Inter-specific differences in the dynamics of water use and pulse-response of co-dominant canopy species in a dryland woodland

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A R T I C L E   I N F O

Article history:
Received 19 March 2015
Received in revised form 2 September 2015
Accepted 8 September 2015
Available online xxx

Keywords:
Water potential
Carbon isotopes
Arid
Niche
Acacia

A B S T R A C T

Differences in the magnitude and timing of rainfall pulse-response among plant species in dryland ecosystems may facilitate the maintenance of species diversity, but this hypothesis remains untested among species that appear to occupy the same ecological niche. Here, we examined the pulse-response of co-dominant *Acacia* species forming the canopy of a dryland woodland. Trees received natural pre-cipitation and supplemental irrigation equivalent to a ~105 mm rainfall pulse over four days. The pulse-response was large and rapid irrespective of species or differences in minimum water potential during drought. Branch water potential increased from ~8 MPa to >1 MPa at pre-dawn and foliage relative water content increased from 43.4% to 98.6% within five days of drought-break. Differences in midday water potential among species following the water pulse reflected differences in rates of stomatal conductance (g_s) and likely as a consequence of differences in specific leaf area (SLA). We conclude there is no temporal partitioning of resources among the canopy species in the study woodland. Instead, water relations in these three closely related species were anisohydric and tightly coupled to soil water availability.

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1. Introduction

Examining inter-specific differences in the magnitude and timing of rainfall pulse uptake and use by dryland plants provides insight into the mechanisms that facilitate species persistence and co-occurrence. The survival of plants in dryland environments requires both an ability to persist through prolonged drought as well as a capacity to respond to strongly episodic patterns of water availability (Kimball et al., 2012; Noy-Meir, 1973; Plaut et al., 2013). Rainfall ‘pulses’ are large and unpredictable events that are a key driver of growth and productivity in drylands (Stafford Smith and Morton, 1990). A rapid increase in soil moisture associated with a rainfall pulse can stimulate an almost instantaneous response in both photosynthesis and plant respiration (Huxman et al., 2004; Schwinning and Sala, 2004). However, the linkages and possible trade-offs between physiological traits that facilitate a rapid response to rainfall pulse events and traits that facilitate persistence through prolonged drought are not well understood.

Knowing something of the timing and rate of water use by species is critical for understanding plant survival and predicting responses to environmental change. Resurrection plants, for example, are at the extreme end of pulse response strategies, surviving drought periods with apoplastic water content below 10% and resuming physiological function within hours of a rainfall event (Scott, 2000; Vicré et al., 2004). Long-lived woody species typically are the slowest to respond to rainfall pulses among life forms in arid environments (Gebauer and Ehleringer, 2000). Woody species growing in arid environments, including *Prosopis velutina* Wooton in the Sonoran Desert (Fravolini et al., 2005; Resco et al., 2009) and *Acacia aneura* F. Muell. ex Benth. in central Australian deserts (Slatyer, 1961) typically take between one and three days to resume photosynthesis following rainfall. When severe drought has preceded a pulse, the resumption of carbon fixation may take as long as two weeks (Resco et al., 2009; Shim et al., 2009). However, there is still considerable variation among woody species, which has been variously attributed to broad differences in plants traits and growth strategies, including contrasting strategies of conifers and angiosperms (Limousin et al., 2013) or differences in root distribution and depth (Gwenzi et al., 2013). However, many of the
shrublands and open woodlands of more arid regions tend to be dominated by closely related species or genera with similar plant architecture (Page et al., 2011a; Schenk et al., 2008). In such plant communities dominated by species with similar traits and growth strategies, the mechanisms that facilitate species co-existence are not well understood.

The simultaneous determination of both short-term pulse response dynamics as well as long-term water use of individual plants or populations provides a more thorough understanding of the strategies plants use to be competitive in dryland environments. Foliar $\delta^{13}$C (the ratio of $^{13}$C/$^{12}$C isotopes relative to an international standard), when coupled with measurements of transpiration and carbon fixation, is considered a proxy for long-term water use efficiency (WUE) in C3 plants owing to the controlling influence of stomatal conductance ($g_s$) on the ratio of carbon dioxide concentration inside and outside the leaf ($c_i/c_a$), which in turn influences carbon isotope discrimination ($\Delta^{13}$C) (Cernusak et al., 2008; Farquhar et al., 1989). Reduced $g_s$ associated with water stress increases the proportion of the heavy isotope ($^{13}$C) fixed relative to the light isotope ($^{12}$C) owing to slower exchange of CO$_2$ with the atmosphere. However, measures of whole-leaf $\delta^{13}$C may not reveal short-term responses to a rainfall pulse because new photoassimilates only constitute a small fraction of the overall leaf carbon (Mildner et al., 2014). In contrast, high turnover of new photoassimilates within foliage means that analysis of $\delta^{13}$C of non-structural carbohydrates should provide a better indication of short-term changes in $\Delta^{13}$C as a result of the prevailing environmental conditions e.g., a sudden rainfall event (Brugnoli et al., 1988; Kodama et al., 2008; Mildner et al., 2014). Capturing the short-term and long-term dynamics of water use among species will help understand the extent to which resource partitioning may contribute to species co-occurrence within dryland ecosystems.

In this study, we compared the pulse-response of three closely related species, A. aneura F. Muell. ex Benth., A. aptaneura Maslin & J.E.Reid and Acacia ayersiana Mocanochie, which are part of the Mulga species complex that dominates much of inland Australia (Maslin and Reid, 2012; Miller et al., 2002). These three species, like all members of the Mulga complex, commonly co-occur and are morphologically very similar (Page et al., 2011a). However, subtle differences in phyllode shape and size among Mulga species both at the stand and landscape scales also coincide with differences in minimum water potential during drought (Page et al., 2011b). Here, we measured the timing and magnitude of water use of mature trees of three co-occurring Acacia species following a water pulse in order to: (i) quantify the severity of water stress (leaf water potential and relative water content) among species, and (ii) capture the magnitude of pulse response using leaf water potential, stomatal conductance and $\delta^{13}$C of the water-soluble and total phyllode carbon fractions. We expected that differences in the rate and timing of water use would reflect differences in phyllode shape among species and that trees showing a greater degree of water stress (lower relative water content and water potential) preceding a rainfall pulse would correspond with a slower pulse-response.

2. Materials and methods

2.1. Study site

This study was conducted in June 2008 within a mulga woodland at West Angelas (~23.045S, 118.827E), in the Hamersley Ranges, 135 km northwest of the town of Newman in the Pilbara region of Western Australia. The vegetation community is a low-open woodland, with a canopy of A. aneura, A. aptaneura and A. ayersiana trees approximately 4 m tall over a sparse perennial shrub layer to 1 m (predominantly Eremophila spp.) and a ground layer of hummock grasses (Triodia spp.). The three Acacia species share a similar growth habit but have different phyllode sizes. A. aptaneura has almost cylindrical phyllodes approximately 1 mm wide in cross section, whereas A. aneura phyllodes are about 1 mm thick and 3 mm wide and A. ayersiana much broader (0.5 mm thick and up to 12 mm wide). A previous study of the species in this woodland examined two phyllode shape variants of A. aneura (Page et al., 2011b) that are now described as A. aptaneura and A. aneura following the recent revision of the species by Maslin and Reid (2012).

We selected nine trees that included three replicates of each of the three species (Fig. 1). Three sub-plots spread over 100 m were selected to contain one representative of each species ($n = 3$). Soils at the site were clay 20%, silt 15% and sand 65% and are thus considered light sandy clay loams (UC1.43; Northcote, 1971). The clay fraction in Pilbara soils is typically high in smectite and other swelling clay minerals. Thus a typical gravimetric moisture content of ~6% during a dry period as measured in this study equate to extremely negative soil water potentials. Moisture release curves from similar Pilbara soils have an inflection point at ~15% gravimetric soil moisture and ~100 kPa and a soil matric potential far exceeding ~1500 kPa when gravimetric soil moisture is below 10% (Ford et al., 2007; McIntyre et al., 2009).

Episodic rainfall events of $\geq$80 mm are infrequent at the study

![Fig. 1. Trees and corresponding phyllode shapes measured for this experiment. (a) A. aptaneura, (b) A. aneura, and (c) A. ayersiana. Each of these three species had three replicate trees, one of which is pictured here. Phyllode sizes in picture inserts are relative. All three trees are of similar height (4–5 m).](image-url)
site but not uncommon (Dogramaci et al., 2012). For example, weather data recorded at Newman (2000—2013) show that there were on average ~five days per year that received >20 mm of rainfall in 24 h and events ≥80 mm occurred once every two years (Bureau of Meteorology, 2014). Mean annual minimum temperature at Newman is 16.1 °C and mean maximum temperature is 31.9 °C (Bureau of Meteorology, 2014). Daily rainfall data were collected at the West Angelas airstrip, 16.5 km southwest of the study site, between January 2005 and December 2008. Monthly rainfall totals and monthly average air temperature and vapour pressure deficit (VPD) for the period January 2006—December 2008 are provided in Fig. 2. Total rainfall in 2005 was 237 mm, 2006 was 781.8 mm and in 2007 was 154.6 mm. In 2008, 165 mm of rain fell between January and March and a further 25 mm in June; 21 mm on the 10th of June 2008 (four days before irrigation), and 4 mm two days before irrigation on the 12th of June 2008 (Fig. 2).

2.2. Design of irrigation experiment

We used a “before/after” water “pulse” design for our experiment to compare the relative pulse-response of different species and phylloide morphologies. We simulated a ~105 mm rainfall pulse by applying approximately 1000 L of water to the base of each tree on the 14th of June 2008 that supplemented 25 mm of natural precipitation received by the site between the 10th and 14th of June. The design did not include “control” trees, limiting the capacity to determine the relative influence of natural precipitation prior to irrigation. Thus, the interpretation of results in this experiment is restricted to considering the influence of the total pulse volume (105 mm) over four days on the physiological response of trees. Days of sampling are specified relative to the first day rainfall was recorded at the site (‘drought break’), the 10th of June (Day0). Supplemental water was applied on Day4.

Soil moisture content (v/v) was measured at midday for the duration of the experiment to check for consistency in water application among trees, using a Trase 1 TDR (Time Domain Reflectometry) system (Soil Moisture Equipment Corp., Santa Barbara, CA). Uncoated probes (15 cm) were inserted into the soil at three equally spaced locations that were 1 m from the base of each tree. The permittivity of the soil was converted to volumetric moisture content using a calibration against gravimetric moisture content of a soil sample collected from the site and transported back to the laboratory (Whalley, 1993). Volumetric soil moisture content was the same among all trees over the course of the experiment and showed the same pattern of wetting up and drying down. Soil moisture content was 6.5% (v/v) on Day12 (Table 1), peaking at 20% (v/v) when supplemental water was applied on Day4 and declined to 15% (v/v) by Day11 (Table 2). Maximum daily air temperature and vapour pressure deficit (VPDmax) were relatively high prior the water pulse (Day12: VPDmax 3.05 kPa, maximum air temperature 26.8 °C). The daily maximum air temperature and VPDmax had decreased following the rainfall on Day2 (VPDmax 1.40 kPa, average daily temperature maximum 19.6 °C Day3 to Day11); these conditions closely resemble the monthly average (Fig. 2). Therefore, the meteorological conditions over the period Day0 to Day4 were more representative of conditions during a natural precipitation event than a scenario in which only artificial irrigation was applied.

2.3. Tree water use

Three replicates of branch water potential were measured on each tree 1 h before dawn and at midday on Day−1, Day0, Day1, Day3, Day5, Day7, Day9, and Day11 using a Scholander type pressure chamber (PMS Instrument Co., Albany, OR). Branches of ~50 cm length were cut from the outer canopy at 2 m above the ground using tree pruners. Terminal branchlets (approximately 10 cm in length) were then cut with a razor blade and branch water potential measured immediately. Relative water content (RWC) was determined from measurements of five phyllodes removed from each branch both at predawn and midday. After total fresh weight (FW) was determined for all five phyllodes they were transferred to a zip-lock bag and kept cool until transported back to the laboratory at The University of Western Australia (UWA) in Perth. All five phyllodes were then submerged in deionised water in the dark overnight, gently blotted on absorbent paper and weighed to determine turgid weight (TW). Submerging foliar material may over-estimate TW because of infiltration of water into air spaces. An alternative method of attaining TW by rehydrating phyllodes through the cut petiole was also attempted but proved unsuccessful as phyllodes failed to absorb water. Dry weight (DW) was then determined after phyllodes were dried at 80 °C for 48 h. RWC was determined using Equation (1).

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

(1)

Stomatal conductance (gs) was measured on five randomly selected fully expanded phyllodes on each tree every 2 h between
P-values derived from a Markov chain Monte Carlo sampling procedure, that all three phyllode types exhibited similar responses to the simulated rainfall squared likelihood ratio test. Models with only one intercept and slope are for the Fishers Honest Significance measure made over a seven day period following a water pulse equivalent to Table 2

Mean values given with 1 standard error in parentheses. n = 3 for all measurements. F-values and P-values are results of one-way ANOVA models. When P-values were <0.05, Fishers Honest Significant Difference post-hoc tests were applied and significant groupings indicated by superscript letters (a, b). All measurements exceeded the 10 MPa limit of the PMS Model 1600 pressure chamber and so were assigned the value of –11 MPa thus no standard error could be calculated.

Specific leaf area (SLA) and the sapwood area/one sided leaf area ratio (Huber value, $H_w$) were measured for all nine trees during this experiment. One branch was sampled at 2 m height from each tree; each branch was within a range of 70–100 cm long and 5–8 mm in diameter at the cut end. All phyllodes were collected from each branch for measurement with a leaf area meter (LI-3100, LI-COR Inc., Lincoln, NE). SLA was calculated as the one-sided leaf area divided by the oven dry weight of foliage after being dried at 80 °C for 48 h. $H_w$ was calculated by dividing the branch cross sectional area by the one-sided leaf area.

### Table 1

<table>
<thead>
<tr>
<th>Soil moisture (%)</th>
<th>A. aneura</th>
<th>A. aptaneura</th>
<th>A. ayersiana</th>
<th>F-value</th>
<th>P-value</th>
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<tr>
<td>6.40 (0.22)</td>
<td>6.14 (0.37)</td>
<td>6.72 (0.21)</td>
<td>1.11 (0.39)</td>
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<td>Pre-dawn RWC (%)</td>
<td>54.4 *</td>
<td>47.1 ab</td>
<td>42.7 b</td>
<td>8.98 (0.02)</td>
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<td>(2.55)</td>
<td>(1.50)</td>
<td>(1.69)</td>
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<td>Mid-day RWC (%)</td>
<td>46.6 (2.55)</td>
<td>41.7 (2.24)</td>
<td>41.9 (2.24)</td>
<td>1.07 (0.26)</td>
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<td>$\delta^{13}$C (%$d_0$)</td>
<td>–26.9 (0.62)</td>
<td>–28.0 (0.46)</td>
<td>–26.6 (0.46)</td>
<td>1.85 (0.24)</td>
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<td>$\delta^{13}$C$_{\text{soluble}}$ (%)</td>
<td>–26.5 (0.50)</td>
<td>–27.8 (0.36)</td>
<td>–27.3 (0.36)</td>
<td>2.52 (0.16)</td>
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<td>$\Psi_{\text{ps}}$ (MPa)</td>
<td>–6.91 * (0.51)</td>
<td>–8.93 b (0.14)</td>
<td>–4.2 b (0.4)</td>
<td>12.1 &lt;0.001</td>
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<td>(0.51)</td>
<td>(0.14)</td>
<td>(0.4)</td>
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<td>$\Psi_{\text{rel}}$ (MPa)</td>
<td>–8.13 a (0.41)</td>
<td>–10 b (0.21)</td>
<td>–10 b (0.08)</td>
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<td>(0.41)</td>
<td>(0.21)</td>
<td>(0.08)</td>
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<td>$g_{\text{max}}$ (mmol m$^{-2}$ s$^{-1}$)</td>
<td>25.6 (5.85)</td>
<td>12.5 (4.82)</td>
<td>26.5 (1.33)</td>
<td>3.13 0.12</td>
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<tr>
<td>(5.85)</td>
<td>(4.82)</td>
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<tr>
<td>SLA (m$^2$ kg$^{-1}$)</td>
<td>1.45 a (0.06)</td>
<td>1.81 a (0.21)</td>
<td>2.86 b (0.08)</td>
<td>30.3 0.01</td>
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<td>(0.06)</td>
<td>(0.21)</td>
<td>(0.08)</td>
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<td>$H_v$ (m$^2$ m$^{-2}$ x10$^{-4}$)</td>
<td>27.7 * (8.36)</td>
<td>12.8 ab (1.77)</td>
<td>7.3 b (0.36)</td>
<td>4.54 0.06</td>
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<td>(8.36)</td>
<td>(1.77)</td>
<td>(0.36)</td>
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<th>Estimate</th>
<th>S.E.</th>
<th>p-value</th>
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### Table 2

Parameter estimates of linear mixed effects models (LMM) fitted to ecophysiological measurements made over a seven day period following a water pulse equivalent to ~105 mm of precipitation.

2.4. Foliar $\delta^{13}$C analysis

The water-soluble carbon fraction was measured as an estimate of recent photoassimilates (sugars) following a simplified version of the method of Brugnoli et al. (1988). Each measurement of $\delta^{13}$C of the water-soluble carbon fraction ($\delta^{13}$C$_{\text{Soluble}}$) and total phyllode ($\delta^{13}$C$_{\text{Total}}$) used material from one bulk sample of 20 phyllodes per tree that had been sampled from the three branches used to measure water potential at midday on each day of sampling (n = 3). Phyllodes were stored in paper seed envelopes and kept cool until transported to the laboratory. Phyllodes were then oven dried at 60 °C for 48 h and ground to a fine powder in a ball mill. Subsamples ~5 mg were weighed into 6 × 4 mm tin capsules (SerCon Ltd., Crewe, UK) for analysis of $\delta^{13}$C$_{\text{Total}}$. The water-soluble carbon fraction was extracted by adding 50 mg of powder to 1 mL of milliQ water and vortexed then incubated at 70 °C for 1 h. Samples were centrifuged at 13 000 g for 10 min and 300 μL of the supernatant was dried down at 70 °C in an 11 × 4 mm tin capsule and then analysed.

We also tested whether a delay between collection and sample preparation influenced the $\delta^{13}$C values of both total phyllode ($\delta^{13}$C$_{\text{Total}}$) and water-soluble carbon ($\delta^{13}$C$_{\text{Soluble}}$). Foliage from five A. aneura trees were collected into paper bags and transported back to the laboratory by air on the same day of sampling. Foliage from each tree was stored in three different ways; (1) frozen at –80 °C and then freeze-dried, (2) microwaved on high in a 1000 W microwave oven for 30 s to denature any enzymes and oven dried at 60 °C for 48 h and (3) left untreated at 25 °C for 14 days and then dried at 60 °C for 48 h. All phyllode samples were then oven dried at 60 °C for 48 h, ground to a fine powder in a ball mill and prepared for analysis of the water-soluble C ($\delta^{13}$C$_{\text{Soluble}}$) and total phyllode C ($\delta^{13}$C$_{\text{Total}}$) as above.

Slope and intercept parameters are listed for the best-fit LMM determined by a chi-squared likelihood ratio test. Models with only one intercept and slope are for the null model and were not improved by the inclusion of a ‘type’ parameter indicating that all three phyllode types exhibited similar responses to the simulated rainfall pulse. P-values derived from a Markov chain Monte Carlo sampling procedure, where P ≤ 0.05 indicates a significant estimate. Parameter estimates in multi-level models ($g_{\text{max}}$ and $\Psi_{\text{rel}}$) are interpreted by adding estimates together. For example, the $g_{\text{max}}$ intercept parameter for A. aneura is 65.3, and A. aptaneura is 65.3 + 4.93 = 70.2.
All phyllode samples were analysed for stable carbon isotope composition (δ¹³C, VPDB ‰) and carbon concentration (% by weight) using a 20/20 isotope ratio mass spectrometer (IRMS) connected with an ANCA-S1 preparation system (Europa Scientific Ltd., Crewe, UK), at the West Australian Biogeochemistry Centre at The University of Western Australia (http://www.wabc.uwa.edu.au). Samples were quantitatively consumed in the ANCA-S1 Elemental Analyser at 1700–1800 °C (Skrzypek and Paul, 2006). Yielded gases are carried in a stream of He through water traps and a GC column before CO₂ is introduced into the IRMS as transient peaks. The stable carbon isotope composition is reported in the standard δ-notation in permil [‰] after normalization of raw isotope data to VPDB scale using multi-point normalization based on four standards (USGS24, NBS22, USGS41, USGS40) each replicated twice (Paul et al., 2007). Values for δ¹³C standards were as calibrated by the Commission on Isotopic Abundances and Atomic Weights (Coplen et al., 2006). The uncertainty associated with stable isotope analyses (1σ – standard deviation) was not more than 0.20‰.

2.5. Data analysis

Differences in physiological parameters among the three species of Acacia (species) before the water pulse (Day−11) were tested using one-way ANOVA models in R 2.11.0 (R Development Core Team, 2010). When P < 0.05, post-hoc tests were performed using Fisher’s Honest Significant Difference test from the ‘multcomp’ package (Hothorn et al., 2008).

Repeated measures analysis was used to test for differences in soil moisture, Ψpd, Ψmd, ΔΨ, gs max, RWC, δ¹³Ctotal and δ¹⁸O in the three groups of trees following the onset of the water pulse. Specifically, linear mixed effects models (LMM) were fitted to the longitudinal data (data for days 5−11 in R using the ‘lme4’ package. A null model (equation (2)), an additive model (equation (3)), and an interaction model (equation (4)) were fitted, where ‘day’ is a continuous variable defined by N(0, σ²) ‘species’ is a factor with the three levels ‘A. aneur’, ‘A. aptaneura’, and ‘A. ayseniana’, μi ~N(0, σ²i) is a random intercept term for each tree and εij ~N(0, σε) are the within group errors for each tree i at each value of day j. A quadratic term and a cubic term for ‘day’ were also included if they improved the model fit.

\[
y = day + μ_i + ε_{ij}
\] (2)

\[
y = day + type + μ_i + ε_{ij}
\] (3)

\[
y = day + type + (day × type) + μ_i + ε_{ij}
\] (4)

Models were compared using both a pair-wise chi-squared likelihood ratio test and a comparison of Akaike’s Information Criterion (AIC). When the chi-squared P < 0.05 and AIC values for the models differed by > 10 the model with the smallest AIC value was chosen as the best fit, otherwise the most parsimonious model was preferred (Bolker et al., 2009). Differences among the three ‘species’ were determined as a P < 0.05 produced for the parameter estimates using Markov chain Monte Carlo sampling from the posterior distribution of the parameters with the pvals. Fnc function in the ‘languageR’ package (Baayen et al., 2008).

3. Results

3.1. Ψ and phyllode relative water content

At Day−11, A. aptaneura was significantly less negative in both Ψpd (−6.91 ± 0.51 MPa) and Ψmd (−8.13 ± 0.41 MPa) compared to the other two (more broad phyllode) species, which were generally −9.2 MPa at pre-dawn and −<10 MPa at midday (one-way ANOVA P < 0.001, Table 1). All trees had begun to rehydrate following a 21 mm rainfall event on Day0. Trees continued to hydrate the following day owing to the greater surface soil moisture coupled with lower evaporation losses due to heavy cloud cover. Consequently, branch water potential was the same among all trees (P > 0.05) and was similar between pre-dawn (−3.07 ± 0.25 MPa) and midday (−2.46 ± 0.27 MPa) on Days (P > 0.05).

Ψpd did not differ among trees for the seven days following the onset of the water pulse (Table 2). All trees were −1 MPa at pre-dawn on Day−1 and became progressively less negative (more hydrated) over the course of the next six days (Table 2, Fig. 3a). However, branch water potential at midday (Ψmd) following the water pulse was consistently less negative in A. aptaneura (−1.8 MPa) compared to A. aneur and A. ayseniana (−2.5 MPa) (Table 2, Fig. 3b). The decrease in water potential between pre-dawn and midday was significant in all three phyllode types from Day−5 to Day−11 (ΔΨ, Table 2). A. aptaneura had the smallest change (0.85 MPa) compared to A. aneur (1.11 MPa) and A. ayseniana (1.39 MPa). ΔΨ values were larger than 1.10 MP before the water pulse in A. aneur and A. ayseniana; absolute values are unknown as Ψmd was beyond the measurement limits of the equipment (<−10 MPa; PMS Model 1000 pressure chamber). ΔΨ of A. aptaneura before the water pulse was 1.22 MPa.

Phyllode relative water content at pre-dawn was less than 55% at Day−11 for all species. However, A. aptaneura were significantly more hydrated (54.4 ± 2.55%) than A. ayseniana (42.7 ± 1.69%) or A. aneur (47.1 ± 1.50%) (P = 0.02, Table 1). By mid-day all phyllodes had dehydrated further and RWC was the same among all three species (43.4%, P > 0.05, Table 1). Following the water pulse, foliage in all three species had completely rehydrated at pre-dawn every day (96.7%), and did not change over the seven days (Table 2). RWC dropped to ~90% at midday each day, but was completely rehydrated again the following morning (Table 2). While the method employed to measure RWC may overestimate turgid weight (TW; see methods), we are confident that our measurements are accurate as RWC was very close to 100% following the water pulse.

3.2. Daily maximum stomatal conductance, gs max

Two weeks before the water pulse (Day-11), all trees had consistently low stomatal conductance (mean gs max = 21.5 mmol m⁻² s⁻¹, P = 0.12; Table 1). Stomatal conductance had increased sharply in all trees following the final day of the water pulse on Day-14. Stomatal conductance of the broader phyllode A. ayseniana (−150 mmol m⁻² s⁻¹) was greater than either A. aneur or A. aptaneura (−100 mmol m⁻² s⁻¹) for the seven days following the water pulse but did not differ between A. aneur and A. aptaneura (Fig. 3e, Table 2). Stomatal conductance in all trees continued to increase until reaching a peak at approximately four days after the end of the water pulse (Day-8) before gradually decreasing to rates equivalent to Day-5 (Fig. 3e, Table 2).

3.3. Patterns of water soluble and total phyllode δ¹³C

Phyllode δ¹³Ctotal values were similar among all three species at Day−11 (−27.2 ± 0.33‰, Table 1). δ¹³Ctotal did not change significantly during the following two weeks (−26.7 ± 0.38‰ on Day−3). However, δ¹³Ctotal was then rapidly diluted by −0.2% per day over a period of 7 days following the initiation of the water pulse, reaching −28.3 ± 0.33‰ on Day−11 (Table 2, Fig. 3c). In contrast, phyllode δ¹⁸O was consistent both before and after the water pulse (−27.0 ± 0.13‰, Table 1, Table 2). The δ¹³Ctotal was...
significantly less negative in A. aptaneura compared to either A. aneura or A. ayersiana for the seven days following the onset of the water pulse when analysed with a linear model. Neither $\delta^{13}$C$_\text{total}$ nor $\delta^{13}$C$_\text{soluble}$ were correlated with any of the other measured physiological parameters, including $g_s$ ($P > 0.05$).

4. Discussion

Our results demonstrate that the magnitude and rate of response of shallow-rooted, long-lived Acacia in the arid zone to a major pulse in water supply is large and rapid (i.e., within five
days). Branch water potential increased from −−8 MPa at pre-dawn to > −1 MPa, irrespective of species or differences in drought severity among trees (minimum branch water potential). Rapid rehydration coupled with increases in gs illustrates the central role which in turn generates a larger increase photosynthesis. Larger phyllodes transpire more water, and total or soluble fractions. When considered as a whole, our results over night causing low (i.e., two months of drought), trees are unable to fully rehydrate persist in regions where droughts can last for periods in excess of DJ.

Carbon fixation (measured as net ecosystem CO2 exchange) has been demonstrated to resume within three to five days after a rainfall pulse across a range of environments, including in a Mongolian Steppe (Wohlfahrt et al., 2008), a Mojave Desert shrubland, USA (Wohlfahrt et al., 2008) and semi-arid grasslands in Arizona, USA (Potts et al., 2006). However, while our study and others have shown a range of woody species can utilise the frequent and unpredictable rainfall inputs that characterise the semi-arid zone, the uniformity of response among species that we show here does not, on its own, provide evidence of the resource partitioning or niche differentiation that we had hypothesized might be facilitating the co-occurrence of these species.

While the response to rehydration was uniform and rapid among all trees, ΔΨ demonstrated key differences in rates of water use among species. The species with the most terete phyllodes, A. aptaneura, had consistently less negative Ψmd than either A. ayersiana (broad) or A. aneura (sub-terete) (Table 2). These differences among species (and phyllode types) are likely a consequence of a larger Hv and lower gs (Table 1) that translated into lower overall rates of water use. However, differences in ΔΨ following irrigation were not reflected by differences in δ13C (either total or soluble fractions). When considered as a whole, our results demonstrate a pulse response strategy in A. aneura, A. aptaneura and A. ayersiana that includes rapid rehydration that would also increase photosynthesis. Larger phyllodes transpire more water, which in turn generates a larger ΔΨ. Once soil water reserves are low (i.e., two months of drought), trees are unable to fully rehydrate overnight causing Ψpd to decrease. This response demonstrates these species are anisohydric and gas exchange is thus strongly coupled to soil water availability. However, it is unknown how these species respond to or withstand the longer droughts that are common in the semi-arid deserts of Australia. Mulga woodlands persist in regions where droughts can last for periods in excess of two years (Pritchard and Mills, 1986). It may be that pulse-response is delayed under more severe drought conditions, a scenario that is fascinating to consider given the already extreme values of minimum water potential (more negative than −10 MPa) recorded here. Furthermore, the differences in minimum water potential among species during the drought period prior to the water pulse suggest that long-term responses to drought may provide some explanation for the persistence of very similar species within the woodland.

The natural precipitation received at the woodland prior to the supplemental irrigation may have influenced the timing and thus overall pulse-response in this experiment. The magnitude of hydraulic response of vegetation to a rainfall pulse depends on both the size of the rainfall pulse as well as the length and severity of the preceding drought period (Resco et al., 2009; Shim et al., 2009). While does not on its own, provide evidence of the resource partitioning or niche differentiation that we had hypothesized might be facilitating the co-occurrence of these species.
role in the ability of A. aneura, A. aptaneura and A. ayersiana to rapidly rehydrate and resume physiological activity after withstanding prolonged periods of cellular dehydration. For example, cyclotols including D-Pinitol, have been identified as significant osmolytes that increase in concentration under drought stress in arid zone species of Acacia (Liu et al., 2008; Merchant et al., 2006), and A. aneura is also known to accumulate the glycone-betaine (Erskine and Pate, 1996), a methyl amine known to be an osmo-regulator. Further investigation of the role of compatible solutes in the growth and survival of arid Acacia spp. would elucidate the mechanisms that facilitate survival and rapid response in species exposed to severe cellular dehydration.

The remarkable magnitude and rate of recovery from very low (<−10 MPa) water potentials observed in these Acacia species highlights the urgent need for further investigation of the structure and function of the xylem pathway in long-lived woody species in the arid zone. There is currently a very active debate surrounding the mechanisms of embolism formation and repair in xylem (Trifilo et al., 2015; Wheeler et al., 2013; Zwieniecki and Holbrook, 2009). In particular, the mechanisms involved in refilling the xylem while adjacent vessels remain under negative tension has long posed a methodological challenge. We did not measure hydraulic conductivity at this experiment, although speculate that even in these Acacia species with very dense wood, there would be a significant reduction in hydraulic conductivity at the extremely negative water potentials measured during the dry period (<−10 MPa). Therefore, future investigation of the dynamics of embolism formation and repair in these species when exposed to natural cycles of drought and rehydration following pulse rainfall will help to greatly inform the debate regarding the role of xylem structure and function in either resistance to, or tolerance and repair of, embolism formation under drought stress.

Acknowledgements

We gratefully acknowledge project funding and logistical assistance from Rio Tinto Pty Ltd, in particular the efforts of Sally Madden and Neville Havelberg. Field and laboratory technical assistance was provided by members of the Ecosystems Research Group at The University of Western Australia, particularly Kaitlyn Height and Chloe Flaherty. Stable isotope analysis was conducted by Grzegorz Skrzypek and Douglas Ford at the West Australian Biogeochemistry Centre. Thanks to David Williams, Derek Eamus and Rod Fensham for comments on an early draft. We also thank two anonymous reviewers for helping to improve the quality this paper.

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