought tolerant species (Bartlett *et al*., 2012). Nevertheless, variation in LMA in *A. marina* was largely a consequence of the increasing thickness of the lamina required to increase both extracellular and intracellular water storage in response to increasing salinity and aridity. These two storage compartments contributed approximately equally to total leaf water storage, but were operational over differ ranges of leaf hydration. Indeed, when placed in context with the soil water salinities of the growth conditions, the PV curves revealed that access to alternative water sources was required to achieve full hydration or turgor. This requirement could be met by foliar water uptake under moist atmospheric conditions as leaves of *Avicennia* can absorb liquid water via trichomes (Nguyen *et al*., 2016) and salt secretion glands (Tan *et al.*, 2013). These results merit further study as they may help to define safety margins analogous to those of cloud forests (Oliveira *et al.*, 2014) for the maintenance of favourable hydration and leaf function with natural variation in soil salinity and atmospheric moisture through the progression of wet and dry seasons or exposure to extreme drought conditions. In the latter case, increasing soil salinity in association with drought would reduce the hydration state that could be achieved through supply of soil water from the roots, while a dry atmosphere could limit the supply of water obtained via foliar water uptake. Indeed, such combined effects may have contributed to drought-induced die-back of mangroves growing in hypersaline soils (Lovelock *et al.*, 2009), and may underlie the recent massive die-back of a 700 km stretch of mangrove forest in northern Australia following unusually hot and dry conditions (Duke *et al.*, 2016). Thus the results of the present study underscore the importance of understanding leaf features that may provide a means of assessing responses of key vegetation types to climate change.

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References

Allen C.D., Macalady A.K., Chenchouni H., Bachelet D., McDowell N., Vennetier M., . . . Cobb N. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, **259**, 660-684.

Ball M.C. (1988) Ecophysiology of mangroves. *Trees*, **2**, 129-142.

Ball M.C. & Anderson J.M. (1986) Sensitivity of photosystems II to NaCl in relation to salinity tolerance. Comparative studies with thylakoids of the salt tolerant mangrove, *Avicennia marina*, and the salt-sensitive pea, *Pisum sativum*. *Functional Plant Biology*, **13**, 689-698.

Ball M.C., Cowan I.R. & Farquhar G.D. (1988) Maintenance of leaf temperature and the optimization of carbon gain in relation to water loss in a tropical mangrove forest. *Australian Journal of Plant Physiology*, **15**, 263-276.

Ball M.C. & Farquhar G.D. (1984a) Photosynthetic and stomatal responses of the grey mangrove, *Avicennia marina*, to transient salinity conditions. *Plant Physiology*, **74**, 7-11.

Ball M.C. & Farquhar G.D. (1984b) Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina*, to long term salinity and humidity conditions. *Plant Physiology*, **74**, 1-6.

Bartlett M.K., Scoffoni C. & Sack L. (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology Letters*, **15**, 393-405.

Bazihizina N., Colmer T.D. & Barrett-Lennard E.G. (2009) Response to non-uniform salinity in the root zone of the halophyte *Atriplex nummularia*: growth, photosynthesis, water relations and tissue ion concentrations. *Annals of Botany*, **104**, 737-745.

Binks O., Meir P., Rowland L., da Costa A.C., Vasconcelos S.S., de Oliveira A.A., . . . Mencuccini M. (2016) Plasticity in leaf-level water relations of tropical rainforest trees in response to experimental drought. *New Phytologist*, **211**, 477-488.

Blackman C.J. & Brodribb T.J. (2011) Two measures of leaf capacitance: insights into the water transport pathway and hydraulic conductance in leaves. *Functional Plant Biology*, **38**, 118-126.

Brodribb T.J. & Holbrook N.M. (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology*, **132**, 2166-2173.

Cheung Y.N.S., Tyree M.T. & Dainty J. (1975) Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. *Canadian Journal of Botany*, **53**, 1342-1346.

Choong M.F., Lucas P.W., Ong J.S.Y., Pereira B., Tan H.T.W. & Turner I.M. (1992) Leaf fracture toughness and sclerophylly: their correlations and ecological implications. *New Phytologist*, **121**, 597-610.

Clough B.F. & Sim R.G. (1989) Changes in gas exchange characteristics and water use efficiency of mangroves in response to salinity and vapor-pressure deficit. *Oecologia*, **79**, 38-44.

Constable L. (2014) *Nocturnal top-down rehydration: contribution of atmospheric moisture to water status of the mangrove, Avicennia marina*. Honours Thesis, The Australian National University.

Downton W.J.S. (1982) Growth and osmotic relations of the mangrove *Avicennia marina*, as influenced by salinity. *Functional Plant Biology*, **9**, 519-528.

Duke N., Ball M. & Ellison J. (1998) Factors influencing biodiversity and distributional gradients in mangroves. *Global Ecology & Biogeography Letters*, **7**, 27-47.

Duke N., Kovacs J., Griffiths A., Preece L., Hill D., Oosterzee P.v., . . . Burrows D. (2016) Large-scale dieback of mangroves in Australia's Gulf of Carpentaria: a severe ecosystem response, coincidental with an unusually extreme weather event. *Marine and freshwater research*

Eller C.B., Lima A.L. & Oliveira R.S. (2013) Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *New Phytologist*, **199**, 151-162.

Ewers F.W., Lopez-Portillo J., Angeles G. & Fisher J.B. (2004) Hydraulic conductivity and embolism in the mangrove tree *Laguncularia racemosa*. *Tree Physiology*, **24**, 1057-1062.

Feller I.C., Lovelock C.E., Berger U., McKee K.L., Joye S.B. & Ball M.C. (2010) Biocomplexity in Mangrove Ecosystems. *Annual Review of Marine Science*, **2**, 395-417.

Flowers T.J. (1972) Salt tolerance in *Suaeda maritima* (L.) Dum: The effect of sodium chloride on growth, respiration, and soluble enzymes in a comparative study with *Pisum sativum* L. . *Journal of Experimental Botany*, **23**, 310-321.

Flowers T.J. & Colmer T.D. (2008) Salinity tolerance in halophytes. *New Phytol*, **179**, 945-963.

Flowers T.J., Troke P.F. & Yeo A.R. (1977) The mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology*, **28**, 89-121.

Goldsmith G.R. (2013) Changing directions: the atmosphere–plant–soil continuum. *New Phytologist*, **199**, 4-6.

Harvey H.W. (1966) *The chemistry and fertility of sea waters*. Cambridge University Press, Cambridge.

Jefferies R.L. (1981) Osmotic adjustment and the response of halophytic plants to salinity. *BioScience*, **31**, 42-46.

Lawlor D.W. & Cornic G. (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell, and Environment*, **25**, 275-294.

Lechthaler S., Robert E.M.R., Tonné N., Prusova A., Gerkema E., Van As H., . . . Windt C.W. (2016) Rhizophoraceae mangrove saplings use hypocotyl and leaf water storage capacity to cope with soil water salinity changes. *Frontiers in Plant Science*, **7**.

Li X., Duke N.C., Yang Y., Huang L., Zhu Y., Zhang Z., . . . Shi S. (2016) Re-evaluation of phylogenetic relationships among species of the mangrove genus *Avicennia* from Indo-West Pacific based on multilocus analyses. *PLOS ONE*, **11**, e0164453.

Lopez-Portillo J., Ewers F.W. & Angeles G. (2005) Sap salinity effects on xylem conductivity in two mangrove species. *Plant Cell and Environment*, **28**, 1285-1292.

Lovelock C.E., Ball M.C., Feller I.C., Engelbrecht B.M.J. & Ewe M.L. (2006) Variation in hydraulic conductivity of mangroves: influence of species, salinity, and nitrogen and phosphorus availability. *Physiologia Plantarum*, **127**, 457-464.

Lovelock C.E., Ball M.C., Martin K.C. & C. Feller I. (2009) Nutrient enrichment increases mortality of mangroves. *PLoS ONE*, **4**, e5600.

McDowell N.G. & Allen C.D. (2015) Darcy's law predicts widespread forest mortality under climate warming. *Nature Clim. Change*, **5**, 669-672.

McKee K.L. (1993) Soil physicochemical patterns and mangrove species distribution--reciprocal effects? *Journal of Ecology*, **81**, 477-487.

Mediavilla S., Garcia-Ciudad A., Garcia-Criado B. & Escudero A. (2008) Testing the correlations between leaf life span and leaf structural reinforcement in 13 species of European Mediterranean woody plants. *Functional Ecology*, **22**, 787-793.

Melcher P.J., Goldstein G., Meinzer F.C., Yount D.E., Jones T.J., Holbrook N.M. & Huang C.X. (2001) Water relations of coastal and estuarine *Rhizophora mangle*: xylem pressure potential and dynamics of embolism formation and repair. *Oecologia*, **126**, 182-192.

Naidoo G. (1989) Seasonal plant water relations in a South African mangrove swamp. *Aquatic Botany*, **33**, 87-100.

Nguyen H.T., Meir P., Wolfe J., Mencuccini M. & Ball M.C. (2016) Plumbing the depths: extracellular water storage in specialized leaf structures and its functional expression in a three-domain pressure-volume relationship. *Plant, Cell & Environment*, n/a-n/a.

Nguyen H.T., Stanton D.E., Schmitz N., Farquhar G.D. & Ball M.C. (2015) Growth responses of the mangrove *Avicennia marina* to salinity: development and function of shoot hydraulic systems require saline conditions. *Annals of Botany*, **115**, 397-407.

Niinemets Ü. (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, **82**, 453-469.

Oliveira R.S., Eller C.B., Bittencourt P.R.L. & Mulligan M. (2014) The hydroclimatic and ecophysiological basis of cloud forest distributions under current and projected climates. *Annals of Botany*, **113**, 909-920.

Paliyavuth C., Clough B. & Patanaponpaiboon P. (2004) Salt uptake and shoot water relations in mangroves. *Aquatic Botany*, **78**, 349-360.

Passioura J.B., Ball M.C. & Knight J.H. (1992) Mangroves may salinize the soil and in so doing limit their transpiration rate. *Functional Ecology*, **6**, 476-481.

Payne R.W., Murray, D.A., Harding, S.A., Baird, D.B. & Soutar, D.M. (2014) GenStat for Windows 16th Edition. VSN International, Hemel Hempstead, UK.

Poorter H., Niinemets Ü., Poorter L., Wright I.J. & Villar R. (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, **182**, 565-588.

Rada F., Goldstein G., Orozco A., Montilla M., Zabala O. & Azocar A. (1989) Osmotic and turgor relations of three mangrove ecosystem species. *Functional Plant Biology*, **16**, 477-486.

Reef R., Markham H.L., Santini N.S. & Lovelock C.E. (2015) The response of the mangrove Avicennia marina to heterogeneous salinity measured using a split-root approach. *Plant and Soil*, **393**, 297-305.

Rossatto D.R., Kolb R.M. & Franco A.C. (2015) Leaf anatomy is associated with the type of growth form in Neotropical savanna plants. *Botany*, **93**, 507-518.

Rowland L., da Costa A.C.L., Galbraith D.R., Oliveira R.S., Binks O.J., Oliveira A.A.R., . . . Meir P. (2015) Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature*, **528**, 119-122.

Scholander P.F. (1968) How mangroves desalinate seawater. *Physiologia Plantarum*, **21**, 251-261.

Scholander P.F., Hammel H.T., Hemmingsen E.A. & Bradstreet E.D. (1964) Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. *Proceedings of the National Academy of Sciences*, **52**, 119-125.

Sobrado M.A. (2007) Relationship of water transport to anatomical features in the mangrove *Laguncularia racemosa* grown under contrasting salinities. *New Phytologist*, **173**, 584-591.

Sperry J.S., Tyree M.T. & Donnelly J.R. (1988) Vulnerability of xylem to embolism in a mangrove vs an inland species of Rhizophoraceae. *Physiologia Plantarum*, **74**, 276-283.

Sternberg L.d.S.L., Ish-Shalom-Gordon N., Ross M. & O'Brien J. (1991) Water relations of coastal plant communities near the ocean/freshwater boundary. *Oecologia*, **88**, 305-310.

Suarez N. & Sobrado M.A. (2000) Adjustments in leaf water relations of mangrove (*Avicennia germinans*) seedlings grown in a salinity gradient. *Tree Physiology*, **20**, 277-282.

Suarez N., Sobrado M.A. & Medina E. (1998) Salinity effects on the leaf water relations components and ion accumulation patterns in *Avicennia germinans* (L.) L. seedlings. *Oecologia*, **114**, 299-304.

Tan W.-K., Lin Q., Lim T.-M., Kumar P. & Loh C.-S. (2013) Dynamic secretion changes in the salt glands of the mangrove tree species *Avicennia officinalis* in response to a changing saline environment. *Plant, Cell & Environment*, **36**, 1410-1422.

Turner N. (1988) Measurement of plant water status by the pressure chamber technique. *Irrigation Science*, **9**, 289-308.

Tyree M.T. & Hammel H.T. (1972) The measurement of the turgor pressure and the water relations of plants by the Pressure-bomb technique. *Journal of Experimental Botany*, **23**, 267-282.

Walker W. (2014) *The role of leaf hydraulics in the photosynthetic performance of the mangrove Avicennia marina growing in seawater and hyper-saline habitats*. Honours Thesis, The Australian National University.

Table 1: Abbreviations

Abbreviations	Symbol	Unit
Plant materials		
A. marina subsp. *australasica* grown at 28, 40, 49 ppt seawater	Au_{28}, Au_{40}, Au_{49}	
A. marina subsp. *eucalyptifolia* grown at 30 ppt seawater	Eu_{30}	
A. marina subsp. *marina* grown at 46 ppt seawater	Ma_{46}	
Parameters		
Bulk modulus of elasticity	ε	MPa
Difference	Δ	
Fractional difference in cellular volume	$\Delta V/V$	
Leaf area	S	cm^2, m^2
Leaf dry mass	DM	g
Leaf dry mass per area	LMA	g m^{-2}
Leaf fresh mass	FM	g
Leaf water content	WC	g
Leaf water content per area	WCA	g m^{-2}
Leaf water content per dry mass	WCD	g g^{-1}
Leaf water potential	Ψ_{leaf}	MPa
Osmotic potential	Ψ_{π}	MPa
Relative water content	RWC	%
Turgor pressure	P	MPa
Water storage capacitance	Q	mmol m^{-2} MPa^{-1}
Water storage (per unit leaf area)	W	mol m^{-2}
Subscript meaning		
Maximum value	max	
(Calculated for) Domain 1	D1	
(Calculated for) Domain 2	D2	
(Measured at the point of) Full turgor	ft	
(Measured at) Turgor loss point	tlp	
Total	tot	

Table 2. Physical properties of leaves of the three subspecies of *A. marina*: subsp. *australasica* (Au), subsp. *eucalyptifolia* (Eu), and subsp. *marina* (Ma) grown under temperate, wet tropical, and arid tropical climates, respectively, in salinities ranging from 28 to 49 ppt. These salinities are given as a subscript following the two letter subspecies designations. Values are means ± se (n= 5). Superscript letters denote significant differences among subspecies as determined by one-way ANOVA with *post hoc* Tukey test when $P \leq 0.05$. Effects of salinity on leaf physical properties were tested within subsp. *australasica* (grey block). For other comparisons, note that effects of subspecies were confounded with environment. Data for Au_{49} was reproduced from Nguyen *et al.* (2016).

Parameter	**Symbol**	**Unit**	**Eu_{30}**		**Au_{28}**		**Au_{40}**		**Au_{49}**		**Ma_{46}**	
			Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
Salinity		ppt	30	0	28	0.4	40	0.4	49	0.6	46	0.7
Leaf area	S	cm^2	14.4^a	0.9	21.2^c	1.2	19.5^b	1.1	14.8^a	1.1	19.9^{bc}	0.6
Leaf dry mass per area	LMA	$g\ m^{-2}$	156^a	5	212^b	5	226^{bc}	10	256^c	14	325^d	11
Maximum water content per area	WCA_{max}	$g\ m^{-2}$	292^a	16	411^b	11	404^b	16	447^{bc}	13	501^c	19
Maximum water content per dry mass	WCD_{max}	$g\ g^{-1}$	1.89^{ab}	0.15	1.94^b	0.04	1.79^{ab}	0.03	1.76^{ab}	0.08	1.54^a	0.05

Table 3. Anatomical features of leaves of the three subspecies of *A. marina*: subsp. *australasica* (Au), subsp. *eucalyptifolia* (Eu), and subsp. *marina* (Ma) grown under temperate, wet tropical, and arid tropical climates, respectively, in salinities ranging from 28 to 49 ppt. These salinities are given as a subscript following the two letter subspecies designations. Values are means ± se (n= 5). Superscript letters denote significant differences among subspecies as determined by one-way ANOVA with *post hoc* Tukey test when P ≤ 0.05. Effects of salinity on leaf physical properties were tested within subsp. *australasica* (grey block). For other comparisons, note that effects of subspecies were confounded with environment. Part of data for Au$_{49}$ was reproduced from Nguyen *et al.* (2016).

Parameter	**Symbol**	**Unit**	**Eu$_{30}$**		**Au$_{28}$**		**Au$_{40}$**		**Au$_{49}$**		**Ma$_{46}$**	
			Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
Lamina thickness	Lth	µm	418^{a}	16	566^{b}	24	565^{b}	14	569^{b}	13	761^{c}	26
Contribution to lamina thickness												
Adaxial epidermis	UEP	%	3	0	3	0	3	0	3	0	3	0
Hypodermis	HP	%	31^{a}	2	38^{b}	1	38^{b}	2	36^{ab}	1	38^{b}	0
Palisade mesophyll	PP	%	30	1	29	1	26	1	30	1	29	1
Spongy mesophyll	SP	%	16	1	13	1	14	1	12	0	15	1
Trichome	TP	%	19^{b}	1	17^{ab}	0	19^{b}	1	19^{b}	1	15^{a}	0
Number of cell layers												
Hypodermis	HN		5.8^{a}	0.4	8.0^{b}	0.5	8.0^{b}	0.3	8.0^{b}	0.0	9.2^{b}	0.2
Palisade mesophyll	PN		3.6^{ab}	0.2	3.6^{ab}	0.2	3.2^{a}	0.2	3.0^{a}	0.0	4.0^{b}	0.0
Spongy mesophyll	SN		4.2	0.4	4.6	0.2	5.0	0.3	5.0	0.3	4.8	0.2
Number of cells per unit area												
Adaxial epidermis	UED	mm^{-2}	$3,091^{a}$	251	$3,613^{ab}$	154	$3,861^{b}$	147	$2,997^{a}$	22	$3,459^{ab}$	225
Hypodermis	HD	mm^{-2}	$1,442^{b}$	135	$1,302^{ab}$	54	$1,569^{b}$	57	$1,420^{b}$	125	962^{a}	56
Palisade mesophyll	PD	mm^{-2}	$2,598^{b}$	206	$2,110^{ab}$	190	$2,340^{ab}$	75	$2,352^{ab}$	205	$1,663^{a}$	120
Spongy mesophyll	SD	mm^{-2}	4,357	505	3,389	158	4,000	472	4,265	494	3,160	124
Trichome												
Number per unit area	TD	mm^{-2}	$2,827^{b}$	115	$2,690^{b}$	48	$2,729^{b}$	82	$2,188^{a}$	88	$1,863^{a}$	79
Internal lumen diameter	µm	µm	24	1	20	2	22	1	20	2	19	1
Length	µm	µm	81^{a}	3	96^{ab}	3	107^{b}	2	109^{b}	5	114^{b}	4
Volume*	TV	µm^{3}	24,181	3,226	16,054	2,602	22,374	2,578	20,460	3,288	15,903	2,794

Table 4. Water relations parameters derived from three-domain PV curves constructed from leaves of the three subspecies of *A. marina*: subsp. *australasica* (Au), subsp. *eucalyptifolia* (Eu), and subsp. *marina* (Ma) grown under temperate, wet tropical, and arid tropical climates, respectively, in salinities ranging from 28 to 49 ppt. These salinities are given as a subscript following the two letter subspecies designations. Values are means ± se (n= 5). Superscript letters denote significant difference among subspecies as determined by one-way ANOVA with *post hoc* Tukey test when P ≤ 0.05. Effects of salinity on leaf physical properties were tested within subsp. *australasica* (grey block). For other comparisons, note that effects of subspecies were confounded with environment. Part of data for Au$_{49}$ was reproduced from Nguyen *et al*. (2016).

Parameter	**Symbol**	**Unit**	**Eu$_{30}$**		**Au$_{28}$**		**Au$_{40}$**		**Au$_{49}$**		**Ma$_{46}$**	
			Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
Leaf water potential at full turgor	Ψ_{ft}	MPa	-0.69^{a}	0.04	-0.87^{b}	0.03	-0.82^{ab}	0.04	-0.85^{ab}	0.03	-0.86^{ab}	0.04
Osmotic potential at full turgor	Ψ_{π}^{ft}	MPa	-3.3^{a}	0.2	-3.7^{ab}	0.1	-4.0^{bc}	0.1	-4.2^{c}	0.1	-4.2^{c}	0.1
Water potential at turgor loss point	Ψ_{tlp}	MPa	-4.1^{a}	0.0	-4.5^{b}	0.1	-4.7^{c}	0.0	-5.1^{d}	0.1	-4.9^{cd}	0.0
RWC at full turgor	RWC_{ft}	%	85	1	88	1	87	1	87	1	87	1
RWC at turgor loss point	RWC_{tlp}	%	71^{a}	1	78^{b}	1	76^{ab}	1	75^{ab}	3	73^{ab}	1
Modulus of elasticity for domain 2	ε_{D2}	MPa	18	3	26	3	27	5	26	4	21	2
Water storage capacitance for domain 1	Q_{D1}	mol m^{-2} MPa^{-1}	3.57	0.27	3.13	0.38	3.61	0.44	3,83	0.35	4.23	0.56
Water storage capacitance for domain 2	Q_{D2}	mol m^{-2} MPa^{-1}	0.66	0.13	0.64	0.08	0.64	0.07	0.72	0.09	1.00	0.11
Total water storage	W_{tot}	mol m^{-2}	4.63^{a}	0.37	4.85^{a}	0.18	5.60^{a}	0.25	6.28^{ab}	0.63	7.56^{b}	0.44
Water storage for domain 1	W_{D1}	mol m^{-2}	2.43^{a}	0.05	2.56^{a}	0.12	2.95^{ab}	0.25	3.26^{ab}	0.34	3.62^{b}	0.28
Water storage for domain 2	W_{D2}	mol m^{-2}	2.20^{a}	0.37	2.23^{a}	0.28	2.65^{ab}	0.15	3.02^{ab}	0.36	4.00^{b}	0.42

Table 5. Estimation of the time that stored water obtained from soil or alternative sources could contribute to gas exchange in leaves of the three subspecies of *A. marina*: subsp. *australasica* (Au), subsp. *eucalyptifolia* (Eu), and subsp. *marina* (Ma) grown under temperate, wet tropical, and arid tropical climates, respectively, in salinities ranging from 28 to 49 ppt. Calculations were based on the distribution of water stored over a different ranges of water potentials as shown in Fig. 8 assuming an evaporation rate of 1 mmol H_2O m^{-2} s^{-1}. Values are means ± se (n= 5). Superscript letter denoted significant difference among subspecies as determined by one-way ANOVA with *post hoc* Tukey test when P ≤ 0.05. Effects of salinity on leaf physical properties were tested within subsp. *australasica* (grey block). For other comparisons, note that effects of subspecies were confounded with environment. Data for Au_{49} was reproduced from Nguyen *et al*. (2016).

Source of stored water	**Environmental source of water**	**Time**	Ψ_{leaf}	Eu_{30}		Au_{28}		Au_{40}		Au_{49}		Ma_{46}	
				Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
Domain 1 (mostly extracellular)	Alternative	min	$\Psi_{ft} < \Psi_{leaf}$	41^a	1	43^a	3	49^{ab}	4	54^{ab}	6	60^b	5
Domain 2 (cellular)	Alternative	min	$\Psi_{soil} < \Psi_{leaf} < \Psi_{ft}$	14^{ab}	2	9^a	2	18^{bc}	3	21^{bc}	2	22^c	1
	Soil	min	$\Psi_{tlp} < \Psi_{leaf} < \Psi_{soil}$	22	5	29	3	26	4	30	5	44	7
Total		min		77^a	6	81^a	3	93^a	4	105^{ab}	11	126^b	7

Figure legends

Figure 1. Long-term average monthly rainfall, minimum and maximum air temperature, and relative humidity at 3 pm at weather stations nearest the three study sites along the tidal margins of Batemans Bay, New South Wales (temperate oceanic, solid square), Daintree River, Queensland (wet tropics, open triangle), and Giralia Bay, Western Australia (arid tropics, open circle). All data were collected by the Australian Bureau of Meteorology at Low Isles lighthouse (No. 031037) for Daintree River, Learmonth airport (No. 005007) for Giralia Bay, and Catalina Country Club (No. 069134) for Batemans Bay.

Figure 2. An exemplary PV curve of *A. marina* subsp. *marina* constructed with leaf relative water content (RWC) as a function of leaf water potential (ψ_{leaf}). The curve shows three domains: D1 dominated by extracellular water, D2, dominated by decline in turgor, and D3 dominated by osmotic effects after turgor loss (Nguyen *et al.* 2016). Open diamond symbols indicate the points of leaf saturation (100% RWC), full turgor (ψ_{ft}, RWC_{ft}), and turgor loss (ψ_{tlp}, RWC_{tlp}). The ranges in leaf water potential ($\Delta\Psi_{leaf}$) and relative water content (ΔRWC) that span domains 1 and 2 are indicated by subscripts D1 and D2, respectively.

Figure 3. Physical properties of field-grown sun leaves of three subspecies of *Avicennia marina* as a function of soil pore water salinity. Panels show (a) leaf area, (b) leaf dry mass, (c) leaf water content, (d) leaf mass per area (LMA), (e) leaf maximum water content per dry mass (WCD_{max}), and (e) leaf maximum water content per area (WCA_{max}). Symbols: subsp. *eucalyptifolia* (Eu_{30} - open triangle), subsp. *australasica* grown at salinities of 28 ppt (Au_{28} - solid triangle), 40 ppt (Au_{40} - solid square), and 49 ppt (Au_{49} - solid circle), and subsp. *marina* (Ma_{46} - open circle). Each point represents one leaf from one of the five trees that were chosen for the experiment. Lines drawn by linear regression show relationships for subsp. *australasica* (dashed line, solid black symbols) and for all three subspecies (solid line, all symbols).

Figure 4. Transverse sections through the leaf lamina of the three subspecies of *A. marina*: (a) subsp. *eucalyptifolia*, (b) subsp. *australasica*, and (c) subsp. *marina* grown under temperate, wet tropical, and arid tropical climates, respectively, in salinities ranging from 28 to 49 ppt. Bars are 50 µm. Abbreviations: UE: upper (adaxial) epidermis, H: hypodermis, P: palisade mesophyll, S: spongy mesophyll, T: trichome layer. Notice the differences in lamina thickness between subspecies, especially in the number of cell layers of the hypodermis.

Figure 5. Lamina thickness and its components in field grown sun leaves of three subspecies of A. *marina*. (a) The fractional distribution of five major tissues comprising the leaf lamina. Subspecies designations as in Fig 3. Fillings indicate upper epidermis (UE, dash), hypodermis (H, hatch), palisade mesophyll (P, grey), spongy mesophyll (S, white), and trichome layer (T, black). Letters denote significant differences between tissue types among subspecies as determined by one-way ANOVA with *post hoc* Tukey test when $P \leq 0.05$. (b) Lamina thickness as a function of maximum water content per unit leaf area (WCA_{max}). (c) LMA as a function of maximum water content per unit leaf area (WCA_{max}). Symbols as given in Fig 3. Each point represents one leaf from one tree, n= five replicate trees per group. Lines drawn by linear regression show significant relationships for subsp. *australasica* (dashed line, solid black symbols) and for all three subspecies (solid line, all symbols).

Figure 6. (a) Osmotic potential at full turgor Ψ_{π}^{ft} and (b) bulk modulus of elasticity as functions of osmotic potential at the turgor loss point (ψ_{tlp}) when salinity was the source of variation in subsp. *australasica* (dashed line, black symbols) and when subspecies combined with environmental factors were the sources of variation (solid line, all symbols). Symbols as given in Fig. 3. Lines drawn by linear regression only for relationships with $P \leq 0.05$. Data from panels (a) and (b) were replotted, respectively, in panels (c) and (d) relative to a meta-analysis (Bartlett *et al*., 2012).

Figure 7. Variation in water content per unit leaf area with dehydration in the three subspecies of *A. marina*. (a) Exemplary curves of water content per unit leaf area (WCA) as a

function of leaf water potential (Ψ_{leaf}) during air-drying. Data are shown for two pairs of leaves, with each pair contrasting subspecies grown under similar soil pore water salinities but different climatic conditions. Symbols: subsp. *eucalyptifolia* (Eu_{30} - open triangle), subsp. *australasica* grown at salinities of 28 ppt (Au_{28} - solid triangle), and 49 ppt (Au_{49} - solid circle), subsp. *marina* (Ma_{46} - open circle). Arrows show WCA at full turgor (open arrows) and at the turgor loss points (solid arrows). (b) Water content per unit leaf area at the turgor loss point (WCA_{tlp}) as a function of maximum water content per unit leaf area (WCA_{max}) when salinity was the source of variation in subsp. *australasica* (dashed line, black symbols) and when subspecies combined with environmental factors were the sources of variation (solid line, all symbols). Symbols as given in Fig. 3. Lines drawn by linear regression only for relationships with $P \leq 0.05$.

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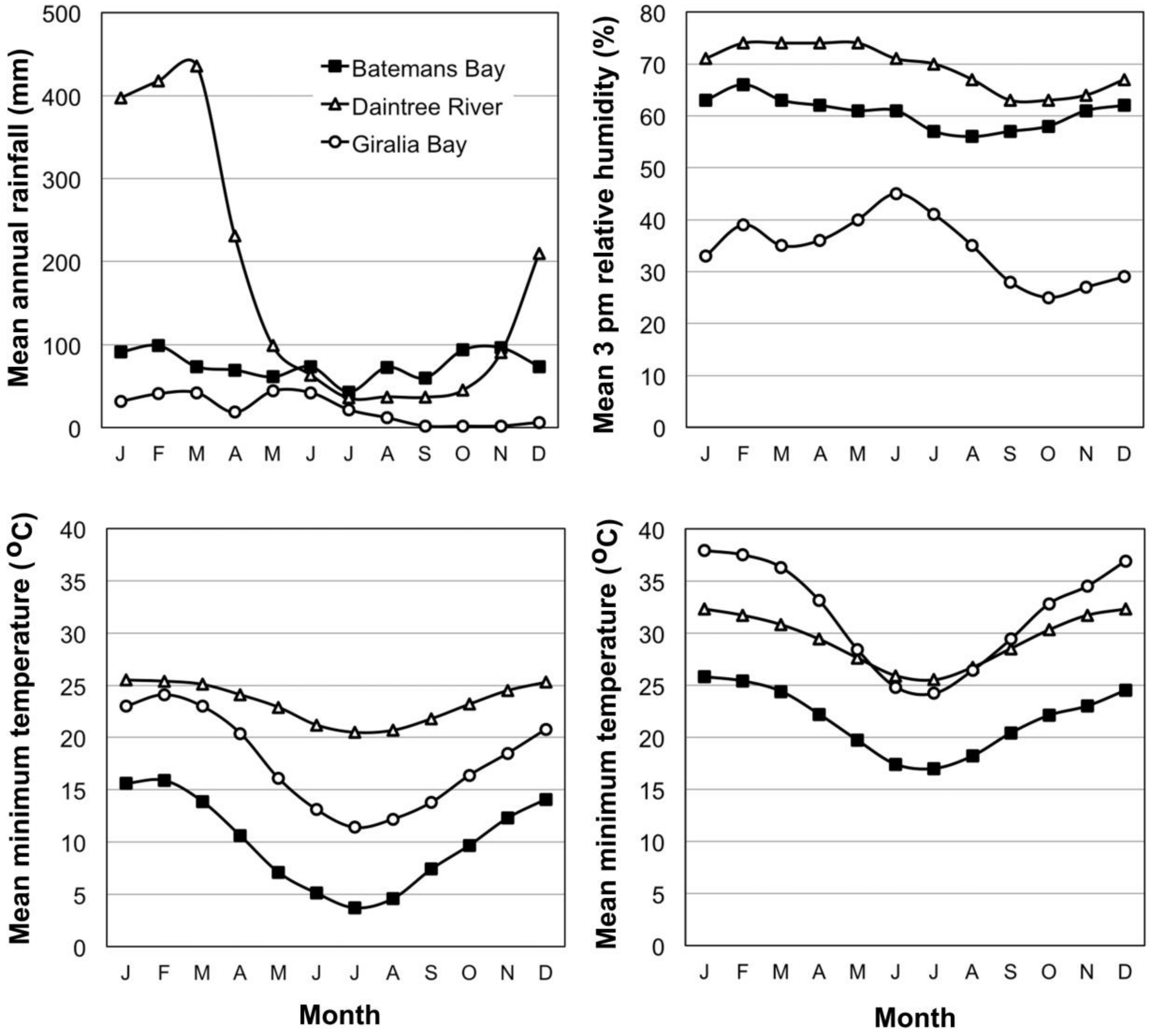

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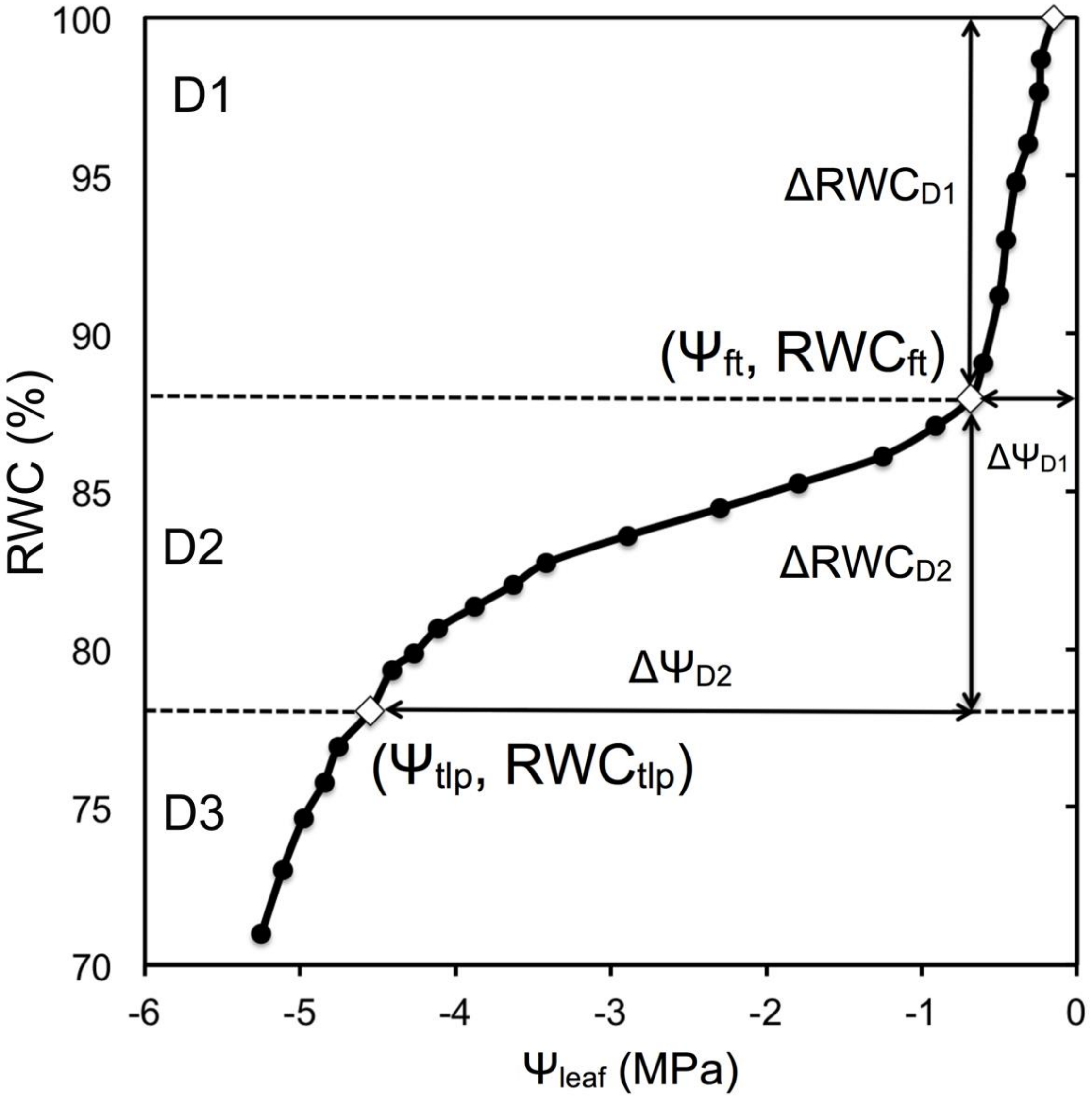

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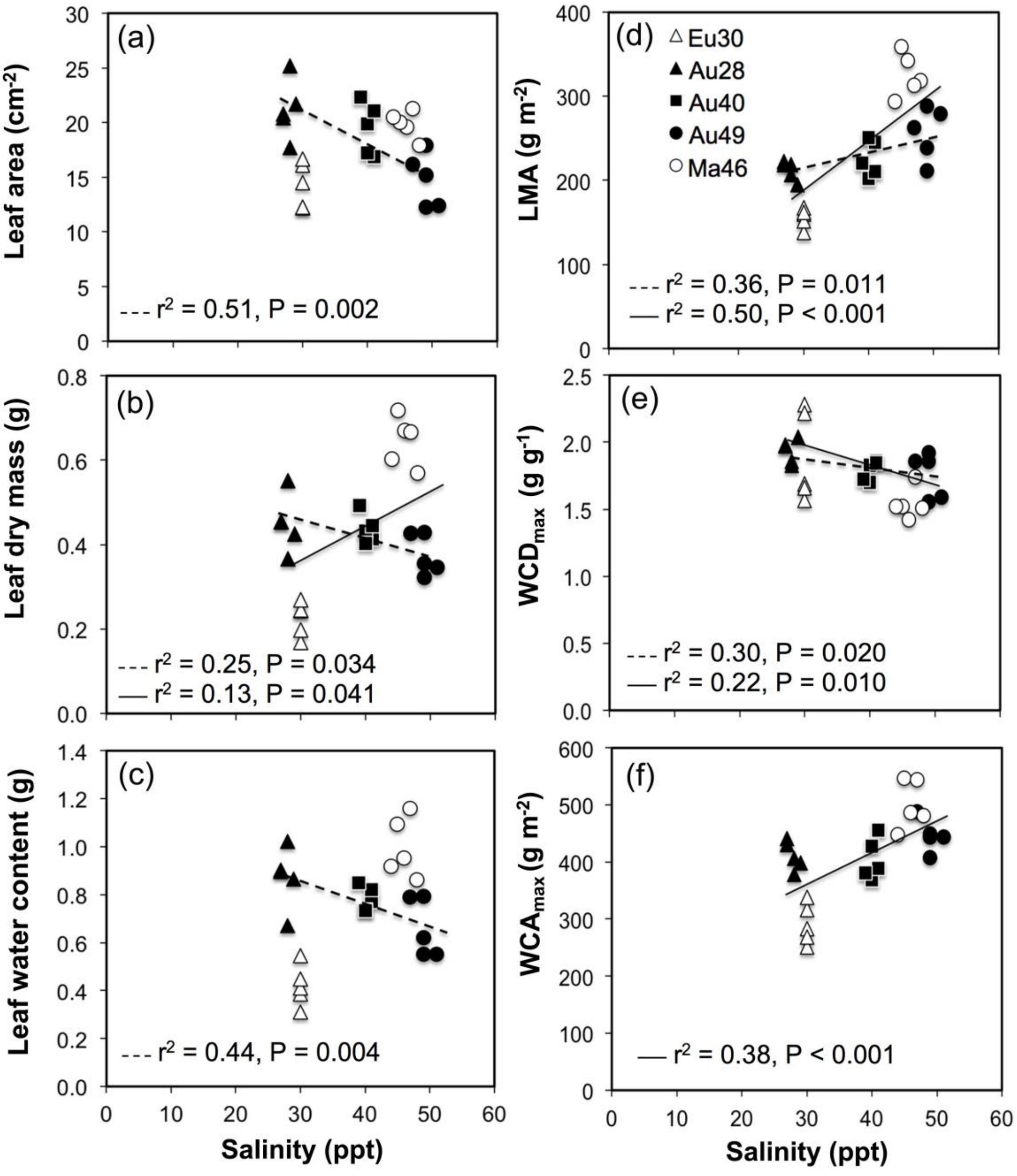

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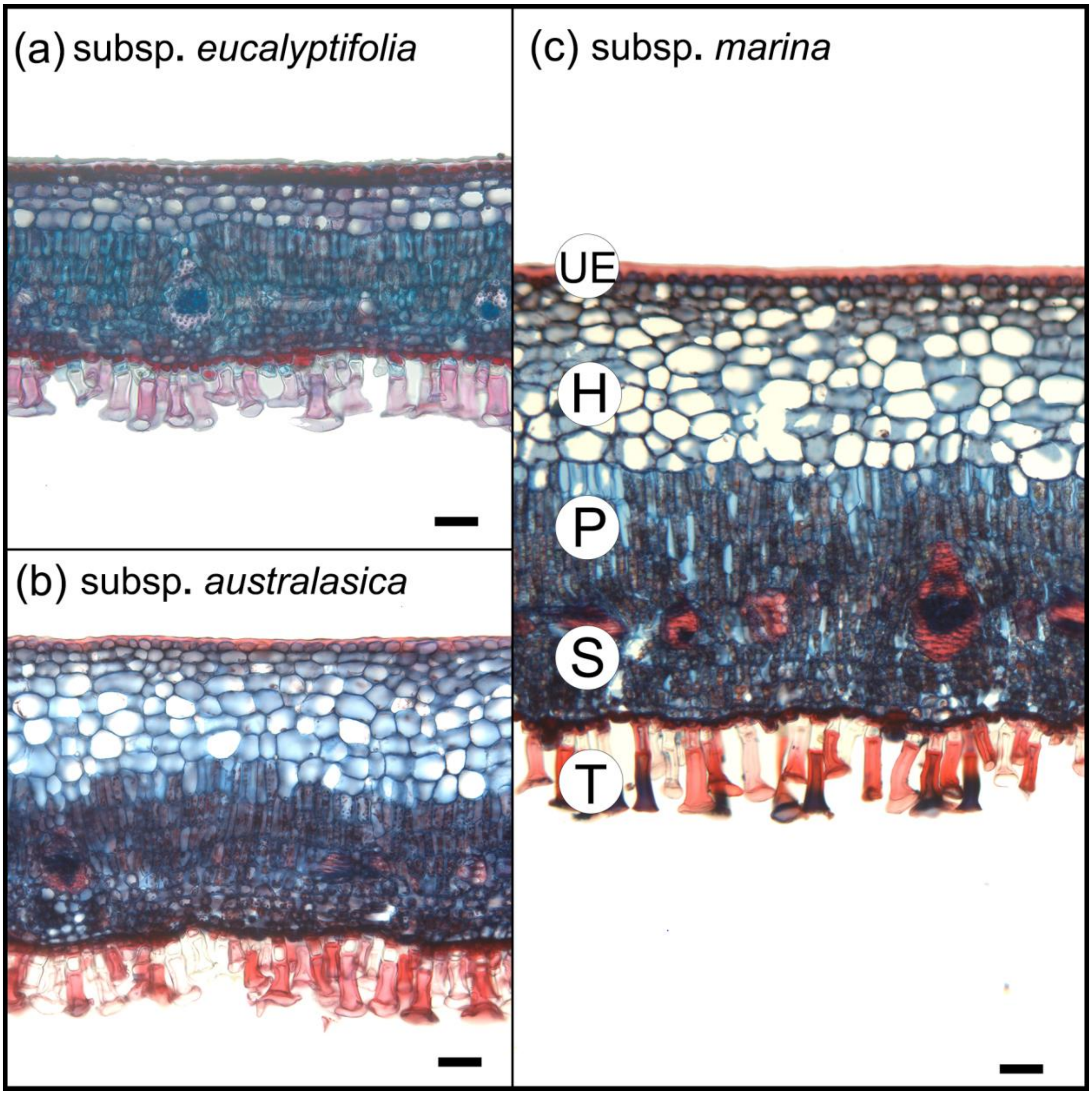

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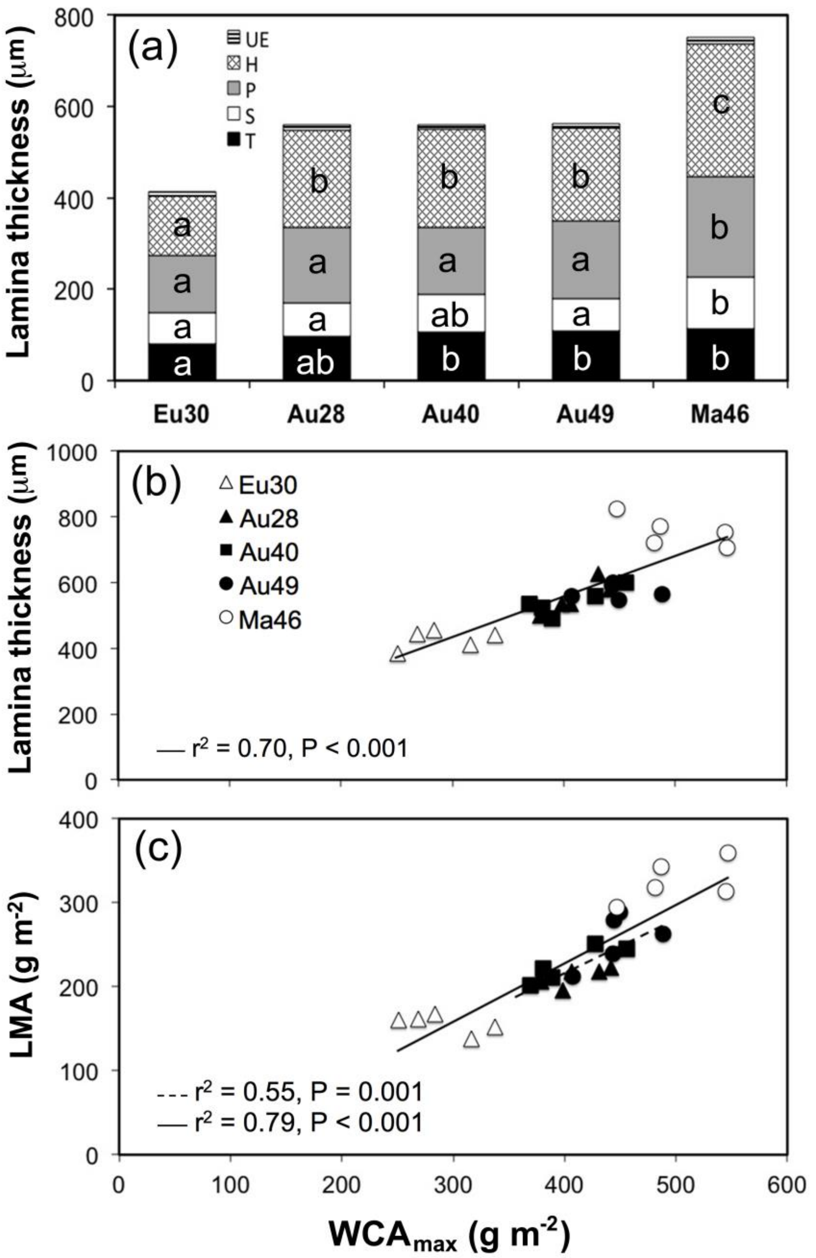

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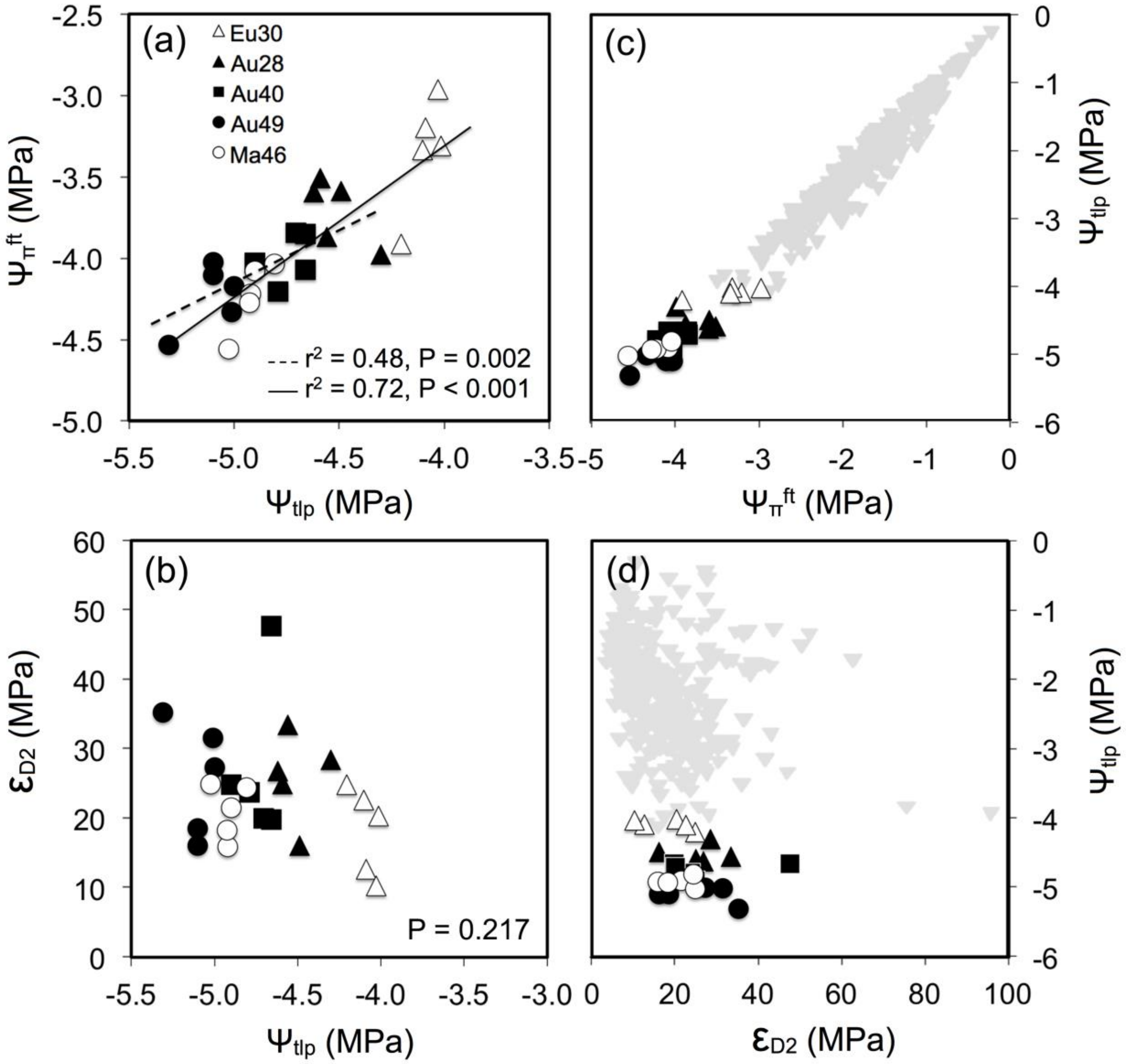

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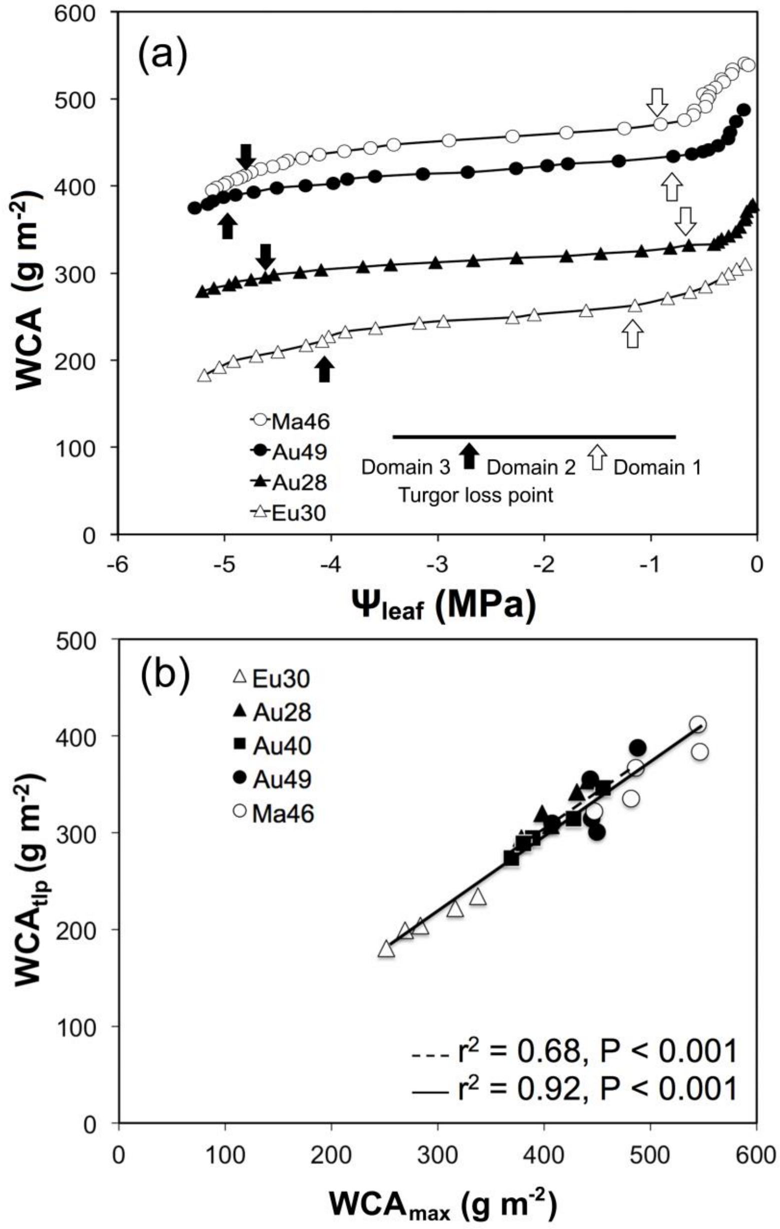

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