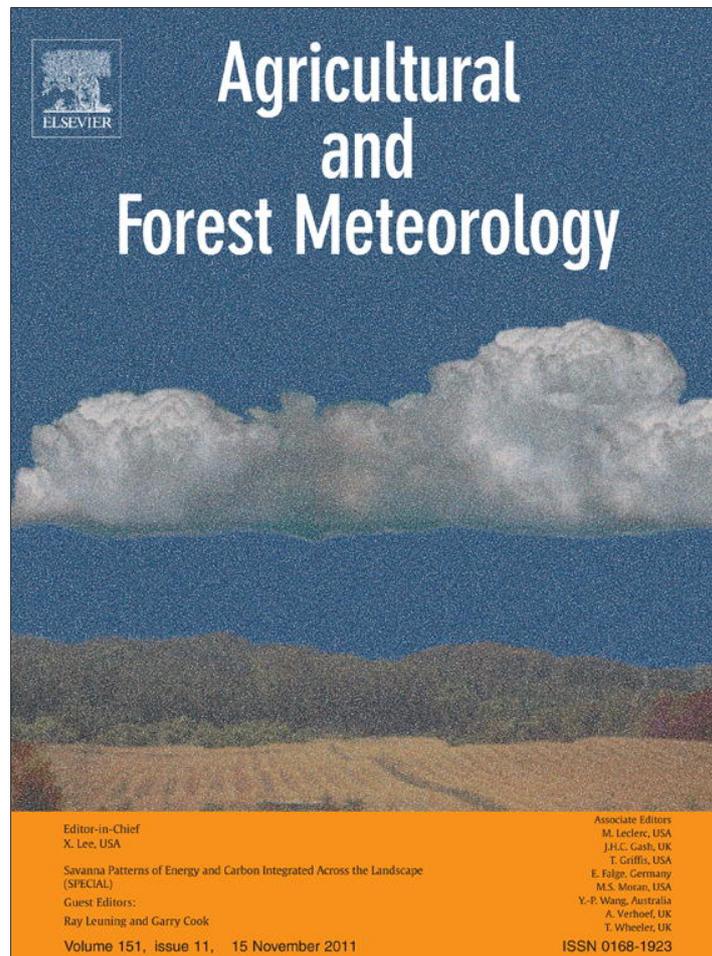


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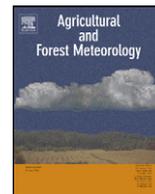
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# Photosynthetic physiology of eucalypts along a sub-continental rainfall gradient in northern Australia

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## ABSTRACT

Leaf-level photosynthetic parameters of species in the closely related genera *Eucalyptus* and *Corymbia* were assessed along a strong rainfall gradient in northern Australia. Both instantaneous gas exchange measurements and leaf carbon isotope discrimination indicated little variation in intercellular CO<sub>2</sub> concentrations during photosynthesis ( $c_i$ ) in response to a decrease in mean annual precipitation from ~1700 mm to ~300 mm. Correlation between stomatal conductance and photosynthetic capacity contributed toward the maintenance of relatively constant  $c_i$  among the sampled leaves, when assessed at ambient CO<sub>2</sub> concentration and photon irradiance similar to full sunlight. Leaf mass per area was the most plastic leaf trait along the rainfall gradient, showing a linear increase in response to decreasing mean annual precipitation. The maximum Rubisco carboxylation velocity,  $V_{cmax}$ , expressed on a leaf-area basis, showed a modest increase in response to decreasing rainfall. This modest increase in  $V_{cmax}$  was associated with the strongly expressed increase in leaf mass per area. These results suggest that variation in ecosystem-level gas exchange during the dry season in north-Australian savannas will likely be dominated by changes in leaf area index in response to increasing aridity, rather than by changes in photosynthetic performance per unit leaf area.

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## 1. Introduction

Understanding physiological responses of plants to environmental resource gradients is important for predicting spatial variation in ecosystem processes and for predicting ecosystem responses to climate change (Koch et al., 1995; Schulze et al., 1996). A strong rainfall gradient exists in northern Australia (Table 1 and Fig. 1), with highest mean annual precipitation (MAP) occurring near the coast and decreasing from north to south, roughly at a rate of 1 mm km<sup>-1</sup> (Bowman and Connors, 1996; Cook and Heerdegen, 2001; Koch et al., 1995). This precipitation gradient is associated with structural and floristic changes in locally dominant vegetation communities (Bowman and Connors, 1996; Hutley et al., 2011; Williams et al., 1996). North of the 500 mm yr<sup>-1</sup> isohyet, dominant vegetation communities tend to be savannas, with a discontinuous tree canopy overlying a grassy understory. Eucalypts, comprising species in the closely related genera *Eucalyptus* and *Corymbia*, dominate the over-stories of these savannas. The height and abundance of the over-story eucalypts decrease with

declining MAP from north to south (Hutley et al., 2011; Schulze et al., 1998; Williams et al., 1996). Where MAP declines to less than about 500 mm, the eucalypt-dominated savannas tend to give way to *Acacia*-dominated shrublands (Bowman and Connors, 1996). Eucalypts are still found in these communities, but generally occur as scattered individuals, rather than canopy-dominant components.

Previous investigations of leaf-level responses of eucalypts to variation in MAP in the Northern Territory of Australia have focussed on measurements of stable carbon isotope discrimination ( $\Delta^{13}C$ ) in leaf dry matter (Miller et al., 2001; Schulze et al., 1998). Leaves were collected at sites ranging from approximately 12°S to 25°S latitude, encompassing MAP ranging from approximately 1800 to 200 mm. Over most of this range, little variation was observed in leaf  $\Delta^{13}C$ , with a marked response only becoming apparent when MAP declined to below about 400 mm (Miller et al., 2001; Schulze et al., 1998). A similarly flat response of eucalypt  $\Delta^{13}C$  to variation in MAP has also been observed in south-western Australia (Schulze et al., 2006a; Turner et al., 2008). These results contrast with a recent global meta-analysis of tree leaf  $\Delta^{13}C$ , which showed an approximately linear increase in  $\Delta^{13}C$  with increasing MAP between 200 and 2000 mm. Over this range, the average  $\Delta^{13}C$  of evergreen angiosperms increased by more than 5% (Diefendorf

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**Table 1**

Summary data for the six study sites. Mean annual precipitation estimates are based on the years 2000 to 2007, and were computed from interpolated rainfall surfaces. Mean annual temperature estimates are averages of mean minimum and mean maximum temperatures for the years 1981–2009, using data from the long term weather station nearest to each study site (<http://www.bom.gov.au/index.shtml>).

| Site           | Location                  | Species  | Elevation (m) | Mean annual precipitation (mm) | Mean annual temperature (°C) |
|----------------|---------------------------|--|---------------|--------------------------------|------------------------------|
| Howard Springs | 12°29'07"S<br>131°08'46"E | <i>Eucalyptus miniata</i><br><i>Eucalyptus tetradonta</i>  | 37            | 1714                           | 27.8                         |
| Adelaide River | 13°04'37"S<br>131°07'04"E | <i>Eucalyptus tectifica</i><br><i>Corymbia latifolia</i>   | 75            | 1532                           | 27.0                         |
| Daly River     | 14°09'33"S<br>131°23'17"E | <i>Eucalyptus tetradonta</i><br><i>Corymbia latifolia</i>  | 73            | 1170                           | 27.2                         |
| Dry Creek      | 15°15'32"S<br>132°22'14"E | <i>Eucalyptus tetradonta</i><br><i>Corymbia terminalis</i> | 167           | 958                            | 27.1                         |
| Sturt Plains   | 17°07'59"S<br>133°19'44"E | <i>Eucalyptus pruinosus</i><br><i>Eucalyptus coolabah</i>  | 228           | 672                            | 26.8                         |
| Boulia         | 22°59'40"S<br>139°56'43"E | <i>Corymbia terminalis</i><br><i>Corymbia aparrerinja</i>  | 151           | 291                            | 24.9                         |

et al., 2010). Notably, this meta-analysis did not include data for any trees sampled in Australia.

Analysis of  $\Delta^{13}\text{C}$  of leaf dry matter provides a convenient method for investigating leaf physiology because it relates to the ratio of intercellular to ambient  $\text{CO}_2$  mole fractions ( $c_i/c_a$ ) during photosynthesis (Farquhar et al., 1982, 1989). Thus, the  $\Delta^{13}\text{C}$  provides a proxy measurement of an important leaf gas exchange characteristic. Analysis of dry matter  $\Delta^{13}\text{C}$  has the further advantage that leaves can be collected, dried, and stored for measurement at a later time. In  $\text{C}_3$  plants, the  $\Delta^{13}\text{C}$  relates to  $c_i/c_a$  according to the following equation (Farquhar et al., 1982; Farquhar and Richards, 1984):

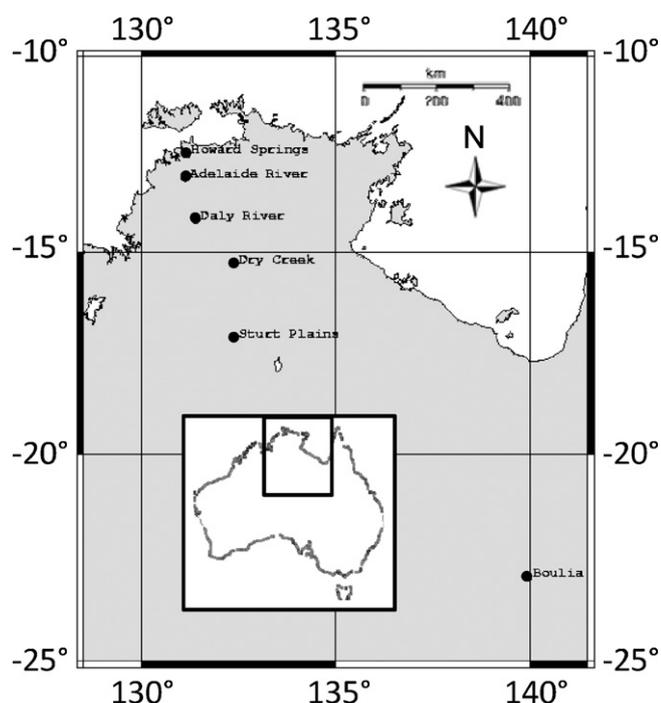
$$\Delta^{13}\text{C} = a - d + (b - a) \frac{c_i}{c_a}, \quad (1)$$

where  $a$  is the  $^{13}\text{C}/^{12}\text{C}$  fractionation that occurs during diffusion of  $\text{CO}_2$  through the stomata (4.4‰),  $b$  is the discrimination against  $^{13}\text{C}$  by carboxylating enzymes (29‰ for Rubisco), and  $d$  is a composite term that includes fractionations caused by dissolution of  $\text{CO}_2$ , liquid phase diffusion, photorespiration, and day respiration

(Farquhar et al., 1989). The  $d$  was previously estimated to have a mean value of about 3‰ in 15 tropical tree species (Cernusak et al., 2008), with other estimates in the literature ranging from about 0 to 4‰ (Cernusak et al., 2007; Hubick, 1990; Hubick and Farquhar, 1989; Hubick et al., 1986).

The  $c_i/c_a$  is controlled by the balance between photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ). For a given  $g_s$ , an increase in  $A$  will cause a decrease in  $c_i/c_a$ . Similarly for a given  $A$ , a decrease in  $g_s$  will cause a decrease in  $c_i/c_a$ . Thus, while the relatively flat response of leaf  $\Delta^{13}\text{C}$  to MAP in eucalypts in northern Australia suggests relatively constant  $c_i/c_a$ , this pattern does not indicate whether  $A$  and  $g_s$  are relatively constant along the precipitation gradient, or whether the two decline in concert as MAP declines. A further ambiguity associated with interpreting the  $\Delta^{13}\text{C}$  of leaf dry matter is that the signal recorded is likely dominated by the canopy  $c_i/c_a$  at the time when the leaf dry matter was synthesized. Therefore, it is unclear whether the observed pattern in leaf dry matter  $\Delta^{13}\text{C}$  in north-Australian eucalypts might provide information about  $c_i/c_a$  only during favourable conditions in the wet season when canopy photosynthesis is most active, or whether it provides information about year-round variation in  $c_i/c_a$  along the precipitation gradient, including during the dry season when drought stress is most severe. Although they represent only a snapshot in time, instantaneous measurements of leaf gas exchange can help to resolve these ambiguities by providing complementary information to that provided by leaf  $\Delta^{13}\text{C}$ .

Leaf photosynthetic capacity is an important parameter in biogeochemical models that predict canopy C and water exchange (Baldocchi and Amthor, 2001). Leaf-level photosynthesis can be predicted according to the biochemical model of Farquhar et al. (1980). A key parameter in this model is the maximum Rubisco carboxylation velocity ( $V_{\text{cmax}}$ ). Thus,  $V_{\text{cmax}}$  provides a measure of photosynthetic capacity that can be readily incorporated into canopy process models. Maximum leaf photosynthesis rates ( $A_{\text{max}}$ ) are generally related to leaf N concentrations (Field and Mooney, 1986), although the relationship between the two can vary among taxa and among regions (Wright et al., 2005). Establishing global relationships between photosynthetic parameters like  $V_{\text{cmax}}$  and  $A_{\text{max}}$  and more easily measured leaf traits like N concentration and leaf mass per area (LMA) offers a promising avenue toward parameterization of global biogeochemical models (Reich et al., 2007). To prevent regional or taxon-specific biases in such global relationships, it is important to include measurements from regions and vegetation types that are under-represented to date (Domingues et al., 2010). Photosynthetic capacity has not previously been assessed by leaf gas exchange analysis in north-Australian eucalypts along the approximately 1500 km north to south gradient in MAP.



**Fig. 1.** A map showing the locations of the study sites in northern Australia. Additional information about each site is provided in Table 1.

Rainfall in our study region of northern Australia is highly seasonal, with a pronounced dry season extending from May until September/October. In savanna communities near Darwin, at the northern end of the precipitation gradient, the soil water that accumulates during the wet season is sufficient to maintain transpiration rates at constant, or even higher, rates in savanna eucalypts throughout the dry season compared to rates in the wet season (Eamus et al., 1999, 2000; Hutley et al., 2000, 2001; O'Grady et al., 1999). Measurements of predawn water potential suggested that these trees have access to available water even at the end of the dry season, presumably from roots that extend to several meters depth in the soil profile (Eamus et al., 2000; Hutley et al., 2000). On the other hand, predawn water potential became more negative during the dry season with increasing distance from the coast (Eamus et al., 2000), suggesting that latitudinal variation in seasonal drought stress along the gradient in MAP in northern Australia should be most pronounced toward the end of the dry season.

In this study, we measured leaf gas exchange in *Eucalyptus* and *Corymbia* species at six sites in northern Australia ranging in MAP from about 1700 mm to about 300 mm. Measurements were carried out as part of a larger campaign to investigate biogeochemical cycling in north-Australian savannas (Beringer et al., 2011). Most measurements took place in September, when we expected latitudinal variation in drought stress to be most pronounced. Our objectives were (1) to investigate the apparent lack of variation in  $c_i/c_a$  along the precipitation gradient, as suggested by previous measurements of leaf dry matter  $\Delta^{13}\text{C}$ , using instantaneous measurements of leaf gas exchange and (2) to assess variation in leaf photosynthetic capacity along the precipitation gradient.

## 2. Materials and methods

Leaf gas exchange measurements were made at six sites along a sub-continental rainfall gradient in northern Australia (Fig. 1). The sites covered a latitudinal range from approximately 12°S to 23°S, with corresponding MAP ranging from approximately 1700 mm to 300 mm (Table 1). Two canopy tree species characteristic of each site were selected for measurements. All of the selected species were from the genus *Eucalyptus*, or the closely related genus *Corymbia* (Table 1). Two individuals of each of the two species at each site were selected for measurements. A total of six leaves were sampled from each species at each site. Canopy access was achieved with a 16 m elevated work platform at the four more northerly sites, and with ladders or from the ground at the two more southerly sites where trees were shorter.

Two portable photosynthesis systems (Li-6400, LiCor Inc., Lincoln, NE, USA) were used to measure the response of photosynthesis to intercellular  $\text{CO}_2$  concentration ( $A$ - $c_i$  curve) and to light (light response curve). The two systems were cross calibrated, and gave unbiased results with respect to each other. The measurement sequence for each sampled leaf started with the  $A$ - $c_i$  curve and then proceeded to the light response curve. The  $\text{CO}_2$  concentration ( $\mu\text{mol mol}^{-1}$ ) entering the leaf gas exchange cuvette was altered in the following sequence: 400, 280, 230, 150, 70, 40, 230, 400, 640, 980, and 1200. A measurement of photosynthesis was logged approximately 2 min after each step change in  $\text{CO}_2$  concentration entering the cuvette. Irradiance during the  $A$ - $c_i$  curve measurements was set at 2000  $\mu\text{mol photosynthetically active radiation (PAR) m}^{-2} \text{s}^{-1}$ , supplied by an artificial light source (6400-02B LED, LiCor Inc.). At the conclusion of the  $A$ - $c_i$  curve, the light response curve was measured on the same leaf. The  $\text{CO}_2$  concentration of air entering the cuvette was maintained at 1200  $\mu\text{mol mol}^{-1}$ , and irradiance ( $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ ) was altered in the following sequence: 2000, 1500, 1000, 500, 200, 120, 70, 40, 20, and 0. A measurement

of photosynthesis was logged approximately 2 min after each step change in irradiance.

We endeavoured to maintain the leaf-to-air vapour pressure difference ( $D$ ) and the leaf temperature ( $T$ ) constant across sites and within a site for each sample leaf during  $A$ - $c_i$  and light response measurements. The target  $D$  was 2.5 kPa and the target  $T$  was 33°C. Mean site values (mean  $\pm$  1 SD) for  $T$  during measurements ranged from 32.3  $\pm$  0.6°C at the Howard Springs site to 33.9  $\pm$  2.0°C at the Boullia site. Mean site values during measurements for  $D$  ranged from 2.1  $\pm$  0.2 kPa at the Howard Springs site to 3.1  $\pm$  0.7 kPa at the Boullia site. Measurements at all sites except the Boullia site took place between 2 and 15 September 2008. Measurements at the Boullia site took place between 5 and 8 December 2008. Precipitation at the Boullia site does not show as highly pronounced a seasonal variation as the more northerly sites. A cumulative precipitation of 56 mm was recorded at the Boullia Airport in the nine months preceding the measurement campaign (<http://www.bom.gov.au/index.shtml>). Gas exchange measurements were taken between 0800 h and 1700 h local time at each site.

At the conclusion of the gas exchange measurements, each leaf was collected and leaf area was determined with an image analysis system (Delta-T Scan, Delta-T Devices, Cambridge, UK). Leaves were dried at 70°C to constant mass, and leaf dry mass was determined to the nearest mg. Leaves were then ground to a fine, homogeneous powder. The nitrogen concentration and leaf carbon isotope ratio a subsample of approximately 3 mg leaf material were determined using a stable isotope ratio mass spectrometer (Delta XP, Finnigan MAT, Bremen, Germany), coupled to an elemental analyser (ECS 4010, Costech Analytical Technologies, Valencia, CA, USA). These analyses were performed in the Stable Isotope Core Laboratory, Washington State University, Pullman, WA, USA. Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) in leaf dry matter was calculated as  $\Delta^{13}\text{C} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p)/(1 + \delta^{13}\text{C}_p)$ , where  $\delta^{13}\text{C}_p$  is  $\delta^{13}\text{C}$  of leaf dry matter, and  $\delta^{13}\text{C}_a$  is that of atmospheric  $\text{CO}_2$ . We assumed a value of -8‰ for  $\delta^{13}\text{C}_a$ . Phosphorus concentration was determined by ashing 100 mg of leaf dry matter at 550°C, followed by dissolution in 1 M  $\text{H}_2\text{SO}_4$  and phosphate detection by automated molybdate colorimetry (Lachat Quickchem 8500, Hach Ltd, Loveland, CO, USA).

The  $A$ - $c_i$  curves were analysed using the spreadsheet utility (version 2007.1) provided by Sharkey et al. (2007). The spreadsheet utility fits the photosynthesis model of Farquhar et al. (1980) to the observed  $\text{CO}_2$  response curve. Estimates are generated of the maximum Rubisco carboxylation rate,  $V_{\text{cmax}}$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); the electron transport rate,  $J$  ( $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$ ); triose phosphate use,  $TPU$  ( $\mu\text{mol triose phosphate m}^{-2} \text{ s}^{-1}$ ); day respiration,  $R_d$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); and mesophyll conductance to  $\text{CO}_2$ ,  $g_m$  ( $\mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ ). The spreadsheet utility provides estimates of these parameters normalized to 25°C to facilitate comparisons with measurements made at other leaf temperatures.

The light response curves were analysed by fitting the observed data to a non-rectangular hyperbola:

$$A = \frac{\phi I + A_{\text{max}} - \sqrt{(\phi I + A_{\text{max}})^2 - 4\theta\phi A_{\text{max}}I}}{2\theta} - R_D \quad (2)$$

where  $\phi$  is the apparent quantum yield ( $\text{mol CO}_2 \text{ mol}^{-1} \text{ PAR}$ ),  $I$  is the irradiance ( $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ),  $A_{\text{max}}$  is the light-saturated gross photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  $\theta$  is the slope of the curve (dimensionless), and  $R_D$  is the dark respiration rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Values for  $\phi$  and  $R_D$  were fitted first by linear regression for measurements made at  $I$  of 0, 20, and 40  $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ . The  $\theta$  and  $A_{\text{max}}$  were then estimated by fitting Eq. (1) to the light response curve using the non-linear regression routine in Systat 12 (Systat Software Inc., Chicago, IL, USA). Because the light response curves were measured with

the CO<sub>2</sub> concentration in air entering the leaf gas exchange cuvette set at 1200 μmol mol<sup>-1</sup>, we assume that the A<sub>max</sub> values estimated from Eq. (1) represent both CO<sub>2</sub> and light saturated photosynthesis.

We tested for variation among sites and between species within a site in photosynthetic parameters using a nested analysis of variance. Post hoc tests for pair-wise differences between species were carried out by Tukey's method. Relationships between continuous variables were tested using least-squares linear regression analyses. Results were considered significant at  $P < 0.05$ . Statistical analyses were performed in Systat 12 (Systat Software Inc.).

### 3. Results

Leaf mass per area, LMA, increased with decreasing MAP along the rainfall gradient (Fig. 2A). The MAP explained 60% of variation in LMA in a linear regression analysis ( $R^2 = 0.60$ ,  $P < 0.001$ ,  $n = 81$ ). There was also variation in LMA between species within several of the sites (Fig. 2A). Thus, species nested within site was a significant term in the analysis of variance ( $P < 0.001$ ,  $n = 81$ ). There was significant variation in mass-based leaf N concentration ( $N_{\text{mass}}$ ) among sites and between species within some of the sites, but there was no overall trend in this parameter with MAP (Fig. 2B). Leaf N concentration expressed on an area basis ( $N_{\text{area}}$ ) increased with decreasing MAP ( $R^2 = 0.35$ ,  $P < 0.001$ ,  $n = 81$ ), due to the strong trend in LMA with MAP. Mass-based leaf P concentration ( $P_{\text{mass}}$ ) decreased significantly with increasing MAP ( $R^2 = 0.06$ ,  $P = 0.02$ ,  $n = 81$ ), and varied between species at some of the sites (Fig. 2C). The decreasing trends in  $P_{\text{mass}}$  and LMA with increasing MAP also resulted in decreasing area-based leaf P concentration ( $P_{\text{area}}$ ) with increasing MAP ( $R^2 = 0.40$ ,  $P < 0.001$ ,  $n = 81$ ). Leaf dry matter  $\Delta^{13}\text{C}$  was not related to MAP ( $P = 0.36$ ,  $n = 81$ ). Analysis of variance indicated that there was significant variation among some of the sites and between species within some sites for leaf  $\Delta^{13}\text{C}$ , but this variation showed no overall trend with MAP (Fig. 2D). Leaf  $\Delta^{13}\text{C}$  did not correlate with  $N_{\text{mass}}$  ( $P = 0.38$ ,  $n = 81$ ) or  $N_{\text{area}}$  ( $P = 0.87$ ,  $n = 81$ ), but did correlate significantly with both  $P_{\text{mass}}$  ( $R^2 = 0.14$ ,  $P < 0.001$ ,  $n = 81$ ) and  $P_{\text{area}}$  ( $R^2 = 0.20$ ,  $P < 0.001$ ,  $n = 81$ ).

Instantaneous measurements of stomatal conductance ( $g_s$ ), made at  $c_a$  of approximately 380 μmol mol<sup>-1</sup> and irradiance of 2000 μmol PAR m<sup>-2</sup> s<sup>-1</sup>, tended to decrease with decreasing MAP, but the trend was not statistically significant ( $P = 0.08$ ,  $n = 76$ ). Mean values for  $g_s$  for each species at each site are shown in Table 2. Net photosynthesis measured under the same conditions showed a similar trend, with a weak dependence on MAP ( $R^2 = 0.06$ ,  $P = 0.03$ ,  $n = 76$ ). Species within a site differed from each other at two of the sites for net photosynthesis under these conditions (Table 2). Variation in net photosynthesis was closely correlated with variation in  $g_s$  when measured at  $c_a$  of 380 μmol mol<sup>-1</sup> and irradiance of 2000 μmol PAR m<sup>-2</sup> s<sup>-1</sup> (Fig. 3). A linear relationship between net photosynthesis and  $g_s$  would imply a constant  $c_i/c_a$  across the set of measurements. Accordingly,  $c_i/c_a$  was relatively constant across the sampled leaves, with only *Eucalyptus tetradonta* at Daly River and *Corymbia aparrerinja* at Boulia differing from each other in the pair-wise comparisons (Table 2). The  $c_i/c_a$  was independent of variation in MAP ( $P = 0.67$ ,  $n = 76$ ).

The  $c_i/c_a$  showed a linear and relatively weak dependence on  $g_s$  for gas exchange measurements made at  $c_a$  of 380 μmol mol<sup>-1</sup> and irradiance of 2000 μmol PAR m<sup>-2</sup> s<sup>-1</sup> ( $R^2 = 0.11$ ,  $P = 0.003$ ,  $n = 76$ ), as shown in Fig. 4. The dashed line in Fig. 4 shows the predicted relationship between  $c_i/c_a$  and  $g_s$  if  $V_{\text{cmax}}$ ,  $J$ ,  $g_m$ , and other parameters in the Farquhar et al. (1980) photosynthesis model were to remain constant across the range of  $g_s$  encountered in the study. The departure of the solid regression line in Fig. 4 from the predicted dashed line suggests that  $c_i/c_a$  declined less at low  $g_s$  than

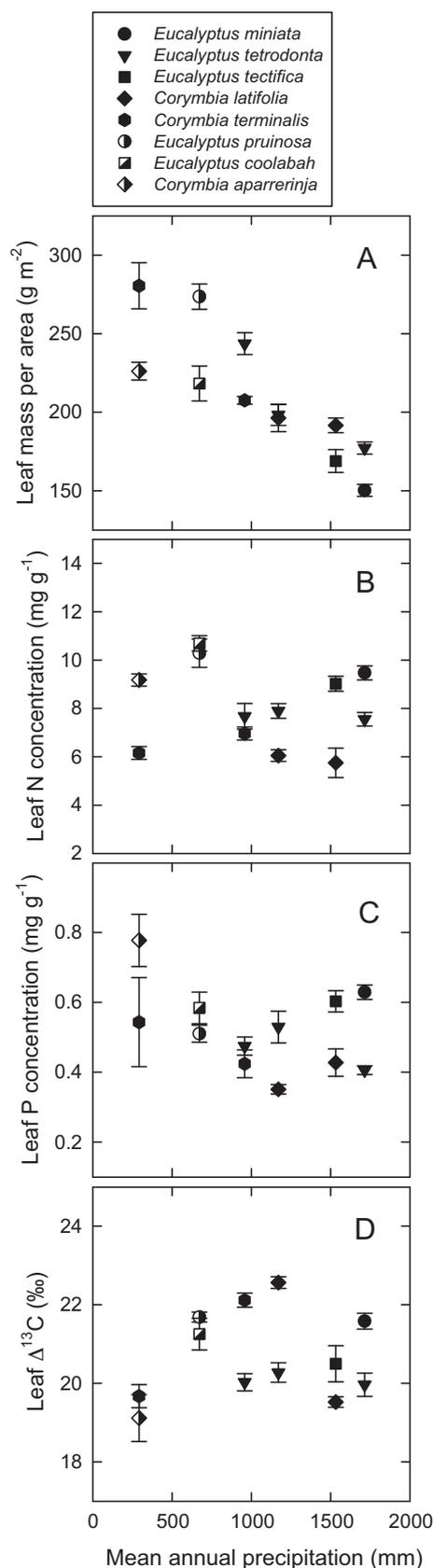
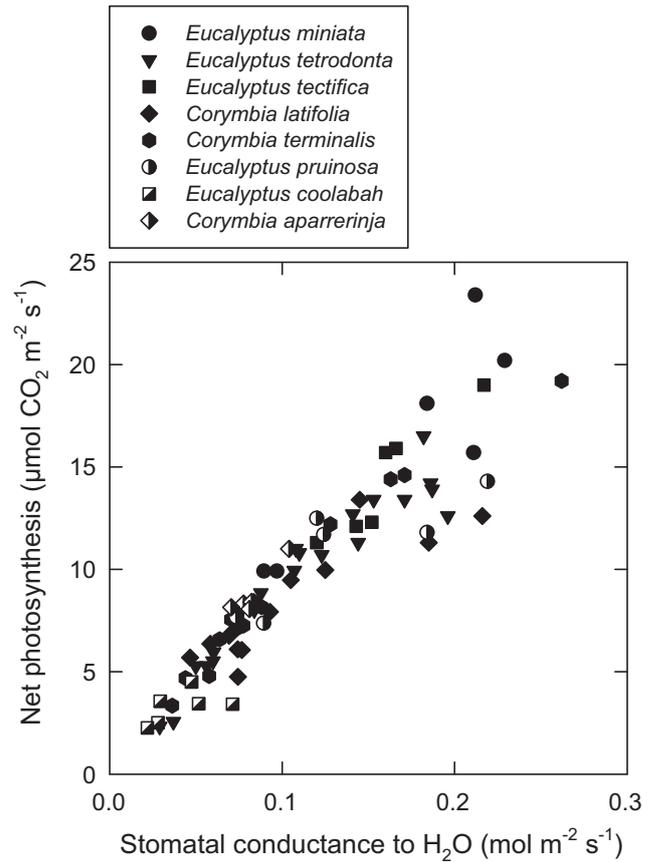


Fig. 2. Leaf mass per area (A), leaf nitrogen concentration (B), leaf phosphorus concentration (C), and carbon isotope discrimination in leaf dry matter (D) plotted as functions of mean annual precipitation. Each symbol represents the mean of six to eight leaves. Error bars are one standard error.

**Table 2**

Gas exchange parameters for eucalypt leaves along a rainfall gradient in northern Australia. Stomatal conductance, net photosynthesis, and the ratio of the internal to ambient CO<sub>2</sub> mole fractions (c<sub>i</sub>/c<sub>a</sub>) were measured at irradiance of 2000 μmol photosynthetically active radiation (PAR) m<sup>-2</sup> s<sup>-1</sup> and c<sub>a</sub> of approximately 380 μmol mol<sup>-1</sup>. The maximal Rubisco carboxylation velocity (V<sub>cm<sub>max</sub></sub>) and electron transport rate (J) were determined at irradiance of 2000 μmol PAR m<sup>-2</sup> s<sup>-1</sup> from CO<sub>2</sub> response curves. Apparent quantum yield (φ) and maximum photosynthetic rate (A<sub>max</sub>) were determined from light response curves at c<sub>a</sub> of approximately 1170 μmol mol<sup>-1</sup>. Values shown are the mean of six to eight leaves for each species at each site ± 1 standard deviation. Values within a column letter differed from each other at P < 0.05.

| Site           | Species                      | Stomatal conductance (mol m <sup>-2</sup> s <sup>-1</sup> ) | Net photosynthesis (μmol m <sup>-2</sup> s <sup>-1</sup> ) | c <sub>i</sub> /c <sub>a</sub> | V <sub>cm<sub>max</sub></sub> at 25 °C (μmol m <sup>-2</sup> s <sup>-1</sup> ) | J at 25 °C (μmol m <sup>-2</sup> s <sup>-1</sup> ) | Apparent quantum yield, φ (mol mol <sup>-1</sup> ) | A <sub>max</sub> (μmol m <sup>-2</sup> s <sup>-1</sup> ) |
|----------------|------------------------------|---|--|--------------------------------|--|--|--|--|
| Howard Springs | <i>Eucalyptus miniata</i>    | 0.170 ± 0.062 a   | 16.2 ± 5.5 a   | 0.54 ± 0.06 a,b                | 77.6 ± 6.4 a,b   | 85.0 ± 14.8 a,b,c                                  | 0.067 ± 0.017 a                                    | 34.0 ± 7.2 a,b,c   |
|                | <i>Eucalyptus tetrodonta</i> | 0.085 ± 0.028 a,b   | 8.0 ± 2.3 b,c  | 0.53 ± 0.03 a,b                | 62.8 ± 12.4 b  | 69.6 ± 9.4 b,c                                     | 0.059 ± 0.013 a,b                                  | 23.6 ± 5.7 b,c   |
| Adelaide River | <i>Eucalyptus tectifia</i>   | 0.160 ± 0.032 a   | 14.4 ± 3.0 a,b   | 0.57 ± 0.03 a,b                | 84.1 ± 15.9 a,b  | 103.3 ± 20.3 a,b                                   | 0.057 ± 0.012 a,b                                  | 39.5 ± 9.2 a,b   |
|                | <i>Corymbia latifolia</i>    | 0.078 ± 0.034 a,b   | 7.4 ± 3.0 b,c  | 0.55 ± 0.07 a,b                | 64.4 ± 20.0 b  | 83.4 ± 22.8 a,b,c                                  | 0.049 ± 0.013 a,b                                  | 29.3 ± 7.8 a,b,c   |
| Daly River     | <i>Eucalyptus tetrodonta</i> | 0.151 ± 0.049 a   | 11.9 ± 3.2 a,b   | 0.59 ± 0.04 a,b                | 93.4 ± 14.3 a,b  | 98.0 ± 12.0 a,b                                    | 0.060 ± 0.013 a,b                                  | 44.0 ± 8.9 a   |
|                | <i>Corymbia latifolia</i>    | 0.133 ± 0.056 a   | 9.3 ± 2.8 a,b,c  | 0.63 ± 0.06 a                  | 73.2 ± 14.9 b  | 80.2 ± 14.1 a,b,c                                  | 0.049 ± 0.012 a,b                                  | 29.8 ± 4.0 a,b,c   |
| Dry Creek      | <i>Eucalyptus tetrodonta</i> | 0.107 ± 0.061 a   | 9.3 ± 5.2 b,c  | 0.59 ± 0.05 a,b                | 84.6 ± 22.2 a,b  | 85.3 ± 22.0 a,b,c                                  | 0.047 ± 0.015 a,b                                  | 28.9 ± 16.8 a,b,c  |
|                | <i>Corymbia terminalis</i>   | 0.081 ± 0.039 a,b   | 7.7 ± 3.1 b,c  | 0.54 ± 0.04 a,b                | 94.1 ± 34.1 a,b  | 95.0 ± 32.2 a,b                                    | 0.059 ± 0.009 a,b                                  | 42.3 ± 8.1 a   |
| Sturt Plains   | <i>Eucalyptus pruinosa</i>   | 0.135 ± 0.056 a   | 10.9 ± 2.8 a,b   | 0.59 ± 0.07 a,b                | 82.1 ± 12.2 a,b  | 91.1 ± 15.5 a,b,c                                  | 0.041 ± 0.018 b                                    | 31.7 ± 7.6 a,b,c   |
|                | <i>Eucalyptus coolabah</i>   | 0.042 ± 0.019 b   | 3.3 ± 0.8 c  | 0.59 ± 0.1 a,b                 | 65.0 ± 9.1 b   | 58.4 ± 7.6 c                                       | 0.051 ± 0.006 a,b                                  | 18.8 ± 2.9 c   |
| Boulia         | <i>Corymbia terminalis</i>   | 0.125 ± 0.080 a   | 10.7 ± 5.7 a,b   | 0.57 ± 0.03 a,b                | 109.6 ± 24.1 a   | 113.1 ± 27.7 a                                     | 0.063 ± 0.009 a,b                                  | 39.2 ± 14.3 a,b  |
|                | <i>Corymbia aparreerinja</i> | 0.083 ± 0.011 a,b   | 8.7 ± 1.1 b,c  | 0.51 ± 0.02 b                  | 77.1 ± 9.7 a,b   | 100.1 ± 11.1 a,b                                   | 0.059 ± 0.004 a,b                                  | 31.0 ± 2.7 a,b,c   |

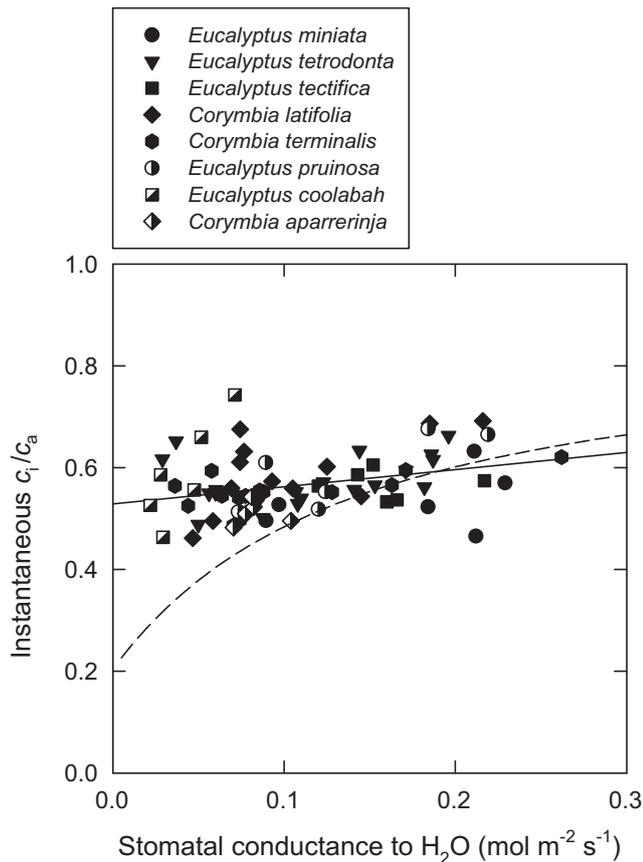


**Fig. 3.** Net photosynthesis plotted against stomatal conductance for eucalypt leaves sampled along a rainfall gradient in northern Australia. Measurements were made at external CO<sub>2</sub> concentration of 380 μmol mol<sup>-1</sup> and irradiance of 2000 μmol photosynthetically active radiation m<sup>-2</sup> s<sup>-1</sup>.

would be expected in the case for invariant photosynthetic capacity and g<sub>m</sub>.

The V<sub>cm<sub>max</sub></sub> normalized to 25 °C and expressed on an area basis showed a weak increasing trend with decreasing MAP (R<sup>2</sup> = 0.07, P = 0.02, n = 79). This relationship was the result of increasing LMA with decreasing MAP; when expressed on a mass basis, V<sub>cm<sub>max</sub></sub> showed an opposite trend of increasing with increasing MAP (R<sup>2</sup> = 0.10, P = 0.004, n = 79). The V<sub>cm<sub>max</sub></sub> was not related to leaf N concentration, neither with the two parameters expressed on an area basis (P = 0.34, n = 79), nor on a mass basis (P = 0.78, n = 79). Similarly, V<sub>cm<sub>max</sub></sub> was not related to leaf P concentration, neither for mass-based (P = 0.86, n = 79) nor area-based (P = 0.44, n = 79) comparisons. However, area-based V<sub>cm<sub>max</sub></sub> was positively correlated with LMA (Fig. 5). Area-based V<sub>cm<sub>max</sub></sub> also correlated with g<sub>s</sub> measured at c<sub>a</sub> of 380 μmol mol<sup>-1</sup> and PAR of 2000 μmol m<sup>-2</sup> s<sup>-1</sup> (R<sup>2</sup> = 0.15, P < 0.001, n = 76). The electron transport rate, J, correlated positively with V<sub>cm<sub>max</sub></sub>. The regression equation with the two parameters normalized to 25 °C was J = 0.91 V<sub>cm<sub>max</sub></sub> + 14.4 (R<sup>2</sup> = 0.76, P < 0.001, n = 79). Mean values of V<sub>cm<sub>max</sub></sub> and J for each species at each site are given in Table 2.

The apparent quantum yield, φ, estimated from the initial slope of the light response curves, showed little variation across the sampled leaves (Table 2). The light-saturated gross photosynthetic rate, A<sub>max</sub>, was positively correlated with V<sub>cm<sub>max</sub></sub> (Fig. 6), suggesting that the two measurements provided consistent indications of variation in photosynthetic capacity. Area-based A<sub>max</sub> was unrelated to MAP (P = 0.90, n = 74), whereas mass-based A<sub>max</sub> increased with increasing MAP (R<sup>2</sup> = 0.16, P < 0.001, n = 74). The A<sub>max</sub> showed no significant relationship with leaf N concentra-

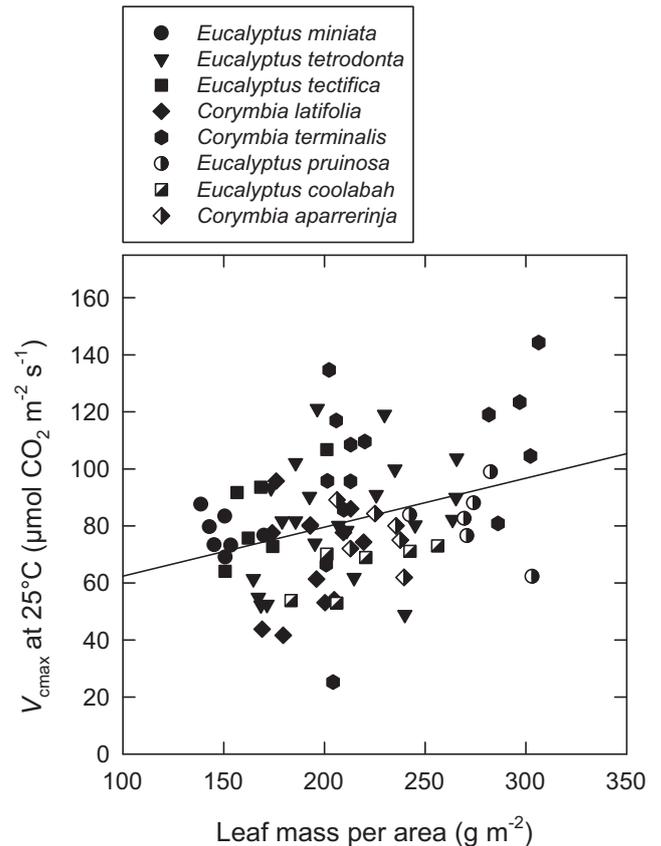


**Fig. 4.** The ratio of intercellular to ambient CO<sub>2</sub> mole fraction plotted against stomatal conductance. The solid line is a linear regression through the data ( $R^2 = 0.11$ ,  $P = 0.003$ ,  $n = 76$ ). The dashed line is the predicted relationship between the two parameters if the maximum Rubisco carboxylation velocity, the electron transport rate, and mesophyll conductance were assumed constant over the observed range of stomatal conductance.

tion or with leaf P concentration. The  $A_{\max}$  correlated with  $g_s$  measured at  $c_a$  of  $380 \mu\text{mol mol}^{-1}$  and PAR of  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $R^2 = 0.39$ ,  $P < 0.001$ ,  $n = 69$ ). The parameter,  $\theta$ , representing the slope of the light response curve, did not vary among sites or between species within a site. The mean value across all sampled leaves was  $0.17 \pm 0.06$  (mean  $\pm$  1SE,  $n = 74$ ). The dark respiration rate measured during the light response curves varied as a function of  $A_{\max}$  according to the regression equation  $R_D = 0.028A_{\max} + 0.61$  ( $R^2 = 0.19$ ,  $P < 0.001$ ,  $n = 74$ ). The gross photosynthetic rate at irradiance of  $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  was on average 81% of  $A_{\max}$ , suggesting that photosynthesis at  $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  was not light-saturated. Mean  $A_{\max}$  for each species at each site is given in Table 2.

#### 4. Discussion

Measurements of leaf dry matter  $\Delta^{13}\text{C}$  for savanna eucalypts in this study were similar to previous observations, showing little variation in response to a strong rainfall gradient in northern Australia (Miller et al., 2001; Schulze et al., 1998). This pattern contrasts with relatively strong relationships between leaf dry matter  $\Delta^{13}\text{C}$  and MAP for trees sampled in North America and Asia (Diefendorf et al., 2010), and in Africa (Midgley et al., 2004). In the present study, we were able to confirm, using instantaneous gas exchange measurements, that  $c_i/c_a$  was nearly constant in savanna eucalypts along the strong rainfall gradient in northern Australia. This observation took place at the end of the dry season, when we expected latitudinal variation in drought stress to be

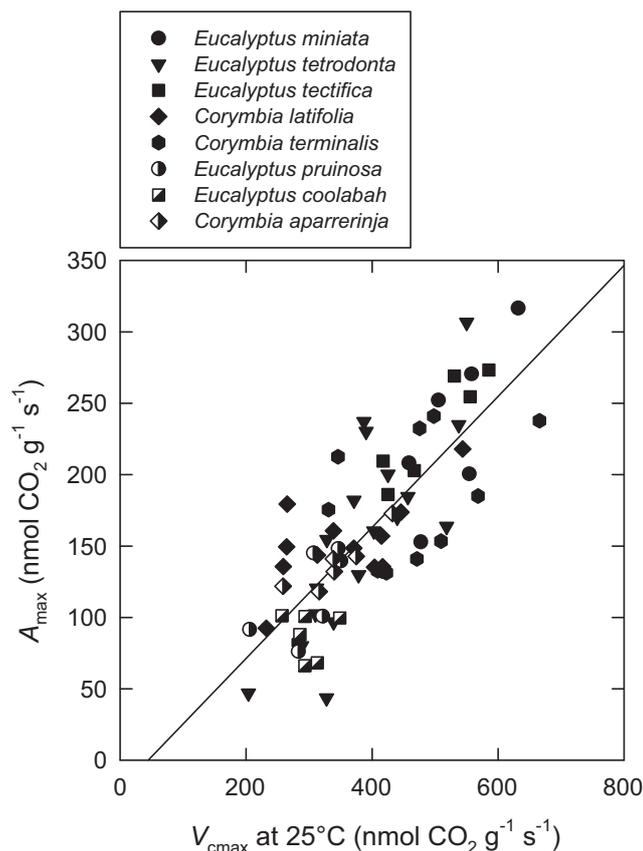


**Fig. 5.** The maximum Rubisco carboxylation velocity plotted against the leaf mass per area for eucalypt leaves sampled along a rainfall gradient in northern Australia. The solid line is a regression line:  $V_{\text{cmax}} = 0.17\text{LMA} + 45$  ( $R^2 = 0.11$ ,  $P = 0.003$ ,  $n = 79$ ).

most pronounced. The nearly constant  $c_i/c_a$  observed in this study resulted at least partly from a correlation between photosynthetic capacity and stomatal conductance. A close coupling between photosynthetic capacity and stomatal conductance has been previously observed in a range of C<sub>3</sub> and C<sub>4</sub> plant species (Brodribb and Feild, 2000; Cernusak and Marshall, 2001; Hubbard et al., 2001; Wong et al., 1978, 1979, 1985).

We found three lines of evidence suggesting that photosynthetic capacity in the leaves that we sampled was linked to stomatal conductance. Firstly, estimates of  $V_{\text{cmax}}$  correlated with  $g_s$  measured at ambient CO<sub>2</sub> concentration and irradiance of  $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ . Secondly, the  $A_{\max}$  estimated from light response curves conducted at saturating CO<sub>2</sub> concentration correlated with  $g_s$  measured at ambient CO<sub>2</sub> and irradiance of  $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ . And thirdly, the observed  $c_i/c_a$  decreased less with decreasing  $g_s$  than was expected theoretically for the case of invariant  $V_{\text{cmax}}$ ,  $J$ , and  $g_m$  (Fig. 4). These observations suggest that modelling approaches for predicting photosynthesis in north-Australian savanna trees may benefit by linking photosynthetic capacity to  $g_s$ . Several authors have formulated models that apply this concept (Ball et al., 1987; Buckley et al., 2003; Farquhar and Wong, 1984; Jarvis and Davies, 1998; Leuning, 1990; Leuning, 1995), although the mechanistic basis for coupling between photosynthetic capacity and  $g_s$  is still uncertain (von Caemmerer et al., 2004).

A correlation between  $g_s$  and mesophyll conductance,  $g_m$ , could have also contributed to the apparent correlation between photosynthetic capacity and  $g_s$ , if  $g_m$  were lower in leaves with low  $g_s$  than in leaves with high  $g_s$ . Although the spreadsheet utility that we used to estimate  $V_{\text{cmax}}$  also provides estimates of  $g_m$  (Sharkey et al., 2007), this method for estimating  $g_m$  was found to be less reliable



**Fig. 6.** Maximum gross photosynthesis determined from light response curves plotted against the maximum Rubisco carboxylation velocity determined from CO<sub>2</sub> response curves. Both parameters are expressed on a mass basis. The solid line is a linear regression:  $A_{\max} = 0.46 V_{\text{cmax}} - 21$  ( $R^2 = 0.63$ ,  $P < 0.001$ ,  $n = 72$ ).

than methods that apply chlorophyll fluorescence or on-line  $\Delta^{13}\text{C}$  measurements (Pons et al., 2009). We did observe a correlation between the estimated  $g_m$  and  $g_s$  measured at ambient CO<sub>2</sub> concentration and 2000  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ , but the relationship was weak ( $R^2 = 0.09$ ,  $P = 0.01$ ,  $n = 76$ ). The mean estimate of  $g_m$  across all leaves in the study was 10  $\mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$ . This estimate is considerably higher than values determined previously for a range of Australian plant species with sclerophyllous leaves (Niinemets et al., 2009). Consistent with previous observations (Flexas et al., 2008; Hassiotou et al., 2009), we also observed a negative correlation between  $g_m$  and LMA ( $R^2 = 0.26$ ,  $P < 0.001$ ,  $n = 79$ ). This suggests that  $g_m$  should decrease with decreasing MAP due to the increase in LMA. A more detailed investigation of variation in  $g_m$  in savanna eucalypts along the north-Australian rainfall gradient using chlorophyll fluorescence or on-line  $\Delta^{13}\text{C}$  methods in combination with gas exchange would be helpful to verify this trend.

The relatively flat responses of leaf dry matter  $\Delta^{13}\text{C}$  and instantaneous  $c_i/c_a$  to a strong rainfall gradient in northern Australia observed here and previously (Miller et al., 2001; Schulze et al., 1998) contrasts with results of two studies in eastern Australia (Stewart et al., 1995; Wright et al., 2001). The studies in eastern Australia investigated variations in  $\Delta^{13}\text{C}$  and  $c_i/c_a$  of broader vegetation communities in response to MAP, whereas our study only investigated species within the closely related genera of *Eucalyptus* and *Corymbia*. However, the north-Australian transect study of Schulze et al. (1998) included a broad taxonomic range of woody plant species, and these authors still only observed a weak community-level response of  $\Delta^{13}\text{C}$  to MAP. Seasonality of rainfall could be a feature contributing to these contrasting results for responses of  $\Delta^{13}\text{C}$  and  $c_i/c_a$  to MAP in northern versus east-

ern Australia (Miller et al., 2001; Schulze et al., 1998). Rainfall in monsoonal northern Australia shows a much stronger seasonal distribution than rainfall in eastern Australia. Within eucalypts more generally, some species appear to show no response of  $\Delta^{13}\text{C}$  to variation in MAP, whereas other species show a more plastic response, with  $\Delta^{13}\text{C}$  increasing in response to increasing MAP (Cernusak et al., 2005; Miller et al., 2001; Schulze et al., 2006a,b; Turner et al., 2010; Warren et al., 2006).

The most plastic leaf trait in response to MAP assessed in our study was the leaf mass per area. The LMA decreased with increasing MAP at a rate of 70 g leaf dry mass  $\text{m}^2$  leaf area  $\text{m}^{-1}$  mean annual precipitation. Because leaf  $N_{\text{mass}}$  was independent of MAP, leaf  $N_{\text{area}}$  increased as MAP decreased due to the variation in LMA. The  $V_{\text{cmax}}$ , expressed per unit leaf area, also increased as MAP decreased, although the increase was not as pronounced as that for leaf  $N_{\text{area}}$ . This change in LMA, leaf  $N_{\text{area}}$ , and photosynthetic capacity with decreasing MAP is consistent with theoretical predictions (Farquhar et al., 2002), and with previous observations in this and other ecosystems (Midgley et al., 2004; Mooney et al., 1978; Schulze et al., 1998; Wright et al., 2001).

Leaf photosynthetic capacity is often observed to correlate with leaf N concentration (Domingues et al., 2010; Field and Mooney, 1986; Wright et al., 2004). This has been explained on the basis of the proportionally large allocation of N to photosynthetic enzymes and light-harvesting pigments in leaves (Evans, 1989). In the present study we did not observe a significant relationship between leaf N concentration and  $V_{\text{cmax}}$ ,  $A_{\max}$ , or photosynthesis at ambient CO<sub>2</sub> concentration. This lack of correlation most likely resulted from the relatively narrow range of leaf N concentration encountered in the study (Fig. 2B). Leaf N concentrations in the leaves that we sampled were low compared to the range of values typically observed in angiosperm trees (Reich and Oleksyn, 2004), but similar to observations for other eucalypts (Bell and Williams, 1997; Prior et al., 2003; Schulze et al., 1998).

In order to cover a larger range of leaf N concentrations, we combined  $V_{\text{cmax}}$  data from the present study with observations recently reported for seedlings of north-Australian tree species grown in a shade house on the Charles Darwin University campus (Orchard et al., 2010). Mean values for each species at each site were taken as data points. A significant correlation resulted between  $V_{\text{cmax}}$  at 25 °C ( $\text{nmol g}^{-1} \text{s}^{-1}$ ) and leaf  $N_{\text{mass}}$  ( $\text{mg g}^{-1}$ ):  $V_{\text{cmax}} = 41.2 N_{\text{mass}} + 151$  ( $R^2 = 0.67$ ,  $P < 0.001$ ,  $n = 22$ ). In the dataset, leaf  $N_{\text{mass}}$  ranged from 5.7 to 33.7  $\text{mg g}^{-1}$ , and  $V_{\text{cmax}}$  at 25 °C from 301 to 1380  $\text{nmol g}^{-1} \text{s}^{-1}$ . This demonstrates that even though we did not observe a relationship between  $V_{\text{cmax}}$  and leaf  $N_{\text{mass}}$  in the present study, leaf  $N_{\text{mass}}$  is still a useful predictor of  $V_{\text{cmax}}$  when a larger range of leaf  $N_{\text{mass}}$  is taken into account. The  $V_{\text{cmax}}-N$  slope of the relationship obtained from this combined analysis of 41.2  $\mu\text{mol CO}_2 \text{g}^{-1} \text{N s}^{-1}$  is in the middle range of  $V_{\text{cmax}}-N$  slopes observed in a range of previous studies (Kull, 2002).

It has been previously observed that leaf photosynthetic capacity correlated with leaf P concentration in some P-limited tropical ecosystems (Domingues et al., 2010; Meir et al., 2007), and that leaf photosynthesis–N slopes can be influenced by leaf P concentrations (Reich et al., 2009). We did not observe any strong relationship between leaf gas exchange rates and leaf P concentrations in our dataset. In common with the N comparisons, this likely resulted from a limited range of variation in P concentrations among the experimental leaves (Fig. 2C). We did, however, observe a significant correlation between leaf  $\Delta^{13}\text{C}$  and leaf  $P_{\text{area}}$ , which hints at the possibility that leaf  $P_{\text{area}}$  could have been influencing photosynthetic capacity in the leaves that we sampled. The average N:P ratio across the dataset was 16.4  $\text{g g}^{-1}$ . According to a simple interpretation of leaf N:P ratios, this would be consistent with co-limitation of productivity in over-story eucalypts along the rainfall gradient by both N and P (Aerts and Chapin, 2000; Güsewell, 2004; Koerselman

and Meuleman, 1996; Reich and Oleksyn, 2004). However, some observations suggest that interpretation of leaf N:P ratios may be more complex (Ågren, 2008; Cernusak et al., 2010; Craine et al., 2008; Garrish et al., 2010). Thus, further investigation is required to determine the relative extents to which N and P availability constrain photosynthesis and growth of eucalypts in savannas of northern Australia.

The light response curves that we measured suggested that photosynthesis was not yet light saturated at  $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  in eucalypt leaves in northern Australia. The gross photosynthetic rate measured at this irradiance was on average 81% of the rate estimated from the asymptote of the light response curves. This explains why the slope between  $J$  and  $V_{\text{cmax}}$  for our dataset determined at  $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  was low in comparison to  $J_{\text{max}}-V_{\text{cmax}}$  slopes reported in other studies. The  $J-V_{\text{cmax}}$  slope determined in our study was 0.91, whereas  $J_{\text{max}}-V_{\text{cmax}}$  slopes reported by others range from about 1.3–1.7 (Meir et al., 2007; Midgley et al., 2004; Wullschlegel, 1993). If we assume that  $J$  estimates at irradiance of  $2000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$  in our study were 81% of  $J_{\text{max}}$ , we would obtain a  $J_{\text{max}}-V_{\text{cmax}}$  slope of 1.1. This value is the same as the  $J_{\text{max}}-V_{\text{cmax}}$  slope recently estimated for West African woodlands (Domingues et al., 2010).

Previous observations of leaf gas exchange in *E. tetradonta* growing at three sites in the Northern Territory with MAP ranging from 1650 to 950 mm suggested modest adjustment of area-based leaf gas exchange rates in response to declining MAP (Prior et al., 2005). In that study, increases in area-based photosynthetic capacity at the drier sites were associated with increases in LMA. We observed a similar trend for multiple eucalypt species at sites spanning a wider range of MAP. In our study, the area-based  $V_{\text{cmax}}$  showed a modest increase with decreasing MAP in association with increasing LMA. Comparative data for wet season gas exchange rates would be helpful to determine whether a similar pattern holds when soil moisture availability is uniformly high along the north to south transect. Furthermore, there is likely a suite of traits related to hydraulic architecture and desiccation tolerance that allows the eucalypt species with more southerly distributions to maintain similar rates of area-based leaf gas exchange to the northerly species during the late dry season. Identifying such traits would provide valuable insight into eucalypt ecophysiology. For the dry season at least, our results suggest that variation in ecosystem-level gas exchange in north-Australian savannas will be largely driven by leaf area index, rather than by marked variation in gas exchange rates per unit leaf area.

## 5. Conclusions

In conclusion, we observed relatively subtle variation in leaf-level physiological processes along a strong rainfall gradient in northern Australia at the end of the dry season, when we expected the north to south variation in drought stress to be most pronounced. Leaf dry matter  $\Delta^{13}\text{C}$  and instantaneous measurements of  $c_i/c_a$  did not vary as functions of MAP. The LMA was the trait that showed the most plasticity along the rainfall transect, increasing in response to decreasing precipitation. The photosynthetic parameter  $V_{\text{cmax}}$  also showed a modest increase in response to decreasing MAP. This increase in  $V_{\text{cmax}}$  was apparently modulated by increasing LMA. Stomatal conductance,  $g_s$ , showed a weak tendency toward decreasing with decreasing MAP. Although  $g_s$  did not show a strong systematic response to MAP, it did vary among leaves in the study, and this variation was correlated with variation in photosynthetic capacity. Our results suggest that variation in ecosystem level gas exchange during the dry season in north-Australian savannas will likely be dominated by changes in leaf area index in response to increasing aridity, rather than by changes in photosynthetic performance per unit leaf area.

## Acknowledgments

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