Contrasting regulation of leaf gas exchange of semi-arid tree species under repeated drought

Tonantzin Tarin[1,][*] , Derek Eamus[2], Nadia S. Santini[3], Rachael H. Nolan[2,4]

[1]Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City 04510, Mexico
[2]Terrestrial Ecohydrology Research Group, School of Life Sciences, University of Technology Sydney, Sydney, NSW 2007, Australia
[3]Instituto de Geología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Mexico City, 04510, Mexico
[4]Hawkesbury Institute for the Environment, Western Sydney University, Science Rd. Penrith, NSW 2751, Australia

*Corresponding author (ttarin@iecologia.unam.mx)

Handling Editor: Maurizio Mencuccini

Predicting how plants respond to drought requires an understanding of how physiological mechanisms and drought response strategies occur, as these strategies underlie rates of gas exchange and productivity. We assessed the response of 11 plant traits to repeated experimental droughts in four co-occurring species of central Australia. The main goals of this study were to: (i) compare the response to drought between species; (ii) evaluate whether plants acclimated to repeated drought; and (iii) examine the degree of recovery in leaf gas exchange after cessation of drought. Our four species of study were two tree species and two shrub species, which field studies have shown to occupy different ecohydrological niches. The two tree species (*Eucalyptus camaldulensis Dehnh.* and *Corymbia opaca* (*D.J.Carr & S.G.M.Carr*) *K.D.Hill & L.A.S.Johnson*) had large reductions in stomatal conductance (g_s) values, declining by 90% in the second drought. By contrast, the shrub species (*Acacia aptaneura Maslin & J.E.Reid* and *Hakea macrocarpa A.Cunn. ex R.Br.*) had smaller reductions g_s in the second drought of 52 and 65%, respectively. Only *A. aptaneura* showed a physiological acclimation to drought due to small declines in g_s versus ψ_{pd} (0.08 slope) during repeated droughts, meaning they maintained higher rates of g_s compared with plants that only experienced one final drought (0.19 slope). All species in all treatments rapidly recovered leaf gas exchange and leaf mass per area following drought, displaying physiological plasticity to drought exposure. This research refines our understanding of plant physiological responses to recurrent water stress, which has implications for modelling of vegetation, carbon assimilation and water use in semi-arid environments under drought.

Graphical Abstract
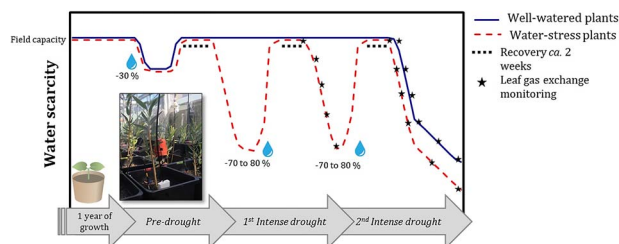
Key words: *Acacia*, Australia, carbon flux, photosynthesis, stomatal conductance, water-use efficiency, water-use strategy.

Introduction

Drought is globally the most widespread climate extreme, with a large influence on the plant carbon cycle (Reichstein et al. 2014, Frank et al. 2015). The Southern Hemisphere experienced a reduction in net primary production (NPP, the difference between photosynthesis and plant respiration), with reduced NPP across 70% of the vegetated land areas due to drought over 2000–2009 (Chapin et al. 2006, Zhao and Running 2010). Globally, a large loss in NPP is projected by the end of the 21st century as a consequence of drought (Cao et al. 2022). However, our understanding of how photosynthesis and respiration respond to drought, notably repeated drought, remains limited. This knowledge gap is especially pronounced for species inhabiting arid and semi-arid ecosystems, which are presumed to be adapted to dry conditions, and therefore drought.

Plants exhibit varied strategies to respond to drought, including differing responses in gas exchange (Peñuelas et al. 2001, Adams et al. 2009, Allen et al. 2010). However, drought studies seldom explore how plants respond to repeated droughts, and whether acclimation to drought may alter gas exchange during and following release from drought (Vandegeer et al. 2020). There are several ways to assess acclimation responses by plants to drought: (i) to compare the response from an initial drought to a second drought of the same plants or (ii) comparing plants exposed to a single drought versus species with well-watered conditions (Lemoine et al. 2018). Testing various ways plants

respond to droughts is laborious and limited to experimental studies.

In theory, stomata close in response to both declining soil water availability and increasing atmospheric vapour pressure deficits (VPD; Duursma et al. 2014, Sperry and Love 2015). Stomatal closure regulates leaf water potential (ψ), preventing rapid declines in ψ and subsequent cavitation, which can lead to plant death (McDowell et al. 2022). There is a continuum of water-use strategies with two extreme states: isohydric and anisohydric (Martinez-Vilalta et al. 2014). Isohydric species use rapid and early declines in stomatal conductance (g_s) to tightly regulate ψ in the early stages of drought. Anisohydric species tolerate a larger decline of ψ, thereby allowing the maintenance of g_s further into drought (Martinez-Vilalta et al. 2014). In reality, applying the iso/anisohydric paradigm is challenging because most plants fall somewhere between the two behaviours. Furthermore, plant water-use strategies can be dynamic and defined by multiple traits (i.e., hydroscapes; Kannenberg et al. 2022).

An important trait that characterizes plant water-use strategies is water-use efficiency (WUE). WUE describes the trade-off between carbon gain and water loss that occurs when plants photosynthesize. Intrinsic WUE (WUE$_i$) can be defined as the ratio of net photosynthetic assimilation (A_n) to g_s. WUE$_i$ responds to (i) stomatal closure and (ii) photosynthesis through changes in photosynthetic parameters such as the maximum rate of carboxylation (V_{cmax}; maximum rate of the Rubisco activity) and the maximum rate of electron transport (J_{max}) (Flexas et al. 2006, Galmes et al. 2007, Sperry and Love 2015). Rates of V_{cmax} and J_{max} provide important insights into plant functioning, in which the Rubisco enzyme and chlorophyll are responsible for A_n in C$_3$ plants (Cernusak et al. 2011). Typically, values of V_{cmax} are higher when water is more readily available than under drought (Zhou et al. 2016); by contrast, values are reduced during short-term water stress. V_{cmax} can maintain the same values during drought if species can acclimate to drought by modifying the Rubisco activity, thus leading to a higher protein content allocated to the Rubisco (Zhou et al. 2016).

Reductions in A_n due to drought affect the whole leaf carbon balance, changing the respiratory loss of CO_2 by plants that account for up to 30–80% of the daily carbon uptake, realized as dark respiration (R_d) (Gimeno et al. 2010, Gauthier et al. 2014). Reductions in R_d usually occur to a lesser extent than reductions in A_n. In addition to physiological responses to drought, plants can also exhibit morphological responses to drought. Leaf mass per area (LMA) is one such trait. High LMA relates to leaf toughness, less air space, high density and packed cells, hence leaf and plant survival (Poorter et al. 2009), and nitrogen content is generally higher in leaves with high LMA (Dong et al. 2022). Therefore, LMA can be an adaptive response to prolonged water stress in plants.

The aim of this study was to compare the responses of key leaf physiological and morphological traits during repeated droughts in diverse co-occurring semi-arid species. We chose four species that represent the main tree genera within central Australia: *Eucalyptus*, *Corymbia*, *Acacia* and *Hakea*. These genera exhibit evergreen sclerophyllous foliage and a set of different hydraulic traits (O'Grady et al. 2009, Santini et al. 2015, Nolan et al. 2017a). *Eucalyptus* and *Corymbia* are members of the Myrtaceae family which are tall, deep-rooted trees and are known to access deep soil water content (SWC) and groundwater in drylands (O'Grady et al. 2006, Rumman

et al. 2018). This means they may rarely experience extremes of low soil moisture content. In contrast, *Acacia* spp., especially those from the Mulga complex, which dominate ~20 to 25% of the semi-arid Australian continent, are highly tolerant to very low soil moisture content (Page et al. 2011, Eamus et al. 2013, Cleverly et al. 2016). *Acacia* spp. and *Hakea* spp. are shrubs that have shallow root systems, possessing specific hydraulic traits, including narrow xylem vessels and small diameter roots, to withstand low water availability (Lamont 1993, Groom et al. 1994, Page et al. 2011, Nolan et al. 2017b). In particular, *Hakea* have been widely observed to generate root clusters. Root clusters play a beneficial role as they effectively increase the surface area available for water uptake (Lamont 2003).

We hypothesized the following: (i) species with a faster growth rate (i.e., Myrtaceae) will have larger declines in A_n and g_s during the development of drought, than species with lower growth rates (i.e., *Acacia* and *Hakea* spp.). (ii) Within species, plants that have experienced drought previously will be less sensitive to subsequent droughts. The sensitivity or acclimatation to drought will be observed as smaller reductions of A_n and g_s as leaf water potential declines during repeated droughts. (iii) The degree of recovery in leaf-scale gas exchange variables (especially A_n and g_s) after repeated droughts will be larger in the Mulga and *Hakea* species than in *Eucalyptus* and *Corymbia* species because of the range of drought-resistant traits exhibited in the former species but not the latter two species.

Materials and methods
Plant taxa and plant growth conditions
Seedlings of *Acacia aptaneura* Maslin & J.E.Reid (also known as Mulga), *Corymbia opaca* (D.J.Carr & S.G.M.Carr) K.D.Hill & L.A.S.Johnson, *Eucalyptus camaldulensis* Dehnh. var. *obtusa* and *Hakea macrocarpa* A.Cunn. ex R.Br. were germinated from seeds in the winter of 2015 in a glasshouse at the University of Technology Sydney, Sydney, Australia. Seeds were obtained from Nindethana Seed Service (Albany, Western Australia), which had been collected from wild populations. These species were selected due to their dominance in semi-arid central Australia (Maslin and Reid 2012, Eamus et al. 2013, Cleverly et al. 2016), due to their representativeness within two important biomes, the Mulga woodland and *Corymbia* savanna (Tarin et al. 2019, 2020), and because of their contrasting functional traits (Table 1). A pre-treatment with boiling water was applied to *A. aptaneura* seeds to break seed dormancy. Around 20 seeds per species were placed in Petri dishes with 4–8% agar and located in a glasshouse at ~25 °C until germination occurred (ca 3–30 days). Petri dishes were placed within a glass cabinet with a sunlit polythene-covered lid that transmitted 70% of sunlight. Seedlings were transplanted from the agar plates to trays (20 × 30 cm) with the same reduced sunlight until they were >5 cm tall. These trays were filled with sterilized soil to prevent contamination from microbes/fungi. Once seedlings were >10 cm tall, they were planted in 18 L pots (one plant per pot) with a soil mix consisting of 50% native mix with low-phosphorus (Greenlife Native Mix) and 50% river sand. We selected pots of this size to maximum the potential area available for root exploration. Nevertheless, we acknowledge that potted plant studies may limit the potential size of roots. High SWC was maintained until initiation of

Table 1. Plant species description from the literature. Note *A. aptaneura* was previously named *A. aneura* (Maslin and Reid 2012).

Family/ species	Plant functional type	Life form	Leaf morphology	Xylem attributes	Rooting attributes	References
Myrtaceae/ *Eucalyptus camaldulensis*	Riparian evergreen, sclerophyll angiosperm tree	Tree >15 m	Broad leaves	Low wood density	Deep-rooted	Santini et al. (2015)
Myrtaceae/ *Corymbia opaca*	Savanna, evergreen angiosperm tree	Tree >15 m	Broad leaves	Large hydraulic conductivity, relatively low wood density	Deep-rooted	Santini et al. (2015) O'Grady et al. (2006, 2009)
Proteaceae/ *Hakea macrocarpa*	Evergreen, angiosperm tree	Shrub 1–3 m	Broad and terete leaves	Low wood density	Strongly dimorphic	Groom et al. (1994)
Fabaceae/ *Acacia aptaneura*	Evergreen, angiosperm tree	As shrub <2 m As tree from 2 to 15 m	Evergreen phyllodes with high leaf density and thickness	Complex xylem vessel network with small vessels size Large wood density with low hydraulic conductivity	Shallow root system with taproot and feeder roots N_2-fixing species	Page et al. (2011) Santini et al. (2015) O'Grady et al. (2009)
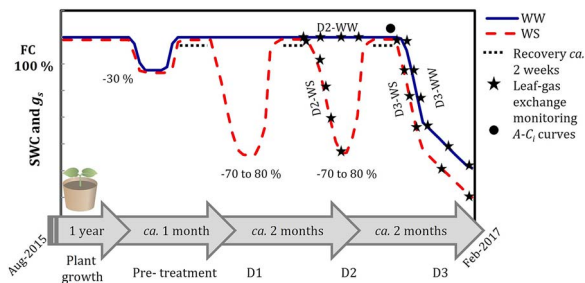
Figure 1. Drought experiment design for well-watered (WW) and water-stressed (WS) treatments. Stomatal conductance (g_s) and SWC were repeatedly monitored during the experiment after the first year of plant growth. Percentages indicate the decline in g_s of the maximum. Experimental droughts are indicated by D1, D2 and D3.

the drought treatments when seedlings were >12 months old. Pots were irrigated with an automated drip water system every 2 days. Environmental conditions were natural, where daily air temperature ranged from 13 to 38 °C, and daytime VPD (D) was on average ca 1.9 kPa (Nolan et al. 2017c).

Experimental design

This study consisted of three repeated droughts to test the effect of pre-exposure to drought (Nolan et al. 2017c). The drought-imposed experiment had eight replicates per treatment (well-watered: WW and water-stressed: WS) per species. Plants were assigned randomly to either WS or WW treatments to avoid differences in plant height across treatments. Prior to the imposition of the three experimental droughts, a pre-treatment mild water stress was imposed to all species and all individuals during July–August 2016 (Figure 1), as described in Nolan et al. (2017c). Application of mild water stress is frequently applied to seedlings in nurseries to 'drought harden' species, i.e., make them more resistant to drought, and subsequently decrease seedling mortality rates (Landis 1999). This mild water stress was imposed by completely ceasing irrigation until g_s dropped by ~30% of maximal g_s and there were concomitant declines in SWC with respect to field capacity (FC).

Following the drought pre-treatment, three sequential experimental droughts (D1, D2 and D3) were applied to all individuals (including WW individuals but only for D3) between September 2016 and February 2017 (the Austral spring/summer season, Figure 1). SWC, measured gravimetrically, and g_s were monitored for all plants. Droughts D1 and D2 were stopped when g_s was ~20 to 30% of g_s control values (Figure 1). The length of each drought varied among species, due to differences in the rate of decline in g_s. The shortest drought events were imposed for *E. camaldulensis* (21–22 days), and the longest drought was imposed for *C. opaca* (37–38 days). Drought length for *A. aptaneura* and *H. macrocarpa* was 26–31 days. During the entire ~5 month experiment, pots were repeatedly moved every ~3 weeks to reduce the impact of any variation in micro-climate across benches within the glasshouse.

Leaf gas exchange

Leaf gas exchange measurements were made with an infrared gas analyser (IRGA) system (Li-6400XT, Li-Cor Inc., Lincoln, NE, USA) coupled to a 2 × 3 cm broadleaf chamber (6400-02B LED Light Source; Li-Cor Inc.). Two leaves per individual were measured on five replicate plants per treatment per species during the second and third experimental droughts every 2 days. Leaf dark respiration (R_d) was measured during drought D3 on a different leaf each time; this was one leaf per plant (n = 4) per treatment. Leaves for R_d were wrapped in aluminium foil prior to sunrise. Gas exchange measurements, including R_d, were made between 09:00 and 11:00 h; each measurement took between 5 and 10 min, until stomatal conductance approached steady-state conditions. Temperature was maintained on average at 33 ± 2.5 °C during measurements. CO_2 concentration was set at 400 p.p.m. inside of the leaf chamber, and photosynthetic photon flux density (PPFD) was 1500 μmol m^{-2} s^{-1} and 0 μmol m^{-2} s^{-1} when measuring R_d. Projected leaf area was measured using an image analysis system (WinDIAS 3, Delta-T Devices, Cambridge, UK), and leaf gas exchange values were area-corrected.

Photosynthetic responses to sub-stomatal CO_2 concentration (A–C_i curves) measurements were made before the start

of drought D3, once plants had recovered from the previous drought, usually within 1 day (D2) after FC was applied to the soil. This was additionally confirmed when g_s was measured and compared with previous g_s measurements at FC (Figure 1). CO_2 concentrations in the leaf cuvette were set at 400, 200, 100, 50, 40, 400, 400, 500, 600, 800, 1000, 1200, 1400 and 1700 p.p.m. ($n = 4$ per species, per treatment) using a 3-min time step between measurements. The plantecophys R package (Duursma 2015) was used in R 3.2.1 (R Development and Core Team, 2016) to estimate J_{max} and V_{cmax} from the fitted A–C_i curves using the Farquhar et al. (1980) model. Leaf temperature was corrected to 25 °C, and D was held as constant as possible during the measurements at 2.3 ± 0.8 kPa.

Plant water status

Pre-dawn leaf water potential (ψ_{pd}; in negative values) was measured on the same days as leaf gas exchange measurements. Leaves/phyllodes were selected randomly before sunrise between 05:00 and 06:00 h ($n = 4$ per species per treatment). Excised leaves were immediately placed in Ziploc bags and sealed and transported to the laboratory in an insulated cooler, and their water potential was measured within the following hour in a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA).

Gas exchange sensitivity to water stress

To estimate the sensitivity of A_n and g_s rates to drought, the value of SWC at 50% loss of leaf gas exchange was calculated following the method of Domec and Gartner (2001). A_n and g_s were normalized to 100% using maximum values. To fit the leaf gas exchange curves as SWC declined, the following Weibull function was used:

$$y \left(\% \text{ of max } g_s \text{ or } A_n \right) = 100 - \left(100/(1 + e^{(a*(\text{SWC}-b))}) \right) \quad (1)$$

where y is either A_n or g_s (normalized values), a describes the slope of the curve and b is the SWC at 50% of A_n or g_s (A_{n50} and g_{s50}, respectively).

LMA and plant growth

Leaf mass per area (LMA: g m^{-2}) was calculated by assessing four individuals ($\sim$10 leaves each) per species of the same individuals for all previous measurements (leaf gas exchange and leaf water potentials). Leaf areas of fresh leaves were measured in a leaf area scanner (WinDIAS 3, Delta-T Devices, Cambridge, UK). The same leaves were then oven-dried at 65 °C and dry weights were obtained after 72 h. Additionally, plant growth was monitored at the start of each experimental drought (from D1 to D3) by measuring the number of leaves, stem diameter and plant height.

Statistical analyses

All statistical analyses were undertaken using the R 3.1.1 Project software (R Development Core Team 2016). We used simple averages for leaf gas exchange measurements that were taken in two leaves per individual. To test plant recovery and differences between species in leaf gas exchange variables and plant growth, the interaction term species × treatment was tested using two-way analysis of variance (ANOVA). Tukey's HSD post-hoc tests were applied to test for significant differences between species. Linear regressions were applied per species to determine the relationship of: ψ_{pd} between A_n and g_s. Differences in slopes were tested with two approaches

separately: (i) for every treatment between species and (ii) within species between treatments. The first approach allowed us to differentiate rates in declining leaf gas exchange variables as drought progressed between species within the same treatment, either WW or WS across droughts. The second approach allowed us to differentiate changes in rates with repeated droughts, for example, to compare plants from D2 versus plants from D3 within species. Slope differences were tested using a standardized major axis method in the SMATR package in R and multiple comparison pair-wise test comparisons among species (Warton et al. 2012). A nonlinear least squares 'nls' function in R was used to evaluate the response of g_s and A_n to SWC.

Results

Plant growth

Plant height ranged between 30 and >100 cm (Figure 2). Throughout the experiment, *E. camaldulensis* was the tallest species, followed by *A. aptaneura* (Figure 2a; $P < 0.05$; see Table S1 available as Supplementary data at *Tree Physiology* Online), while *C. opaca* was the smallest (30–60 cm) species after 1 year of growth. Within species, there were significant differences between treatments in height and diameter values ($P < 0.05$, see Table S1 available as Supplementary data at *Tree Physiology* Online).

There was a considerable reduction in the numbers of leaves for the WS and WW in *E. camaldulensis* during D3 ($P < 0.05$). Stem diameter was considerably large in *E. camaldulensis* during the entire experiment (4–8 mm) but was similar within the remaining three species (2–5 mm) for both treatments WS and WW.

Temporal trends in leaf gas exchange

In all species, g_s and A_n decreased as drought progressed (Figure 3). Among species there was a large range of values of g_s (0.02–1.3 mol m^{-2} s^{-1}; Figure 3a–d), A_n (0.84–35.32 μmol m^{-2} s^{-1}; Figure 3e–h) and WUE$_i$ (8–380 μmol mol^{-1}) during the drought experiments of D2-WS, D3-WS and D3-WW. During drought, the largest rates of g_s were observed in *E. camaldulensis* with 1.07 and 0.80 mol m^{-2} s^{-1} and *C. opaca* with 1.26 and 0.86 mol m^{-2} s^{-1} in droughts D2 and D3, respectively. In contrast, the largest A_n, hence the largest maximum assimilation rate was observed in *A. aptaneura* (32.32 μmol m^{-2} s^{-1}) with minimal differences across the remaining three species (A_n = 28.2, 26.6 and 22.5 μmol m^{-2} s^{-1} for *H. macrocarpa*, *C. opaca* and *E. camaldulensis*, respectively).

Generally, across all four species, WUE$_i$ showed varied responses as drought progressed (Figure 3i and l). WUE$_i$ increased in some, but not all, treatments, and this was most pronounced in *A. aptaneura* and *E. camaldulensis* (Figure 3i and l). In *C. opaca* and *E. camaldulensis*, moderate reductions in SWC, from 0.2 to 0.1 m^3 m^{-3}, resulted in increased WUE$_i$ but further reductions in SWC decreased WUE$_i$ (Figure S1k and l available as Supplementary data at *Tree Physiology* Online).

SWC values associated with a 50% loss in gas exchange

With the normalized values of g_s and A_n (normalized to 100% using maximum values of g_s and A_n at high SWC), SWC values associated with a 50% loss in g_s and A_n were
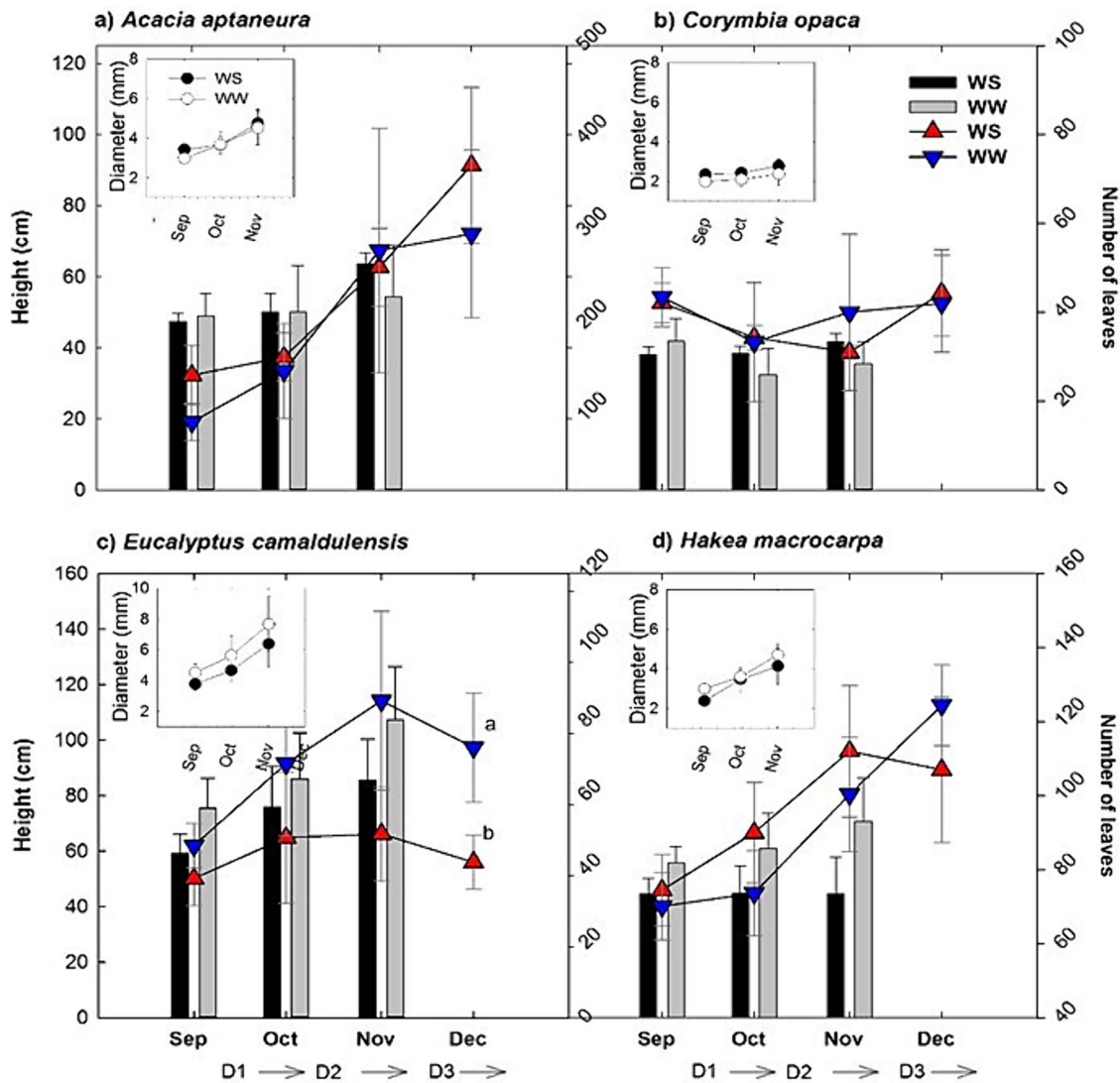
Figure 2. Plant growth during drought. Plant height (bars), number of leaves (triangles) and stem diameter (circles) changes for well-watered (WW) and water-stressed (WS) treatments during September–December 2016 after 1 year of growth. *X*-axes also indicate estimated starts of each experimental drought (D1, D2, D3; dates vary for each species). (a and b) *A. aptaneura*, (c and d) *Corymbia opaca*, (e and f) *Eucalyptus camaldulensis* and (g and h) *Hakea macrocarpa*. Error bars represent ±1 SE (*n* = 4).

calculated (Figures S1 and S2 available as Supplementary data at *Tree Physiology* Online). During D2, values of SWC associated with a 50% of loss in g_s (g_{s50}) and A_n (A_{n50}) were larger in *H. macrocarpa* (0.15 m^3 m^{-3}) and *C. opaca* (0.13 m^3 m^{-3}) than *E. camaldulensis* (0.08 m^3 m^{-3}) and *A. aptaneura* (0.07 m^3 m^{-3}). In *H. macrocarpa* g_{s50} and A_{n50} were twice as large for the D2-WS (0.15 m^3 m^{-3}) as for the D3-WS (0.07 m^3 m^{-3}). For *E. camaldulensis* plants subject to repeated water stress, there was a 10% decline in the value of SWC associated with a 50% decline in g_{s50} and A_{n50} between the second and third droughts (from 0.08 to 0.07 m^3 m^{-3}). In contrast, for *C. opaca* plants subject to repeated water stress, there was an increase in the value of SWC associated with a 50% decline in g_{s50} and A_{n50}, from 0.13 to 0.18 m^3 m^{-3}. *Acacia aptaneura* did not show changes in either g_{s50} or A_{n50} (ca 0.07 m^3 m^{-3}).

Across all species in the final drought, the value of SWC associated with a 50% decline in gas exchange values was either similar or lower for the plants subject to repeated water stress (Figures S1 and S2 available as Supplementary data

at *Tree Physiology* Online). The largest difference of g_{s50} between D3-WW (0.12 m^3 m^{-3}) and D3-WS (0.07 m^3 m^{-3}) was observed in *E. camaldulensis*, followed for *H. macrocarpa* and *A. aptaneura*, with no changes in *C. opaca* (0.18 m^3 m^{-3}).

Gas exchange following release from drought

To compare leaf gas exchange among species following recovery and under moist conditions (during the last recovery period after D2; see Figure 1), averages of g_s, A_n and WUE$_i$ were calculated separately for the four species per treatment (Table 2). Contrasting gas exchange values were observed among species, yet the interaction species × treatment was not significantly different (*P* > 0.05) for g_s, A_n, R_d/A_n and WUE$_i$ (Table S1 available as Supplementary data at *Tree Physiology* Online).

The most water-use efficient plants were *H. macrocarpa* (88.8 ± 9.0 μmol mol^{-1}), *A. aptaneura* (82.9 ± 4.6 μmol mol^{-1}) and *C. opaca* (80.7 ± 7.8 μmol mol^{-1}), all in the WS treatment. However, *A. aptaneura* had low WUE$_i$ in the WW treatment (64.1 ± 4.6 μmol mol^{-1}) and was not
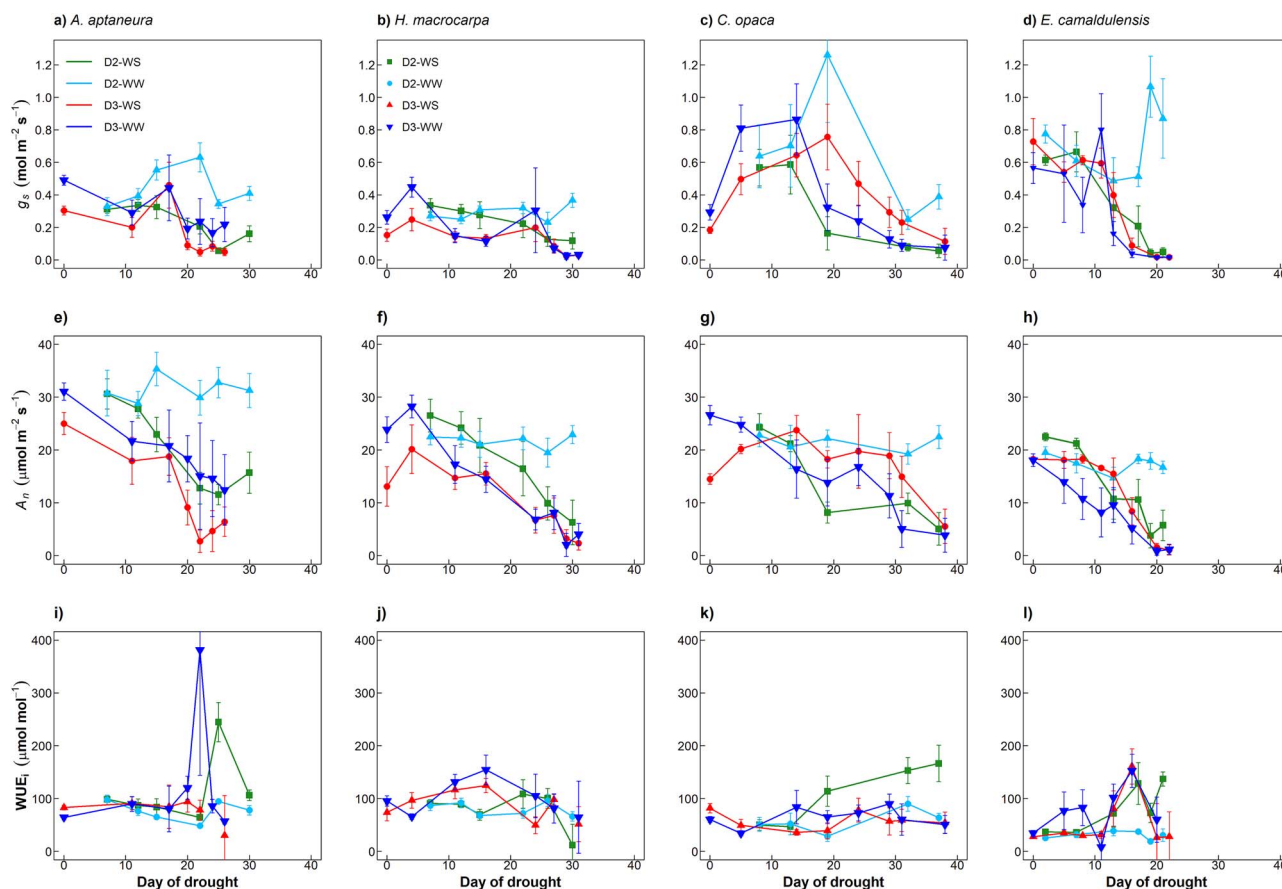
Figure 3. Effect of drought on leaf gas exchange: Stomatal conductance (g_s; a–d), net assimilation (A_n; e–h) and intrinsic water-use-efficiency (WUE$_i$; i–l). Treatments are well-watered (WW) and water-stress (WS) for 2nd and 3rd droughts (D2 and D3; see Figure 1). Error bars represent ±1 SE. Data points are the means of measurements within each treatment ($n = 5$).

Table 2. Summary of plant species comparisons of mean values of leaf mass per area (LMA; g m^{-2}), net assimilation (A_n; μmol m^{-2} s^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}), intrinsic water-use efficiency (WUE$_i$; μmol mol^{-1}), the ratio of night-time respiration to daytime net assimilation (R_d/A_n), velocities of carboxylase (V_{cmax}; maximum rate of the rubisco activity; μmol m^{-2} s^{-1}) and the maximum rate of electron transport (J_{max}; μmol m^{-2} s^{-1}) for each treatment: Well-watered (WW) and water-stressed (WS). Parameters such as A_n, g_s, WUE$_i$, LMA, V_{cmax} and J_{max} correspond to the last recovery period of the experiment. R_d/A_n ratio values were averaged from different point measurements during the third drought per treatment (WS and WS). Errors represent ±1 SE ($n = 5$ and 4 for V_{cmax} and J_{max}). Within columns, different letters indicate that means were significantly different at $P < 0.05$ among species with two-way ANOVAs and the iteration species × treatment (Table S1 available as Supplementary data at *Tree Physiology* Online); the Tukey's HSD post-hoc tests for significant differences was applied for differences between species.

| Species	Treatment	LMA	A_n	R_d/A_n	g_s	WUE$_i$	V_{cmax}	J_{max}
A. aptaneura	WS	543 ± 38.6a	25.0 ± 2.1a	0.10 ± 0.04b	0.30 ± 0.03b	82.9 ± 4.6b	85 ± 14.7b	287 ± 53.4c
	WW	385 ± 93.3a	31.0 ± 1.2a	0.10 ± 0.3b	0.49 ± 0.03b	64.1 ± 4.6b	95 ± 7.8b	283.4 ± 31.7c
C. opaca	WS	286 ± 34.1bc	14.5 ± 1.0b	0.18 ± 0.07b	0.18 ± 0.01b	80.7 ± 7.8b	110 ± 3.4b	–
	WW	220 ± 53.2bc	16.5 ± 2.4b	0.21 ± 0.07b	0.28 ± 0.06b	63.5 ± 6.3b	91 ± 6.5b	294 ± 14bc
E. camaldulensis	WS	135 ± 16.7b	18.3 ± 0.4b	0.07 ± 0.01b	0.73 ± 0.06a	27.9 ± 3.9a	68 ± 2.4a	137 ± 13.7bc
	WW	130 ± 10.6b	18.1 ± 1.2b	0.12 ± 0.03b	0.57 ± 0.10a	34.7 ± 5.0a	60 ± 7.5a	119 ± 8.5b
H. macrocarpa	WS	498 ± 54.2ac	16.7 ± 4.3b	0.83 ± 0.22a	0.18 ± 0.03b	88.0 ± 8.5b	76 ± 5.7ab	180 ± 29.5bc
	WW	285 ± 52.5ac	19.8 ± 2.8b	0.52 ± 0.14a	0.23 ± 0.03b	88.9 ± 9.0b	86 ± 5.7ab	254 ± 23.2bc

significantly different from the WUE$_i$ of *C. opaca* WW ($63.5 \pm 6.3\ \mu$mol mol^{-1}). The lowest WUE$_i$ was for *E. camaldulensis* ($27.9 \pm 3.9\ \mu$mol mol^{-1} and $34.7 \pm 5.0\ \mu$mol mol^{-1}) in both WS and WW, respectively.

After the second drought, R_d/A_n was largest in *H. macrocarpa* (WS = 0.83 ± 0.22 and WW = 0.52 ± 0.14) and significantly different from the remaining three species (Table 2). The smallest ratio was observed in *A. aptaneura* (WS = 0.10 ± 0.04 and WW = 0.10 ± 0.3) and *E. camaldulensis* (WS = 0.07 ± 0.01 and WW = 0.12 ± 0.03).

For WW treatment, *H. macrocarpa* also had the largest R_d/A_n value and was statically different from *E. camaldulensis*, *C. opaca* and *A. aptaneura*.

Plant sensitivity to leaf pre-dawn water potentials

Variations in g_s and A_n (log10 transformed) were linearly correlated with ψ_{pd} in all four species (Figures 4 and 5 for g_s and A_n, respectively). In D2 imposed on the WS treatment plants (D2-WS), *E. camaldulensis* showed the largest slope (m) in g_s ($m = 0.50$, Figure 4c and Table 3) and A_n
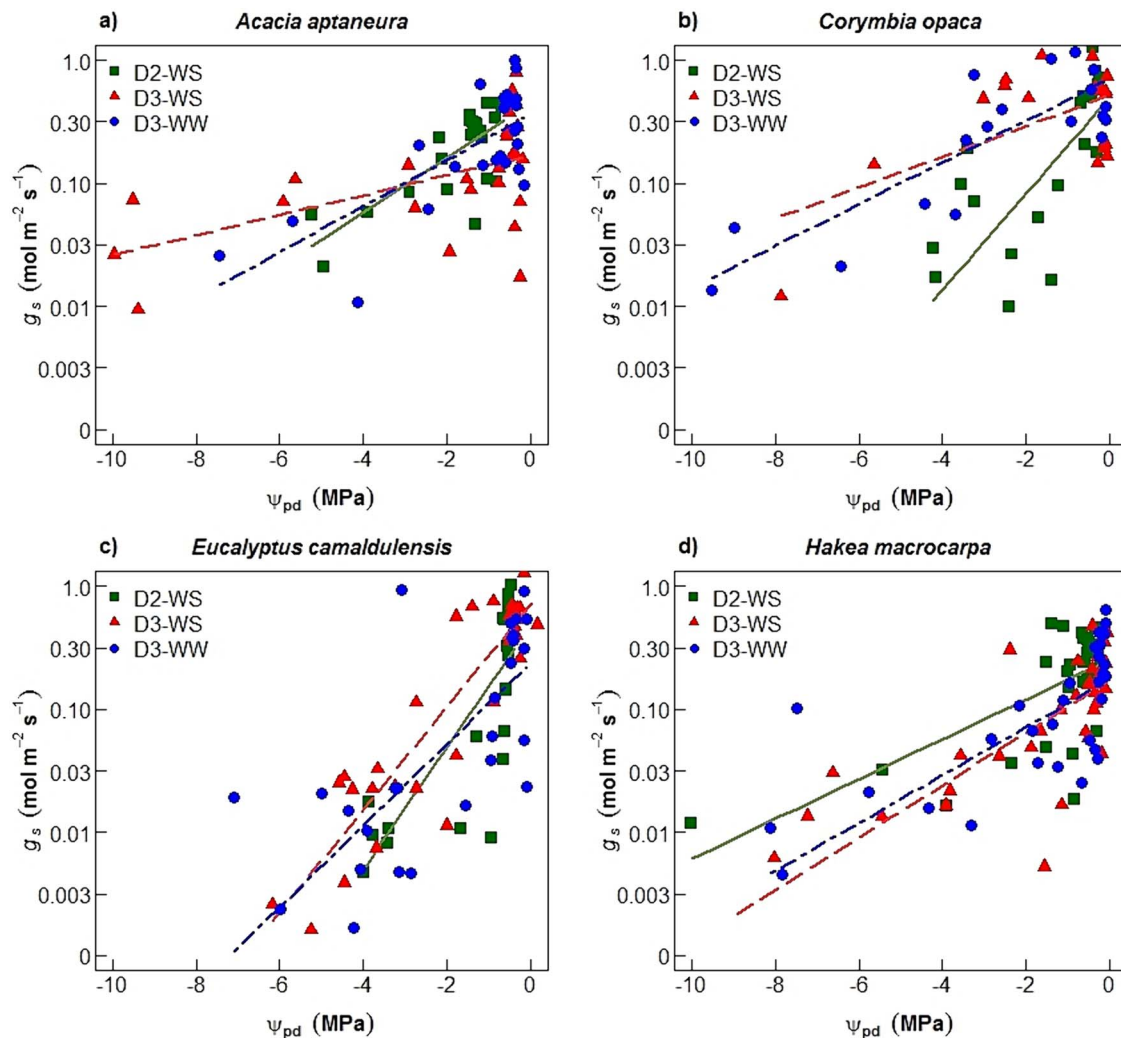
Figure 4. Stomatal conductance (log10(g_s) scale; Mol m^{-2} s^{-1}) versus leaf pre-dawn water potentials (ψ_{pd}) for all four species (a–d). Symbols indicate treatments as: squares for 2nd drought (D2-WS; water-stressed treatment), triangles for 3rd drought (D3-WS) and circles for D3-WW (well-watered treatment). Regression line colour corresponds to treatment symbol colour. Regression coefficients are shown in Table 3.

(m = 0.40, Figure 5c), followed by *C. opaca* for g_s (m = 0.39, Figure 4b) and *H. macrocarpa* for A_n (m = 0.26, Figure 5b). *Hakea macrocarpa* had the smallest slopes for g_s (m = 0.16; Figure 4d) in D2-WS and *A. aptaneura* for A_n (m = 0.15; Figure 5a) in D2-WS.

First, we tested plant response to drought by comparing D2-WS (second drought to same plants; see Figure 1) with D3-WS (third drought to same plants). Results showed a decrease in the slopes of the response of g_s to ψ_{pd} of 18, 64 and 68% for *E. camaldulensis*, *A. aptaneura* and *C. opaca*, respectively, from D2-WS to D3-WS (Table 3). In contrast, an increase was observed in the slope (of g_s to ψ_{pd}) of 31% in *H. macrocarpa* (Figure 4d). The slope of the regression of A_n versus ψ_{pd} plotted on a semi-log plot significantly decreased between D2-WS and D3-WS treatment for all species except *H. macrocarpa* (m = 0.26 and m = 0.25, D2-WS and D3-WS, respectively; Table 3).

Second, we tested plant response to drought by comparing D3-WS (three droughts, same plants; see Figure 1) with D3-WW (plant that only experienced one drought at the end of the experiment). Differences between the treatments (D3-WS and D3-WW) during the final drought were observed in *A. aptaneura* and *E. camaldulensis*. There were significant

differences in slopes only in *A. aptaneura* for the plants that experienced three droughts (D3-WS) and plants that experienced only the final drought (D3-WW; Table 3). In *A. aptaneura*, the slope of D3-WS (m = 0.08) was 58% smaller than that of D3-WW (m = 0.19). The slope (A_n versus ψ_{pd}) in *A. aptaneura* of D3-WS (m = 0.09; repeated drought) was 36% smaller than D3-WW (m = 0.14), whereas in *E. camaldulensis* the difference was 31% smaller in D3-WS (m = 0.22) compared with the D3-WW (0.32) treatment (Table 3). *Corymbia opaca* did not show significant differences between slopes (D3-WS and D3-WW).

Responses of V_{cmax}, J_{max} and LMA to drought

Mean values for rates of carboxylation (V_{cmax}; μmol m^{-2} s^{-1}) and the maximum rate of electron transport (J_{max}; μmol m^{-2} s^{-1}) estimated from A–C_i curves are presented in Table 2 (see also for A–C_i curves Figure 6). The interaction species × treatments were not significantly different for V_{cmax} (P > 0.05), but they were for J_{max} (Table S1 available as Supplementary data at *Tree Physiology* Online).

Acacia aptaneura and *E. camaldulensis* had the smallest differences in J_{max} between treatments. The largest difference between treatments was in *H. macrocarpa* (180 ± 29.5
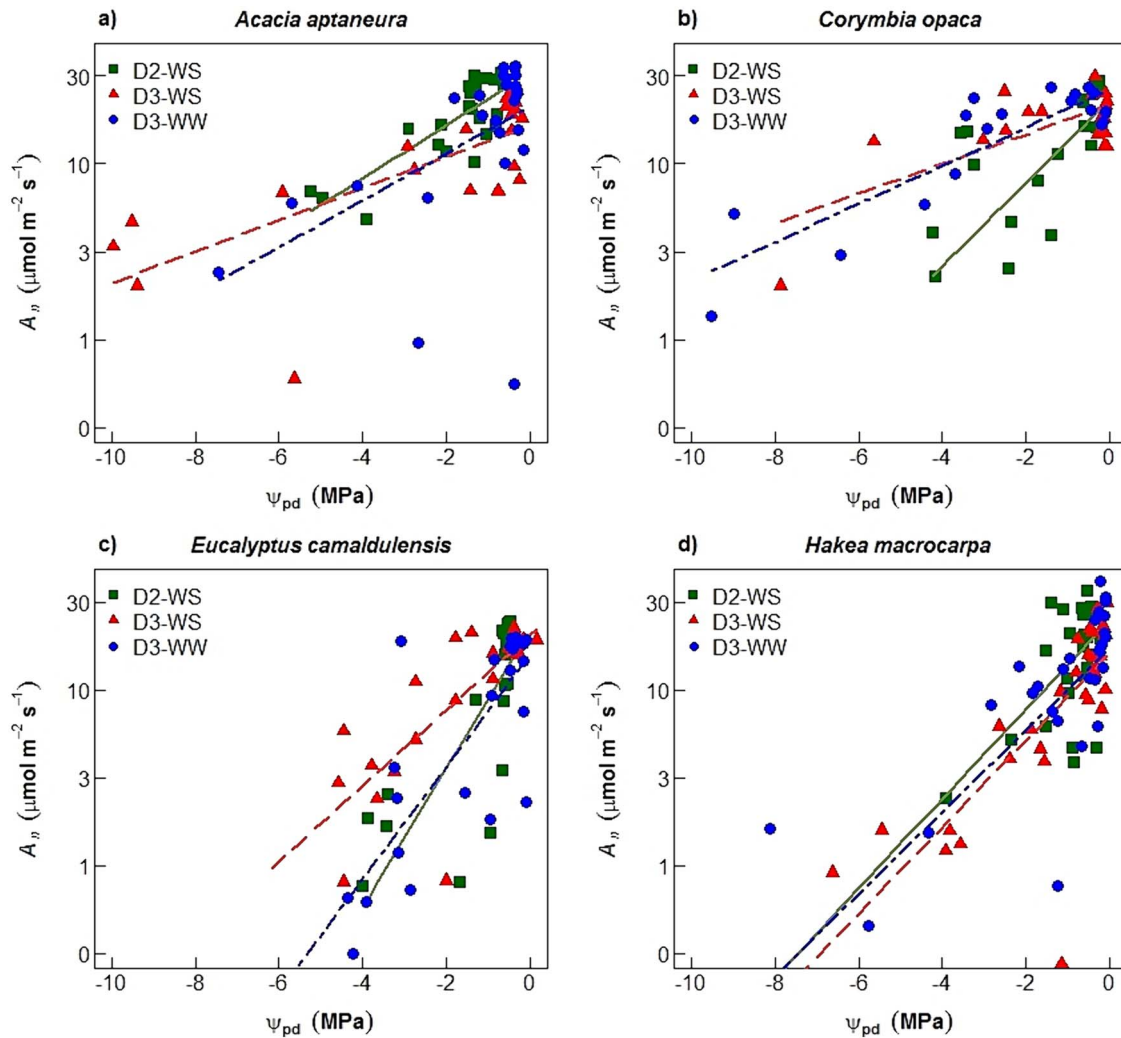
Figure 5. Net assimilation (log10(A_n) scale; μmol m^{-2} s^{-1}), versus leaf pre-dawn water potentials (ψ_{pd}). Symbols indicate treatments as: squares for 2nd drought (D2-WS; water-stressed treatment), triangles for 3rd drought (D3-WS) and circles for D3-WW (well-watered treatment). Regression line colour corresponds to treatment symbol colour. Regression coefficients are shown in Table 3.

Table 3. Coefficients ($\pm$1 SE) of the linear regressions between pre-dawn water potentials (ψ_{pd}; MPa) and stomatal conductance (Figure 4, log10(g_s) scale; Mol m^{-2} s^{-1}) and ψ_p versus net assimilation (Figure 5, log10(A_n) scale; μmol m^{-2} s^{-1}), for each species and treatment. Within columns, asterisk indicate that slopes were significantly different (P-value < 0.05) between treatments within same species and lower-case letters among species and the same treatment (either WS or WW) as tested separately using a standardized major axis method.

Species	Treatment	g_s versus Ψ_{pd}; Figure 4			A_n versus Ψ_{pd}; Figure 5		
		Slope	r^2	P-value	Slope	r^2	P-value
A. aptaneura	D2-WS	0.22 ± 0.04 a	0.55	<0.001	0.15 ± 0.02 a	0.63	<0.001
	D3-WS	0.08 ± 0.02 a*	0.27	<0.001	0.09 ± 0.02 a*	0.51	<0.001
	D3-WW	0.19 ± 0.04 a	0.51	<0.001	0.14 ± 0.02 a	0.68	<0.001
C. opaca	D2-WS	0.39 ± 0.09 b	0.45	<0.001	0.24 ± 0.07 b	0.35	0.003
	D3-WS	0.12 ± 0.04 ac	0.31	0.01	0.08 ± 0.02 a	0.49	<0.001
	D3-WW	0.17 ± 0.02 ac	0.67	<0.001	0.11 ± 0.01 b	0.76	<0.001
E. camaldulensis	D2-WS	0.50 ± 0.08 b	0.63	<0.001	0.40 ± 0.06 b	0.65	<0.001
	D3-WS	0.41 ± 0.03 b	0.81	<0.001	0.22 ± 0.03 b	0.61	<0.001
	D3-WW	0.33 ± 0.06 b	0.51	<0.001	0.32 ± 0.04 c	0.68	<0.001
H. macrocarpa	D2-WS	0.16 ± 0.03 a	0.43	<0.001	0.26 ± 0.07 b	0.32	<0.001
	D3-WS	0.21 ± 0.03 c	0.62	<0.001	0.25 ± 0.03 b	0.7	<0.001
	D3-WW	0.19 ± 0.03 c	0.53	<0.001	0.23 ± 0.05 a	0.41	<0.001
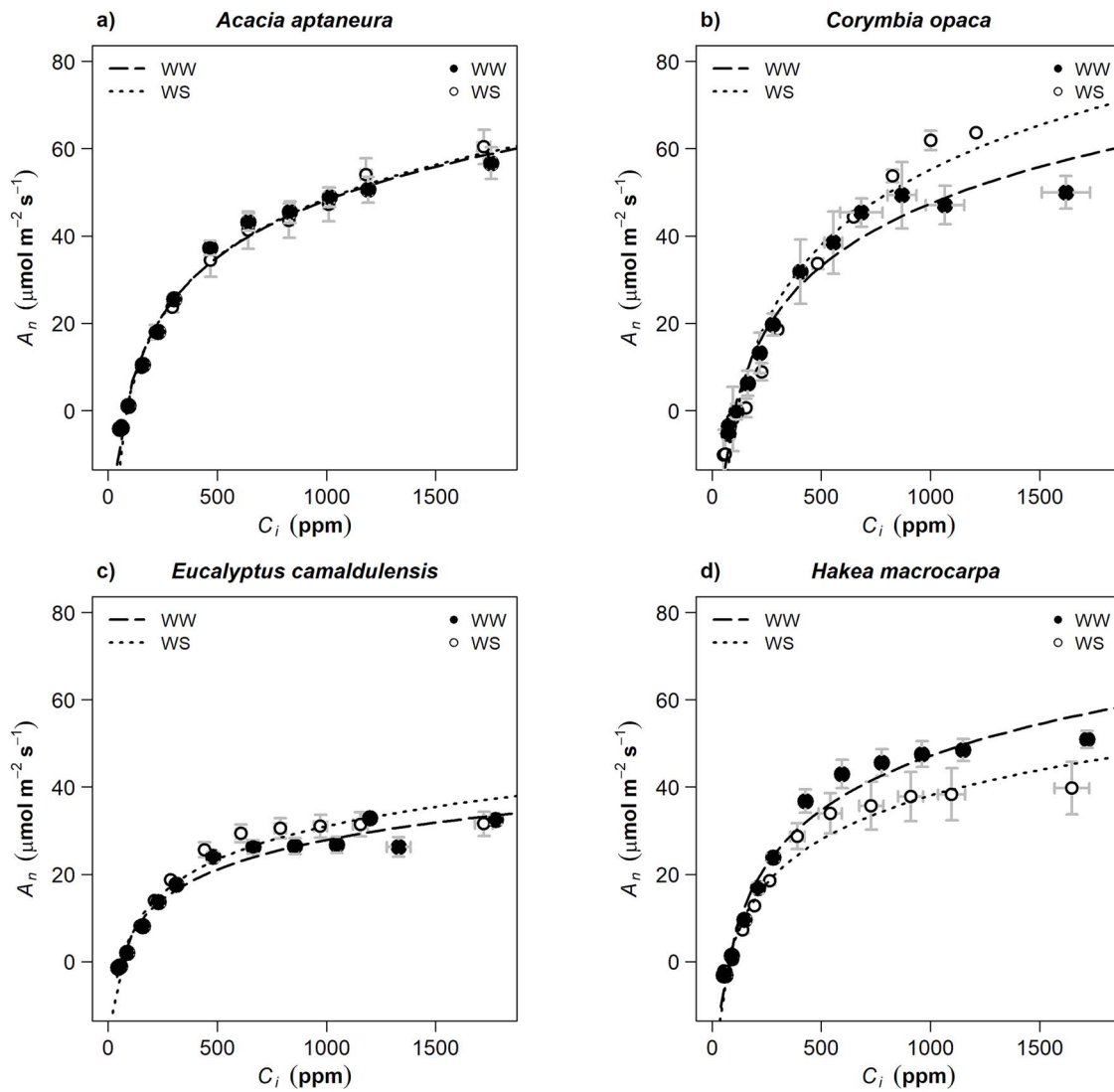
Figure 6. The response of net assimilation (A_n) to intercellular CO_2 (C_i). Open white circles correspond to WW treatment and closed black symbols to WS treatment. Error bars represent ± 1SE ($n = 4$).

and 254 ± 23.2 μmol m^{-2} s^{-1}; WS and WW, respectively; Table 2). *Corymbia opaca* had the highest V_{cmax} values (110 ± 3.4 μmol m^{-2} s^{-1}) in WS treatment, while *A. aptaneura* had the highest V_{cmax} value (95 ± 7.8 μmol m^{-2} s^{-1}) in WW treatment (Table 2). Minimum V_{cmax} values for both WS and WW were observed in *E. camaldulensis*, but these were not statistically different from *H. macrocarpa* in both treatments, WW for *C. opaca* and both treatments for *A. aptaneura*. Additionally, *C. opaca* and *E. camaldulensis* had the smallest LMA compared with the remaining two species. *Acacia aptaneura* and *H. macrocarpa* had the largest differences (in LMA), but differences were not statistically significant between treatments (Table 2, Table S1 available as Supplementary data at *Tree Physiology* Online).

Discussion

In this study, we examined the effects of repeated droughts on four co-occurring semi-arid species that exhibited contrasting physiological strategies observed in their natural habitat (O'Grady et al. 2009, Santini et al. 2015, Nolan et al. 2017*a*,

Rumman et al. 2018). We examined leaf gas exchange, photosynthetic capacity, LMA and plant growth to track the effects of repeated droughts and recovery after releases of drought. Consistent with our hypotheses, we observed differing responses to repeated drought among our four study species; these results are discussed in detail in the following sections.

Coordination in leaf gas exchange and leaf water potential

In semi-arid field conditions, *E. camaldulensis* and *C. opaca* are tall, deep-rooted trees, that access groundwater (O'Grady et al. 2009, Cleverly et al. 2016); hence, we hypothesized that these species with relatively fast growth rates would have larger declines in leaf gas exchange than *A. aptaneura* and *H. macrocarpa*. In agreement with our hypothesis, results showed that *E. camaldulensis* and *C. opaca* had the largest slope values in the regression of g_s and A_n versus ψ_{pd}, for plants that experienced the second drought (during D2-WS; Table 2). Indeed, *E. camaldulensis* and *C. opaca* had greater declines in g_s versus ψ_{pd} (more than 100%) compared with *H. macrocarpa*, and 77% greater for *C. opaca* and 130% for

E. camaldulensis compared with *A. aptaneura* in drought D2-WS (Table 3). Likewise, large declines were observed in the regression A_n versus ψ_{pd}, yet to a lesser degree compared with g_s. Declines of g_s for a given decline in ψ_{pd} can provide an insight into plant water-use strategies, indicating a more iso-hydric or anisohydric behaviour (Tardieu and Thierry 1998, Klein 2014). We found that *E. camaldulensis* and *C. opaca* had large declines in g_s and A_n as drought progressed, meaning they had a tight regulation of leaf gas exchange. This stomatal control decreases plant water use and thus prevents ψ_{pd} from declining as soil water availability declines, which makes these plants more isohydric. Although we did not measure midday ψ, a related glasshouse study on *E. camaldulensis* and *A. aptaneura* (Nolan et al. 2017c) found that pre-dawn and midday ψ were tightly correlated in both drought and control plants ($r^2 > 0.93$). Furthermore, Nolan et al. (2017c) found that *E. camaldulensis* had a higher turgor loss point (expressed in negative values as for leaf water potentials), and a shallower slope in the relationship between pre-dawn and midday ψ compared with *A. aptaneura*, and concluded that *E. camaludelensis* was relatively more isohydric than *A. aptaneura*. The existence of isohydric behaviour is still debatable (Martínez-Vilalta and Garcia-Forner 2017, Feng et al. 2019), although most isohydric species use rapid and early declines in g_s to tightly regulate leaf water potentials in the early stages of drought, resulting in decreasing A_n (Meinzer et al. 2009, Limousin et al. 2013, Nolan et al. 2017b).

Consistent with our results, Santini et al. (2015, 2017) found that the Myrtaceae species (*C. opaca* and *E. camaldulensis*) exhibit larger xylem vessel diameters, lower wood density and larger hydraulic conductivity than *Acacia* species (*A. aneura* and *A. aptaneura*). Wide vessel size makes the xylem more vulnerable to embolism; however, stomatal closure can prevent hydraulic failure when water availability declines and therefore delay drought-induced mortality (Eamus et al. 2000, McDowell et al. 2008). Plant water use is also a function of the dynamics of the below-ground environment, which is crucial to the above-ground process, such as leaf gas exchange Kannenberg et al. 2022). In terms of plant growth, we observed that *E. camaldulensis* had the widest stem diameter (and hence sapwood area) and was the tallest of all four species examined (Figure 2c). Given all species were germinated from seed at the same time and grown for the same period prior to this study (12 months), these data reflects the faster growth rate of *E. camaldulensis* compared with the other species. A larger sapwood area and plant size are indicative of larger rates of water flow and overall plant water-use, all of which are related and coordinate to some extent the leaf gas exchange in plants (Katul et al. 2003, Sperry and Love 2015). *Corymbia opaca* did not grow as tall as *E. camaldulensis* in this study (Figure 2b), which may be explained by the difference in a number of traits between these species. *Corymbia opaca* has higher wood density, lower root hydraulic conductance and smaller vessel diameter than those observed in *E. camaldulensis* (Santini et al. 2015, 2017). Investing in high wood density often occurs at the expense of plant height and diameter growth (Enquist et al. 1999, O'Grady et al. 2009). Investing carbohydrates in plant maintenance and hydraulic architecture, such as deep roots and high venation density (to enhance water transport), is often made at the expense of leaf traits (Brodribb and Jordan 2011, Poorter et al. 2012, Yin et al. 2018). We observed in the final drought that *E. camaldulensis* reduced its numbers

of leaves in the water-stressed (WS) treatment, but this was not observed in *C. opaca* (Figure 2c). One possible reason is the higher LMA in *C. opaca* compared with *E. camaldulensis*, with higher LMA associated with longer leaf life-spans (Wang et al. 2022). This suggests that allocation of assimilated carbohydrates was preferably given to anchorage and a tall structure for *E. camaldulensis*, rather than maintaining leaves while droughts were imposed (data was not collected for below-ground traits). Although we did not study the below-ground plant behaviour, above-ground growth can provide insights into plant water-use strategies in variation to root-deep, rood hydraulics and root structure, which ultimately determine water flow, transport and loss in plants.

Our results support our first hypothesis: *A. aptaneura* showed the smallest slope-values in the regression of g_s and A_n versus ψ_{pd} during the last drought (DS-WS; Table 3). *Hakea macrocarpa* only exhibited moderate declines (slopes) for a wider range of water potential values (Figures 4 and 5). Smaller declines in the relationship of g_s versus ψ_{pd} compared with *C. opaca* and *E. camaldulensis* implies that *A. aptaneura* had more risky stomatal regulation for a wider range of leaf water stress (and more negative water potential values). Previous studies have identified *A. aptaneura* as an anisohydric species (Page et al. 2016, Nolan et al. 2017c). Anisohydric species tolerate a larger decline of leaf water potentials, thereby allowing the maintenance of g_s and A_n further into drought (Martinez-Vilalta et al. 2014). *Acacia aptaneura* continued to photosynthesize at much lower ψ_{pd} (Figure 5a). Both *A. aptaneura and H. macrocarpa* are known for being highly tolerant to drought and high temperatures (Table 1). For example, higher wood density was observed within the two species (*A. aptaneura* and *H. macrocarpa*) than species from the Myrtaceae family (Santini et al. 2015). High wood density protects against xylem cavitation, leading to a superior resistance to cell wall collapse, thereby allowing species with high wood density to tolerate low water potentials (Santiago et al. 2004) and to maintain to some extent leaf gas exchange. The response to progressive water stress observed in the four studied species was in agreement with studies that have sought to understand the response of leaf gas exchange to drought in C_3 plants (Flexas et al. 2004, Galmes et al. 2007, Cano et al. 2014).

Changes in WUE during drought

In our study, WUE_i was maintained at more or less constant low values in the early stages of droughts for all four species (Figure 3i–l). However, as drought progressed, WUE_i increased for *A. aptaneura* and *E. camaldulensis* but only for a short period, followed by declines in WUE_i towards the end of the droughts. Although not all species had a significant increase in WUE_i, all species showed a significant decline in WUE_i at the end of the droughts, excepting drought D2-WS. This biphasic pattern, an increase in WUE_i as soil water stress increases, but consistent decline in WUE_i under extreme water stress has been observed across multiple arid and semi-arid plant species (Manzoni et al. 2011, Limousin et al. 2013, 2015). There are different plausible explanations for declining WUE_i under extreme drought. One explanation is that stomatal closure results in assimilation rates that are zero or close to them; however, residual conductance via cuticular transpiration and/or leaky stomata results in continued water loss (Petrík et al. 2023). Another explanation is that stomata remain open to provide evaporative cooling, but assimilation

does not occur. However, this decoupling of g_s from A_n is likely to only occur under heat wave conditions (Marchin et al. 2023), which were not present in our study. Species that show high WUE$_i$, such as *A. aptaneura* demonstrate a competitive advantage over the other three species to extreme soil water deficits or droughts.

The increase in WUE$_i$ was driven mainly by decreasing g_s rather than A_n as drought progressed, except for *H. macrocarpa*. For example, reductions of g_s were up to 31%, 38% and 46% (for *A. aptaneura*, *C. opaca* and *E. camaldulensis*, respectively) more than the reductions for A_n in all drought and periods as the soils dried down (Table 3 and Figure S2 available as Supplementary data at *Tree Physiology* Online). For *H. macrocarpa* WUE$_i$ was pretty much constant during all droughts and only decreased at the end of the droughts due to a decrease in A_n. *Hakea macrocarpa* showed greater reductions in A_n than in g_s (for a given reduction in ψ_{pd}) but only up to 20%, specifically in drought D3-WS. The decoupling of g_s from A_n showed in *H. macrocarpa* suggests transpiration cooling during soil drying as a strategy to resists drought, or perhaps is related to leaky stomata (Duursma et al. 2019). Plants that experienced drought and have reductions in A_n tend to consume non-structural carbohydrates to maintain cellular survival, respiratory mechanism and osmotic adjustment (McDowell et al. 2008).

Remarkably, it was observed that *H. macrocarpa* significantly increased R_d, which led to an increase in the ratio of R_d/A_n that experienced drought (Table 2). Large values of R_d have been previously observed in field conditions in other Australian species such as *Acacia lasiocarpa* (Atkin et al. 2015, Falster et al. 2021). Both stomatal closure and the 20% decrease in A_n as drought progressed were important drivers for the increase in R_d for *H. macrocarpa* and hence the ratio of R_d/A_n. This change in the plant carbon balance is a well-understood mechanism in plant mortality (Vandegeer et al. 2020, McDowell et al. 2022). Assessing the response and adaptation of plants to drought is crucial, and understanding the mechanisms inducing a decrease or increase in WUE$_i$ will ultimately provide information on which species would be more resistant to drought.

The effect of repeated droughts and plant recovery

We observed an acclimation response to drought in *A. aptaneura* (Table 3), i.e., less declines in gas exchange with repeated drought. *Acacia aptaneura* significantly reduced the rate at which g_s and A_n were declining for a given decline in ψ_{pd} in plants from D3-WS by 57% for g_s and 35% for A_n compared with individuals of D3-WW. Changes in exogenous hormones (i.e., ABA) may explain this behaviour, since the accumulation of ABA that is expected to occur during droughts enhances stomatal sensitivity to a low water potential levels in repeated droughts (Eamus and Narayan 1989, Thomas et al. 2000, Nolan et al. 2017c). Our previous study (Nolan et al. 2017c) showed a 'peaking-type' response, where ABA increased in the early-mid stages of the drought, followed by an abrupt decline once a threshold leaf water potential of −3.9 MPa was reached. Another explanation is the possible accumulation of osmotically active solutes as the soil dries, which allows species to adjust the turgor loss point at which leaf cells lose turgor and close stomatal (Bartlett et al. 2014, Nolan et al. 2017c). A lower turgor loss point (i.e., more negative values) was observed in *A. aptaneura* following repeated drought in our previous study (Nolan et al. 2017c).

These physiological mechanisms allow *A. aptaneura* to maintain relatively higher rates of gas exchange during repeated droughts. Consequentially, *A. aptaneura* showed the largest values of WUE$_i$ (Figure 3i) by declining g_s more than A_n, which is supported by observations done in field conditions, where *Acacia* species tend to maintain large rates of primary productivity even outside of the rainy season, while maintaining high WUE values as soil water declines (Tarin et al. 2019, 2020). All this may explain the high dominance of Mulga in the Australian continent, with these species occupying 20–25% of the continent, particularly *A. aptaneura* and *A. aneura* (Eamus et al. 2013). Mulga dominates in central Australia, where there is high rainfall variability (Cleverly et al. 2016).

Interestingly, we also observed that the rate of declines in leaf gas exchange for *C. opaca* were in the same range as *A. aptaneura* in the third drought and both treatments (D3-WS and D3-WW, Table 2). This suggests that *C. opaca* has the plasticity to exhibit different plant water-use strategies when exposed to repeated droughts and highlights the importance of examining physiological responses to soil dryness under repeated exposure to drought. Plasticity has been observed in plant water-use strategies, showing other species like *Larrea tridentata* to have a partial isohydric behaviour during drought conditions but was extremely anisohydric in wet conditions (Guo et al. 2020). Another interesting result was observed in *H. macrocarpa,* which did not show significant differences between treatments during the final drought (Table 3); however, the point at which both g_s and A_n were reduced by >50% differed from ∼0.15 m³ m⁻³ (during the second drought, Figure S3) to ∼ 0.07 m³ m⁻³ (Figure S2d available as Supplementary data at *Tree Physiology* Online; during the final drought). It may be possible that root clusters, which are common across *Hakea* spp. (Lamont 2003), benefited plant–water relations. Root clusters (which in fact were observed at the end of the experiment) can increase the soil volume explored by a factor of up to 300, and release deeply sourced water at night for subsequent uptake the following day through the process of hydraulic lift (Lamont 2003). This may explain why there were no changes in rate declines of g_s and A_n when regressed with ψ_{pd} between treatments. Similarly, for *E. camaldulensis*, plants from the final drought did not show differences between treatments, with plants from both treatments exhibiting large photosynthetic rates and stomatal conductance values (Figure 3). In field conditions across different rainfall gradients, eucalypts have shown little or no variation in leaf water potentials among wet and dry seasons due to their ability to access groundwater (Eamus et al. 2000, Mitchell et al. 2014, Nolan et al. 2017a). Thus, hydraulic traits discussed above explain why *E. camaldulensis* was not sensitive to repeated droughts compared to the *Acacia* species examined in the present study. In conclusion, although we observed differences between treatments with these three species (*C. opaca*, *E. camaldulensis* and *H. macrocarpa*), there was a lack of memory for repeated droughts, and none of them showed an acclimation for individuals that experienced three consecutive droughts.

Plant responses to drought have been extensively examined in relation to limitations in leaf gas exchange, the response of mesophyll conductance and biogeochemical limitations occurring at different time-scales during periods of drought (Flexas et al. 2004, 2006, Limousin et al. 2013, Zhou et al. 2016). Thus, plant recovery from droughts depends on species ability to diminish these limitations when water subsequently

becomes available (Galmes et al. 2007). We showed full recovery in A_n and g_s during the last re-watering cycle (between the second and final droughts) within the first 3 days after SWC was increased to FC for all species (Table 2). There were no significant differences in leaf gas exchange between WW and WS treatments after cessation of drought, indicating a complete recovery in all four species. Full plant recovery following alleviation of drought has been observed in other C_3 plants, in which the period of recovery varied from 1 to 3 days (Flexas et al. 2006, Resco et al. 2009, Cano et al. 2014). As observed in Flexas et al. (2006), usually photosynthesis recovers in 1 day at mild water stress, but photosynthesis can have a slower recovery if the biochemical or hydraulic pathway is damaged (Resco et al. 2009).

The photosynthetic response to drought that occurs independent of stomatal responses can be assessed by evaluating changes in the capacities of carboxylation (V_{cmax}) and electron transport (J_{max}) (Vogan and Maherali 2014). We observed small increases in V_{cmax} and J_{max} in *A. aptaneura* and *H. macrocarpa*, but they were not statistically significant. Maintenance of V_{cmax} and J_{max} may indicate that plant species have large resistance to drought in support to our third hypothesis (Table 2). Our results showed that *A. aptaneura* (which is a N_2-fixing species) had the largest LMA values for both treatments (Table 2; Cook and Dawes-Gromadzki 2005, Page et al. 2011). N_2-fixing species usually have large foliar nitrogen contents, and foliar nitrogen is positively correlated with LMA (Poorter et al. 2009). Interestingly, *A. aptaneura* showed an increase of 41% and *H. macrocarpa* of 75% in LMA from the WW to the WS treatment (Table 2). This reflects the high plasticity of LMA of these two species when exposed to drought. High LMA values >400 g m^{-2} have been previously reported in other species, such as *Hakea acuminata*, *A. aptaneura*, *Eucalyptus cyanophylla* and *E. haemastoma*, according to the AusTrait database (Lamont et al. 2002; Dong et al. 2022; Falster et al. 2021). LMA increases when rainfall or water availability is limited to reduce cell expansion rates under drought, and high LMA is also a result of expensive leaf construction and low nutrient content, as semi-arid Australia is well known for having low nitrogen content in its soils.

Conclusions

Our work evaluated the response to repeated experimental droughts using eleven physiological and morphological traits related to leaf gas exchange and plant growth and evaluated plant recovery of four co-occurring species of central Australia. Results showed contrasting physiological behaviours among the four species, but also within species depending on whether plants had been previously exposed to drought. Our results highlight the potential for acclimatation and plasticity in drought responses. By understanding functional attributes of dominant species in the face of climate change through refining metrics of plants physiology, this work has the potential to contribute to reducing uncertainties and improve vegetation and ecosystem modelling approaches for the central Australian region and potentially other similar arid regions across the globe.

Acknowledgments

Authors' contributions

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

Funding

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

Adams HD, Guardiola-Claramonte M, Barron-Gafford GA, Villegas JC, Breshears DD, Zou CB, Troch PA, Huxman TE. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. Proc Natl Acad Sci USA. 106(17):7063–7066. https://doi.org/10.1073/pnas.0901438106.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage. 259(4):660–684. https://doi.org/10.1016/j.foreco.2009.09.001.

Atkin OK, Bloomfield KJ, Reich PB, Tjoelker MG, Asner GP, Bonal D, Bönisch G, Bradford MG, Cernusak LA, Cosio EG et al. 2015. Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. New Phytol. 206:614–636. https://doi.org/10.1111/nph.13253.

Bartlett MK, Zhang Y, Kreidler N, Sun S, Ardy R, Cao K, Sack L. 2014. Global analysis of plasticity in turgor loss point, a key drought tolerance trait. Ecol Lett. 17(12):1580–1590. https://doi.org/10.1111/ele.12374.

Brodribb TJ, Jordan GJ. 2011. Water supply and demand remain balanced during leaf acclimation of *Nothofagus cunninghamii* trees. New Phytol. 192(2):437–448. https://doi.org/10.1111/j.1469-8137.2011.03795.x.

Cano FJ, Lopez R, Warren CR. 2014. Implications of the mesophyll conductance to CO_2 for photosynthesis and water-use efficiency during long-term water stress and recovery in two contrasting eucalyptus species. Plant Cell Environ. 37(11):2470–2490. https://doi.org/10.1111/pce.12325.

Cao D, Zhang J, Han J, Zhang T, Yang S, Wang J. 2022. Projected increases in global terrestrial net primary productivity loss caused by drought under climate change. Earths Future. 10:e2022EF002681. https://doi.org/10.1029/2022EF002681.

Cernusak LA, Hutley LB, Beringer J, JAM H, Turner BL. 2011. Photosynthetic physiology of eucalypts along a sub-continental rainfall gradient in northern Australia. Agric For Meteorol. 151(11): 1462–1470. https://doi.org/10.1016/j.agrformet.2011.01.006.

Chapin FS, Woodwell GM, Randerson JT, Rastetter EB, Lovett GM, Baldocchi DD, Clark DA, Harmon ME, Schimel DS, Valentini R et al. 2006. Reconciling carbon-cycle concepts, terminology, and methods. Ecosystems. 9(7):1041–1050. https://doi.org/10.1007/s10021-005-0105-7.

Cleverly J, Eamus D, Van Gorsel E, Chen C, Rumman R, Luo Q, Coupe NR, Li L, Kljun N, Faux R et al. 2016. Productivity and evapotranspiration of two contrasting semiarid ecosystems following the 2011 global carbon land sink anomaly. Agric For Meteorol. 220:151–159. https://doi.org/10.1016/j.agrformet.2016.01.086.

Cook GD, Dawes-Gromadzki TZ. 2005. Stable isotope signatures and landscape functioning in banded vegetation in arid-Central Australia. Landsc Ecol. 20(6):649–660. https://doi.org/10.1007/s10980-005-0069-1.

Domec JC, Gartner BL. 2001. Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. Trees. 15(4):204–214. https://doi.org/10.1007/s004680100095.

Dong N, Prentice IC, Wright IJ, Wang H, Atkin OK, Bloomfield KJ, Domingues TF, Gleason SM, Maire V, Onoda Y et al. 2022. Leaf nitrogen from the perspective of optimal plant function. J Ecol. 110:2585–2602. https://doi.org/10.1111/1365-2745.13967.

Duursma RA. 2015. Plantecophys–an R package for analysing and modelling leaf gas exchange data. PloS One. 10(11):e0143346. https://doi.org/10.1371/journal.pone.0143346.

Duursma RA, Barton CVM, Lin YS, Medlyn BE, Eamus D, Tissue DT, Ellsworth DS, McMurtrie RE. 2014. The peaked response of transpiration rate to vapour pressure deficit in field conditions can be explained by the temperature optimum of photosynthesis. Agric For Meteorol. 189-190:2–10. https://doi.org/10.1016/j.agrformet.2013.12.007.

Duursma RA, Blackman CJ, Lopéz R, Martin-StPaul NK, Cochard H, Medlyn BE. 2019. On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. New Phytol. 221:693–705. https://doi.org/10.1111/nph.15395.

Eamus D, Narayan AD. 1989. The influence of prior water stress and abscisic acid foliar spraying on stomatal responses to CO_2, IAA, ABA, and calcium in leaves of *Solanum melongena*. J Exp Bot. 40(214):573–579.

Eamus D, O'Grady AP, Hutley LB. 2000. Dry season conditions determine wet season water use in the wet-dry tropical savannas of northern Australia. Tree Physiol. 20:1219–1226.

Eamus D, Cleverly J, Boulain N, Grant N, Faux R, Villalobos-Vega R. 2013. Carbon and water fluxes in an arid-zone Acacia savanna woodland: an analyses of seasonal patterns and responses to rainfall events. Agric For Meteorol. 182-183:225–238. https://doi.org/10.1016/j.agrformet.2013.04.020.

Enquist B, West GB, Charnov EL, Brown JH. 1999. Allometric scaling of production and life-history variation in vascular plants. Nature. 401:907–911.

Falster D, Gallagher R, Wenk EH, Wright IJ, Indiarto D, Andrew SC, Baxter C, Lawson J, Allen S, Fuchs A et al. 2021. AusTraits, a curated plant trait database for the Australian flora. Sci Data. 8(1):254. https://doi.org/10.1038/s41597-021-01006-6.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C_3 species. Planta. 149:78–90.

Feng X, Ackerly DD, Dawson TE, Manzoni S, McLaughlin B, Skelton RP, Vico G, Weitz AP, Thompson SE. 2019. Beyond isohydricity: the role of environmental variability in determining plant drought responses. Plant Cell Environ. 42(4):1104–1111. https://doi.org/10.1111/pce.13486.

Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6(3):269–279. https://doi.org/10.1055/s-2004-820867.

Flexas J, Ribas-Carbo M, Bota J, Galmes J, Henkle M, Martinez-Canellas S, Medrano H. 2006. Decreased rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. New Phytol. 172(1):73–82. https://doi.org/10.1111/j.1469-8137.2006.01794.x.

Frank D, Reichstein M, Bahn M, Thonicke K, Frank D, Mahecha MD, Smith P, van der Velde M, Vicca S, Babst F et al. 2015. Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. Glob Chang Biol. 21(8):2861–2880. https://doi.org/10.1111/gcb.12916.

Galmes J, Medrano H, Flexas J. 2007. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. New Phytol. 175(1):81–93. https://doi.org/10.1111/j.1469-8137.2007.02087.x.

Gauthier PP, Crous KY, Ayub G, Duan H, Weerasinghe LK, Ellsworth DS, Tjoelker MG, Evans JR, Tissue DT, Atkin OK. 2014. Drought increases heat tolerance of leaf respiration in *Eucalyptus globulus* saplings grown under both ambient and elevated atmospheric [CO_2] and temperature. J Exp Bot. 65(22):6471–6485. https://doi.org/10.1093/jxb/eru367.

Gimeno TE, Sommerville KE, Valladares F, Owen KA. 2010. Homeostasis of respiration under drought and its important consequences for foliar carbon balance in a drier climate: insights from two contrasting *Acacia* species. Funct Plant Biol. 37:323–333.

Groom PK, Lamont BB, Kupsky L. 1994. Contrasting morphology and mcophysiology of co-occurring broad and terete leaves in *Hakea trifurcata* (Proteaceae). Aust J Bot. 42:307–320.

Guo JS, Hultine KR, Koch GW, Kropp H, Ogle K. 2020. Temporal shifts in iso/anisohydry revealed from daily observations of plant water potential in a dominant desert shrub. New Phytol. 225:713–726. https://doi.org/10.1111/nph.16196.

Kannenberg SA, Guo JS, Novick KA, Anderegg WR, Feng X, Kennedy D, Konings AG, Martínez-Vilalta J, Matheny AM. 2022. Opportunities, challenges and pitfalls in characterizing plant water-use strategies. Funct Ecol. 36:24–37. https://doi.org/10.1111/1365-2435.13945.

Katul G, Leuning R, Oren R. 2003. Relationship between plant hydraulic and biochemical properties derived from a steady-state coupled water and carbon transport model. Plant Cell Environ. 26:339–350.

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. https://doi.org/10.1111/1365-2435.12289.

Lamont BB. 1993. Why are hairy root clusters so abundant in the most nutrient-impoverished soils of Australia? Plant Soil. 155(156):269–272.

Lamont BB. 2003. Structure, ecology and physiology of root clusters - a review. Plant Soil. 248:1–19.

Lamont BB, Groom PK, Cowling RM. 2002. High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. Funct Ecol. 16:403–412.

Landis CW. 1999. Seedling propagation. The container tree nursery manual, Vol. 4. USDA Forest Service, WA, USA.

Lemoine NP, Griffin-Nolan RJ, Lock AD. 2018. Drought timing, not previous drought exposure, determines sensitivity of two short-grass species to water stress. Oecologia. 188:965–975. https://doi.org/10.1007/s00442-018-4265-5.

Limousin JM, Bickford CP, Dickman LT, Pangle RE, Hudson PJ, Boutz AL, Gehres N, Osuna JL, Pockman WT, NG MD. 2013. Regulation and acclimation of leaf gas exchange in a piñon-juniper woodland exposed to three different precipitation regimes. Plant Cell Environ. 36(10):1812–1825. https://doi.org/10.1111/pce.12089.

Limousin JM, Yepez EA, McDowell NG, Pockman WT, Tjoelker M. 2015. Convergence in resource use efficiency across trees with differing hydraulic strategies in response to ecosystem precipitation manipulation. Funct Ecol. 29(9):1125–1136. https://doi.org/10.1111/1365-2435.12426.

Manzoni S, Vico G, Katul G, Fay PA, Polley W, Palmroth S, Porporato A. 2011. Optimizing stomatal conductance for maximum carbon

gain under water stress: a meta-analysis across plant functional types and climates. Funct Ecol. 25(3):456–467. https://doi.org/10.1111/j.1365-2435.2010.01822.x.

Marchin RM, Medlyn BE, Tjoelker MG, Ellsworth DS. 2023. Decoupling between stomatal conductance and photosynthesis occurs under extreme heat in broadleaf tree species regardless of water access. Glob Chang Biol. 29:6319–6335. https://doi.org/10.1111/gcb.16929.

Martínez-Vilalta J, Garcia-Forner N. 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant Cell Environ. 40(6): 962–976.

Martinez-Vilalta J, Poyatos R, Aguade D, Retana J, Mencuccini M. 2014. A new look at water transport regulation in plants. New Phytol. 204(1):105–115. https://doi.org/10.1111/nph.12912.

Maslin BR, Reid JE. 2012. A taxonomic revision of Mulga (*Acacia aneura* and its close relatives: Fabaceae) in Western Australia. West Aust Herb. 22(4):129–267.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG et al. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol. 178(4):719–739. https://doi.org/10.1111/j.1469-8137.2008.02436.x.

McDowell NG, Sapes G, Pivovaroff A, Adams HD, Allen CD, Anderegg WRL, Arend M, Breshears DD, Brodribb T, Choat B et al. 2022. Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. Nat Rev Earth Environ. 3(5):294–308. https://doi.org/10.1038/s43017-022-00272-1.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR. 2009. Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. Funct Ecol. 23(5):922–930. https://doi.org/10.1111/j.1365-2435.2009.01577.x.

Mitchell PJ, O'Grady AP, Tissue DT, Worledge D, Pinkard EA. 2014. Co-ordination of growth, gas exchange and hydraulics define the carbon safety margin in tree species with contrasting drought strategies. Tree Physiol. 34(5):443–458. https://doi.org/10.1093/treephys/tpu014.

Nolan RH, Fairweather KA, Tarin T, Santini NS, Cleverly J, Faux R, Eamus D. 2017a. Divergence in plant water-use strategies in semiarid woody species. Funct Plant Biol. 44:1134–1146. https://doi.org/10.1071/fp17079.

Nolan RH, Tarin T, Fairweather KA, Cleverly J, Eamus D. 2017b. Variation in photosynthetic traits related to access to water in semi-arid Australian woody species. Funct Plant Biol. 44(11):1087–1097. https://doi.org/10.1071/fp17096.

Nolan RH, Tarin T, Santini NS, McAdam SAM, Ruman R, Eamus D. 2017c. Differences in osmotic adjustment, foliar abscisic acid dynamics, and stomatal regulation between an isohydric and anisohydric woody angiosperm during drought. Plant Cell Environ. 40: 3122–3134. https://doi.org/10.1111/pce.13077.

O'Grady AP, Eamus D, Cook PG, Lamontagne S. 2006. Groundwater use by riparian vegetation in the wet–dry tropics of northern Australia. Aust J Botany. 54(2):145–154. https://doi.org/10.1071/bt04164.

O'Grady AP, Cook PG, Eamus D, Duguid A, Wischusen JD, Fass T, Worledge D. 2009. Convergence of tree water use within an arid-zone woodland. Oecologia. 160(4):643–655. https://doi.org/10.1007/s00442-009-1332-y.

Page GF, Liu J, Grierson PF. 2011. Three-dimensional xylem networks and phyllode properties of co-occurring *Acacia*. Plant Cell Environ. 34(12):2149–2158. https://doi.org/10.1111/j.1365-3040.2011.02411.x.

Page GFM, Merchant A, Grierson PF. 2016. Inter-specific differences in the dynamics of water use and pulse-response of co-dominant canopy species in a dryland woodland. J Arid Environ. 124: 332–340. https://doi.org/10.1016/j.jaridenv.2015.09.004.

Peñuelas J, Lloret F, Montoya R. 2001. Severe drought effects on Mediterranean woody flora in Spain. Forest Sci. 47(2):214–218.

Petrík P, Petek-Petrik A, Mukarram M, Schuldt B, Lamarque LJ. 2023. Leaf physiological and morphological constraints of water-use efficiency in C3 plants. AoB Plants. 15(4):plad047. https://doi.org/10.1093/aobpla/plad047.

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182(3):565–588. https://doi.org/10.1111/j.1469-8137.2009.02830.x.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193(1):30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.Rproject.org/.

Reichstein M, Bahn M, Mahecha MD, Kattge J, Baldocchi DD. 2014. Linking plant and ecosystem functional biogeography. Proc Natl Acad Sci USA. 111(38):13697–13702. https://doi.org/10.1073/pnas.1216065111.

Resco V, Ewers BE, Sun W, Huxman TE, Weltzin JF, Williams DG. 2009. Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C3 woody legume, *Prosopis velutina*. New Phytol. 181(3):672–682. https://doi.org/10.1111/j.1469-8137.2008.02687.x.

Rumman R, Cleverly J, Nolan RH, Tarin T, Eamus D. 2018. Speculations on the application of foliar 13C discrimination to reveal groundwater dependency of vegetation, provide estimates of root depth and rates of groundwater use. Hydrol Earth Syst Sci Discuss. 1–25:4875–4889. https://doi.org/10.5194/hess-2017-540.

Santiago LS, Goldstein G, Meinzer FC, Fisher JB, Machado K, Woodruff D, Jones T. 2004. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. Oecologia. 140(4):543–550. https://doi.org/10.1007/s00442-004-1624-1.

Santini NS, Cleverly J, Faux R, Lestrange C, Rumman R, Eamus D. 2015. Xylem traits and water-use efficiency of woody species co-occurring in the Ti Tree Basin arid zone. Trees. 30(1):295–303. https://doi.org/10.1007/s00468-015-1301-5.

Santini NS, Cleverly J, Faux R, McBean K, Nolan R, Eamus D. 2017. Root xylem characteristics and hydraulic strategies of species co-occurring in semi-arid Australia. IAWA J. 39(1):43–62. https://doi.org/10.1163/22941932-20170188.

Sperry JS, Love DM. 2015. What plant hydraulics can tell us about responses to climate-change droughts. New Phytol. 207(1):14–27. https://doi.org/10.1111/nph.13354.

Tardieu F, Thierry S. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot. 49(Issue Special Issue):419–432. https://doi.org/10.1093/jxb/49.Special_Issue.419.

Tarin T, Nolan RH, Medlyn BE, Cleverly J, Eamus D. 2019. Water-use efficiency in a semi-arid woodland with high rainfall variability. Glob Chang Biol. 26(2):496–508. https://doi.org/10.1111/gcb.14866.

Tarin T, Nolan RH, Eamus D, Cleverly J. 2020. Carbon and water fluxes in two adjacent Australian semi-arid ecosystems. Agric For Meteorol. 281:107853. https://doi.org/10.1016/j.agrformet.2019.107853.

Thomas DS, Eamus D, Shanahan S. 2000. Influence of season, drought and xylem ABA on stomatal responses to leaf-to-air vapour pressure difference of trees of the Australian wet–dry tropics. Aust J Bot. 48: 143–151.

Vandegeer RK, Tissue DT, Hartley SE, Glauser G, Johnson SN. 2020. Physiological acclimation of a grass species occurs during sustained but not repeated drought events. Environ Exp Bot. 171:103954. https://doi.org/10.1016/j.envexpbot.2019.103954.

Vogan PJ, Maherali H. 2014. Increased photosynthetic capacity as a mechanism of drought adaptation in C3 plants. Int J Plant Sci. 175(9):1033–1041. https://doi.org/10.1086/678088.

Wang H, Prentice CI, Wright IJ, Warton DI, Qiao S, Xu X, Zhou J, Kikuzawa K, Stenseth NC. 2022. Leaf economics fundamentals explained by optimality principles. Sci Adv. 9:eadd5667. https://doi.org/10.1126/sciadv.add5667.

Warton DI, Duursma RA, Falster DS, Taskinen S. 2012. smatr 3- an R package for estimation and inference about allometric lines. Methods Ecol Evol. 3(2):257–259. https://doi.org/10.1111/j.2041-210X.2011.00153.x.

Yin Q, Wang L, Lei M, Dang H, Quan J, Tian T, Chai Y, Yue M. 2018. The relationships between leaf economics and hydraulic traits of woody plants depend on water availability. Sci Total Environ. 621: 245–252. https://doi.org/10.1016/j.scitotenv.2017.11.171.

Zhao M, Running S. 2010. Drought-induced reduction in global terrestrial net primary production from 2000 throught 2009. Science. 329:940–943.

Zhou SX, Medlyn BE, Prentice IC. 2016. Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian *Eucalyptus* species. Ann Bot. 117(1):133–144. https://doi.org/10.1093/aob/mcv161.