

# Homeostasis of respiration under drought and its important consequences for foliar carbon balance in a drier climate: insights from two contrasting *Acacia* species

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**Abstract.** Under drought, when photosynthesis ( $A$ ) is impaired, foliar respiration ( $R$ ) becomes crucial to estimate plant carbon balance. However, the effects of drought on  $R$  remain unclear and little is known about differences between congeners with divergent anatomy. In this study we compared the physiological response to imposed drought in plants of two *Acacia* species: *Acacia pycnantha* Benth. and *Acacia floribunda* (Vent.) Willd. in a controlled environment. We subjected half of the plants to two desiccation cycles. Relative water content (RWC), the ratio of variable to maximum fluorescence ( $F_v/F_m$ ), phyllode dark respiration ( $R_{\text{dark}}$ ), stomatal conductance to water ( $g_s$ ), light-saturated photosynthesis ( $A_{\text{sat}}$ ) were monitored. Drought significantly reduced RWC,  $g_s$ ,  $F_v/F_m$ , and  $A_{\text{sat}}$ ; increased the instantaneous water use efficiency in the species with higher foliage mass per area (FMA) (*A. pycnantha*) and did not have any significant effect on  $R_{\text{dark}}$  but increased the  $R_{\text{dark}}/A_{\text{sat}}$  ratio. Although the shape of the response to drought of both species was similar, the two species differed in the magnitude in the increase of the  $R_{\text{dark}}/A_{\text{sat}}$  ratio, with drought-mediated increases in  $R_{\text{dark}}/A_{\text{sat}}$  being greater in *A. floribunda* than in *A. pycnantha*; the latter also showing greater recovery of photosynthesis. Collectively, our results highlight the extent to which drought alters the carbon balance of the two selected species. The homeostasis of  $R_{\text{dark}}$  under drought is particularly relevant within a climate change scenario where more severe and frequent drought episodes are predicted to occur.

**Additional keywords:** *Acacia floribunda*, *Acacia pycnantha*, fluorescence, photosynthesis, stomatal conductance to water, water-stress.

## Introduction

Mitochondrial respiration ( $R$ ) provides plant cells with the energy and carbon skeletons necessary to grow and survive. Respiratory energy (ATP and reducing equivalents) is required for cellular maintenance and growth, whereas TCA cycle intermediates provide the carbon skeletons necessary for the synthesis of cellular and tissue components (Amthor 2000; Cannell and Thornley 2000; Lambers *et al.* 2000; Padmasree *et al.* 2002; Wright *et al.* 2006). Coupled to the production of respiratory products is the release of large amounts of  $\text{CO}_2$ ; 30–80% of the photosynthetic carbon fixed taken up each day is released by whole-plant  $R$ , with approximately half of whole-plant  $R$  taking place in leaves (Poorter *et al.* 1990; Atkin *et al.* 1996; Wright *et al.* 2006). Collectively, respiratory  $\text{CO}_2$  released by plants represents 30–65% of total ecosystem respiration (Reichstein *et al.* 2002), resulting in 50–60 Gt carbon per year being released into the atmosphere by plant  $R$ ; this is a large flux, being near 10 times the amount of carbon released by fossil fuel burning (Canadell *et al.* 2000; Prentice *et al.* 2000; Atkin *et al.* 2005).

Given the importance of  $R$  for the carbon economy of individual plants and global atmospheric  $\text{CO}_2$  concentrations, it is essential that we establish how key environmental drivers impact on specific rates of  $R$  and the proportion of photosynthetic  $\text{CO}_2$  fixed ( $A$ ) that is respired (i.e.  $R/A$ ). In recent years strong emphasis has been placed on unravelling the effects on plant  $R$  of several environmental factors such as temperature (Atkin *et al.* 2005), irradiance (Sims and Pearcy 1989; Turnbull *et al.* 1993; Noguchi and Terashima 1997; Zaragoza-Castells *et al.* 2007), increased atmospheric  $\text{CO}_2$  concentration (Urban 2003; Gonzalez-Meler *et al.* 2004), and nutrient availability (Ryan *et al.* 1996; Reich *et al.* 2008). Although some attention has been given to the impacts of water deficit on plant  $R$  (Ribas-Carbo *et al.* 2005; Galmes *et al.* 2007; Slot *et al.* 2008), there is currently no consensus on how rates of  $R$  vary in response to increasing severity of drought (Chaves *et al.* 2003; Flexas *et al.* 2005, 2006; Atkin and Macherel 2009).

In most plant species, drought has an inhibitory effect on stomatal opening and photosynthetic  $\text{CO}_2$  fixation

(Flexas *et al.* 2001, 2006; Gulías *et al.* 2002; Lawlor and Cornic 2002; Chaves *et al.* 2003). Reduced rates of *A* are likely to reduce substrate supply to mitochondria, with the result that rates of foliar *R* in darkness ( $R_{\text{dark}}$ ) might be lower under drought conditions (e.g. Haupt-Herting *et al.* 2001; Ribas-Carbo *et al.* 2005; Galmes *et al.* 2007). Alternatively, drought could increase the demand for respiratory ATP to support cellular metabolism (Flexas *et al.* 2005; Atkin and Macherel 2009), with the result that foliar  $R_{\text{dark}}$  increases under drought (e.g. Collier and Cummins 1993; Gratani *et al.* 2007; Slot *et al.* 2008). By contrast, drought has little effect on foliar  $R_{\text{dark}}$  in other species (Galmes *et al.* 2007). Given such variability and the need to more accurately account for drought-mediated changes in foliar  $R_{\text{dark}}$  in large scale predictive models (e.g. Cox *et al.* 2000), it is important that further studies quantify the responses of foliar  $R_{\text{dark}}$  to drought in additional plant species.

Vegetation-climate models require empirical studies linking structural and physiological plant traits with species relative abundances and ecosystem properties, such as carbon and water fluxes (Garnier *et al.* 2004; Wright *et al.* 2005; Poorter *et al.* 2009). Detailed analyses of the differences among species in both morphological traits and physiological features can provide sound foundations to scale up plant processes to vegetation dynamics and ecosystem functioning, which is crucial for modelling in turn how biogeochemical cycles and vegetation boundaries will be affected by land-use and climate change (Wright *et al.* 2004).

Here, we investigated whether two closely-related *Acacia* species of the subgenus Phyllodineae exhibit different respiratory and photosynthetic responses to drought. The Phyllodineae subgenus is the most widely distributed in Australia, being found in both high and low rainfall habitats; therefore, it offers an opportunity to study drought sensitivity in closely related species that have adapted to contrasting environmental conditions (Maslin *et al.* 2003). For our comparison we chose one species with thick, coriaceous phyllodes (*Acacia pycnantha* Benth.), and another species that exhibits thinner and narrower phyllodes (*Acacia floribunda* (Vent.) Willd.). Specifically, we sought to determine whether there are differences in the shape and timing of respiratory and photosynthetic responses to drought between these two contrasting *Acacia* species. Our working hypotheses were (i) that in both species, the onset of drought leads to declines in both *A* and phyllode  $R_{\text{dark}}$  and, (ii) in drought treated plants, the proportional decline in *A* is greater than  $R_{\text{dark}}$  leading to a rise in the  $R_{\text{dark}}/A$  ratio. To gain additional insights into how drought impacted on *A*, we also quantified a range of fluorescence parameters for both species.

## Materials and methods

### *Plant material, growth conditions and experimental design*

The study species were: *Acacia pycnantha* Benth. (golden wattle), a tall shrub/tree with thick coriaceous and glabrous phyllodes, widely distributed in coastal and inland south-eastern Australia. The other species, *Acacia floribunda* (Vent.) Willd. (gossamer wattle), is a spreading shrub/tree with thin, flexible, linear phyllodes; it is widespread in forests and woodlands along the coastal and subcoastal regions of south-eastern Australia.

Seeds of *A. floribunda* were collected from Bega, NSW in south-eastern Australia (36°40'S, 149°54'E). Seeds of *A. pycnantha* were collected from Clare, SA in southern Australia (33°50'S, 138°36'E). Seeds were placed in water at 80°C to break the impervious testa and promote germination. We planted 200 seeds of each species in Yates seed raising mix (Yates, Padstow, NSW, Australia). Seedlings were later transferred to 6 L pots and grown in a potting mix with added sand and mushroom compost. Plants were grown outdoors in a common garden adjacent to the glasshouses at the Australian National University, Canberra, for one year before experimentation and fertilised with Osmocote (Scotts-Sierra Horticultural Products, Marysville, OH, USA) native plant slow release fertiliser and an iron chelate solution.

Leading up to the experimental period starting in spring (November) 2008, plants were placed in a temperature-controlled glasshouse (mean daytime temperature of 25°C) according to a randomised split block-plot design. Plants were watered daily to field capacity. The experimental design consisted of four blocks each containing seven plants of each species (i.e. 28 plants of each species in total). In each block, four plants of each species were randomly assigned to the drought treatment and three to the control treatment. This rendered 16 drought plants and 12 control plants of each species evenly distributed in four blocks. Drought treatment consisted of progressive desiccation achieved by cessation of daily watering for defined periods (see below), followed by a period of re-watering/recovery from drought. Control plants were watered to field capacity throughout. Two drought treatment cycles were imposed: for *A. pycnantha*, the first cycle lasted 5 days and the second 8 days; for *A. floribunda*, the first cycle lasted 7 days and the second 10 days. Increasing drought severity was monitored daily by measuring stomatal conductance to water ( $g_s$ ) (considered indicative of phyllode water stress, regardless of the factor causing the stress (Gulías *et al.* 2002)), as part of the more general gas exchange parameters (see below); additionally during the second desiccation cycle, drought severity was also measured by phyllode relative water content (RWC; (Lawlor and Cornic 2002)). RWC was calculated according to:  $((\text{fresh mass} - \text{dry mass}) / (\text{fully hydrated mass} - \text{dry mass})) \times 100$ . Fresh mass was obtained weighing the phyllodes immediately after being removed from the plant. Thereafter, fully hydrated mass was determined after phyllodes had been kept in a water-saturated environment at 21°C with the petiole immersed in water for 24 h. Finally, phyllodes were oven-dried at 68°C for 48 h to obtain the dry mass. Given the destructive nature of the RWC measurements, those were taken only during the second desiccation cycle, to avoid altering the root/shoot ratio. In both drought treatment cycles and species, cessation of watering was maintained until loss of turgor was observed (Slot *et al.* 2008); we then re-watered plants for four days before the start of the next desiccation cycle.

### *Physiological measurements: fluorescence and gas exchange*

We took all physiological measurements at the same time period every day along both drought treatment cycles. Phyllode gas-exchange parameters were measured in the morning (5 h after sun

rise) and fluorescence measurements were taken in the afternoon (8 h after sun rise). Due to limitations in the total number of plants that could be measured each day, we imposed drought treatments initially on two blocks on the first day, followed by the remaining two blocks one day later. In doing so, we were able to measure all plants at the same water status over two consecutive days (measuring eight plants per day). For each plant, we selected one of the most recently fully-expanded phyllodes for foliar gas-exchange and fluorescence measurements, we followed the same phyllodes for the whole extent of each desiccation cycle. Phyllodes used for gas-exchange measurements were harvested at the end of the experiment to calculate the foliage mass per unit foliage area (FMA).

Plants were dark-adapted for 30 min before measurement with a Heinz Waltz GmbH IMAGING-PAM chlorophyll fluorometer (Effeltrich, Germany) with standard head. Phyllodes were exposed to a weak modulated measuring beam to obtain the minimal fluorescence ( $F_o$ ) where all PSII reaction centres are open. Phyllodes were then given a short pulse of light ( $2400 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 0.8 s) in order to obtain the maximal fluorescence ( $F_m$ ) where all PSII reaction centres are closed. The maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ) was then calculated according to:  $F_v/F_m = (F_m - F_o)/F_m$ .

Phyllode gas-exchange parameters were measured at a phyllode temperature of near  $20^\circ\text{C}$  using a LI-6400 infrared gas analyser (Li-Cor, Lincoln, NE, USA). For all measurements, relative humidity was kept close to ambient (between 55 and 65%). Measurements of *A* were first made under light-saturating/ambient- $\text{CO}_2$  ( $A_{\text{sat}}$ ) and then under light-saturating/ $1000 \mu\text{mol mol}^{-1}\text{-CO}_2$  ( $A_{1000}$ ) conditions. Preliminary light-response curves were made to establish the irradiance necessary to achieve light-saturation ( $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  for both species). Measurements of  $A_{\text{sat}}$  were made at ambient  $\text{CO}_2$  concentration ( $400 \mu\text{mol mol}^{-1}$ ), with the subsequent measurements at an atmospheric  $\text{CO}_2$  concentration of  $1000 \mu\text{mol mol}^{-1}$  ( $A_{1000}$ ) resulting in photosynthesis being near  $\text{CO}_2$ -saturated (as determined from photosynthesis *v.* intercellular  $\text{CO}_2$  concentration ( $A - C_i$ ) curves performed in the same species; data not shown). Finally, we measured dark respiration ( $R_{\text{dark}}$ ) (in the presence of  $400 \mu\text{mol mol}^{-1} \text{CO}_2$ ) after each phyllode had been placed in darkness for 30 min, keeping temperature, relative humidity and  $\text{CO}_2$  concentration constant and close to ambient. Finally we calculated instant water use efficiency (iWUE) according to:  $\text{iWUE} = A_{\text{sat}}/g_s$  (Flexas *et al.* 2001).

### Statistical analysis

All statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). To test for normality we used the Kolmogorov–Smirnov test and the Levene's test for homogeneity of variance (Quinn and Keough 2002). As drought intensity increased with time during both desiccation cycles, we tested for significant differences between treatments and different days using a repeated-measures ANOVA; differences between particular days and treatments were explored using a post-hoc Tukey's HSD test. Different species

and drought treatment cycles were analysed separately. Finally, linear regression analysis was used to explore correlations between the physiological parameters and RWC.

## Results

### Foliage mass per unit foliage area (FMA)

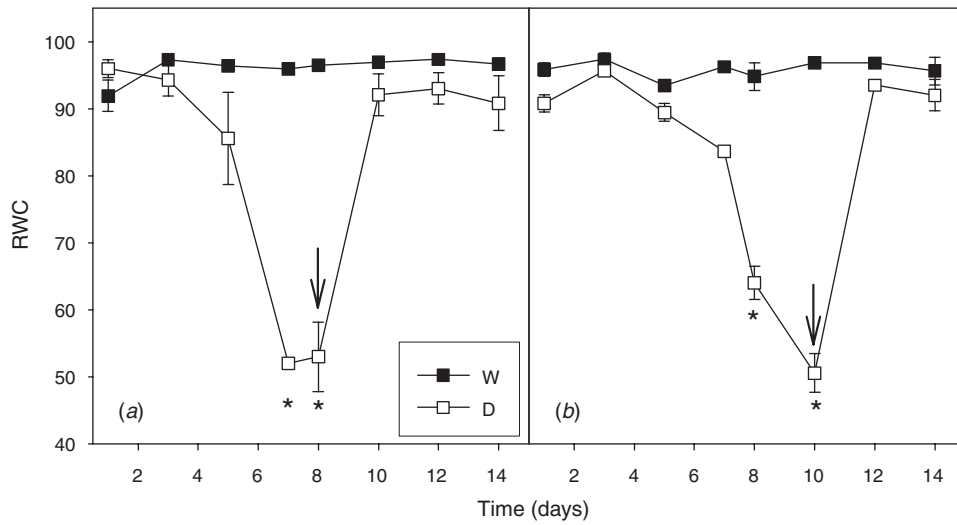
Within each species, FMA values were not significantly different between plants that were well watered or drought-treated (for plants sampled at the end of the treatment cycles). A one-way ANOVA revealed the overall average FMA values were significantly different between the two species (*A. pycnantha*:  $152.3 \pm 7.0 \text{ g m}^{-2}$  ( $n=24$ ,  $\pm$  s.e.); *A. floribunda*:  $123.2 \pm 4.9 \text{ g m}^{-2}$  ( $n=23$ ,  $\pm$  s.e.);  $F=11.22$ ,  $P=0.002$ ).

### Water status

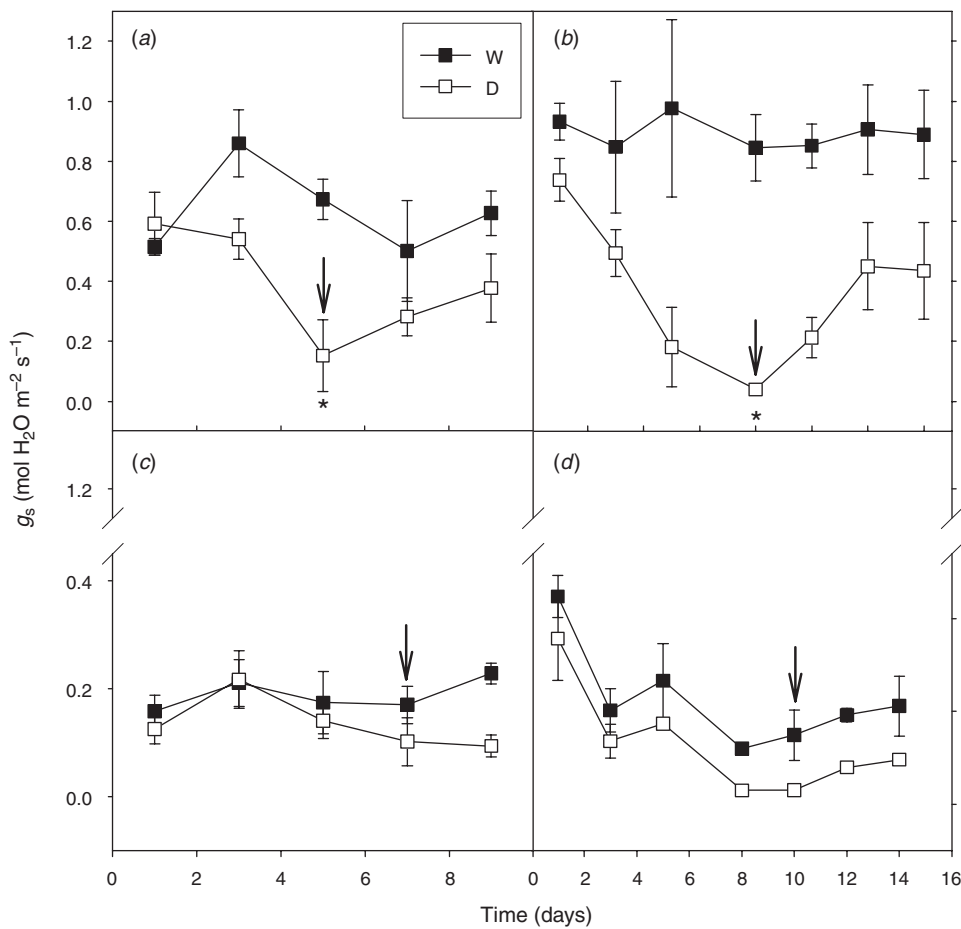
To provide an overview of how cessation of watering impacted on phyllode water status of each species, we measured the RWC (2nd desiccation cycle only) and stomatal conductance to water (both cycles). Figure 1 shows the impact of drought on RWC during the second desiccation cycle. Significant declines in RWC were observed 7–8 days after cessation of watering in *A. pycnantha* (reaching a RWC of  $53.0 \pm 5.2\%$ ,  $n=4$  on day 8; Fig. 1a). Similarly, significant declines in RWC were observed in *A. floribunda* (Fig. 1b); however, the decline in RWC was more gradual than in *A. pycnantha*, with RWC not reaching its minimum ( $50.5 \pm 2.9\%$ ,  $n=4$ ) until 10 days after the cessation of watering. Re-watering resulted in large and rapid increases in RWC of phyllodes in both species (Fig. 1). Drought treatment also had a significant effect on  $g_s$  (measured under ambient  $\text{CO}_2$  and saturating light) on both species in both desiccation cycles (Fig. 2). In *A. pycnantha*,  $g_s$  declined from  $0.59 \pm 0.11 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$  (mean  $\pm$  s.e.,  $n=4$ ) to  $0.15 \pm 0.12 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$  in the first desiccation cycle and from  $0.74 \pm 0.07 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$  to  $0.04 \pm 0.02 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$  in the second. In *A. floribunda*,  $g_s$  declined less dramatically in the first desiccation cycle, from  $0.13 \pm 0.03 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$  to  $0.10 \pm 0.04 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ , whereas in the second it declined from  $0.36 \pm 0.09 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$  to  $0.03 \pm 0.01 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ . In addition to the significant drought treatment effects, the repeated-measures ANOVA revealed that  $g_s$  was significantly affected by time (in *A. pycnantha* in the first cycle and in *A. floribunda* in the second cycle) and by the time  $\times$  treatment interaction (in *A. pycnantha* in the first cycle) (see Table S1 available as an Accessory Publication to this paper). Post-hoc Tukey's HSD test showed that significant differences, for both RWC and  $g_s$ , were found between treatments for the days of maximum water-stress (Figs 1, 2). In both desiccation cycles, re-watering did not result in a full recovery of  $g_s$  in either species, with re-watering have almost no effect on  $g_s$  in *A. floribunda* (Fig. 2c, d). Nevertheless, overall the watering regimes resulted in a broad range of water status phenotypes with which we could test hypotheses concerning the impact of drought on phyllode  $R_{\text{dark}}$  and the balance between  $R_{\text{dark}}$  and *A*.

### Photosynthetic responses to drought

As expected, drought significantly decreased rates of photosynthesis measured under light-saturating ( $A_{\text{sat}}$ ) and



**Fig. 1.** Impact of watering regimes on phyllode relative water content (RWC in %;  $\pm$  s.e.,  $n = 4$  for all days except for day 7:  $n = 2$ ) through time in the second drought cycle for the two species: (a) *Acacia pycnantha* and (b) *Acacia floribunda*. Closed symbols: well watered plants (W), open symbols: drought plants (D). Significant differences, according to Tukey's HSD test, between treatments are indicated: \* $P < 0.05$ . Arrows indicate re-watering.



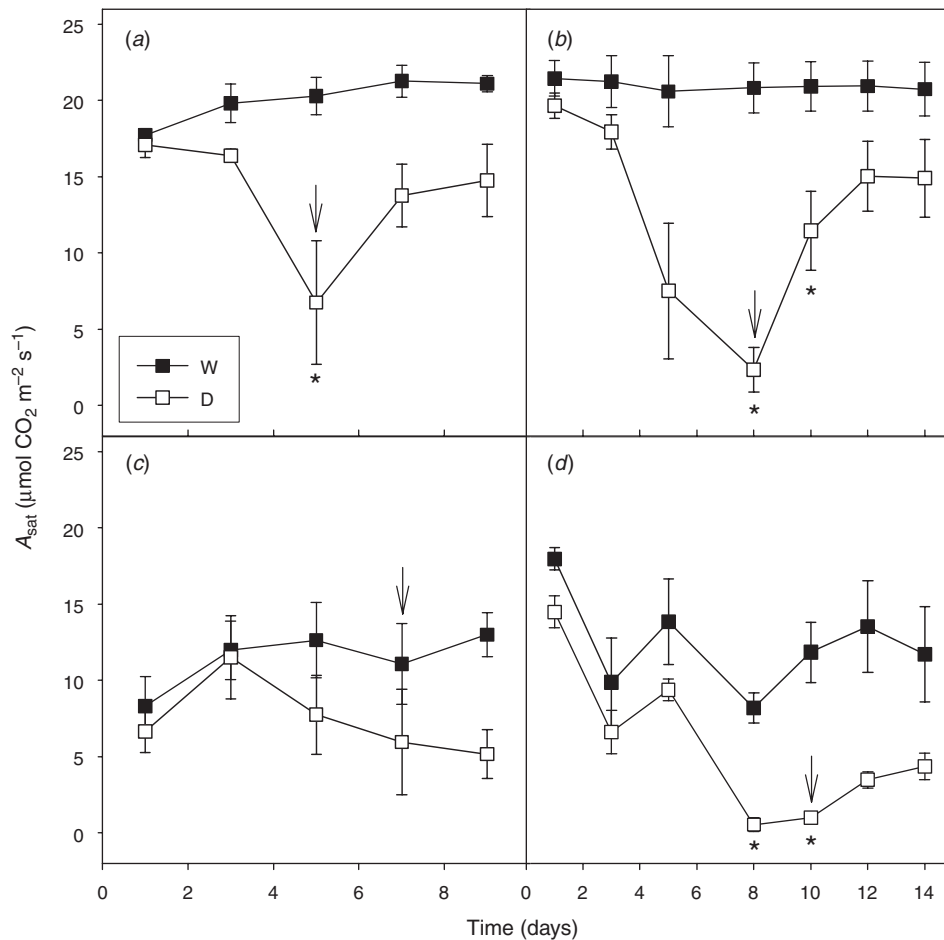
**Fig. 2.** Impact of watering regimes on phyllode stomatal conductance ( $g_s$ ;  $\pm$  s.e.,  $n = 4$ ), measured at ambient CO<sub>2</sub> and saturating light intensity, for (a, b) *Acacia pycnantha* and (c, d) *Acacia floribunda* during the (a, c) first and (b, d) second drought cycles. Closed symbols: well watered plants (W), open symbols: drought plants (D). Significant differences, according to Tukey's HSD test, between treatments are indicated: \* $P < 0.05$ . Arrows indicate re-watering.

light-saturating/1000  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  ( $A_{1000}$ ) conditions (Figs 3, 4; Table S1) for both species in both drought cycles. In drought treated plants,  $A_{\text{sat}}$  and  $A_{1000}$  of both species increased after re-watering, although not to the rates exhibited by the well watered controls. There was a significant effect of time on  $A_{\text{sat}}$  and  $A_{1000}$  in both species in the 2nd drought cycle, and on  $A_{1000}$  in the 1st cycle. In *A. pycnantha*, the effect of drought on  $A_{\text{sat}}$  and  $A_{1000}$  varied with time (as indicated by a significant time  $\times$  treatment interaction). By contrast, no such interaction was found when considering  $A_{\text{sat}}$  and  $A_{1000}$  of *A. floribunda*, with the effects of drought being consistent through time. Importantly, in both drought cycles, significant differences in rates of  $A_{\text{sat}}$  and  $A_{1000}$  were found between well watered and drought treated plants on the days of maximum water stress for both species, as revealed by post-hoc Tukey HSD tests (Figs 3, 4). Finally, we found that iWUE significantly increased with imposition of drought in *A. pycnantha*, but not in *A. floribunda* (Table S1). Again differences were observed on the days of maximum water stress: for *A. pycnantha*, in the first desiccation cycle on the fifth day iWUE was higher in drought treated plants (mean  $\pm$  s.e.,  $n=4$ :

$70.13 \pm 14.11 \mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ ,  $n=4$ ) than in well watered plants ( $29.5 \pm 3.4 \mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ ); the same was observed in the second desiccation cycle on the eighth day ( $72.3 \pm 9.9$  v.  $26.1 \pm 3.8 \mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ ). For *A. floribunda* we did not find any significant differences in iWUE between drought treated and well water plants in either the first or second desiccation cycles (Tables S1, S2).

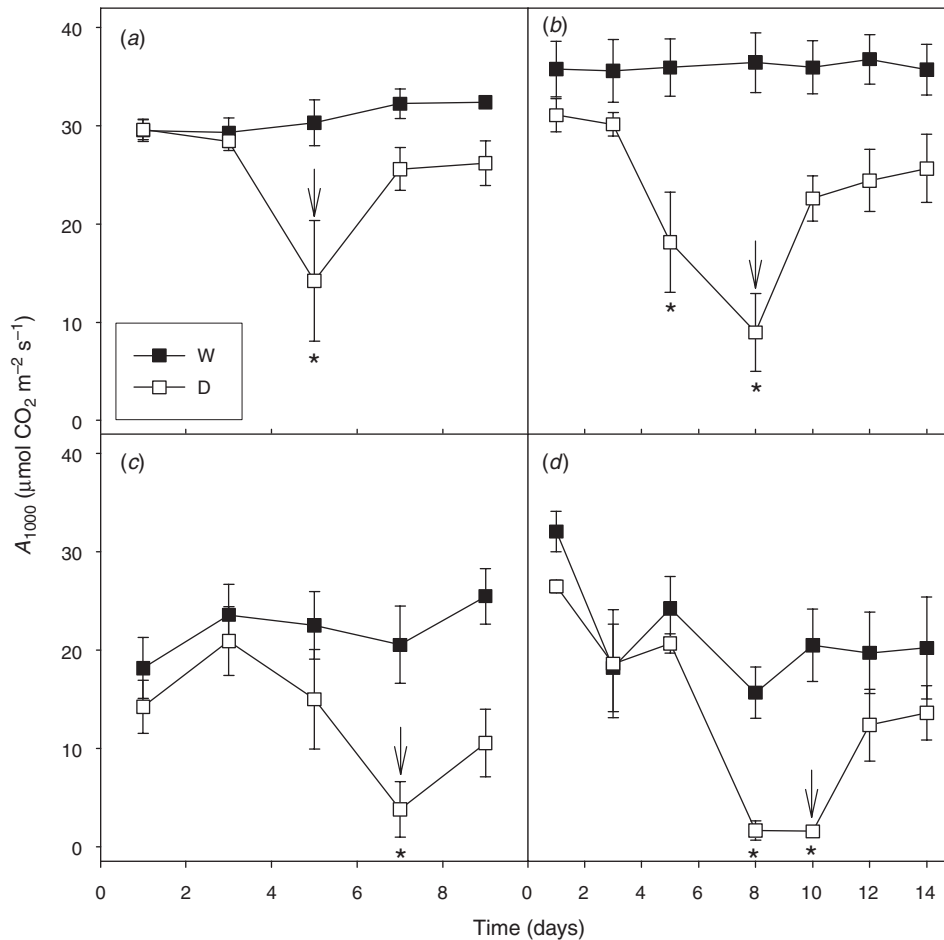
To explore whether variations in  $A_{\text{sat}}$  were associated with changes in internal  $\text{CO}_2$  concentration ( $C_i$ ), we correlated  $A_{\text{sat}}$  with  $C_i$  for both species and drought cycles; we found that  $A_{\text{sat}}$  and  $C_i$  were significantly correlated in both species and desiccation cycles (Table 1). However, we also found that in the presence of a near non-limiting  $\text{CO}_2$  concentration (i.e.  $1000 \mu\text{mol mol}^{-1}$ ), rates of  $A_{1000}$  also decreased in response to drought; suggesting that impaired photosynthesis, due to water scarcity, was due not only to limited  $\text{CO}_2$  availability caused by stomatal closure, but also to changes in Rubisco activation state and/or rates of RuBP regeneration (which might occur in response to photoinhibition of PSII).

To address whether the impacts of drought and re-watering on *A* were associated with changes in the quantum efficiency of



**Fig. 3.** Rates of light saturated photosynthesis ( $A_{\text{sat}}$ ;  $\pm$  s.e.,  $n=4$ ) for (a, b) *Acacia pycnantha* and (c, d) *Acacia floribunda* during the (a, c) first and (b, d) second drought cycles. Closed symbols: well watered plants (W), open symbols: drought plants (D). Significant differences, according to Tukey's HSD test, between treatments are indicated:  $*P < 0.05$ . Arrows indicate re-watering.





**Fig. 4.** Rates of light-saturated photosynthesis in phyllodes provided with  $1000 \mu\text{mol mol}^{-1} \text{CO}_2$  ( $A_{1000}$ ;  $\pm$  s.e.,  $n=4$ ) for (a, b) *Acacia pycnantha* and (c, d) *A. floribunda* during the (a, c) first and (b, d) second drought cycles. Closed symbols: well watered plants (W), open symbols: drought plants (D). Significant differences, according to Tukey's HSD test, between treatments are indicated: \* $P < 0.05$ . Arrows indicate re-watering.

**Table 1.** Correlations between physiological parameters and percentage of relative water content (RWC) for *Acacia pycnantha* and *Acacia floribunda* in the second desiccation cycle

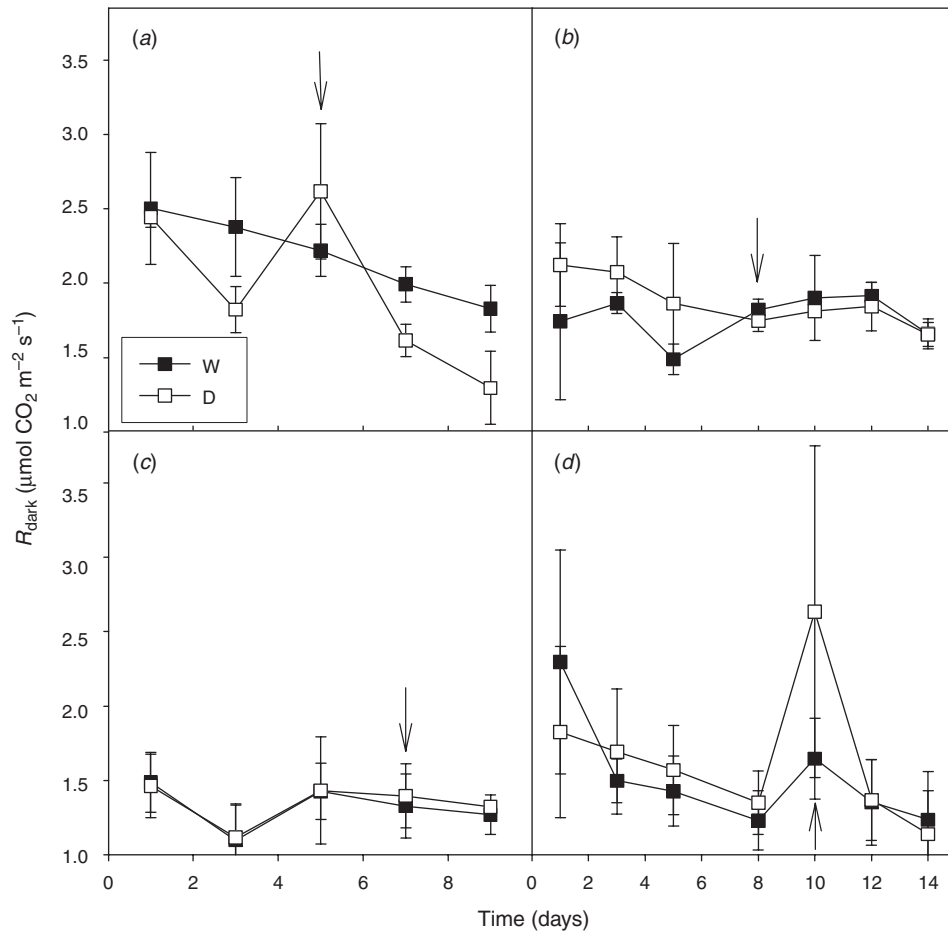
$A_{1000}$ , light-saturated photosynthesis in phyllodes provided with  $1000 \mu\text{mol mol}^{-1} \text{CO}_2$ ;  $A_{\text{sat}}$ , light saturated photosynthesis;  $R_{\text{dark}}$ , dark respiration;  $g_s$ , stomatal conductance; iWUE, instant water use efficiency;  $F_v/F_m$ , maximum quantum efficiency of PSII photochemistry;  $\Delta$ , residual variance exceeds variance of the response variable. Significant differences are indicated: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$

Correlation	<i>A. pycnantha</i>		<i>A. floribunda</i>	
	$r^2$	$P$	$r^2$	$P$
$A_{1000}$ vs RWC	0.703	<0.0001***	0.453	0.0042**
$A_{\text{sat}}$ vs RWC	0.703	<0.0001***	0.453	0.0042**
$R_{\text{dark}}$ vs RWC	0.016	0.6367	0.226	0.0628
$R_{\text{dark}}/A_{\text{sat}}$ vs RWC	0.295	0.0297*	0.013	0.6768
iWUE vs RWC	0.006	0.7736	0.21	0.5931
$g_s$ vs RWC	0.439	0.0051	0.280	0.0384*
$F_v/F_m$ vs RWC	$\Delta$	0.832	0.685	<0.001***

PSII, we plotted the dark-adapted  $F_v/F_m$  values against time for each species. *A. floribunda* exhibited a significant decrease in  $F_v/F_m$  on the day of greatest drought stress in each cycle (Table S1). There was a significant interaction between treatment and time for  $F_v/F_m$  in *A. floribunda* in both cycles (Table S1). By contrast, *A. pycnantha* exhibited no significant changes in  $F_v/F_m$  on the day of greatest stress, although cessation of water supply did induce a significant decline in  $F_v/F_m$  during the second drought cycle (Table S1). Taken together, these data suggest that the large changes in  $A_{\text{sat}}$  and  $A_{1000}$  were unlikely to be the result of large changes in the efficiency of PSII.

#### Impact of drought on respiratory $\text{CO}_2$ release and R/A ratios

In contrast to the large drought-induced declines in  $A_{\text{sat}}$  and  $A_{1000}$  (Figs 3, 4), rates of phyllode dark respiration ( $R_{\text{dark}}$ ) were not significantly affected by the drought treatment in either species or desiccation cycles (Fig. 5; Table S1). Although  $R_{\text{dark}}$  in



**Fig. 5.** Rates of phyllode respiration measured in darkness ( $R_{\text{dark}}$ ;  $\pm$  s.e.,  $n=4$ ) for (a, b) *Acacia pycnantha* and (c, d) *Acacia floribunda* during the (a, c) first and (b, d) second drought cycles. Closed symbols: well watered plants (W), open symbols: drought plants (D). Arrows indicate re-watering.

*A. pycnantha* did decline with time in the 1st cycle (in both treatments), no time-based changes in  $R_{\text{dark}}$  were observed in the 2nd cycle (in either species), or in *A. floribunda* in the 1st cycle.

Given the lack of drought-induced changes in  $R_{\text{dark}}$  (Fig. 5) and the large drought-induced decreases in  $A_{\text{sat}}$  (Fig. 3), we would expect the ratio of  $R_{\text{dark}}/A_{\text{sat}}$  to increase in response to drought. When considered collectively within a two-way ANOVA,  $R_{\text{dark}}/A_{\text{sat}}$  was not significantly affected by the drought treatment *per se* (except for *A. floribunda* in the first desiccation cycle). However, there was a significant effect of time and of the time  $\times$  treatment interaction in the 2nd cycle, suggesting that drought did alter  $R_{\text{dark}}/A_{\text{sat}}$  on specific days (Table S1). Post-hoc Tukey's HSD tests showed that drought significantly increased  $R_{\text{dark}}/A_{\text{sat}}$  ratios on the days of maximum water stress in both species in the 2nd desiccation cycle, and in *A. pycnantha* in the 1st cycle (Fig. 5). Taken together, these results suggest that carbon losses by respiration begin to outstrip rates of photosynthetic carbon gain under conditions of severe drought.

#### Relationship between physiological parameters and RWC

Phyllode gas-exchange parameters that were significantly affected by drought ( $A_{\text{sat}}$ ,  $A_{1000}$  and  $g_s$ ) were significantly

correlated with measured RWC in the 2nd desiccation cycle in both species (Table 1). Similarly,  $R_{\text{dark}}/A_{\text{sat}}$  ratios were significantly correlated with RWC in *A. pycnantha* but not in *A. floribunda* (Table 1).

As we did not have a measure of RWC for the 1st desiccation cycle, we used  $g_s$  as a measure of plant water status and correlated it to the rest of the gas-exchange parameters monitored. We found that  $g_s$  was significantly correlated with both  $A_{\text{sat}}$  and  $A_{1000}$  in both species and desiccation cycles (Table 2). Similar to our findings with RWC,  $R_{\text{dark}}$  was not correlated with  $g_s$ ; however, the  $R_{\text{dark}}/A_{\text{sat}}$  ratio was significantly correlated with  $g_s$  in *A. floribunda* in the 2nd cycle (Table 2).

When considering the fluorescence parameters, RWC explained very little of the variation in *A. pycnantha*. Although there was a significant increase in  $F_o$  with RWC in *A. pycnantha*, the regression explained only 18% of the variation in  $F_o$  (data not shown). By contrast, in *A. floribunda* there was a significant positive relationship between  $F_v/F_m$  and RWC (Table 1).

#### Discussion

We sought to establish if drought leads to lower rates of phyllode respiration in two *Acacia* species and whether drought alters

**Table 2. Correlations between gas-exchange parameters for *Acacia pycnantha* and *Acacia floribunda* and desiccation cycles**

$A_{1000}$ , light-saturated photosynthesis in phyllodes provided with  $1000\ \mu\text{mol mol}^{-1}\ \text{CO}_2$ ;  $A_{\text{sat}}$ , light saturated photosynthesis;  $R_{\text{dark}}$ , dark respiration; iWUE, instant water use efficiency;  $g_s$ , stomatal conductance to water;  $C_i$ , intercellular  $\text{CO}_2$  concentration. Significant differences are indicated: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$

Correlation	Cycle 1				Cycle 2			
	<i>A. pycnantha</i>		<i>A. floribunda</i>		<i>A. pycnantha</i>		<i>A. floribunda</i>	
	$r^2$	$P$	$r^2$	$P$	$r^2$	$P$	$r^2$	$P$
$A_{1000}$ vs $g_s$	0.436	<0.0001***	0.532	<0.0001***	0.567	<0.0001***	0.712	<0.0001***
$A_{\text{sat}}$ vs $g_s$	0.673	<0.0001***	0.833	<0.0001***	0.644	<0.0001***	0.784	<0.0001***
$R_{\text{dark}}$ vs $g_s$	0.051	0.1613	0.003	0.7375	<0.001	0.9632	0.040	0.1733
$R_{\text{dark}}/A_{\text{sat}}$ vs $g_s$	0.004	0.7106	0.070	0.1291	0.001	0.8021	0.127	0.0130**
iWUE vs $g_s$	0.042	0.5702	0.002	0.7919	0.017	0.6309	0.021	0.3219
$A_{\text{sat}}$ vs $C_i$	0.486	<0.0001***	0.697	<0.0001***	0.424	<0.0001***	0.371	<0.0001***

the balance between  $R_{\text{dark}}$  and photosynthesis. By using two contrasting *Acacia* species with different phyllode morphology, we also sought to gain insights into whether drought-mediated changes in  $A$  and  $R_{\text{dark}}$  differ between the two *Acacia* species. In contrast to a majority of other studies investigating the effect of drought on foliar  $R_{\text{dark}}$  (Haupt-Herting *et al.* 2001; Ribas-Carbo *et al.* 2005; Galmes *et al.* 2007; Atkin and Macherel 2009), we found that  $R_{\text{dark}}$  was insensitive to drought in phyllodes of both *Acacia* species. However, due to large drought-mediated decreases in photosynthesis, the ratio of  $R_{\text{dark}}$  to  $A_{\text{sat}}$  (i.e.  $R/A$ ) increased during the most severe periods of drought, demonstrating that the carbon balance of the two *Acacia* species was severely affected by water stress. Under this situation the plant carbon gain would be seriously compromised and if maintained it would eventually lead to carbon starvation (Flexas *et al.* 2006; Valladares and Sanchez-Gomez 2006). Moreover, we found that such responses were observed in both *Acacia* species, irrespective of their inherent differences in phyllode morphology and FMA values.

#### Respiratory and photosynthetic response to drought

In a recent review, Atkin and Macherel (2009) pointed out that in mature fully expanded leaves,  $R_{\text{dark}}$  responses to drought can be more variable than in other organs. In two-thirds of the reviewed experiments,  $R_{\text{dark}}$  decreased with drought and no change was observed in almost all the remainder, with only a few exhibiting an increase. In most of the studies where a decrease of  $R_{\text{dark}}$  with drought was observed, the study species were crop (González-Meler *et al.* 1997; Ghashghaie *et al.* 2001; Haupt-Herting *et al.* 2001; Ribas-Carbo *et al.* 2005) and herbaceous species (e.g. Collier and Cummins 1993; Galmes *et al.* 2007). In studies where an increase in  $R_{\text{dark}}$  with drought was observed severe levels of water stress were imposed and the species involved were crop and herbaceous (Ghashghaie *et al.* 2001; Bartoli *et al.* 2005; Slot *et al.* 2008); which agrees with the biphasic response of  $R_{\text{dark}}$  to drought proposed by Flexas *et al.* (2005). According to Flexas *et al.* (2005) the energetic cost of cellular maintenance is increased under severe water stress. This results in an increase in  $R_{\text{dark}}$ ; as has been recently reported by Slot *et al.* (2008). Slot *et al.* (2008) observed increased  $R_{\text{dark}}$  with the onset of drought coupled to the onset of foliar wilting and likely increase demand for respiratory ATP. In contrast to these results, studies reporting no change

in  $R_{\text{dark}}$  with water stress mostly targeted evergreen species with high FMA (e.g. Flexas *et al.* 2005; Galmes *et al.* 2007). Our results accord with others, such as Galmes *et al.* (2007), and suggest that evergreen perennial shrubs and trees, whose lifetime leaf productivity occurs over longer time periods, might respond slower to water stress and not adjust their  $R/A$  in the short-term. By contrast, short-lived species that need to optimise their carbon gain over shorter periods may respond quicker to water scarcity and lower their  $R_{\text{dark}}$  rates.

Our finding that rates of  $R_{\text{dark}}$  in *Acacia* phyllodes were maintained under water stress conditions (Fig. 5) suggests that respiratory metabolism was unlikely to have been limited by substrate supply and/or that the demand for respiratory products (ATP, NADH, carbon-skeletons) was maintained (Atkin and Macherel 2009). Maintenance of  $R_{\text{dark}}$  under water stress caused, together with significant reduction of  $A_{\text{sat}}$ , the increase of the  $R/A$  ratio. As expected, photosynthesis was severely impaired by drought in both species. Decreased  $A_{\text{sat}}$  with RWC was accompanied by reduced  $g_s$  and  $C_i$ . However, decreases in photosynthesis were not simply the result of stomatal limitations, as we also found that photosynthesis under near- $\text{CO}_2$  saturated conditions ( $A_{1000}$ ) was also significantly reduced in the days of maximum water stress. Decreases in  $A_{1000}$  were not associated with a major decrease in the maximum efficiency of PSII ( $F_v/F_m$ ). This, together with the fact that stomatal conductance decreased to below a threshold where biochemical limitations potentially arise ( $0.10\ \text{mol H}_2\text{O m}^{-2}\ \text{s}^{-1}$ ) (Flexas *et al.* 2006), suggests that water stress was impairing photosynthesis through non-stomatal limitation such as inactivation of the Rubisco and/or restrictions in regeneration of RuBP due to factors other than PSII efficiency.

#### Differences in the physiological response to drought between species

We expected *A. pycnantha* to be more resistant to drought, due to less foliage area (relative to foliage mass) available for water loss. However, *A. pycnantha* showed a water-spending strategy (McDowell *et al.* 2008) that led to an abrupt decline of RWC to ~50% over 8 days; whereas *A. floribunda* appeared to be more conservative in its initial water use and severe declines in RWC (down to ~50%) were not observed until the tenth day. In contrast, *A. pycnantha* exhibited greater recovery of  $A$  and  $g_s$



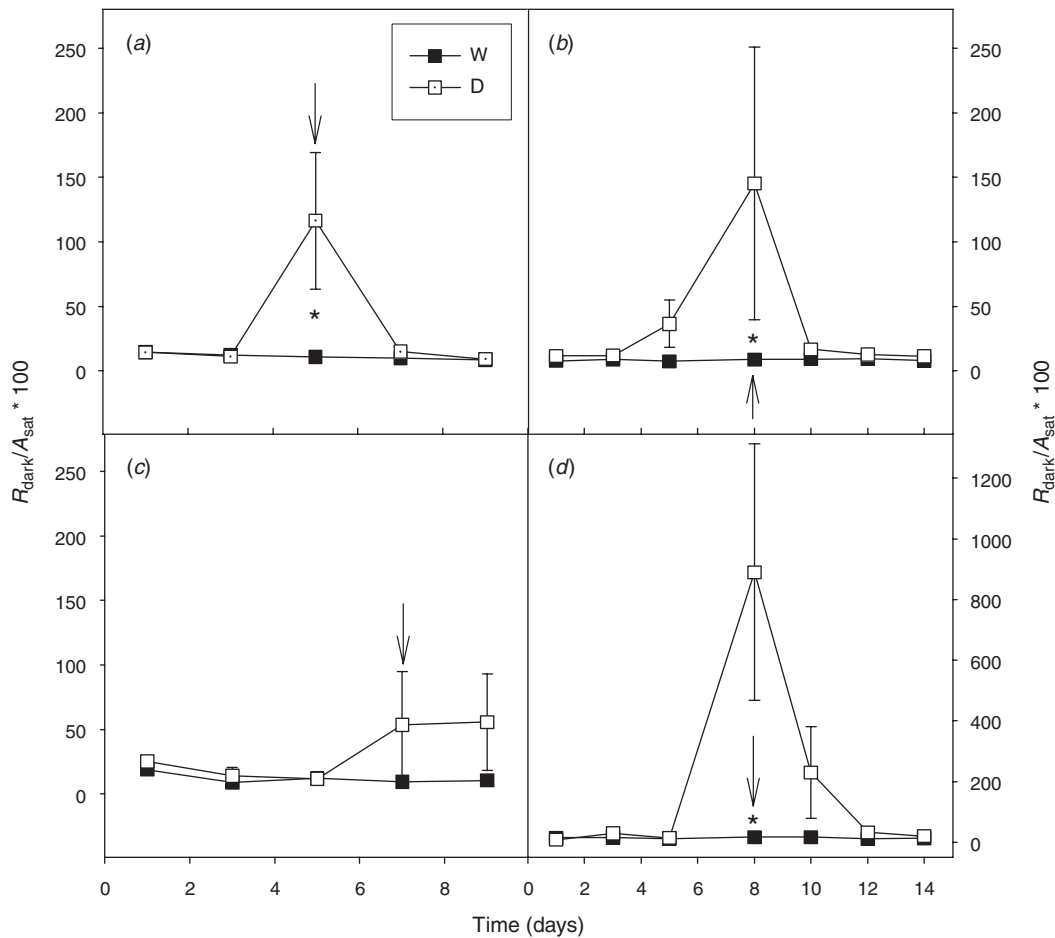
when re-watered and increased iWUE in response to water stress, suggesting that this species might be more resistant to prolonged drought periods with time-spaced rainfall events; whereas, *A. floribunda* would be more resistant to intense and rapid droughts as used in our study. *A. floribunda* would likely suffer more from chronic and prolonged drought: first because it has less recover capacity of photosynthesis and second, because its photosynthetic apparatus seemed to be more vulnerable. This is shown by the significant correlation found for this species between RWC and  $F_v/F_m$  and also by the fact that  $F_v/F_m$  was significantly affected by drought not only during the second (more prolonged and severe, as occurred to *A. pycnantha*) desiccation cycle, but also during the first and shorter desiccation cycle. Further work is needed to establish why *A. pycnantha* exhibits greater sensitivity to water stress in early stages than *A. floribunda*.

Finally, we note that although *A. pycnantha* exhibited a smaller increase in the  $R/A$  ratio, area-based rates of both  $A$  and  $R_{\text{dark}}$  in *A. pycnantha* were consistently higher than in *A. floribunda*. Besides differing in  $\text{CO}_2$  exchange rates, the two

species also differed greatly in their  $g_s$ ; especially during the second desiccation cycle, when control plants of *A. pycnantha* showed  $g_s$  values of  $0.85\text{--}0.98 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , which contrast with the lower values of the control plants of its congener *A. floribunda*:  $0.20\text{--}0.45 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  (Fig. 2; Table S2). These observed  $g_s$  values of *A. pycnantha* are at the upper range of those observed in *Acacia* phyllodes while those of *A. floribunda* are at the lower end of the range. Rates of  $g_s$  observed in non-stressed *Acacia* phyllodes range from  $0.10$  to  $1.36 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  (Cole *et al.* 1994; Nativ *et al.* 1999; Liu *et al.* 2004; Norisada and Kojima 2005; Grigg *et al.* 2008).

#### Effects of drought on carbon balance

Some large scale predictive models (e.g. the Hadley Centre GCM) assume that the  $R/A$  ratio remains constant during the onset of drought (Cox *et al.* 2000). This relies on the tight coupling between variations in photosynthesis and respiration (i.e. the  $R/A$  ratio is homeostatic). However, this tight coupling between photosynthesis and respiration has not been



**Fig. 6.** Ratio of phyllode respiration measured in darkness to light-saturated photosynthesis ( $R_{\text{dark}}/A_{\text{sat}}; \pm \text{s.e.}, n=4$ ) for (a, b) *Acacia pycnantha* and (c, d) *Acacia floribunda* during the (a, c) first and (b, d) second drought cycles. Closed symbols: well watered plants (W), open symbols: drought plants (D). Significant differences, according to Tukey's HSD test, between treatments are indicated:  $*P < 0.05$ . Arrows indicate re-watering.

extensively shown (Gratani *et al.* 2007); rather there is growing evidence from our results (Fig. 6) and previous studies (Haupt-Herting *et al.* 2001; Galmes *et al.* 2007; Slot *et al.* 2008; Atkin and Macherel 2009) that  $R/A$  invariably rises with drought. Thus, it is unlikely to be correct to assume a constant  $R/A$  ratio in large scale, predictive models. Moreover, we have found that, relative to large decreases in  $A_{\text{sat}}$ ,  $R_{\text{dark}}$  actually remains relatively homeostatic under drought; suggesting that maintenance of rates of  $R_{\text{dark}}$  are necessary for foliar functioning under drought. Atkin and Macherel (2009) proposed that the maintenance of  $R_{\text{dark}}$  enables survival and rapid recovery of productivity under water stress conditions. Accounting for increases in  $R/A$  will be vital if GCMs are to accurately predict the impacts of climate change on productivity and vegetation pattern in areas experiencing more frequent and severe droughts.

Our results are especially relevant within a climate change context, where more severe and frequent drought events are predicted to occur (Christensen *et al.* 2007; Medrano *et al.* 2009). Drought is a relevant environmental factor for both chosen species as both are distributed in south-eastern Australia. In this region mean annual temperature is likely to increase, with particularly large increases predicted in the summer, and rainfall patterns are likely to be altered. Species inhabiting this area will be subject to increased aridity (Christensen *et al.* 2007). For both studied species, we have found that drought increases the  $R/A$  ratio, a key component of the plant carbon balance. If more widespread, such findings could have important feedback consequences for the global carbon balance.

## Conclusions

Our results provide further evidence of the insensitivity of  $R_{\text{dark}}$  to drought, with two contrasting species differing in foliar morphological traits responding similarly to water stress. On the one hand, this maintenance of  $R_{\text{dark}}$  under drought is likely to be crucial in ensuring that foliar tissues can survive extended periods of drought and thus be better placed to take advantage of subsequent rainfall events. However, on the other hand, by maintaining rates of  $R_{\text{dark}}$  under drought, respiratory carbon release rapidly begins to exceed photosynthetic carbon gain, with negative implications for the carbon economy of whole plants, rates of ecosystem-level net primary productivity and future atmospheric  $\text{CO}_2$  concentrations. Therefore, understanding how drought impacts on rates of  $R_{\text{dark}}$  is crucial for predicting impacts of future climates on the productivity and performance of individual plants, as well as rates of carbon exchange over wide spatial and temporal scales.

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