

Informing arid region mine-site restoration through comparative ecophysiology of *Acacia* species under drought



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ABSTRACT

Establishing vegetation on disturbed sites in arid environments is difficult due to decreased water availability caused by altered soil properties: depth, compaction, and hydraulic characteristics. Plants cope with moisture stress through a combination of traits, including physiological strategies such as anisohydry and isohydry. We used a typical mine restoration substrate in a glasshouse pot experiment to investigate drought tolerance of nine Pilbara region *Acacia* species classified according to habitat preferences defined by preferred soil type: alluvial (fine textured), sandy, rocky, and generalists without a clear soil preference. Seedlings were examined to (1) determine physiological shoot, and morphological shoot and root traits associated with drought tolerance, and (2) identify if these traits were correlated with species' soil preferences. Species from alluvial, rocky, and one sandy soil species were more anisohydric. These species had higher stomatal conductance at more negative leaf water potentials. Alluvial soil species had greater total biomass allocation to lateral roots, whereas two coarser textured soil species had high allocation to tap roots. Soil preference was a poor predictor of plant water relations, presumably due to complex soil profiles in nature associated with widely differing hydraulic characteristics, and interactions among plant functional traits influencing water uptake, transport and loss.

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1. Introduction

Plant establishment for vegetation restoration on mine-impacted sites is often challenging, especially in arid environments where moisture deficits pose a considerable hindrance. In such conditions, water availability may be further compromised by alteration of soil hydraulic characteristics by machinery (e.g. compaction), and lack of topsoil to construct profiles with the required depth and moisture holding capacity (Sheoran et al., 2010). Better understanding of physiological and morphological adaptations to drought may aid species selection and substrate design resulting in enhanced establishment of plant species in restored areas, such as former mine sites (Grierson et al., 2011). Although studies have presented useful information concerning species response to drought, some knowledge gaps exist regarding the traits and strategies used for assessment of drought tolerance (e.g. Bartlett et al., 2012; Galmés et al., 2007; Valladares and Sánchez-Gómez, 2006). Remaining physiologically active under moisture stress hinges on the ability to tolerate high water tensions in the xylem which enables continued moisture uptake from a

drying soil (Bhaskar and Ackerly, 2006). Although physiological adaptations are essential to drought tolerance, soils and plant-soil interactions strongly affect those adaptations, thus influencing plant available water across climates (Hacke et al., 2000; Jury and Horton, 2004; Sperry and Hacke, 2002).

Plant adaptations to drought in seasonally dry environments are essential for their survival. To maximise long-term carbon gain, and nutrient use efficiency (Gray, 1983), many species in these environments are evergreen and have long-lived leaves with low nutrient concentrations, low leaf area to mass ratios, low photosynthetic rates, and high root:shoot ratios (Ackerly, 2004; Kummerow et al., 1977; Mooney and Dunn, 1970). Another advantage for survival in drought prone environments is high tolerance to low water potentials in xylem tissues to avoid cavitation. High wood density and its associated resistance to cavitation has been shown to be an effective drought adaptation (Hacke et al., 2001). Plants may also cope with moisture stress through physiological controls such as anisohydric and isohydric strategies. Under declining soil water potentials (Ψ_s), anisohydric plants typically have more negative leaf water potentials (Ψ_l), maintain higher stomatal conductance (g_s), and often have wider Ψ_l ranges (Barnes, 1986; McDowell et al., 2008). Conversely, isohydric plants reduce g_s in response to decreasing Ψ_s thus maintaining more constant Ψ_l to

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avoid water transport failure (McDowell et al., 2008; Sperry et al., 2002). However, even within semi-arid to arid systems, plant adaptations to drought vary as different combinations of traits provide drought tolerance across varying soil types and associated hydraulic properties.

Plants grow on a range of soil types that have different hydraulic characteristics with textures ranging from heavy clays to coarse sands (Hacke et al., 2000). Plant water availability is largely a function of soil texture through the influence of pore size, hydraulic conductivity, and moisture retention capacity (Bhaskar and Ackerly, 2006; McDowell et al., 2008). Therefore, varying soil textures present different challenges for plant water extraction (Bristow et al., 1984) and species associated with different soil types often have different morphological traits and water use strategies to facilitate plant water uptake (McDowell et al., 2008; Westoby and Wright, 2006), suggesting a close coordination between plant traits or strategies and soil profiles/textures (Westoby and Wright, 2006). For instance, populations of shrub species growing on fine-textured soils generally have more negative Ψ_1 than populations of the same species growing on coarse textured soils (Sperry and Hacke, 2002). These more negative Ψ_1 reflect the smaller and more abundant pore spaces in fine textured soils that hold water at more negative Ψ_s (Sperry and Hacke, 2002). Additionally, plant rooting depth may be influenced by moisture retention across soil textures (Jackson et al., 2000). Plants on sandy soils are required to grow roots into deeper moist soil layers due to low soil moisture retention capacity of sands (Sperry and Hacke, 2002). Deep growth in coarser textured soils is also supported by greater oxygen availability at depth, and easier root penetration, especially in sandy soils (Sperry and Hacke, 2002). In finer textured soils deep root growth is reduced due to broader Ψ_s ranges as a result of smaller pore spaces, shallower wetting, and impedance to root penetration (Sperry and Hacke, 2002). This stresses the importance of quantifying drought adaptation across soil types within the same water limited environment.

The Pilbara region in NW Australia is semi-arid to arid, and is prone to periods of severe seasonal drought (Van Vreeswyk, 2004). Despite severe moisture deficits it hosts a large plant diversity with 1094 native vascular species, including 150 conservation significant species, on 21 soil groups forming 44 plant-soil associations (EPA, 2014; Van Vreeswyk, 2004). The Pilbara is mostly dominated by *Triodia-Acacia-Eucalyptus* alliances, however, species within these genera vary across sub-regions and soil types (Beard, 1975; Van Vreeswyk, 2004), and thus are expected to exhibit functional differences reflecting adaptations to varying soil types. The region is rich in mineral resources producing more than 90% of Australia's iron ore (DMP, 2012). Active and pending mining tenements cover 91.8% of the Pilbara, and thus large areas will require restoration of native flora due to mining disturbance (EPA, 2014). Because mining restoration substrates differ from naturally occurring soils in depth, compaction, and hydraulic characteristics, physiological and morphological plant responses to drought stress in these substrates are largely unknown. Therefore, it is of value (1) to examine how the ecophysiological and morphological mechanisms of drought tolerance differ among common Pilbara species when grown on a common mine site restoration substrate, and (2) to determine whether potential differences are related to species' preferred soil types. The latter would suggest that specific adaptations to local soil types or profiles occur and are expressed even when species are grown on a common substrate.

In this study, nine Pilbara *Acacia* species were grown in a typical mine site restoration substrate in a glasshouse experiment with the following aims: (1) determine the physiological shoot, and morphological shoot and root traits associated with drought tolerance, and (2) determine if these traits differed between their preferred soil types. We hypothesised that, when faced with soil moisture deficit, *Acacia* species with a tendency to keep stomata

open for longer, allowing further decreases of plant water potentials, would be associated with lower osmotic potentials at full hydration (π_{sat}) and greater biomass investment in lateral roots. We also expected such species to typically occur in more fine-textured alluvial soils than more coarse-textured sandy and rocky soils.

2. Materials and methods

2.1. Study species

Nine Pilbara *Acacia* species from four contrasting preferred soil types were chosen for this study. Species were categorised according to their soil preferences based on evidence gathered from literature, online flora descriptions (Flora Base, <http://florabase.dpaw.wa.gov.au/>) and expert consultations. Soil preference categories are as follows:

- alluvial (fine textured) soil species: *Acacia aneura* Benth., *A. citrinoviridis* Tindale & Maslin, and *A. cowleana* Tate;
- sandy soil species: *A. coriacea* subsp. *pendens* R.S.Cowan & Maslin and *A. stellaticeps* Kodela, Tindale & D.A.Keith;
- rocky soil species: *A. pruinocarpa* Tindale and *A. maitlandii* F.Muell.; and
- generalist species: *A. bivenosa* DC. and *A. ancistrocarpa* Maiden & Blakely.

Although study species were categorised according to these soil preferences, we recognise that within-species variation in distribution patterns exists, e.g. related to ecotypes or interactions with climate. Soil preferences indicated here reflect the preferred soil types in which these species are most commonly found.

2.2. Experimental design

The experiment was conducted in a glasshouse mimicking the climate in the species' native habitats. Air temperature was approximately 32 °C during the day and 25 °C at night (Appendix A). During the growth stage (14 August 2013–14 February 2014), automated shade cloths on the glasshouse structure were raised when outside temperature reached 30 °C or more which reduced sunlight by 60%. During the experimental period (15–25 February 2014) plants received natural light which peaked on average at approximately 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during mid-day; shade cloths were not deployed, however, the glasshouse structure reduced light by 25%. Plants were grown in 2.8 L free-draining pots (diameter 16.5 cm, height 16.5 cm). Pots were lined with a fine synthetic mesh and a thin layer of gravel (0.40 kg) to retain soil, and inhibit root growth out of the pots. Each pot was filled with soil (2.60 kg; see details below), and then topped with a thin layer of gravel (0.30 kg) to limit soil water evaporation. Soil was kept moist and allowed to settle for one week prior to sowing.

Acacia species seeds were collected from the Pilbara and obtained from the Botanical Gardens and Parks Authority (Kings Park, Perth, Western Australia). Seeds were scarified in near-boiling water prior to sowing. Ninety pots were sown with five seeds from one of nine *Acacia* species. Ten additional pots were left without plants to quantify soil evaporation rates. A standard block design was used across five benches in the glasshouse, each *Acacia* species having two pots (one well-watered, one water-stressed) per bench. Pots were randomised within each block. Pots were watered to field capacity during the growing stage, by hand for the first month, and by micro-irrigation three times daily for one minute thereafter. After six months, one healthy seedling (of representative size for its species) was kept per pot, and the extras were cut at the stem-soil interface.

Some mortality occurred during the well-watered phase of the experiment reducing the number of replicates for some species. Each species had five replicates for drought and control treatments, with the exception of *Acacia maitlandii* with four and three plants, respectively, and *Acacia cowleana* and *Acacia stellaticeps* with four

transpiration by leaf area as determined at the final harvest. Leaf area (LA) was determined with a scanner and using WinRHIZO software (Regent Instruments, Quebec, Canada). Standardised daily leaf-area-based transpiration rates for each drought plant across all species were calculated as:

$$\text{Standardised Transpiration} = \frac{\text{Daily Transpiration/LA}}{\text{Mean (Control Plant Daily Transpiration/LA)}} \quad (2-1)$$

plants for both treatments. All pots were hand-watered and weighed to a constant soil water content of 0.1395 g g^{-1} on the afternoon before the start of the experimental treatments (February 15, 2014). Watering then ceased for all drought plants, but continued for control plants which were watered daily to the same initial water content. All plants had phyllodes rather than true leaves during experimental conditions; all physiological measurements were conducted on the youngest fully grown phyllodes. Phyllodes will be referred to as leaves hereafter.

2.3. Soil analysis

Soil for the experiment was collected from a restored waste rock dump at Mount Goldsworthy ($20^{\circ}21'04.6 \text{ S}$, $119^{\circ}31'42.4 \text{ E}$, 92 m a.s.l.) in the Pilbara. The top 50 cm were collected using a backhoe, then transported in barrels to the University of Western Australia Plant Growth Facilities (PGF), Perth, Western Australia ($31^{\circ}59'03.2 \text{ S}$, $115^{\circ}53'10.3 \text{ E}$, 5.2 m a.s.l.). Soil was air dried in a drying room for 7 days and then sieved to discard particles $>5 \text{ mm}$. The soil was analysed in the Wesfarmers CSBP Ltd. Soil and Plant Analysis Laboratory (Bibra Lake, Western Australia) to determine physical and chemical properties, including soil texture (size classes: $0.02 > \text{ sand} < 2.0$, $0.002 > \text{ silt} < 0.02$, $\text{clay} < 0.002 \text{ mm}$; %; Modified Pipette Procedure), electrical conductivity (1:5 soil: water extract), pH (1:5 soil: water extract and 1:5 soil: CaCl_2 0.01 M solution), organic carbon (Walkley-Black method), ammonium nitrogen (extracted in 1 M KCl), nitrate nitrogen (extracted in 1 M KCl), plant available phosphorus (Colwell P, bicarbonate extraction), potassium (Colwell K, bicarbonate extraction), sulphur (extracted in KCl at 40°C), exchangeable cations (aluminium, calcium, magnesium, potassium, and sodium; Mehlich No. 3 test), extractable copper, iron, manganese and zinc (DTPA extraction), and boron (hot CaCl_2 method) (Appendix B). Plants were fertilised twice during the growth period with an application of 0.1 L of liquid fertiliser onto the soil at a concentration of 0.005 kg L^{-1} containing 95 mg of nitrogen, 42 mg of phosphorus, and 79 mg of potassium.

A soil water retention curve was produced for the experimental soil. Water was added to dry soil at concentrations ranging from 0.0041 to 0.104 ml g^{-1} , mixed in air-tight plastic bags, allowed to equilibrate for 24 h, and measured using a WP4C Dewpoint Potentiometer (Decagon Devices Inc., Pullman, WA, USA). Gravimetric soil moisture content (%) of analysed soil was then verified using the oven-drying method (Appendix C).

2.4. Physiological functional traits

Evapotranspiration (ET; water loss from pots with plants), evaporation (E; water loss from pots without plants), and pot water content was determined daily by weighing the pots at the same hour every day during the experimental period. Whole-plant daily transpiration was calculated as $\text{ET} - \text{E}$ for every plant, and leaf area-based transpiration was calculated by dividing whole-plant

transpiration by leaf area as determined at the final harvest. Leaf area (LA) was determined with a scanner and using WinRHIZO software (Regent Instruments, Quebec, Canada). Standardised daily leaf-area-based transpiration rates for each drought plant across all species were calculated as:

During the experimental period, starting 15 February 2014, stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{ s}^{-1}$) was monitored daily during mid-morning (08:00–10:00) on the same position on one fully grown leaf per plant leaf location, using a Leaf Porometer (Decagon Devices Inc., Washington, USA) calibrated before every use. Pot weighing for quantification of ET, E, and pot water content was conducted every afternoon (14:00–16:00). Daily measurements continued until individual drought pots reached a soil water content of 0.035 g g^{-1} (equivalent to -2.18 MPa according to the soil water retention curve), when plants were harvested. The harvest cut-off soil moisture content of 0.035 g g^{-1} was chosen to represent moderate stress, based on a pilot experiment, avoiding the risk of mortality in the least tolerant species. Leaves (one per measurement) for measuring leaf water potentials (Ψ_l ; MPa) and osmotic potential (π ; MPa) were harvested (15:00–17:00) from plants that reached the pre-defined drought water content after pot weighing; these plants were harvested the following morning after the final measurement of g_s . The average soil water content at harvest was 0.0325 g g^{-1} (-2.64 MPa), with some variability among individual plants, as some plants were slightly over, or under the harvest threshold, and were likely to surpass the threshold by the following day. The number of days for each plant to reach pre-defined harvest conditions was recorded.

Leaf water potential was measured using a Pressure Chamber (PMS Instruments, Oregon, USA). Leaves were cut using a scalpel, wrapped in aluminium foil, placed in an airtight plastic bag, and kept cool for immediate transport and analysis in the nearby laboratory. A grass-compressing gland sealing system was used to hold leaves in place as they were not petiolate. Leaves for measurement of π were collected in the same manner. Each leaf sample was then cut in half lengthwise; one half for estimating leaf relative water content (RWC) and the other for osmotic potential. Samples for osmotic potential were frozen in a -80°C freezer immediately after cutting, and thawed in their vials for one hour before sap expression (Turner, 1981). Leaf osmotic potential was measured using a vapour pressure osmometer and sample chambers (PSYPRO CR-7 and C-52, Wescor Inc, Utah, USA). Filter paper discs were wetted in leaf sap then placed into sample chambers at an ambient temperature of 21°C . Osmotic potential at full turgor (π_{sat} ; i.e. at 100% RWC) was calculated using Equation (2-2) (Ludlow et al., 1983).

$$\pi_{\text{sat}} = \pi \times \text{RWC} \quad (2-2)$$

Osmotic adjustment was calculated as the difference in π_{sat} between droughted and control plants. Leaf RWC was measured using the method outlined by Ryser et al. (2008). Leaf fresh mass (FM_{Leaf}) was measured immediately after cutting. Leaf saturated mass (SM_{Leaf}) was measured after leaves were placed between moist paper towels for 24 h and covered with aluminium foil at room temperature. Leaf dry mass (DM_{Leaf}) was determined after samples were dried for 48 h in a 70°C oven. Leaf relative water content was computed using Equation (2-3).

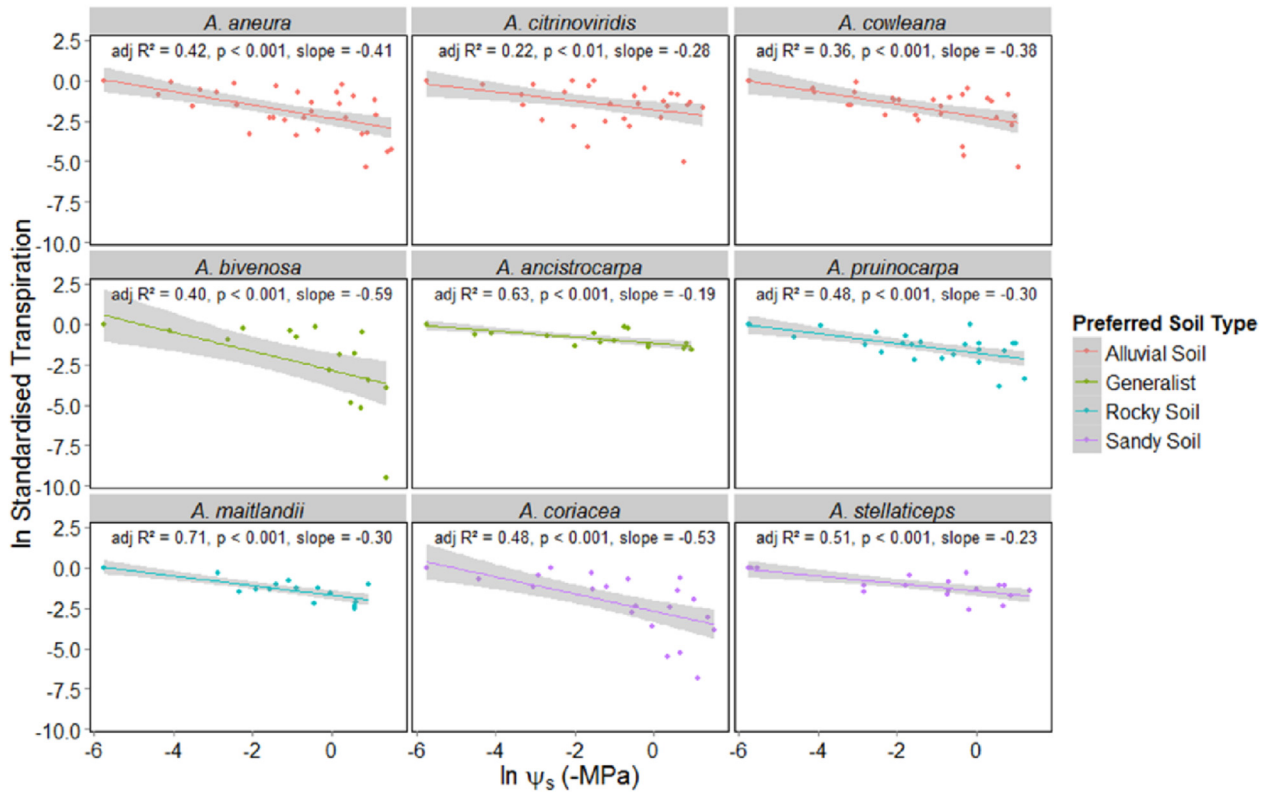


Fig. 3–1. The natural logarithm of daily standardised transpiration during pot drying (transpiration of droughted relative to that of well-watered plants) as a function of the natural logarithm of soil water potential (Ψ_s ; -MPa) for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Grey shaded areas denote 95% confidence bands around the regression line. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

$$RWC = \frac{FM_{\text{Leaf}} - DM_{\text{Leaf}}}{SM_{\text{Leaf}} - DM_{\text{Leaf}}} \quad (2-3)$$

2.5. Morphological functional traits

During harvest aboveground components were separated into leaves and stems. Fresh weights were measured for all aboveground components, and leaves were scanned to determine LA using WinRHIZO software. Roots were carefully washed atop a sieve to avoid loss of fine roots within 3 days of harvest, placed in plastic bags, and kept refrigerated at 5 °C for up to 2 days prior to analysis. The majority of roots had not reached the bottom of the pots; in only a few pots did fine roots make contact with the synthetic fabric. Belowground components were separated into tap and lateral roots and weighed. Root scanning was conducted on sub-samples of each root system, due to their size. Sub-samples constituted of the tap root and lateral roots from one side of the tap root. Root length was determined for tap and lateral roots in each sub-sample using WinRHIZO software. All plant components (leaves, stems, tap roots, and lateral roots) were oven dried at 70 °C for 48 h to determine the dry weight of each component. Specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$) was computed by dividing LA by leaf dry mass. The mass fraction of leaves (LMF; g g^{-1}), stems (SMF; g g^{-1}), tap roots (RMF_{TR}; g g^{-1}), and lateral roots (RMF_{LR}; g g^{-1}) was calculated by dividing its dry weight by total plant dry weight. The tap root to lateral root mass ratio (TR/LR Ratio; g g^{-1}) of each plant was determined for whole root systems by dividing the dry mass of tap roots by the dry mass of lateral roots. The specific root length of lateral roots (SRL_{LR}; cm g^{-1}) for each plant was determined by dividing lateral root length by the dry mass of lateral roots. The root length to leaf area ratio (RL/LA Ratio; cm g^{-1}) was computed by

dividing total root length by LA for each plant.

Stem specific density (SSD; g mm^{-3}) was determined for each plant using the dimensional method (Pérez-Harguindeguy et al., 2013) where the oven-dry mass (at 70 °C for 72 h) of a main stem section including bark (DM_{Stem}) was divided by its fresh volume. Stem volume (V_{Stem}) and SSD were computed as:

$$V_{\text{Stem}} = (0.5D)^2 \times \pi \times L \quad (2-4)$$

$$SSD = \frac{DM_{\text{Stem}}}{V_{\text{Stem}}} \quad (2-5)$$

where D and L are stem diameter and length, respectively, measured with a digital micrometer (Mitutoyo, Honshu, Japan).

2.6. Data analysis

Data were analysed with RStudio statistical software (R v0.98.1028, R Foundation for Statistical Computing, R, 2013). Analysis of covariance (ANCOVA) was conducted to test differences in slopes and intercepts among regression lines for log-transformed standardised transpiration (response variable) with log-transformed Ψ_s (explanatory variable), and either soil type with nested species, or species as the categorical variables. Natural logarithmic transformations were applied to standardised transpiration and Ψ_s to achieve linearity thus satisfying assumptions of the ANCOVA. The same analysis was conducted for g_s (response variable). Tukey's multiple pairwise comparison test was used to analyse differences among species or soil groups if ANCOVA model outputs were significant.

Differences in Ψ_1 , π_{sat} , LMF, SLA, SSD, RMFs, SRLs, TR/LR Ratio,

and RL/LA Ratio for treatment, soil groups with nested species, and species were tested using mixed effects models with weighted variance structures to ensure homogeneity of variance as outlined in Zuur et al. (2009). When testing between soil type preferences, soil type was a fixed factor and species within soil types a random factor. When testing between species the fixed factor was species. Nested models were tested using five variance structures (VarFixed, VarIndent, VarPower, VarExp, and VarConstPower) and Analysis of Variance (ANOVA). Models were evaluated and selected using Akaike's information criterion (AIC; a measure of relative model performance for a given set of data), and plots of standardised residuals. Models with the lowest AIC value, and homogeneity of variance were chosen for further analysis. Selected models were subsequently tested using ANOVA at $\alpha = 0.05$. Tukey's multiple pairwise comparison test was used to analyse differences among species or soil groups if ANOVA model outputs were significant. Mean differences in π_{sat} for osmotic adjustment within species, between control and drought plants were calculated using paired one-tailed t-tests with a 95% confidence.

3. Results

The experimental soil used had no major geochemical restrictions to plant growth (Appendix B). The soil was classified as a sandy loam with a soil moisture retention curve that displayed a gradual decline of Ψ_s with decreasing soil moisture content (Appendix C). Experimental plants appeared healthy throughout the growth period and showed no signs of nutrient deficiency or toxicity.

3.1. Physiological functional traits

Differences in log-transformed standardised transpiration (leaf-area-based transpiration of droughted plants, relative to that of well-watered plants), and the rate of decline (slopes in Fig. 3–1)

were apparent but not significantly different between species ($F_{8, 216} = 1.78$, $P > 0.05$) or preferred soil type with nested species ($F_{2, 4} = 6.85$, $P > 0.05$). Log-transformed standardised transpiration of *Acacia bivenosa* (generalist group) was most sensitive to soil water status, while *Acacia ancistrocarpa* (generalist group) was least sensitive (Fig. 3–1). At the Ψ_s corresponding to 25% of the Ψ_s at field capacity (-2.2 MPa), these two species had reduced transpiration by 31% and 26%, reaching predefined drought conditions after 6 and 7 days, respectively. Overall, species from the rocky soil group tended to be least sensitive to soil water status, with transpiration rates at 25% soil field capacity reduced by 28%. These species took 8 days to reach predefined drought conditions. Plant sizes varied among species and preferred soil types, therefore, the effect of plant size on standardised transpiration is thought to be negligible.

Patterns in the reductions of g_s as a function of decreasing Ψ_s ($R^2 = 0.12$, $P < 0.01$) largely coincided with those for standardised transpiration as a function of decreasing Ψ_s . Unlike transpiration, the rate (slope) of g_s decline as function of drying soil differed significantly among species ($F_{8, 219} = 10.41$, $P < 0.0001$) and was associated with preferred soil type with nested species ($F_{2, 4} = 7.75$, $P < 0.05$). Decline in g_s was highest in the generalist soil group but was only significantly ($P < 0.05$) different from the sandy soil group, and lowest in the rocky soil group with significantly ($P < 0.05$) lower rates compared to the alluvial and sandy soils groups (Fig. 3–2). *Acacia pruinocarpa* of the rocky soil group was least sensitive to decreasing Ψ_s compared to six other experimental species, and continued to have high rates of g_s ($P > 0.05$) at more negative Ψ_s (Fig. 3–2). The absence of a response of early morning g_s to drying in *A. pruinocarpa*, despite a significant decrease in transpiration, may point to stomatal closure later during the day in this species.

To explore if species' responses to drying soils tended towards isohydric or anisohydric behaviour, Fig. 3–3 shows their sensitivity of g_s plotted against their change in Ψ_l . The generalist Group

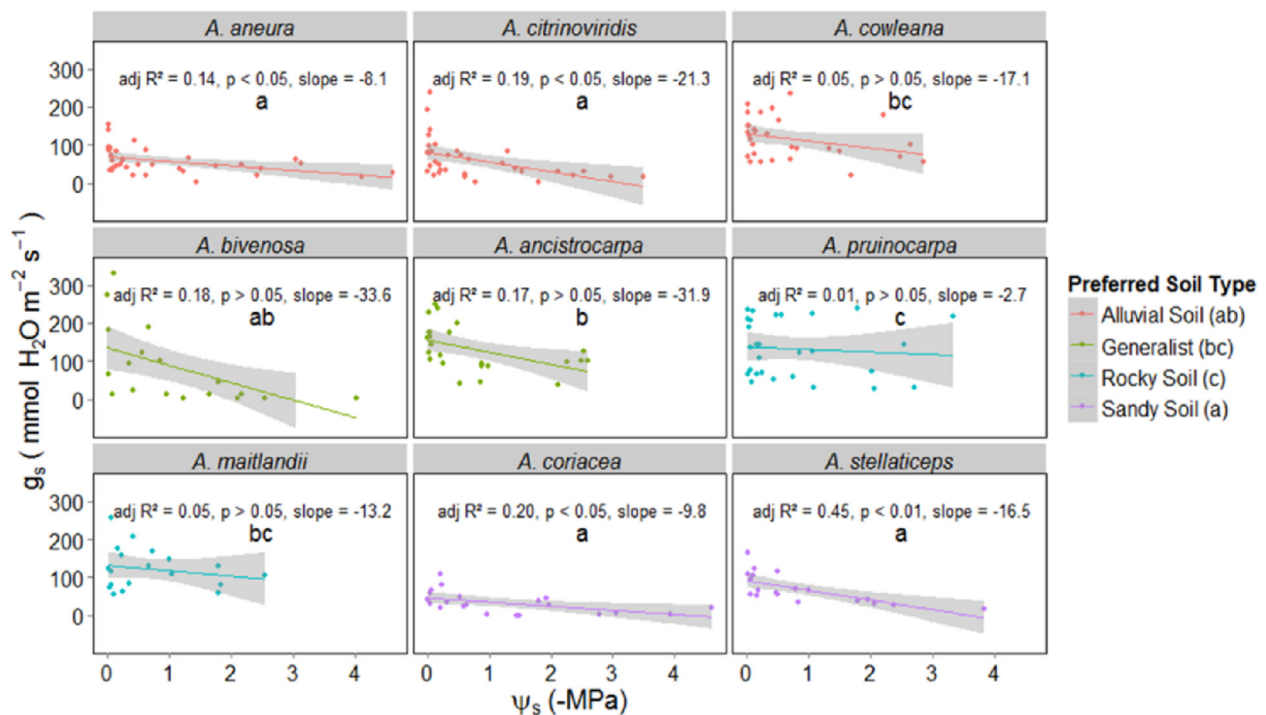


Fig. 3–2. Daily morning (08:00–10:00) stomatal conductance (g_s ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) as a function of soil water potential (Ψ_s ; -MPa) for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Grey shaded areas denote 95% confidence bands around the regression line. Letters indicate significant differences ($P < 0.05$) between species, or preferred soil types. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

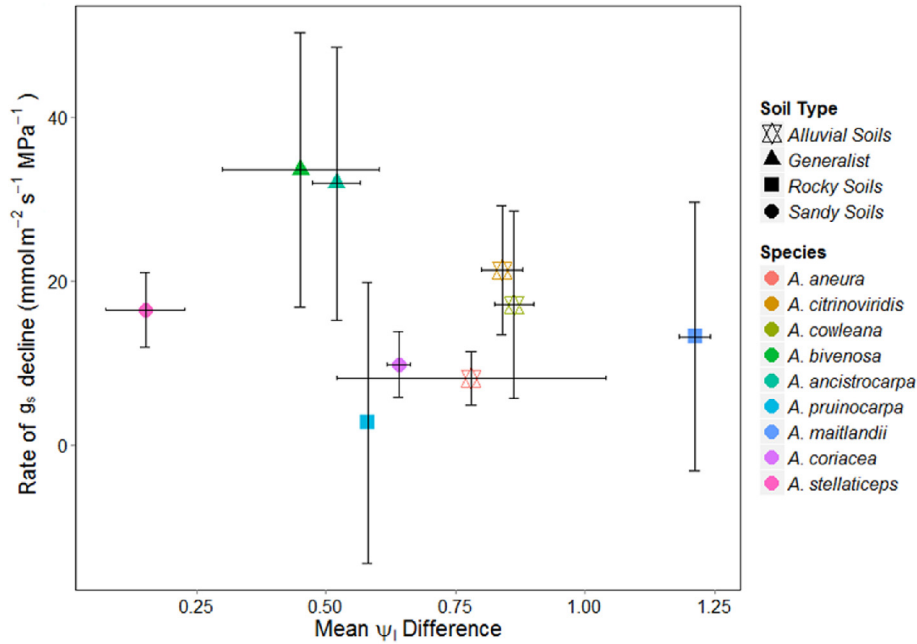


Fig. 3–3. Rate of stomatal conductance (g_s ; $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) decline as a function of drying soil (slope) (\pm SE) plotted against the mean (\pm SE) Ψ_1 difference between control and drought plants for nine *Acacia* species and their preferred soil types from the West Australian Pilbara. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

(*Acacia bivenosa*, *Acacia ancistrocarpa*) had the highest rates of g_s decline, and along with *Acacia stellaticeps* the smallest differences between control and drought Ψ_1 . The tendency to keep Ψ_1 less negative through stomatal closure points to isohydric behaviour. The alluvial soil group (*Acacia aneura*, *Acacia cowleana*, and *A. cowleana*), rocky soil group (*Acacia maitlandii*, *Acacia pruinocarpa*), and *Acacia coriacea* had slow to moderate rates of decline, and the largest Ψ_1 differences between control and drought plants. These species kept their stomata open, reducing Ψ_1 , making them better adapted to extraction at more negative Ψ_s , or soil with higher clay content.

There were significant differences in Ψ_1 between species ($P < 0.05$), with *A. aneura*, and *Acacia stellaticeps* having the most negative and least negative Ψ_1 for drought plants, respectively (Fig. 3–4). However, there was no clear association between soil

groups and Ψ_1 . Difference between control and drought plant Ψ_1 for species in the alluvial soils group were similar, with changes in Ψ_1 ranging from -0.78 to -0.86 MPa (Fig. 3–3, Fig. 3–4), and were coupled with intermediate rates of g_s decline (Fig. 3–3) and transpiration (Fig. 3–1). However, the greatest Ψ_1 difference between control and drought plants occurred in *Acacia maitlandii* from the rocky soils group at -1.21 MPa (Fig. 3–3), but was not significantly more negative compared to other species (Fig. 3–4).

Overall Ψ_1 and leaf osmotic potential at full turgor (π_{sat}) of drought plants were negatively correlated ($R^2 = 0.21$, $P < 0.01$). There were significant differences in π_{sat} between species for drought plants ($P < 0.05$), with *Acacia ancistrocarpa* and *Acacia coriacea* having the least and most negative potentials, respectively (Fig. 3–5). However, there was no clear association between soil group and leaf osmotic potential. Most species tended to have more

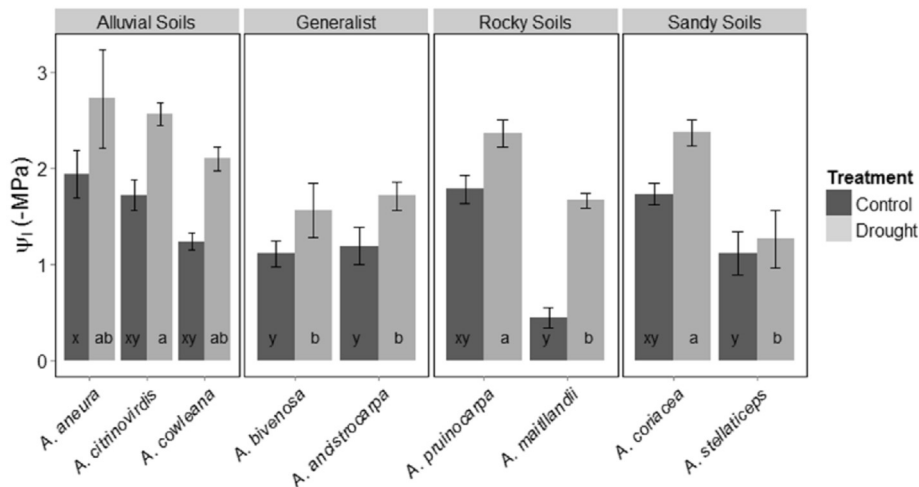


Fig. 3–4. Mean (\pm SE) leaf water potential (Ψ_1 ; -MPa) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Letters indicate significant differences ($P < 0.05$) between species within treatments. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

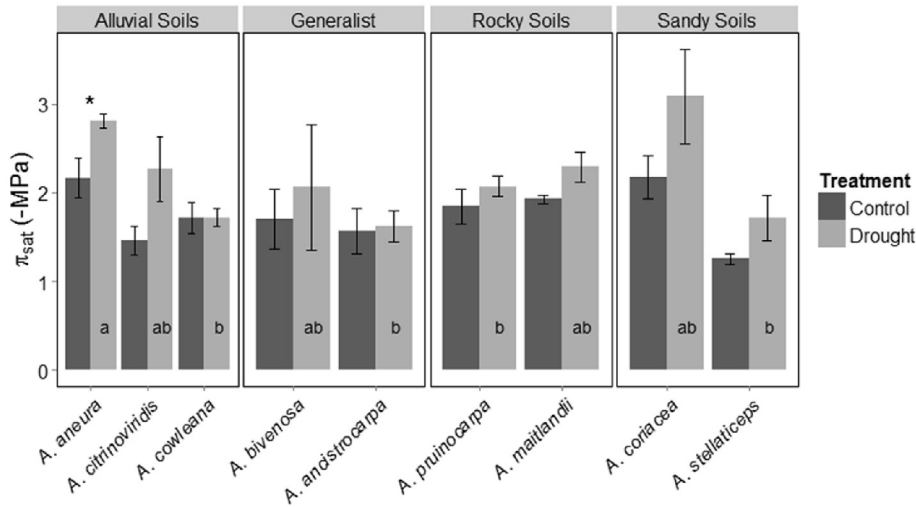


Fig. 3–5. Mean (\pm SE) leaf osmotic potential at full turgor (π_{sat} ; -MPa) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Asterisks indicate significant ($P < 0.05$) osmotic adjustment between control and drought plants within species. Letters denote significant differences ($P < 0.01$) between species for drought plants; there were no significant differences between species for control plants. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

negative osmotic potentials at full turgor in the drought treatment, indicative of osmotic adjustment, although this was only significant for *A. aneura*, whereas several species including *Acacia cowleana* and *A. ancistrocarpa* showed no signs of osmotic adjustment.

3.2. Morphological functional traits

There were significant biomass allocation differences among species and preferred soil type groups. For drought plants, the lateral root mass fractions (RMF_{LR}) of *A. aneura* and *Acacia ancistrocarpa* were the largest and smallest, respectively ($P < 0.05$) (Fig. 3–6). The alluvial soil group RMF_{LR} was significantly ($F_{3, 5} = 6.08$, $P < 0.05$) higher compared to all other soil groups for drought plants only. No other significant ($P < 0.05$) associations between soil group and any other morphological functional trait were found for drought and control plants. For drought plants,

Acacia cowleana and *A. aneura*, had the highest and lowest ($P < 0.05$) leaf mass allocations respectively (Fig. 3–6). The same pattern of leaf mass allocations in *A. cowleana* and *A. aneura* held true for control plants ($P < 0.05$). Stem mass fraction (SMF) and tap root mass fraction (RMF_{TR}) differed between species for drought and control plants ($P < 0.05$). *Acacia coriacea* and *A. cowleana* SMF and RMF_{TR} were the highest and lowest for both treatments, respectively (Fig. 3–6).

Significant ($P < 0.005$) differences in tap root to lateral root mass allocation of drought and control plants were apparent among species. For control and drought plants, *Acacia coriacea* and *Acacia cowleana* allocated the largest and least proportion of belowground biomass to their tap root, respectively. However, there were no clear differences between soil groups in tap root to lateral root mass allocation. A significant interaction between species and treatment was observed ($P < 0.05$, Appendix E), the ratio decrease in response

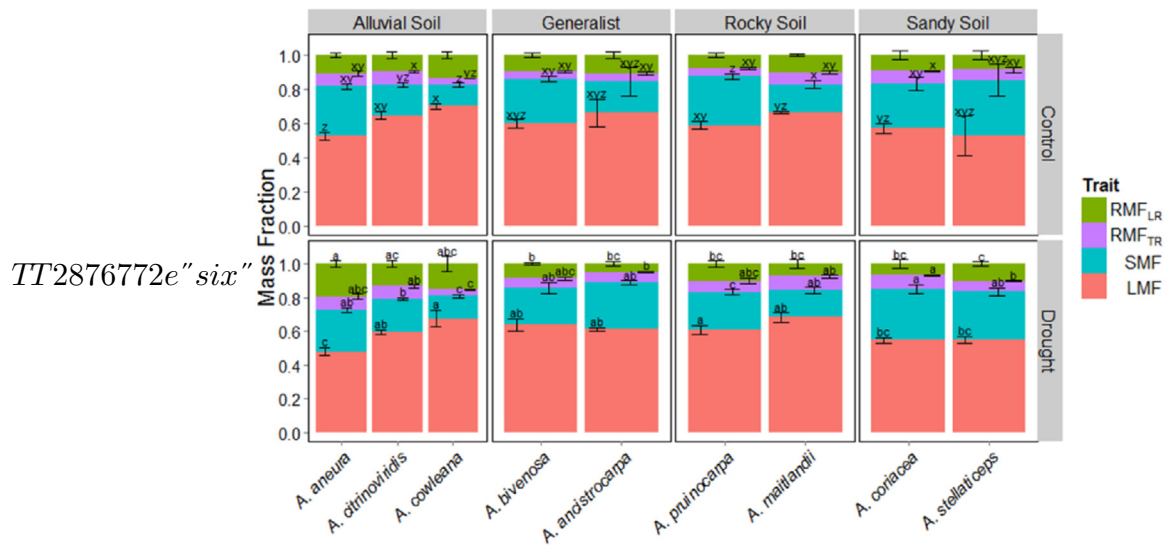


Fig. 3–6. Mean (\pm SE) lateral root, tap root, stem and leaf mass fractions (g g^{-1}) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Letters indicate significant differences ($P < 0.05$) between species within treatments and traits. The alluvial soil group RMF_{LR} was significantly ($P < 0.05$) higher compared to all other soil groups for drought plants only, differences in RMF_{LR} for control plants were non-significant. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

to the drought treatment in two species from the alluvial soil group, whereas in other species there was either no response or an increase (Fig. 3–7).

Significant ($P < 0.05$) differences in specific lateral root length (SRL_{LR}) were detected among species for drought and control plants, but not between soil groups. *Acacia maitlandii* and *Acacia stellaticeps* had the highest SRL_{LR} for drought and control plants, respectively (Appendix D). Conversely, *Acacia coriacea* had the lowest SRL_{LR} in both treatments. Root length to leaf area ratio (RL/LA) was significantly ($P < 0.05$) different among species, but not preferred soil type. *A. aneura* had the highest ratio for both treatments indicating it invested more in below ground water acquisition per unit transpiring leaf area (Appendix D). The lowest ratios were measured in *Acacia bivenosa* and *A. coriacea* for control and drought plants, respectively. Specific leaf area (SLA) differed significantly ($P < 0.05$) amongst species, but was not related to soil group. *A. maitlandii* had the highest SLA for drought and control plants, while *Acacia pruinocarpa* and *A. coriacea* had the lowest SLA for control and drought plants, respectively (Appendix D). Stem specific densities (SSD) differed significantly ($P < 0.05$) between species for drought plants only, but were unrelated to preferred soil type in either treatment. The highest and lowest SSDs were attributed to *A. coriacea* and *Acacia cowleana*, respectively (Appendix D).

3.3. Discussion

Acacia species in this experiment varied markedly in their physiological and morphological responses to drought. Under drought stress species vary in their degree of anisohydry (Tardieu and Davies, 1992; Tardieu and Simonneau, 1998). Anisohydric plants typically maintain higher g_s at more negative Ψ_1 under declining soil water content, and often have a wider range of Ψ_1 (Klein, 2014; McDowell et al., 2008). These plants usually occupy habitats predisposed to drought (McDowell et al., 2008), however, some plants in these regions tend towards isohydric behaviour.

Results from this study are discussed in terms of a continuum from less to more anisohydric.

Species with a higher degree of anisohydry in this experiment tended to be from the alluvial soil group (*A. aneura*, *A. cowleana*, *A. cowleana*), rocky soil group (*A. pruinocarpa* and *A. maitlandii*), and *A. coriacea* from the sandy soil group. In this experiment these species had the highest capacity to extract tightly bound water from the tested mine substrate and thus had the largest “water use envelope” (Sperry et al., 2002) of all experimental species. Their Ψ_1 tended to be more negative for control and drought plants, while g_s remained relatively high at more negative Ψ_1 under drought conditions. Similarly, on a coarse textured natural site Gwenzi et al. (2014) observed that *A. pruinocarpa* Ψ_1 was most negative (–3.5 MPa) with the greatest difference between wet and dry season conditions, and had the highest rates of gas exchange over both seasons compared to co-occurring *A. bivenosa*, *Acacia inaequilatera*, and *Acacia pyrifolia*. Also observations on *Eucalyptus wandoo* and *E. accedens* in the south-west of Western Australia showed that these species had lower Ψ_1 and accessed water held at lower Ψ_s in clay-rich layers, as compared to two other *Eucalyptus* species occurring on coarser textured soil (Poot and Veneklaas, 2013). Low minimum Ψ_1 confer a competitive advantage to these species during seasonal drought by allowing them to access tightly bound water (Poot and Veneklaas, 2013). Unlike *A. coriacea*, however, *A. stellaticeps* in the same sandy soil group exhibited less anisohydric behaviour. This would suggest that the latter species is less able to extract water from more fine-textured soils, and would prefer deep sandy profiles, whereas *A. coriacea* may also occur on shallower sandy profiles underlain by more fine textured soils. Topographic variation within sandy landscapes (such as dunes, interdunes and sandplains), as well as soil hydraulic characteristics, root system distributions, and the interactions between all these factors may influence local water availability and allow a range of strategies to be successful (e.g. Grigg et al., 2008a; Grigg et al., 2008b; Hoy, 2014). For example, Rosenthal et al. (2005) showed that plants on dune ridges had improved water status due to deep

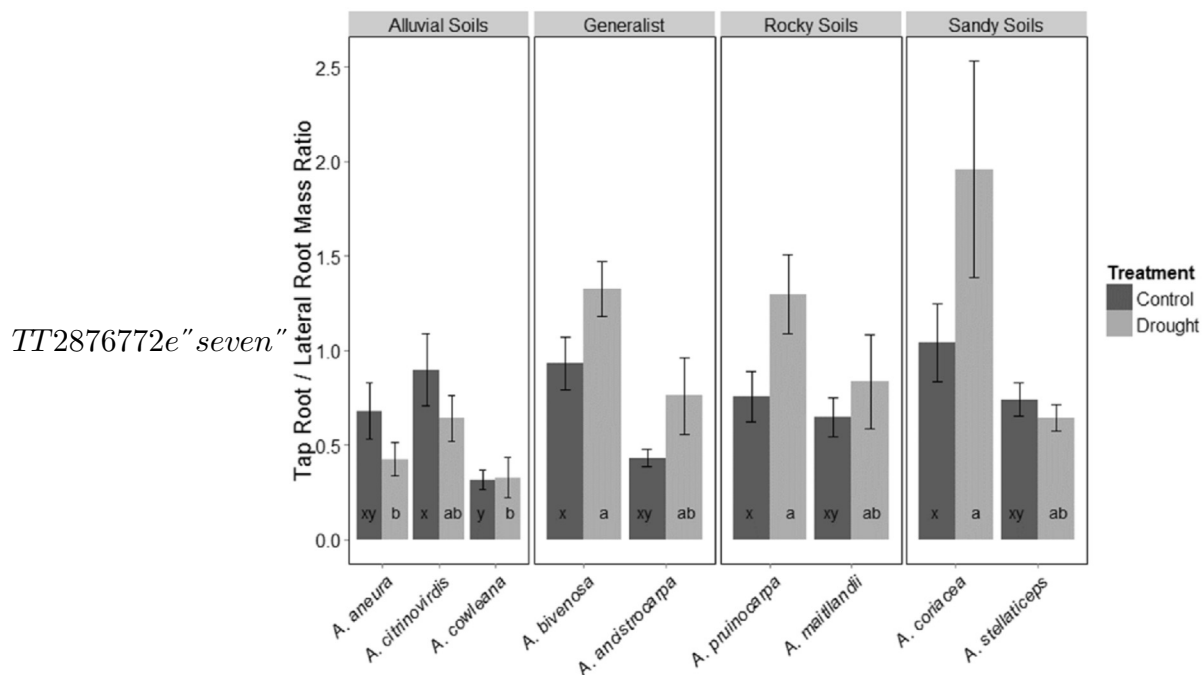


Fig. 3–7. Mean (\pm SE) tap root to lateral root mass ratio ($g\ g^{-1}$) of control (well-watered) plants and of drought plants measured at the pre-defined harvest condition (Ψ_s of –2.18 MPa), for nine *Acacia* species and their preferred soil types from the West Australian Pilbara region. Letters indicate significant differences ($P < 0.05$) between species within treatments. For each species $n = 5$, with the exception of *A. cowleana*, *A. maitlandii* and *A. stellaticeps* where $n = 4$.

roots and increased water availability with depth (Rosenthal et al., 2005), while others found low water stress and high growth of plants with shallower roots in depressed parts of sandy landscapes due to proximity to the ground water table (Gries et al., 2003).

Water uptake by the more anisohydric species *A. aneura*, *A. cowleana*, and *A. coriacea* may have been supported by more negative osmotic potentials and the accumulation of solutes (osmotic adjustment). More negative osmotic potentials are associated with a lower turgor loss point and allow greater tissue dehydration (Bartlett et al., 2012). Species with more negative osmotic potentials also tend to have cell walls that are less elastic under moisture deficit protecting tissue integrity especially during re-hydration (Bartlett et al., 2012; Mitchell et al., 2008). All three aforementioned experimental species had the most negative osmotic potentials, had comparatively lower SLAs, and tended to show osmotic adjustment, although *A. aneura* was the only species with significant adjustment. Similar to other studies, these three species also had the three most negative Ψ_1 , which is largely determined by osmotic potential (Bartlett et al., 2012; Hoy, 2014; Mitchell et al., 2008). However, the positive relationship between π_{sat} and Ψ_1 considering all nine species ($R^2 = 0.23$, $P < 0.001$ data not shown) was considerably weaker than the relationships reported between π_{sat} and Ψ_1 at turgor loss point reported by other authors. For instance, Hoy (2014) found a strong relationship between these two factors for eight species in the Great Sandy Desert of Western Australia, as did Mitchell et al. (2008) in southwestern Western Australia, and Bartlett et al. (2012) across species in a global dataset. It is likely that leaves sampled at the end of our drought treatment had not reached turgor loss points, causing a poor correlation with osmotic potential. Alternatively, the role of tissue properties such as elasticity may have a bigger role in differentiating Ψ_1 among these closely-related plants. Nonetheless, the low π_{sat} of *A. cowleana*, *A. coriacea*, and especially *A. aneura* partly explains their low Ψ_1 and improved water extraction ability.

Leaf physiological responses to drought observed in the alluvial soil group tended towards anisohydry, which was likely associated with high investment in lateral roots. Species in the group had significantly higher RMF_{LR} compared to other soil groups (Fig. 3–6) and tended to have the lowest TR/LR Ratios (Fig. 3–7). Greater total root mass and root biomass allocation to lateral roots reduces the risk of hydraulic failure at negative Ψ_s , especially in superficial fine textured soils. Increasing the number of lateral roots expands the root area capable of water uptake reducing the demand for water uptake per unit area of root (Comas et al., 2013; West et al., 2008). Therefore, more anisohydric physiological behaviour exemplified by the alluvial soil group was likely supported by lateral root characteristics that helped to facilitate continued water extraction under drying soil conditions.

Species displaying more isohydric water relations were from the generalist soil group (*A. bivenosa* and *Acacia ancistrocarpa*), and *A. stellaticeps* (sandy soil group). In agreement with other studies, these species were found to be conservative water users through stomatal closure during water stress resulting in moderately negative Ψ_1 (Grigg et al., 2008a; Hoy, 2014). Conservative water users can survive periods of mild drought providing they have positive carbon status, but cannot access tightly bound water at very negative Ψ_s , leaving them more susceptible to cavitation as soil continues to dry (Szota et al., 2011).

Drought avoidance as soils continue to dry can be facilitated by plant access to water at greater depth through formation of deep tap roots (McDowell et al., 2008; White et al., 2000), or via hydraulic redistribution from deeper tap roots to lateral roots through dimorphic rooting patterns (e.g. *A. ancistrocarpa*; Grigg et al., 2008a). In this study, TR/LR was largest in two coarse textured soil species that were more anisohydric, it was also statistically significant for one

generalist species, *A. bivenosa* that was less anisohydric (Fig. 3–7). Gwenzi et al. (2014) previously reported high tap root biomass investment for *A. bivenosa* with comparatively shallow fine roots in the top 15 cm, and a main tap root that reached approximately 0.5 m-depth. It displayed a dimorphic rooting system, which may be an inherent drought response evolved as an adaptation that may explain *A. bivenosa*'s occurrence in a variety of habitats. Conversely, the high tap root biomass investment of more anisohydric *A. coriacea* and *A. pruinoarpa* from coarse textured soils is likely an adaptation to access water deeper in the profile, which is characteristic of species growing on coarse textured soils (Hacke et al., 2000; Sperry and Hacke, 2002). For instance, on a coarse textured material *A. pruinoarpa* had a lack of fine roots at the surface, but a deep root system with access to deep moisture (Gwenzi et al., 2014). Therefore, high tap root biomass investments of species in this study are likely associated with typical rooting strategies; one supporting a more dimorphic root system (*A. bivenosa*), and the other placing much more emphasis on deep tap roots (*A. coriacea* and *A. pruinoarpa*), characteristic of species from coarse textured soils. However, each above-mentioned species had a counterpart within the same soil group that did not have significantly high tap root allocation. Thus observed seedling rooting patterns were not specific to a particular preferred soil type. This may indicate that (1) there may be high spatial heterogeneity even within habitats classified under a specific soil type, (2) that different trait combinations can lead to successful adaptation to these harsh habitats, (3) that our classification of species into their preferred soil types is not accurate, or (4) that drought responses of seedlings grown in pots are different from those of adult plants in the field. All above explanations would necessitate further research on the reliability of species soil/habitat classification and the local heterogeneity in soil profiles and landscapes.

3.4. Conclusion

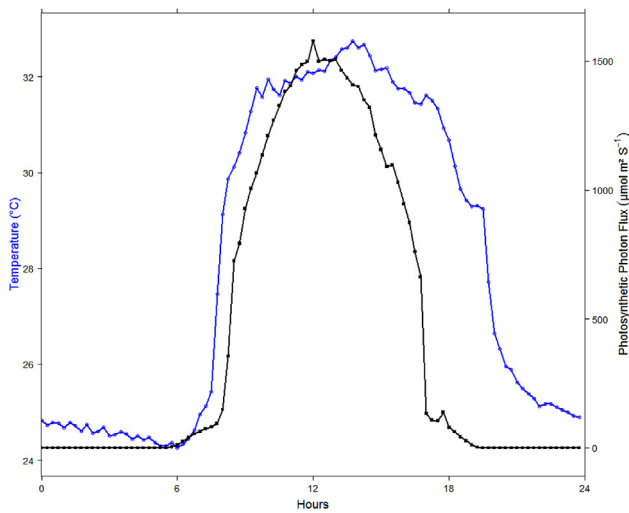
Our data suggest that (1) *Acacia* species from the semi-arid Pilbara of Western Australia differ significantly in a range of traits associated with drought tolerance, showing a continuum from less to more anisohydric behaviour, (2) differences in drought tolerance traits were in most cases poorly related with preferred soil type, and (3) *a priori* classification of species into preferred soil types may help with inferring drought tolerance but is not a good predictor.

This study demonstrates the range of drought tolerance traits within and between preferred soil types, in the genus *Acacia* growing in a mine site restoration substrate under drought conditions. Species groupings into preferred soil types should be completed from site specific empirical data rather than *a priori* classification. In light of results presented here, drought tolerance traits of young *Acacia* seedlings are more likely species-specific than related to soil type preferences when growing in a mine site restoration substrate. It should be noted, however, that the classification based on soil preference used here does not capture all traits that affect plant water availability. Given more accurate empirical evidence, the potential to implement ecophysiological and morphological trait-based selection criteria for revegetation of local native flora on disturbed sites, underpinned by the geotechnical characteristics of the restoration substrate still exists. Trait-based selection criteria could utilise physiological and morphological traits identified in this and other studies to match the most appropriate local native flora to the geotechnical characteristics of a restoration substrate, or conversely, to define the geotechnical conditions required to provide niches for this flora. Land restoration practitioners would benefit from this information as it is key to successful restoration of mined lands in arid to semi-arid systems, improving the overall success of revegetation projects on disturbed lands.

Acknowledgements

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Appendix A



Average daily glasshouse temperatures ($^{\circ}\text{C}$) and photosynthetic photon flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) outside the glasshouse, measured at 15 min intervals through the sampling period (15–25 February 2014).

Appendix B

Physical and chemical properties of restoration substrate sourced from Mt. Goldsworthy, Pilbara, Western Australia.

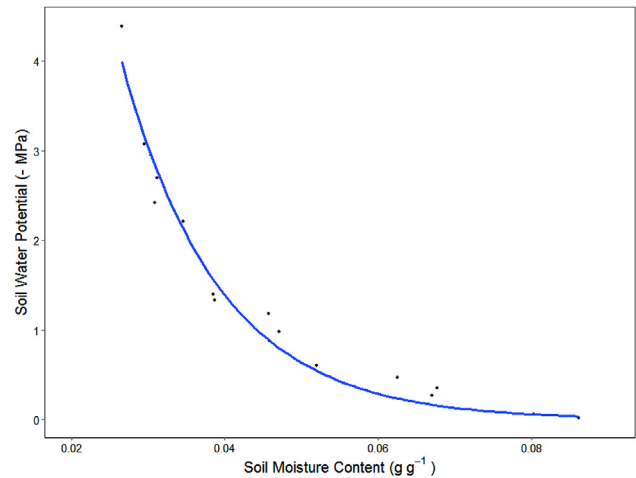
Analyte	Units	Value
Clay	%	13.4
Sand	%	69.7
Silt	%	16.9
pH Level (H_2O)	pH	8.6
pH Level (CaCl_2)	pH	7.9
Conductivity	dS m^{-1}	0.11
Ammonium nitrogen	g kg^{-1}	0.001
Nitrate nitrogen	g kg^{-1}	<0.001
Phosphorus colwell	g kg^{-1}	0.003
Potassium	g kg^{-1}	0.12
Sulphur	g kg^{-1}	0.23
Organic carbon	g kg^{-1}	2.1
Copper	g kg^{-1}	0.0007
Iron	g kg^{-1}	0.006
Manganese	g kg^{-1}	0.005

(continued)

Analyte	Units	Value
Zinc	g kg^{-1}	0.005
Boron	g kg^{-1}	0.005
Aluminium	$\text{meq}/100 \text{ g}$	0.06
Calcium	$\text{meq}/100 \text{ g}$	4.75
Magnesium	$\text{meq}/100 \text{ g}$	1.73
Potassium	$\text{meq}/100 \text{ g}$	0.21
Sodium	$\text{meq}/100 \text{ g}$	0.44

The soil was analysed by the CSBP Soil and Plant Analysis Laboratory (Bibra Lake, WA) to determine physical and chemical properties, including soil texture (classes: $0.02 > \text{sand} < 2.0$, $0.002 > \text{silt} < 0.02$, $\text{clay} < 0.002 \text{ mm}$; %, Modified Pipette Procedure), electrical conductivity (1:5 soil: water extract), pH (1:5 soil: water extract and 1:5 soil: CaCl_2 0.01 M solution), organic carbon (Walkley-Black method), ammonium nitrogen (extracted in 1 M KCl), nitrate nitrogen (extracted in 1 M KCl), plant available phosphorus (Colwell P, bicarbonate extraction), potassium (Colwell, bicarbonate extraction), sulphur (extracted in KCl at 40°C), exchangeable cations (aluminium, calcium, magnesium, potassium, and sodium; Mehlich No. 3 test), extractable copper, iron, manganese and zinc (DTPA extraction), and boron (hot CaCl_2 method).

Appendix C



Soil water potential (Ψ_s ; -MPa) and gravimetric soil moisture content (g g^{-1}) of the soil used in the experiment ($R^2 = 0.95$, $y = 30.897e^{-75.73x}$).

Appendix D

Summary of statistical results for mean differences between species and preferred soil type categories with species nested within preferred soil type categories, for standardised transpiration response to decreasing Ψ_s (MPa^{-1}), response of stomatal conductance (g_s) to decreasing Ψ_s ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$), leaf water potential (Ψ_l ; MPa), osmotic potential at full turgor (π_{sat} ; MPa), specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$), stem specific density (SSD; g cm^{-3}), root length to leaf area ratio (RL/LA Ratio; cm cm^{-2}), leaf mass fraction (LMF; g g^{-1}), stem mass fraction (SMF; g g^{-1}), lateral root mass fraction (RMF_{LR} ; g g^{-1}), tap root mass fraction (RMF_{TR} ; g g^{-1}), total specific lateral root length (SRL_{LR} ; cm g^{-1}), tap root to lateral root ratio (TR/LR Ratio; g g^{-1}), and total plant dry mass (g) of

control (well-watered) and drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species from the West Australian Pilbara region. Letters indicate significant differences ($P < 0.05$) among species or preferred soil type groups within treatments. For each species $n = 5$, with the exception of *Acacia cowleana*, *Acacia maitlandii* and *Acacia stellaticeps* where $n = 4$. Degrees of freedom for species and preferred soil type groups were eight and three, respectively.

(π_{sat} ; MPa), specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$), stem specific density (SSD; g cm^{-3}), root length to leaf area ratio (RL/LA Ratio; cm cm^{-2}), total plant dry mass (g), leaf mass fraction (LMF; g g^{-1}), stem mass fraction (SMF; g g^{-1}), lateral root mass fraction (RMF_{LR}; g g^{-1}), tap root mass fraction (RMF_{TR}; g g^{-1}), total specific lateral root length (SRL_{LR}; cm g^{-1}), and tap root to lateral root ratio (TR/LR Ratio; g g^{-1}) of control (well-watered) and drought plants measured at the pre-defined harvest condition (Ψ_s of -2.18 MPa), for nine *Acacia* species

Factor	ln transpiration (slope)	g_s (slope)	Ψ_l		π_{sat}		SLA		SSD		RL/LA ratio	
Species	Drought	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
<i>A. aneura</i>	-0.41	-8.10 ^a	-1.94 ^x	-2.72 ^{ab}	-2.17	-2.81 ^a	47.2 ^z	47.3 ^c	0.46	0.46 ^{bc}	9.70 ^x	15.1 ^a
<i>A. citrinoviridis</i>	-0.48	-21.30 ^a	-1.72 ^{xy}	-2.56 ^a	-1.46	-2.27 ^{ab}	52.8 ^z	53.6 ^c	0.52	0.62 ^{ab}	3.97 ^{xy}	6.63 ^{ab}
<i>A. cowleana</i>	-0.38	-17.10 ^{bc}	-1.24 ^{xy}	-2.10 ^{ab}	-1.72	-1.72 ^b	96.2 ^x	72.8 ^b	0.38	0.42 ^c	7.77 ^{xy}	7.73 ^{ab}
<i>A. bivenosa</i>	-0.59	-33.60 ^{ab}	-1.11 ^y	-1.56 ^b	-1.7	-2.06 ^{ab}	71.1 ^y	62.6 ^{abc}	0.46	0.49 ^{abc}	2.16 ^y	3.20 ^{abc}
<i>A. ancistrocarpa</i>	-0.19	-31.90 ^b	-1.19 ^y	-1.71 ^b	-1.57	-1.62 ^b	103.0 ^{xyz}	67.8 ^{abc}	0.5	0.44 ^c	5.69 ^{xyz}	4.93 ^{abc}
<i>A. pruinocarpa</i>	-0.3	-2.70 ^c	-1.78 ^{xy}	-2.36 ^a	-1.85	-2.07 ^b	43.0 ^z	48.5 ^c	0.51	0.62 ^{ab}	6.95 ^z	5.19 ^c
<i>A. maitlandii</i>	-0.3	-13.20 ^{bc}	-0.45 ^y	-1.66 ^b	-1.93	-2.29 ^{ab}	119.4 ^x	97.2 ^a	0.63	0.46 ^{bc}	3.97 ^x	5.57 ^a
<i>A. coriacea</i>	-0.53	-9.80 ^a	-1.73 ^{xy}	-2.37 ^a	-2.18	-3.09 ^{ab}	42.7 ^z	43.5 ^c	0.6	0.67 ^a	4.27 ^z	2.91 ^c
<i>A. stellaticeps</i>	-0.23	-16.50 ^a	-1.11 ^y	-1.26 ^b	-1.25	-1.72 ^b	74.7 ^{xyz}	73.3 ^b	0.49	0.54 ^{abc}	4.46 ^{xyz}	4.96 ^b
Soil categories	Drought	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Alluvial soil	-0.42	-15.5 ^{ab}	-1.63	-2.46	-1.78	-2.27	63.2	56.8	0.46	0.51	7.1	9.98
Generalists	-0.39	-32.75 ^{bc}	-1.15	-1.64	-1.63	-1.84	87.4	65.2	0.48	0.47	3.92	4.06
Rocky soil	-0.3	-7.95 ^c	-1.12	-2.01	-1.89	-2.18	71.7	70.1	0.56	0.55	5.83	5.36
Sandy soil	-0.38	-13.15 ^a	-1.42	-1.82	-1.72	-2.4	56.9	56.7	0.58	0.61	4.35	3.82

Factor	LMF		SMF		RMF _{LR}		RMF _{TR}		SRL _{LR}		TR/LR ratio		Total plant dry Mass	
Species	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
<i>A. aneura</i>	0.53 ^z	0.48 ^c	0.29 ^{xy}	0.25 ^{ab}	0.11	0.19 ^a	0.07 ^{xy}	0.08 ^{abc}	3986 ^{xy}	3609 ^{ab}	0.68 ^{xy}	0.42 ^b	1.33 ^{yz}	1.35 ^b
<i>A. citrinoviridis</i>	0.65 ^{xy}	0.60 ^{ab}	0.18 ^{yz}	0.20 ^b	0.1	0.13 ^{ac}	0.08 ^x	0.08 ^{ab}	3157 ^y	3002 ^b	0.90 ^x	0.64 ^{ab}	2.62 ^y	2.99 ^b
<i>A. cowleana</i>	0.70 ^x	0.68 ^a	0.12 ^z	0.13 ^c	0.14	0.15 ^{abc}	0.04 ^{yz}	0.04 ^c	7005 ^x	5421 ^a	0.32 ^y	0.33 ^b	0.45 ^z	1.14 ^b
<i>A. bivenosa</i>	0.62 ^{xyz}	0.61 ^{ab}	0.27 ^{xy}	0.27 ^{ab}	0.06	0.05 ^b	0.05 ^{xy}	0.06 ^{abc}	3289 ^y	5988 ^{ab}	0.93 ^x	1.33 ^a	8.46 ^x	8.95 ^a
<i>A. ancistrocarpa</i>	0.60 ^{xyz}	0.64 ^{ab}	0.26 ^{xyz}	0.22 ^{ab}	0.1	0.09 ^{bc}	0.04 ^{xy}	0.05 ^b	6883 ^x	5019 ^a	0.43 ^{xy}	0.76 ^{ab}	1.15 ^{yz}	1.86 ^b
<i>A. pruinocarpa</i>	0.67 ^{xy}	0.68 ^a	0.16 ^z	0.16 ^c	0.1	0.07 ^{bc}	0.07 ^{xy}	0.08 ^{abc}	3684 ^{xy}	4268 ^a	0.76 ^x	1.30 ^a	2.18 ^y	2.41 ^b
<i>A. maitlandii</i>	0.59 ^{yz}	0.61 ^{ab}	0.28 ^x	0.23 ^{ab}	0.08	0.10 ^{bc}	0.05 ^{xy}	0.07 ^{ab}	6147 ^x	6133 ^a	0.65 ^{xy}	0.84 ^{ab}	0.82 ^{yz}	1.13 ^b
<i>A. coriacea</i>	0.57 ^{yz}	0.55 ^{bc}	0.26 ^{xy}	0.30 ^a	0.09	0.07 ^c	0.08 ^x	0.08 ^a	2189 ^y	2508 ^b	1.04 ^x	1.96 ^a	8.07 ^x	9.54 ^a
<i>A. stellaticeps</i>	0.59 ^{xyz}	0.61 ^{bc}	0.28 ^{xyz}	0.23 ^{ab}	0.08	0.1	0.05 ^{xy}	0.07 ^b	7267 ^x	4399 ^a	0.65 ^{xy}	0.84 ^{ab}	2.35 ^{yz}	3.45 ^{ab}
Soil categories	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Alluvial soil	0.62	0.58	0.2	0.19	0.11	0.16 ^a	0.06	0.06	4552	3910	0.63	0.46	1.54	1.87
Generalists	0.61	0.62	0.26	0.25	0.08	0.07 ^b	0.05	0.06	5086	5503	0.68	1.04	4.81	5.41
Rocky soil	0.63	0.65	0.22	0.19	0.09	0.09 ^b	0.06	0.08	4607	5097	0.7	1.07	1.67	1.84
Sandy soil	0.58	0.58	0.27	0.26	0.08	0.08 ^b	0.06	0.07	4446	3349	0.84	1.4	5.52	6.83

Appendix E

Summary of statistical results between treatment and species for leaf water potential (Ψ_l ; MPa), osmotic potential at full turgor

from the West Australian Pilbara region. For each species $n = 5$, with the exception of *Acacia cowleana*, *Acacia maitlandii* and *Acacia stellaticeps* where $n = 4$. Significance is indicated by ** $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$, and ***** $P < 0.0001$.

Factor	df	F-Values						
		Ψ_l	π_{sat}	SLA	SSD	RL/LA ratio	Total plant dry mass	
Treatment	1	62.58 ^{*****}	11.44 ^{**}	1.78	0.55	0.62	3.05	
Species	8	13.27 ^{*****}	5.81 ^{****}	11.27 ^{*****}	4.19 ^{***}	5.76 ^{*****}	11.54 ^{*****}	
treatment × species	8	1.36	1.18	1.19	1.09	0.93	0.25	
Error	83							

Factor	df	F-Value					
		LMF	SMF	RMF _{LR}	RMF _{TR}	SRL _{LR}	TR/LR Ratio
Treatment	1	2.34	0.93	0.08	2.15	3.21	0.09
Species	8	8.40 ^{****}	19.79 ^{*****}	4.29 ^{***}	19.25 ^{*****}	14.72 ^{*****}	6.35 ^{****}
Treatment × species	8	0.69	1.36	1.52	0.53	2.43	2.1*
Error	83						

References

- Ackerly, D., 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecol. Monogr.* 74, 25–44.
- Barnes, F.J., 1986. Carbon Gain and Water Relations in Pinyon-juniper Habitat Types. New Mexico State University.
- 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. *Ecol. Lett.* 15, 393–405.
- Beard, J.S., 1975. Vegetation Survey of Western Australia: Sheet 5: Pilbara. University of Western Australia Press, Perth.
- Bhaskar, R., Ackerly, D.D., 2006. Ecological relevance of minimum seasonal water potentials. *Physiol. Plant.* 127, 353–359.
- Bristow, K.L., Campbell, G.S., Calissendorff, C., 1984. The effects of texture on the resistance to water movement within the Rhizosphere. *Soil Sci. Soc. Am. J.* 48, 266–270.
- Comas, L.H., Becker, S.R., Cruz, V.M.V., Byrne, P.F., Dierig, D.A., 2013. Root traits contributing to plant productivity under drought. *Front. Plant Sci.* 4, 442.
- DMP, 2012. 2011 WA Mineral and Petroleum Statistics Digest. Department of Mines and Petroleum, Perth.
- EPA E.P.A., 2014. Cumulative environmental impacts of development in the Pilbara region: Advice of the Environmental Protection Authority to the Minister for Environment under Section 16(e) of the Environmental Protection Act 1986. Government of Western Australia, Perth.
- Galmés, J., Ribas-Carbó, M., Medrano, H., Flexas, J., 2007. Response of leaf respiration to water stress in Mediterranean species with different growth forms. *J. Arid Environ.* 68, 206–222.
- Gray, J.T., 1983. Nutrient use by evergreen and deciduous shrubs in southern California: I. Community nutrient cycling and nutrient-use efficiency. *J. Ecol.* 71, 21–41.
- Grierson, C.S., Barnes, S.R., Chase, M.W., Clarke, M., Grierson, D., Edwards, K.J., Jellis, G.J., Jones, J.D., Knapp, S., Oldroyd, G., Poppy, G., Temple, P., Williams, R., Bastow, R., 2011. One hundred important questions facing plant science research. *New Phytol.* 192, 6–12.
- Gries, D., Zeng, F., Foetzi, A., Arndt, S.K., Bruelheide, H., Thomas, F.M., Zhang, X., Runge, M., 2003. Growth and water relations of *Tamarix ramosissima* and *Populus euphratica* on Taklamakan desert dunes in relation to depth to a permanent water table. *Plant Cell Environ.* 26, 725–736.
- Grigg, A.M., Veneklaas, E.J., Lambers, H., 2008a. Water relations and mineral nutrition of closely related woody plant species on desert dunes and interdunes. *Aust. J. Bot.* 56, 27–43.
- Grigg, A.M., Veneklaas, E.J., Lambers, H., 2008b. Water relations and mineral nutrition of *Triodia* grasses on desert dunes and interdunes. *Aust. J. Bot.* 56, 408–421.
- Gwenzi, W., Hinz, C., Bleby, T.M., Veneklaas, E.J., 2014. Transpiration and water relations of evergreen shrub species on an artificial landform for mine waste storage versus an adjacent natural site in semi-arid Western Australia. *Ecophysiology* 7, 965–998.
- Hacke, U.G., Sperry, J.S., Ewers, B.E., Ellsworth, D.S., Schäfer, K.V.R., Oren, R., 2000. Influence of soil porosity on water use in *Pinus taeda*. *Oecologia* 124, 495–505.
- Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis, S.D., McCulloh, K.A., 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126, 457–461.
- Hoy, E., 2014. Plant Form and Function from an Ecohydrological Perspective in the Australian Arid Zone, with Reference to Mine-site Rehabilitation. Department of Plant Biology, The University of Western Australia.
- Jackson, R.B., Sperry, J.S., Dawson, T.E., 2000. Root water uptake and transport: using physiological processes in global predictions. *Trends Plant Sci.* 5, 482–488.
- Jury, W.A., Horton, R., 2004. Soil Physics. John Wiley & Sons.
- Klein, T., 2014. The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. *Funct. Ecol.* 28, 1313–1320.
- Kummerow, J., Krause, D., Jow, W., 1977. Root systems of chaparral shrubs. *Oecologia* 29, 163–177.
- Ludlow, M., Chu, A., Clements, R., Kerslake, R., 1983. Adaptation of species of *Cenrosema* to water stress. *Funct. Plant Biol.* 10, 119–130.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., Yezzer, E.A., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* 178, 719–739.
- Mitchell, P.J., Veneklaas, E.J., Lambers, H., Burgess, S.S.O., 2008. Leaf water relations during summer water deficit: differential responses in turgor maintenance and variation in leaf structure among different plant communities in south-western Australia. *Plant Cell Environ.* 31, 1791–1802.
- Mooney, H.A., Dunn, E.L., 1970. Photosynthetic systems of Mediterranean-climate shrubs and trees of California and Chile. *Am. Nat.* 104, 447–453.
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., van der Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S., Cornelissen, J.H.C., 2013. New handbook for standardised measurement of plant functional traits worldwide. *Aust. J. Bot.* 61, 167–234.
- Poot, P., Veneklaas, E., 2013. Species distribution and crown decline are associated with contrasting water relations in four common sympatric eucalypt species in southwestern Australia. *Plant Soil* 364, 409–423.
- Rosenthal, D.M., Ludwig, F., Donovan, L.A., 2005. Plant responses to an edaphic gradient across an active sand dune/desert boundary in the Great basin desert. *Int. J. Plant Sci.* 166, 247–255.
- Ryser, P., Bernardi, J., Merla, A., 2008. Determination of leaf fresh mass after storage between moist paper towels: constraints and reliability of the method. *J. Exp. Bot.* 59, 2461–2467.
- Sheoran, V., Sheoran, A., Poonia, P., 2010. Soil reclamation of abandoned mine land by revegetation: a review. *Int. J. Soil, Sediment Water* 3, 13.
- Sperry, J.S., Hacke, U.G., 2002. Desert shrub water relations with respect to soil characteristics and plant functional type. *Funct. Ecol.* 16, 367–378.
- Sperry, J.S., Hacke, U.G., Oren, R., Comstock, J.P., 2002. Water deficits and hydraulic limits to leaf water supply. *Plant Cell Environ.* 25, 251–263.
- Szota, C., Farrell, C., Koch, J.M., Lambers, H., Veneklaas, E.J., 2011. Contrasting physiological responses of two co-occurring eucalypts to seasonal drought at restored bauxite mine sites. *Tree Physiol.* 31, 1052–1066.
- Tardieu, F., Davies, W.J., 1992. Stomatal response to abscisic acid is a function of current plant water status. *Plant Physiol.* 98, 540–545.
- Tardieu, F., Simonneau, T., 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *J. Exp. Bot.* 49, 419–432.
- Turner, N., 1981. Techniques and experimental approaches for the measurement of plant water status. *Plant Soil* 58, 339–366.
- Valladares, F., Sánchez-Gómez, D., 2006. Ecophysiological traits associated with drought in mediterranean tree seedlings: individual responses versus interspecific trends in eleven species. *Plant Biol.* 8, 688–697.
- Van Vreeswyk, A.M.E., 2004. An Inventory and Condition Survey of the Pilbara Region. Department of Agriculture, Western Australia.
- West, A.G., Hultine, K.R., Sperry, J.S., Bush, S.E., Ehleringer, J.R., 2008. Transpiration and hydraulic strategies in a piñon–juniper woodland. *Ecol. Appl.* 18, 911–927.
- Westoby, M., Wright, I.J., 2006. Land-plant ecology on the basis of functional traits. *Trends Ecol. Evol.* 21, 261–268.
- White, D.A., Turner, N.C., Galbraith, J.H., 2000. Leaf water relations and stomatal behavior of four allopatric *Eucalyptus* species planted in Mediterranean southwestern Australia. *Tree Physiol.* 20, 1157–1165.
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer.