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# 1 **Gas exchange and water-use efficiency in plant canopies: a** 2 **review**

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## 8 9 **ABSTRACT**

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11 In this review, I first address the basics of gas exchange, water-use efficiency, and carbon isotope  
12 discrimination in C<sub>3</sub> plant canopies. I then present a case study of water-use efficiency in northern  
13 Australian tree species. In general, C<sub>3</sub> plants face a trade-off whereby increasing stomatal  
14 conductance for a given set of conditions will result in a higher CO<sub>2</sub> assimilation rate, but a lower  
15 photosynthetic water-use efficiency. A common garden experiment suggested that tree species which  
16 are able to establish and grow in drier parts of northern Australia have a capacity to use water rapidly  
17 when it is available through high stomatal conductance, but that they do so at the expense of low  
18 water-use efficiency. This may explain why community level carbon isotope discrimination does not  
19 decrease as steeply with decreasing rainfall on the North Australian Tropical Transect as has been  
20 observed on some other precipitation gradients. Next, I discuss changes in water-use efficiency that  
21 take place during leaf expansion in C<sub>3</sub> plant leaves. Leaf phenology has recently been recognized as  
22 a significant driver of canopy gas exchange in evergreen forest canopies, and leaf expansion involves  
23 changes in both photosynthetic capacity and water-use efficiency. Following this, I discuss the role  
24 of woody tissue respiration in canopy gas exchange and how photosynthetic re-fixation of respired  
25 CO<sub>2</sub> can increase whole-plant water-use efficiency. Finally, I discuss the role of water-use efficiency  
26 in driving terrestrial plant responses to global change, especially the rising concentration of  
27 atmospheric CO<sub>2</sub>. In coming decades, increases in plant water-use efficiency caused by rising CO<sub>2</sub>  
28 are likely to partially mitigate impacts on plants of drought stress caused by global warming.  
29

30 **KEY WORDS:** Photosynthesis, stable carbon isotope, stomatal conductance, water-use efficiency

## INTRODUCTION

Large quantities of CO<sub>2</sub> and water vapour are exchanged between terrestrial plant canopies and the atmosphere each year. Estimates of the annual gross primary production (GPP) globally for terrestrial plants, that is the total amount of carbon captured by photosynthesis per annum, range from about 120 to 140 Pg C yr<sup>-1</sup> (Beer et al., 2010; Welp et al., 2011; Jasechko et al., 2013; Le Quere et al., 2013). The energy that is captured and stored by photosynthesis is distributed to other parts of the plant in the form of chemical bonds holding organic molecules together. These bonds are then broken to support growth and maintenance, transferring energy through molecules such as ATP and NADPH, and releasing CO<sub>2</sub> back to the atmosphere. In the steady state, the same quantity of CO<sub>2</sub> will be released to the atmosphere by the terrestrial biosphere each year as is taken up by photosynthesis. About half of the return flux comes from plant respiration, and about half from the decomposition of dead plant material (Waring et al., 1998; Malhi et al., 1999).

In order to carry out photosynthesis, terrestrial plants must open their stomata to allow CO<sub>2</sub> molecules to diffuse eventually into the chloroplast stroma where the enzymes that make up the photosynthetic carbon reduction cycle reside. When stomata open, the moist interior of the leaf is inevitably exposed to a drier atmosphere outside the leaf. As a result, water vapour diffuses from the leaf interior to the atmosphere through the process of transpiration. Estimates of global terrestrial transpiration range between about 20 and 60 Eg H<sub>2</sub>O yr<sup>-1</sup> (Hetherington and Woodward, 2003; Jasechko et al., 2013). Plant water-use efficiency describes the amount of CO<sub>2</sub> taken up by photosynthesis for a given amount water vapour lost to the atmosphere. Assuming intermediate global estimates for photosynthesis (130×10<sup>15</sup> g C yr<sup>-1</sup>) and transpiration (40×10<sup>18</sup> g H<sub>2</sub>O yr<sup>-1</sup>) results in an average leaf-level water-use efficiency for terrestrial plants, expressed in molar units, of about 5 mmol CO<sub>2</sub> mol<sup>-1</sup> H<sub>2</sub>O. Expressed in mass units, this means that, on average, a terrestrial plant loses about 300 g of water to the atmosphere for each g of carbon that it takes up by photosynthesis.

Carbon has two stable isotopes that occur naturally in the environment. The ratio of minor (<sup>13</sup>C) to major (<sup>12</sup>C) isotopes provides a valuable natural tracer that can reveal unique insights into the exchanges of carbon and water between plants and the atmosphere. The stable isotope <sup>13</sup>C contains one neutron more than the much more abundant <sup>12</sup>C, and comprises about 1% of carbon in terrestrial ecosystems (Craig, 1953). The ratio <sup>13</sup>C/<sup>12</sup>C is modified to varying extents during transfer between the atmosphere and plants, and among different components within plants and ecosystems. These modifications are termed fractionations; alternatively the term discrimination can be applied, which specifically denotes the proportional deviation from unity of the isotope ratio of a reactant relative to a product (Farquhar and Richards 1984). Discrimination against <sup>13</sup>C varies according to how plants respond physiologically to changes in their environment. Such variations in isotopic discrimination

can therefore reveal important information about plant and ecosystem responses to global change (Yakir and Sternberg, 2000; Dawson et al., 2002; Maguas and Griffiths, 2003; Cernusak et al., 2013a).

Because the natural variations that occur in stable isotope ratios are very small in absolute terms, the isotopic composition of a material ( $\delta_X$ ) is typically expressed as its relative deviation from that of a standard (Coplen, 2011):

$$\delta_X = \frac{R_X - R_{Std}}{R_{Std}}, \quad (\text{Eqn 1})$$

where  $R_X$  is the isotope ratio of material  $X$ , and  $R_{Std}$  is that of the standard. The resulting  $\delta$  values are often multiplied by 1000, giving them the dimensionless descriptor of per mil (‰), in order to avoid the use of very small numbers. Although different isotope laboratories often use different secondary standards, the  $\delta$  values are always related back to a primary standard, so that values can be compared among laboratories. For example, in the case of  $\delta^{13}\text{C}$ , values are related to the primary standard Pee Dee Belemnite (Craig, 1957), which has a  $^{13}\text{C}/^{12}\text{C}$  ratio of 0.01124.

As noted above, discrimination against  $^{13}\text{C}$  during photosynthetic  $\text{CO}_2$  assimilation ( $\Delta^{13}\text{C}$ ) can also be described as the relative deviation of the product from the reactant; that is, the relative deviation of the  $^{13}\text{C}/^{12}\text{C}$  ratio of plant biomass from the  $^{13}\text{C}/^{12}\text{C}$  ratio of atmospheric  $\text{CO}_2$  (Farquhar and Richards 1984):

$$\Delta^{13}\text{C} = \frac{R_a - R_p}{R_p}, \quad (\text{Eqn 2})$$

where  $R_a$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio of atmospheric  $\text{CO}_2$  and  $R_p$  is that of plant biomass. Analytical determinations of carbon isotope ratios will typically be reported as  $\delta$  values of the form given by Eqn. 1. These  $\delta$  values can be used to calculate  $\Delta^{13}\text{C}$  by combining Eqns. 1 and 2:

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_a - \delta^{13}\text{C}_p}{1 + \delta^{13}\text{C}_p}. \quad (\text{Eqn 3})$$

Here  $\delta^{13}\text{C}_a$  is the  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  and  $\delta^{13}\text{C}_p$  is that of plant biomass. Both sides of Eqn. 3 can be multiplied by 1000 to express the terms as per mil, but this only applies to the numerator on the right side. Thus, if the  $\delta^{13}\text{C}_a$  and  $\delta^{13}\text{C}_p$  are already expressed in per mil, the denominator on the right side of the equation would become  $1 + \delta^{13}\text{C}_p/1000$  and the calculated  $\Delta^{13}\text{C}$  would be in per mil.

## DIFFUSIVE FLUXES OF $\text{CO}_2$ AND WATER VAPOUR

Perhaps the simplest mathematical description of photosynthesis is one based on diffusion of  $\text{CO}_2$  into the leaf. The abbreviation typically applied to photosynthesis is  $A$ , for assimilation of  $\text{CO}_2$ . In order to calculate the diffusive flux of  $\text{CO}_2$  into the leaf, one must take account of both the concentration gradient for  $\text{CO}_2$  between the atmosphere and the leaf interior and resistance to

diffusion of the pathway, or its reciprocal, the conductance. Thus, the equation can be written as (von Caemmerer and Farquhar, 1981),

$$A = g_c(c_a - c_i) - E\bar{c} . \quad (\text{Eqn 4})$$

Here, the  $A$  has dimensions of moles of  $\text{CO}_2$  per area of leaf surface per unit time. Thus, it is a rate variable and the specific units typically employed at the leaf level are  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . The term  $g_c$  represents the conductance to  $\text{CO}_2$  from the atmosphere to the leaf intercellular air spaces inside the leaf, and has the same dimensions as  $A$ , with specific units in this case of  $\text{mol m}^{-2} \text{ s}^{-1}$ . The terms  $c_a$  and  $c_i$  represent  $\text{CO}_2$  concentrations in the atmosphere and intercellular air spaces, respectively, expressed here as mole fractions, with units of  $\mu\text{mol CO}_2 \text{ mol}^{-1}$  air. The final term in the equation is a ternary correction. This correction takes account of the net diffusive flux of water vapour out of the leaf through the stomatal pores. Due to this flux, there are three gases in play:  $\text{CO}_2$ , water vapour and air. As  $\text{CO}_2$  diffuses into the leaf, it can collide with the molecules that comprise air; it can also collide with the additional water vapour molecules that make up the transpiration flux, whereby a higher transpiration rate will slow the diffusion of  $\text{CO}_2$  into the leaf more than a lower transpiration rate (Jarman, 1974; von Caemmerer and Farquhar, 1981; Boyer and Kawamitsu, 2011). The term  $\bar{c}$  is defined as  $(c_a+c_i)/2$ , and thus represents the average  $\text{CO}_2$  concentration between the atmosphere and the intercellular air spaces. A derivation of equation 4 including the ternary effect can be found in von Caemmerer and Farquhar (1981).

A similar diffusive flux equation can be written for transpiration ( $E$ ). In this case, the rate of transpiration is expressed as the product of the conductance to water vapour of the stomata plus boundary layer and the water vapour concentration gradient from the leaf intercellular air spaces to the atmosphere outside the leaf:

$$E = g_t(w_i - w_a) + E\bar{w} , \quad (\text{Eqn 5})$$

where  $g_t$  is the conductance to water vapour of the stomata plus boundary layer,  $w_i$  and  $w_a$  are the water vapour mole fraction in the intercellular air spaces and in the atmosphere, respectively, and  $\bar{w}$  is  $(w_i+w_a)/2$ . Here, the final term again comes about due to the ternary corrections that are applied in the derivation of the diffusive flux equation (Jarman, 1974; von Caemmerer and Farquhar, 1981). A typical value for  $\bar{w}$  would be about  $0.03 \text{ mol H}_2\text{O mol}^{-1}$  air, and the final term therefore represents about 3% of the transpiration rate. Because this is a small proportion, the final term is often neglected and equation 3 expressed as,

$$E = g_t(w_i - w_a) . \quad (\text{Eqn 6})$$

Equations 4 and 6 are generally those that are employed in commercial gas exchange systems to calculate gas exchange parameters from measurements of  $\text{CO}_2$  uptake and water vapour loss from a leaf in a gas exchange cuvette (e.g., Walz, 2005; PP Systems, 2006; LI-COR Biosciences, 2008).

The conductances in equations 4 and 6 refer to the total conductance from the atmosphere to the intercellular air spaces. This total conductance can be decomposed into stomatal and boundary layer components. Inverses of conductances are additive and the total conductance to H<sub>2</sub>O can thus be written as,

$$\frac{1}{g_t} = \frac{1}{g_s} + \frac{1}{g_b}, \quad (\text{Eqn 7})$$

where  $g_s$  is the stomatal conductance to H<sub>2</sub>O and  $g_b$  is the boundary layer conductance to H<sub>2</sub>O. The  $g_t$  is typically determined from measurements of the evaporation rate from a leaf in a cuvette, and  $g_b$  estimated from known values for the air mixing rate within the cuvette. The  $g_s$  can then be calculated from Eqn. 7 once the  $g_t$  and  $g_b$  are known. Analogous equations can be written for conductances to CO<sub>2</sub>, and because the diffusion pathways for CO<sub>2</sub> and water vapour are the same, the conductances for the two gases can be related based on their respective diffusivities in air. The stomatal conductance to CO<sub>2</sub>,  $g_{sc}$ , can be calculated as  $g_s/1.6$ ; the boundary layer conductance to CO<sub>2</sub>,  $g_{bc}$ , can be calculated as  $g_b/1.37$ . The coefficients relating the conductances for the two gases differ between stomata and boundary layer because transport through the stomatal pore is strictly by molecular diffusion, whereas transport through the boundary layer involves a mixture of molecular diffusion and turbulent transport.

The series of calculations that is typically employed when leaf gas exchange is measured is to first use the equations above, along with measurements of water loss from a leaf and boundary layer conductance to water vapour, to solve for the stomatal conductance to water vapour,  $g_s$ . This can then be used along with an equation analogous to Eqn. 7, but written for CO<sub>2</sub>, to calculate the total conductance to CO<sub>2</sub>,  $g_c$ . Once this is obtained, it can be combined with the measured CO<sub>2</sub> uptake rate and equation 4 to solve for  $c_i$ , the intercellular CO<sub>2</sub> mole fraction (Gaastra, 1959; Moss and Rawlins, 1963).

## WATER-USE EFFICIENCY

Dividing equation 4 above by equation 6 leads to an expression for water-use efficiency, expressed as  $A/E$ , the rate of CO<sub>2</sub> uptake for a given rate of water loss. The equation can be simplified by neglecting the ternary corrections and the boundary layer conductances to give the following:

$$\frac{A}{E} = \frac{c_a - c_i}{1.6(w_i - w_a)}. \quad (\text{Eqn 8})$$

Here the ratio of the stomatal conductance to CO<sub>2</sub> and water vapour,  $g_{sc}/g_s$ , has been replaced by the factor 1/1.6. Equation 8 shows that for a given leaf-to-air water vapour mole fraction difference ( $w_i - w_a$ ), the water-use efficiency depends on the extent of drawdown in the CO<sub>2</sub> concentration from the

atmosphere to the leaf interior. Thus, all else equal, a lower intercellular CO<sub>2</sub> concentration during photosynthesis will correspond to a higher water-use efficiency. Equation 8 can be written slightly differently to highlight the term  $c_i/c_a$ , the ratio of intercellular to atmospheric CO<sub>2</sub> mole fractions:

$$\frac{A}{E} = \frac{c_a \left(1 - \frac{c_i}{c_a}\right)}{1.6(w_i - w_a)}. \quad (\text{Eqn 9})$$

Equation 9 can also be multiplied on both sides by  $w_i - w_a$  to derive an equation that is independent of the leaf-to-air water vapour mole fraction difference. In this case, the quantity on the left side of the equation,  $A/g_s$ , is referred to as the intrinsic water use efficiency:

$$\frac{A}{g_s} = \frac{c_a \left(1 - \frac{c_i}{c_a}\right)}{1.6}. \quad (\text{Eqn 10})$$

It is desirable to have  $c_i/c_a$  explicitly in these equations for water-use efficiency because it also relates independently to carbon isotope discrimination. A simplified version of the equation for  $\Delta^{13}\text{C}$  for C<sub>3</sub> plants can be written as (Farquhar et al., 1982a; Farquhar et al., 1982b),

$$\Delta^{13}\text{C} = a + (b - a) \frac{c_i}{c_a}, \quad (\text{Eqn 11})$$

where  $a$  is the fractionation that occurs between <sup>13</sup>CO<sub>2</sub> and <sup>12</sup>CO<sub>2</sub> during diffusion through the stomatal pore (4.4‰), and  $b$  is the fractionation by Rubisco during carboxylation. When expressed in this simplified form, the appropriate value for  $b$  is 27‰ (Farquhar et al., 1982a; Seibt et al., 2008; Cernusak et al., 2013a). Equation 11 is simplified in that it neglects fractionations associated with diffusion through the boundary layer, day respiration, photorespiration, diffusion through the mesophyll, and ternary effects (Farquhar et al., 1982b; Farquhar and Cernusak, 2012; Cernusak et al., 2013a). Combining Eqn. 11 with Eqn. 3, and neglecting the term  $1 + \delta^{13}\text{C}_p$  from the denominator of Eqn. 3 (approximate value 1.03) results in the following:

$$\delta^{13}\text{C}_p = \delta^{13}\text{C}_a - a - (b - a) \frac{c_i}{c_a}. \quad (\text{Eqn 12})$$

Equation 12 predicts a linear relationship between  $\delta^{13}\text{C}_p$  and  $c_i/c_a$ , assuming the  $\delta^{13}\text{C}_a$  remains relatively constant. Because the CO<sub>2</sub> in the atmosphere is generally well mixed, this assumption will usually be reasonably well met in outdoor situations. The predicted linear relationship between  $\delta^{13}\text{C}_p$  and  $c_i/c_a$  generally shows good agreement with observations (Fig. 1). Although Eqn. 12 neglects several processes, the relationship is attractive for its simplicity. However, the neglected processes can also be of interest, depending on the research question being asked, and in such cases the more complete model for carbon isotope discrimination has also proved very useful (Farquhar and Cernusak, 2012; Cernusak et al., 2013a; Ubierna and Farquhar, 2014). A good example of this is its application for estimating mesophyll conductance to CO<sub>2</sub> (Evans et al., 1986; Flexas et al., 2012).

Measurements of  $\delta^{13}\text{C}_p$  provide a means of gaining insight into plant water-use efficiency across time and space. One advantage of sampling plant organic material for  $\delta^{13}\text{C}$  is that the signal

integrates over the period of time during which the tissue was formed. This can have significant advantages when compared to instantaneous measurements of  $A/E$  or  $A/g_s$ , in terms of the signal to noise ratio that can be achieved. Also, because the  $\delta^{13}\text{C}$  signal does not change once set down in organic material, historically preserved plant samples, such as tree rings and herbarium specimens, can also be analysed. In addition, because sampling for  $\delta^{13}\text{C}_p$  typically involves only collecting leaf or stem material and then drying it, field collections can be carried out efficiently and over large distances or in difficult to access locations such as forest canopies. However, a note of caution regarding the use of  $\delta^{13}\text{C}_p$  to infer variation in water-use efficiency is required; this is that in order to estimate  $A/E$ , the  $w_i-w_a$  must be known (Ehleringer, 1993). Thus, when comparing plants grown in different environments and with different leaf temperatures, it is important to take into account the variation in the leaf-to-air water vapour mole fraction difference if leaf-level water-use efficiency is to be explored. Otherwise inferences will be limited to the intrinsic water-use efficiency,  $A/g_s$ .

Water-use efficiency can also be defined for higher levels of organisation than the leaf, namely the whole plant and the ecosystem. Equation 9 can be adapted to provide an expression for whole-plant water-use efficiency (Farquhar and Richards, 1984):

$$W_P = \frac{c_a(1-\frac{c_i}{c_a})(1-\phi_c)}{1.6(w_i-w_a)(1+\phi_w)}. \quad (\text{Eqn 13})$$

Here,  $W_P$  is the ratio of carbon gained to water lost for the whole plant, with the same units as for  $A/E$  of  $\text{mmol C mol}^{-1} \text{H}_2\text{O}$ . A term has been added in the numerator to account for respiratory carbon losses. Thus,  $\phi_c$  is the proportion of carbon gained by photosynthesis that is subsequently lost from the plant by respiration of leaves at night and respiration of stems and roots during day and night. A term has also been added to the denominator to account for unproductive water loss; that is, water loss not associated with photosynthesis. The term  $\phi_w$  represents unproductive water loss as a proportion of water loss associated with photosynthesis. Unproductive water loss mainly comprises transpiration from partially open stomata at night, but also includes evaporation from non-photosynthetic plant tissues during day and night, and can include evaporation from soil associated with the plant depending on the context in which the equation is employed (Farquhar and Richards, 1984).

A final definition of water-use efficiency useful for landscape-level studies of global change is ecosystem water-use efficiency,  $W_E$ , which is defined as GPP divided by evapotranspiration; or in other words, the total carbon gained by an ecosystem through photosynthesis divided by total water lost through both transpiration and soil evaporation.

An important determinant of water-use efficiency in terrestrial plants is the type of photosynthetic pathway that the plant uses:  $\text{C}_3$ ,  $\text{C}_4$ , or CAM (Briggs and Shantz, 1914; Winter et al., 2005; Cernusak et al., 2007a). The ancestral and most widespread photosynthetic pathway is referred



to as  $C_3$ , because reduction of  $CO_2$  by the Calvin cycle results initially in the production of three-carbon sugar phosphate molecules. Globally,  $C_3$  photosynthesis accounts for about 80% of terrestrial GPP, (Still et al., 2003). Nearly all woody plants employ the  $C_3$  photosynthetic pathway, although there are notable exceptions (Percy and Troughton, 1975; Lüttge, 2006; Sage, 2016). As a result, more than 99% of terrestrial plant biomass resides in  $C_3$  plants (Still et al., 2003). Plants that employ the  $C_4$  photosynthetic pathway typically have a higher water-use efficiency than those that employ the  $C_3$  photosynthetic pathway. The  $C_4$  photosynthetic pathway suppresses photorespiration and uses PEP-carboxylase for  $CO_2$  fixation in the mesophyll instead of Rubisco, enabling a lower  $c_i/c_a$  and therefore a higher water-use efficiency. Plants that employ CAM, or Crassulacean Acid Metabolism, have a further advantage in that they perform  $CO_2$  fixation with PEP-carboxylase at night, when  $w_i-w_a$  is less than that during the day. Although water-use efficiency varies among these three photosynthetic pathways, there is also considerable variation within photosynthetic pathways (Winter et al., 2005; Cernusak et al., 2007a). In the following sections, I will focus on variation within the  $C_3$  photosynthetic pathway.

## THE BIOCHEMICAL MODEL OF $C_3$ PHOTOSYNTHESIS

A second approach to mathematically describing photosynthesis is through the biochemical processes that result in  $CO_2$  assimilation. The assimilation of  $CO_2$  during photosynthesis in  $C_3$  plants can potentially be limited by three different rate-limiting reactions: carboxylation by Rubisco, regeneration of RuBP by the electron transport chain, or triose phosphate utilization. The Farquhar-von Caemerrer-Berry photosynthesis model (Farquhar et al., 1980) provides a widely applied description of the  $CO_2$  assimilation rate in  $C_3$  plants as a function of these three processes:

$$A = \min\{W_c, W_j, W_{tp}\} \left(1 - \frac{\Gamma^*}{c_c}\right) - R_d, \quad (\text{Eqn 14})$$

where  $W_c$  is the Rubisco-limited carboxylation rate,  $W_j$  is the RuBP regeneration-limited carboxylation rate,  $W_{tp}$  is the triose phosphate utilization-limited carboxylation rate,  $\Gamma^*$  is the  $CO_2$  compensation point in the absence of day respiration,  $c_c$  is the  $CO_2$  partial pressure in the chloroplast, and  $R_d$  is the day respiration rate. Equation 14 holds for situations when  $c_c$  is larger than  $\Gamma^*$ . The Rubisco-limited carboxylation rate can be defined as

$$W_c = \frac{c_c V_{cmax}}{c_c + K_c \left(1 + \frac{O}{K_o}\right)}, \quad (\text{Eqn 15})$$

where  $V_{cmax}$  is the maximum Rubisco carboxylation rate,  $K_c$  is the Michaelis-Menten constant for carboxylation by Rubisco,  $O$  is the oxygen partial pressure in the chloroplast, and  $K_o$  is the Michaelis-

Menten constant for oxygenation by Rubisco. The RuBP regeneration-limited carboxylation rate can be defined as

$$W_j = \frac{Jc_c}{4c_c + 8\Gamma_*}, \quad (\text{Eqn 16})$$

where  $J$  is potential electron transport rate. Finally, the triose phosphate utilization-limited carboxylation rate can be defined as

$$W_{tp} = \frac{3T_p c_c}{c_c - \Gamma_*}, \quad (\text{Eqn 17})$$

where  $T_p$  is the rate of triose phosphate export from the chloroplast. The chloroplastic  $\text{CO}_2$  partial pressure,  $c_c$ , can be calculated according to the relationship

$$c_c = c_i - \frac{A}{g_i}, \quad (\text{Eqn 18})$$

where  $g_i$  is the internal conductance to  $\text{CO}_2$  transport, also referred to as the mesophyll conductance. It is preferable to use partial pressures for  $c_c$  and  $c_i$  in equation 18, rather than mole fractions, because it describes liquid phase diffusion. The partial pressure of a component gas in a mixture can be converted to a mole fraction by dividing the partial pressure of the component gas by the total pressure of all gases in the mixture.

The biochemical model of photosynthesis and the diffusive flux equation for photosynthesis both express  $A$  as a function of  $c_i$ , in addition to other parameters. This means that for a given  $g_s$ , and assuming set values for the other necessary parameters, there will be a unique value of  $c_i$  that gives the same prediction for  $A$  for both the biochemical model and the diffusive flux equation. This value of  $c_i$  can be solved for iteratively by allowing  $c_i$  to vary until the predictions of  $A$  converge for the biochemical model and the diffusive flux equation. The value of  $c_i$  at which the convergence occurs will depend on the other assigned parameter values such as  $V_{\text{cmax}}$ ,  $J$ ,  $g_i$ , etc. Repeating over a range of  $g_s$  provides insight into how water-use efficiency can be expected to vary as a function of stomatal regulation of water loss. Predictions of this behaviour are shown in Fig. 2 for a given set of assumed environmental and physiological parameters. Some general expectations can be established from this exercise; for example, that  $A$  should increase with increasing  $g_s$  in a curvilinear fashion (Fig. 2A). Transpiration rate,  $E$ , also increases with increasing  $g_s$  (Fig. 2B). The increase is not linear because as  $E$  increases, leaf temperature decreases, all else equal, and therefore  $w_i - w_a$  decreases. This causes the relationship between  $E$  and  $g_s$  to also be curvilinear; however, the curvature is not as much as that between  $A$  and  $g_s$ . As a result, the water-use efficiency,  $A/E$ , decreases with increasing  $g_s$  (Fig. 2C). This is also seen in the increasing  $c_i/c_a$  with increasing  $g_s$  (Fig. 2D), which would correspond to increasing  $\Delta^{13}\text{C}$  as seen from Eqn. 11 and therefore to more negative  $\delta^{13}\text{C}_p$ , all else equal. An important prediction that emerges from this and similar analyses is that there should be a trade-off whereby opening stomata to allow more  $\text{CO}_2$  to diffuse into the leaf increases the photosynthetic rate,

but decreases the water-use efficiency (Cowan, 1978; Farquhar et al., 1989). This trade-off should hold for most combinations of environmental and physiological parameters. A notable exception is that if photosynthetic capacity is sufficiently large and boundary layer conductance sufficiently small,  $A/E$  may actually increase with increasing  $g_s$  (Meinzer et al., 1997); but, this would be an unusual situation.

Given that plants are likely to face a trade-off between increasing  $A$  and decreasing  $A/E$  with increasing  $g_s$ , how should we expect them to behave in water-limited environments? Cowan and Farquhar (1977) suggested that because stomata are dynamic, plants could regulate their stomatal apertures in illuminated leaves such that  $g_s$  would be relatively high when  $w_i-w_a$  was low, and relatively low when  $w_i-w_a$  was high. In such a way, a plant could achieve efficiency with respect to water use by minimising the amount of water that it would spend to assimilate a given amount of  $\text{CO}_2$  over a given period of time. This optimisation approach appears to be consistent at least with diurnal patterns of  $g_s$  in which  $g_s$  tends to decrease in the middle of the day when  $w_i-w_a$  is at a maximum (Schulze et al., 1972; Schulze et al., 1974; Zotz et al., 1995; Aalto et al., 2002).

At a broader, landscape scale, plants from drier habitats tend to have less negative  $\delta^{13}\text{C}_p$ , and therefore lower  $\Delta^{13}\text{C}$ , than those from wetter habitats. This indicates lower  $c_i/c_a$  and higher  $A/g_s$ , suggesting that adaptation and/or acclimation processes favour higher intrinsic water-use efficiency in dry environments. Recent meta-analyses of  $\delta^{13}\text{C}_p$  values collected from the literature show that mean annual precipitation can explain up to half of observed variation globally in  $\delta^{13}\text{C}_p$  of  $\text{C}_3$  plants (Diefendorf et al., 2010; Kohn, 2010; Cornwell et al., 2018). In studies which have specifically targeted aridity gradients,  $\delta^{13}\text{C}_p$  typically increases with increasing aridity, consistent with decreasing  $c_i/c_a$  (Stewart et al., 1995; Schulze et al., 1998; Miller et al., 2001; Midgley et al., 2004; Schulze et al., 2006; Prentice et al., 2011; Givnish et al., 2014). However, there appears to be variation among rainfall gradients in the slope of the relationship between  $\delta^{13}\text{C}_p$  and rainfall or aridity indices at both the species level and at the community level (Stewart et al., 1995; Schulze et al., 1998; Prentice et al., 2011). One of the more intensively studied of these transects is the North Australian Tropical Transect, which runs approximately from Darwin to Alice Springs in the Northern Territory, Australia (Koch et al., 1995; Williams et al., 1996; Hutley et al., 2011). Trees along this transect show relatively modest changes in  $\delta^{13}\text{C}_p$  from the wetter, northern end to the drier, southern end (Schulze et al., 1998; Miller et al., 2001). *In situ* measurements of leaf gas exchange confirmed that these modest changes in  $\delta^{13}\text{C}_p$  were indeed consistent with little to no change in  $c_i/c_a$  and therefore  $A/E$  at a given  $w_i-w_a$  along this rainfall gradient (Cernusak et al., 2011a).

## A CASE STUDY OF WATER-USE EFFICIENCY IN NORTHERN AUSTRALIAN TREE SPECIES

In order to test for inherent differences in water-use efficiency of northern Australian tree species which occupy different parts of the north-to-south rainfall gradient, I grew seedlings of nine species in a common garden in Darwin, Northern Territory, Australia. The seedlings were grown in a shade house on the Charles Darwin University campus during 2009. The species varied broadly in geographic range size, from *Podocarpus grayae*, with a narrow geographic distribution restricted to relatively wet rainforest patches in northeast Queensland and Arnhem Land, to *Eucalyptus camaldulensis*, which is distributed across most of the Australian continent (Fig. 3). The species with larger geographic ranges sizes were those whose distributions extended further south, and therefore into drier habitats. I estimated geographic range sizes from botanical textbooks (Brock, 1993; Brooker and Kleinig, 2004), and from the Atlas of Living Australia (<https://www.ala.org.au/>; accessed October 2014). Using the Atlas of Living Australia, I also extracted the mean annual precipitation and aridity index for all location records for each species. I then calculated the averages of these indices. Northern Australian tree species with larger geographic range sizes have drier distributions on average because of the strong rainfall gradient from north to south in northern Australia. Those which are narrowly distributed in the northern most part of the continent occur in areas of higher rainfall, and those whose distributions extend further south into the continental interior have more occurrences in lower rainfall areas.

When grown in the common garden, the different tree species showed variation in leaf  $\delta^{13}C_p$  and instantaneous gas exchange rates. The relationship between leaf  $\delta^{13}C_p$  and instantaneous measurements of  $c_i/c_a$  is shown in Figure 1. Figure 4B shows that the variation in  $c_i/c_a$  and  $\delta^{13}C_p$  among species was driven by variation in the maximum  $g_s$  achieved by the different species under well-watered conditions. Although  $\delta^{13}C_p$  was linearly related to the rate of  $CO_2$  assimilation, the slope of the relationship is opposite to that which would be expected if photosynthetic capacity were driving variation in  $\delta^{13}C_p$  (Fig. 4A). If this were the case, a higher  $A$  would be expected to be associated with a less negative  $\delta^{13}C_p$ , rather than a more negative  $\delta^{13}C_p$ .

I observed a strong correlation between leaf  $\delta^{13}C_p$  of seedlings grown in the common garden and the natural logarithm of geographic range size (Fig. 5C). Larger geographic ranges were also correlated with higher  $A$  and  $g_s$  for the well-watered seedlings grown in the common garden (Fig. 5A and 5B). Similar patterns were observed when geographic range size was replaced by the range-wide mean annual precipitation for each species (Fig. 6). This indicates that the species which are able to establish and grow in drier, more southerly habitats are able to achieve higher maximum  $g_s$  when

water is plentiful. Due to the trade-off shown in Fig. 2 between increasing  $A$  and decreasing  $A/E$  with increasing  $g_s$ , this results in higher photosynthesis rates and lower water-use efficiency. This suggests that the ability of northern Australian tree species to establish in habitats of progressively lower mean annual precipitation is strongly linked to their ability to use water resources rapidly when they are available, especially in the seedling stage before deep roots have developed. A high maximum  $g_s$  contributes toward this ability, as does, presumably, a suite of traits related to hydraulic architecture and whole-plant hydraulic conductance.

Other researchers have also observed that species or ecotypes from more arid environments tend to have lower water-use efficiency than counterparts from more mesic environments when grown in common gardens (Read and Farquhar, 1991; Comstock and Ehleringer, 1992; Hubick and Gibson, 1993; Lauteri et al., 1997; Guy and Holowachuk, 2001; Searson et al., 2004; Goedhart and Pataki, 2012). However, this is not a universal observation, and examples of the opposite behaviour have also been recorded, where species or ecotypes from more arid environments showed higher water-use efficiency when grown in a common garden than their more mesic comparators (Anderson et al., 1996; Brodribb and Hill, 1998; Li et al., 2000). Nevertheless, on balance, there would appear to be more examples of the former type of behaviour than the latter. This suggests that a capacity for rapid water use tends to be favoured in species and ecotypes that occur in habitats where water sufficiency is an ephemeral condition, and that such rapid water use typically comes at the expense of lower water-use efficiency.

## **EFFECTS OF LEAF PHENOLOGY ON GAS EXCHANGE AND WATER-USE EFFICIENCY**

Plants can also respond to seasonal or annual variation in rainfall by modulating their canopy leaf area through leaf abscission in response to drying soil and leaf flushing when soil moisture availability improves, or in anticipation of such improvement (Eamus, 1999). Such modulations can have important implications for canopy gas exchange. In addition to changing the total leaf area available for exchanging  $\text{CO}_2$  and water vapour with the atmosphere, it has recently been recognized that the proportional distribution of the canopy among different leaf age classes is a key feature for understanding and modelling canopy gas exchange (Beringer et al., 2007; Beringer et al., 2015; Lopes et al., 2016; Wu et al., 2016; Wu et al., 2017). In particular, the proportion of the canopy that is occupied by young expanding foliage can significantly impact upon  $\text{CO}_2$  and water vapour exchange. Thus, it is important to understand how the gas exchange properties of leaves change as they progress from emergence through to maturity and eventually to senescence.

In the initial stages of leaf development, the leaf will be almost entirely reliant on other leaves or storage to provide it with organic molecules for energy and carbon skeletons to drive growth. As leaf expansion proceeds, the photosynthetic competency of the leaf will increase, and the net CO<sub>2</sub> exchange in sunlight will trend from net CO<sub>2</sub> efflux, whereby leaf respiration is larger than gross photosynthesis, to net CO<sub>2</sub> assimilation (Choinski et al., 2003; Cernusak et al., 2006; Cernusak et al., 2009). Thus, as it gains in photosynthetic ability, an expanding leaf will contribute progressively more carbon resources from its own photosynthesis toward its requirement for growth. This progression from heterotrophy to autotrophy as judged from the net CO<sub>2</sub> exchange necessarily means that the intercellular CO<sub>2</sub> concentration will trend from being higher than  $c_a$  to being lower than  $c_a$  as  $A$  increases from negative to positive values. Therefore, young, expanding leaves which show positive net CO<sub>2</sub> assimilation could be expected to photosynthesize with a lower water-use efficiency than mature leaves due to a higher  $c_i/c_a$ . This has indeed been observed in a number of species (Francey et al., 1985; Terwilliger, 1997; Terwilliger et al., 2001; Cernusak et al., 2006; Cernusak et al., 2009). Net CO<sub>2</sub> assimilation, stomatal conductance, and intercellular CO<sub>2</sub> concentration are shown for two eucalypt species in Fig. 7 as a function of leaf size for young, expanding leaves and mature leaves. I made these measurements during 2012 at the Tumbarumba OzFlux site (Keith et al., 2012; Beringer et al., 2016).

An interesting question arises as to the cause of the high intercellular CO<sub>2</sub> concentration, and therefore low water-use efficiency, in young, expanding leaves: does it result from a low photosynthetic capacity, or does it result from a high day respiration rate associated with synthetic reactions linked to growth? To address this question, I measured the response of CO<sub>2</sub> assimilation to intercellular CO<sub>2</sub> concentration ( $A$ - $c_i$  curves) in the leaves shown in Fig. 7. The biochemical model of C<sub>3</sub> photosynthesis was fitted to the  $A$ - $c_i$  curves by submitting the data to the website [www.leafweb.org](http://www.leafweb.org) (Gu et al., 2010) to estimate the parameters  $V_{\text{cmax}}$  and  $J_{\text{max}}$  as measures of photosynthetic capacity and the day respiration rate,  $R_d$ . Figure 8 shows these three parameters plotted against leaf size for young, expanding leaves and mature leaves. This analysis suggests that it is a combination of lower photosynthetic capacity in the young leaves, indicated by lower  $V_{\text{cmax}}$  and  $J_{\text{max}}$ , and very high  $R_d$  which causes low net CO<sub>2</sub> assimilation and higher  $c_i$  in young, expanding leaves than in mature leaves (Fig. 8). The pattern for  $R_d$  is noteworthy insofar as growth respiration is known to be a general phenomenon (McCree, 1970; Thornley, 1970; Amthor, 2000). As a result, high  $c_i/c_a$  and low water-use efficiency in expanding leaves may also be general among terrestrial plants. Combined measurements of gas exchange and online carbon isotope discrimination would be helpful to probe whether there are also directional changes in mesophyll conductance associated with leaf expansion. Results available so far indicate that mesophyll conductance in developing leaves likely increases with increases in photosynthetic capacity, but that such increases can also be slowed

by thickening of mesophyll cell walls (Hanba et al., 2001; Miyazawa and Terashima, 2001; Marchi et al., 2008; Tosens et al., 2012).

## WOODY TISSUE RESPIRATION AND PHOTOSYNTHESIS

In addition to leaves, woody tissues can also play a role in controlling whole-plant water-use efficiency. Autotrophic respiration typically consumes about half the carbon fixed by plants through photosynthesis (Gifford 1994). This includes respiratory carbon released from leaves, stems, and roots. In trees, respiration associated with woody tissues comprises a significant fraction of total respiration. For example, in a beech forest in France, it was estimated that annual CO<sub>2</sub> efflux from above-ground woody tissues consumed 26% of annual GPP (Damesin et al., 2002). On the other hand, in a pine forest in the United States, CO<sub>2</sub> efflux from above-ground woody-tissues was estimated to have consumed only 6% of GPP (Law et al., 1999). These results suggest that woody-tissue CO<sub>2</sub> efflux can be a variable, and potentially very important, component of ecosystem carbon balance (Yang et al., 2016). Potentially of relevance to this spread of estimates is that the parenchyma cell fraction in angiosperm wood is significantly higher than that in conifer wood (Morris et al., 2016). Parenchyma cells are the living cells in sapwood that likely play a strong role in modulating CO<sub>2</sub> efflux from woody tissues (Ryan, 1990).

Woody tissues can generally be characterized as having either a smooth bark or a rough bark. Those woody tissues that have a smooth bark often also have a green, chlorophyllous layer of photosynthetic tissue just beneath the bark surface. This photosynthetic tissue refixes CO<sub>2</sub> that would otherwise diffuse from the woody tissue through the bark to the atmosphere (Sprugel and Benecke, 1991; Cernusak and Marshall, 2000; Pfanz et al., 2002). This process has been termed refixation or corticular photosynthesis (Sprugel and Benecke, 1991); it uses sunlight that penetrates the bark cortex and endogenously-produced CO<sub>2</sub> for CO<sub>2</sub> assimilation (Cernusak et al., 2001).

Refixation of respired CO<sub>2</sub> by photosynthetic bark increases plant water-use efficiency (Cernusak and Cheesman, 2015; Vandegehuchte et al., 2015). This is because refixation relies upon internally produced CO<sub>2</sub> to provide its substrate for photosynthesis. Therefore it does not require that the plant expose moist tissues to the drier atmosphere in order to capture CO<sub>2</sub>. In a comparison of photosynthesis in the bark of *Pinus monticola* with that in its leaves, water-use efficiency was found to be 50 times higher for the bark (Cernusak and Marshall, 2000). In a whole-plant context, it is easiest to think of the impact of refixation on water-use efficiency through its influence on  $\phi_c$ . Refixation reduces the loss of respiratory CO<sub>2</sub> from the plant, thereby decreasing  $\phi_c$  and increasing  $W_P$ , as seen in equation 13.

Stable carbon isotope ratios can help to provide insight into refixation rates in photosynthetic bark (Cernusak et al., 2001; Cernusak and Hutley, 2011). Because there are at least two possible fates for respired CO<sub>2</sub> in this case, diffusion to the atmosphere or assimilation by bark chloroplasts, there is opportunity for discrimination against <sup>13</sup>C. The main mechanism is discrimination by Rubisco. A third possible fate for stem-respired CO<sub>2</sub> is to be dissolved in the xylem water and transported toward the canopy in the transpiration stream (Teskey and McGuire, 2002; Teskey et al., 2008). Available evidence suggests that partitioning of CO<sub>2</sub> into this third pathway does not result in detectable fractionation of δ<sup>13</sup>C of CO<sub>2</sub> (Ubierna et al., 2009).

The extent to which Rubisco fractionation is manifested in the δ<sup>13</sup>C of CO<sub>2</sub> efflux and refixed photosynthate depends on the extent of refixation, but the expected trend differs for refixed photosynthate versus CO<sub>2</sub> efflux (Fig. 9). If the proportional refixation rate is high, then there will be little opportunity for discrimination by Rubisco because nearly all available CO<sub>2</sub> will effectively be captured. In this case, refixed photosynthate will show little depletion in δ<sup>13</sup>C compared to dark respired CO<sub>2</sub>. On the other hand, if the proportional refixation rate is low, then the effects of discrimination by Rubisco will be expressed to a larger extent, and refixed photosynthate will show more depletion in δ<sup>13</sup>C. However, the pattern for the δ<sup>13</sup>C of CO<sub>2</sub> efflux is opposite, with a larger effect at higher proportional refixation rates (Figs. 9 and 10). This is because although at a low refixation rate the discrimination by Rubisco will be more complete, only a small proportion of CO<sub>2</sub> will be removed from the efflux and so the overall impact will be small.

A useful application of the refixation signal in the δ<sup>13</sup>C of CO<sub>2</sub> efflux could be to use it to infer the proportional refixation rate within the bark. Rearranging equation 5 of Cernusak et al. (2001) and neglecting very small terms leads to the following simplified expression for the proportional refixation rate,  $P/D$ , as a function of the δ<sup>13</sup>C of net CO<sub>2</sub> efflux:

$$\frac{P}{D} = \frac{\delta^{13}C_n - \delta^{13}C_D}{b - a - (\delta^{13}C_a - \delta^{13}C_n - a) \frac{c_a}{c_i}}, \quad (\text{Eqn 19})$$

where  $P$  is the gross photosynthesis rate in the bark,  $D$  is the day respiration rate of the woody tissue, δ<sup>13</sup>C<sub>n</sub> is the δ<sup>13</sup>C of net CO<sub>2</sub> efflux, δ<sup>13</sup>C<sub>D</sub> is that of day respiration, δ<sup>13</sup>C<sub>a</sub> is that of atmospheric CO<sub>2</sub>,  $b$  is discrimination by Rubisco,  $a$  is diffusional fractionation,  $c_i$  is the CO<sub>2</sub> mole fraction inside the bark, and  $c_a$  is that in the atmosphere. Note that the isotopic terms in Eqn. 19 are not in per mil.

An additional approach for using stable carbon isotopes to investigate the role of refixation in the carbon balance of woody plants is through light exclusion experiments. If sunlight is blocked from absorption by a woody tissue for a period of time of sufficient duration that some tissue growth or sugar production takes place, then the δ<sup>13</sup>C of this tissue or sugars can be compared to a counterpart which did not have sunlight excluded from it. One way of accomplishing this is to cover the woody



tissue with a reflective substance such as aluminium foil. If refixation contributes carbon to the tissue or sugar sampled from the illuminated stem then its  $\delta^{13}\text{C}$  should be more negative than that sampled from the light-excluded stem (Cernusak et al., 2001; Saveyn et al., 2010; Cernusak and Hutley, 2011; Simbo et al., 2013). Cernusak and Hutley (2011) showed that such an analysis could be combined with stable oxygen isotope measurements to estimate both the contribution of refixation to wood production and the flux-weighted proportional refixation rate. They found that refixation contributed 11% of the carbon in wood of a smooth-barked eucalypt, indicating a significant role of refixation for increasing whole-plant water-use efficiency.

In addition to refixation of respired  $\text{CO}_2$ , some stems have stomata and are capable of net photosynthetic uptake of  $\text{CO}_2$  from the atmosphere (Nilsen, 1995; Ávila et al., 2014). This has been most often observed in desert shrubs, particularly in leguminous shrubs. In species which show net uptake of  $\text{CO}_2$  during stem photosynthesis, the water-use efficiency of stem net photosynthesis has also been observed to be higher than that of leaf net photosynthesis (Ehleringer et al., 1987; Comstock and Ehleringer, 1988; Nilsen and Sharifi, 1997; Ávila-Lovera and Tezara, 2018).

## **GAS EXCHANGE AND WATER-USE EFFICIENCY IN RESPONSE TO GLOBAL CHANGE**

Although woody tissues have an important role to play in terms of contributing to variation in  $W_p$ , the overall response of  $W_p$  to global change is best understood by again considering leaves. As can be seen from equation 10, an increase in the atmospheric  $\text{CO}_2$  concentration,  $c_a$ , will cause a proportional increase in  $A/g_s$  of leaves, so long as  $c_i/c_a$  remains approximately constant. This means that, all else equal and with constant  $c_i/c_a$ , water-use efficiency of terrestrial vegetation is expected to increase in direct proportion to increasing atmospheric  $[\text{CO}_2]$ . There is evidence from  $\delta^{13}\text{C}$  in tree rings to suggest that this situation has been approximately realized as  $c_a$  has risen from the industrial revolution to present (Frank et al., 2015; van der Sleen et al., 2015). Observations of the  $\delta^{13}\text{C}$  change in atmospheric  $\text{CO}_2$  from 1765 to 2015 based on direct measurements and measurements of air trapped in ice cores further support this pattern (Keeling et al., 2017). Measurements of ecosystem gas exchange using eddy covariance suggest an even steeper increase in ecosystem-level water-use efficiency in some forests (Keenan et al., 2013; Dekker et al., 2016). The increase in water-use efficiency as a result of rising  $c_a$  has been implicated in greening trends in warm, arid regions of the global land surface over recent decades (Donohue et al., 2013; Zhu et al., 2016). Based on chamber studies, the approximate maintenance of  $c_i/c_a$  as  $c_a$  increases typically involves both an increase in  $A$  and a decrease in  $g_s$  (Cernusak et al., 2011b; Cernusak et al., 2013b; Dalling et al., 2016). The increase

in  $A/g_s$  caused by rising  $CO_2$  is expected to partly mitigate increasing drought stress caused by global warming in coming decades (Roderick et al., 2015; Swann et al., 2016).

Other global change processes such as nitrogen deposition and land cover change can also impact water-use efficiency of plants and ecosystems. Increasing nitrogen availability can increase water-use efficiency both by increasing photosynthesis and in some cases by concomitantly decreasing  $g_s$  (Cernusak et al., 2007b; Cramer et al., 2008; Cramer et al., 2009; Garrish et al., 2010). Modeling efforts suggest that nitrogen deposition has been a weaker, but still important, driver of increases in water-use efficiency compared to atmospheric  $CO_2$  over recent decades across terrestrial ecosystems (Huang et al., 2015; Zhou et al., 2017). Land cover change can alter ecosystem water-use efficiency, for example, by changing the balance between  $C_3$  and  $C_4$  vegetation, by replacing annual with perennial species, or by changing canopy structure and leaf area index in such a way that it impacts the partitioning between evaporation and transpiration at the ecosystem level (Nosetto et al., 2005; VanLoocke et al., 2012; Zhou et al., 2017). Recent estimates suggest that the overall contribution of land cover change since 1900 has been to reduce water-use efficiency of terrestrial ecosystems on average, compared to what it would be in the absence of land cover change (Zhou et al., 2017).

On the global scale, there is currently an imbalance between the amount of  $CO_2$  absorbed by the terrestrial biosphere through photosynthesis and the amount released back to the atmosphere through plant respiration, decomposition and fire, such that there is a net absorption of about  $3 \text{ Pg C yr}^{-1}$  (Le Quere et al., 2018). This terrestrial sink is slowing the rate of increase in atmospheric  $CO_2$  that results from fossil fuel emissions and land use change compared to what it would otherwise be. The terrestrial carbon sink is thought to be the result of stimulation of photosynthesis and water-use efficiency by rising atmospheric  $CO_2$ , increased nitrogen availability due to nitrogen deposition, increased growing season length at high latitudes as a result of global warming, and changes in land management. The strength of the terrestrial sink shows a marked inter-annual variability, with recent evidence suggesting that this results from precipitation-driven shifts in GPP in semi-arid ecosystems, whereas tropical ecosystems make a steadier contribution from year to year (Poulter et al., 2014; Ahlstrom et al., 2015).

Overall, the future, long-term sustainability of the terrestrial carbon sink is highly uncertain (Le Quere et al., 2009; Huntingford et al., 2013; Wenzel et al., 2016). A diminishing terrestrial carbon sink would accelerate the rise in atmospheric  $CO_2$  concentration and therefore quicken the pace of global climate change. This is strongly motivating the scientific community to strive to better understand the processes and characteristics that control the gas exchange and water-use efficiency of terrestrial plant canopies. Recent advances in theoretical modelling (Wang et al., 2017) and meta-analyses of empirical data sets (Medlyn et al., 2017; Cornwell et al., 2018) have demonstrated once

again that stable carbon isotope ratios can provide a unique and powerful tool to help achieve this goal.

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### Legends to the figures:

**Fig. 1.** The carbon isotope ratio ( $\delta^{13}\text{C}$ ) of leaf dry matter plotted against the ratio of intercellular to ambient  $\text{CO}_2$  concentrations ( $c_i/c_a$ ) measured instantaneously with a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc., Lincoln, Nebraska, USA). Measurements were made on seedlings growing in a shade house on the Charles Darwin University campus in 2009. Seedlings were well watered at the time of gas exchange measurements. Gas exchange measurements were conducted between 9am and 12pm local time, with five leaves measured per plant. Light intensity was set at  $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The leaves were sampled in bulk for  $\delta^{13}\text{C}$  and compared to the average  $c_i/c_a$  for the five leaves for each plant. Each point in the figure represents an individual plant. The solid line shows the theoretical relationship according to a simplified model of carbon isotope discrimination. The dashed line shows a least-squares regression line of best fit.

**Fig. 2.** Theoretical predictions of relationships between  $\text{CO}_2$  assimilation rate (A), transpiration rate (B), the ratio of  $\text{CO}_2$  assimilation to transpiration (C), and the ratio of intercellular to ambient  $\text{CO}_2$  concentrations,  $c_i/c_a$  (D) plotted against stomatal conductance. The model was parameterized for tropical conditions typical of northern Australia.

**Fig. 3.** Occurrence record distributions for nine north Australian tree species that were used in a common garden experiment conducted on the Charles Darwin University campus in 2009. Occurrence records and maps were provided by the Atlas of Living Australia (<https://www.ala.org.au/>; accessed October 2014).

**Fig. 4.** The carbon isotope ratio ( $\delta^{13}\text{C}$ ) of leaf dry matter plotted against instantaneous measurements of the  $\text{CO}_2$  assimilation rate (A) and stomatal conductance (B). The seedlings are the same as those shown in Fig. 1. Gas exchange measurements were made when plants were well watered, and exposed to similar air vapour pressure deficits, between 9am and 12pm local time. The variation in stomatal conductance in panel B represents variation among species in maximum  $g_s$  under well-watered conditions, rather than variation imposed by different environmental conditions at the time of measurement. Light intensity for all measurements was  $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The relationships show that variation in leaf  $\delta^{13}\text{C}$  among species was driven by variation in stomatal conductance because the relationship with photosynthesis shown in panel A is opposite to that which would be expected if variation in photosynthetic capacity were driving variation in carbon isotope

discrimination. Each point in the graph represents an individual plant, with measurements on five leaves averaged for each individual.

**Fig. 5.** Geographic range size for nine northern Australian tree species plotted against instantaneous measurements of CO<sub>2</sub> assimilation rate (A) and stomatal conductance (B), and carbon isotope ratio ( $\delta^{13}\text{C}$ ) of leaf dry matter (C) for seedlings grown in a common garden at Charles Darwin University, Darwin, Northern Territory. Note that axes for geographic range size and stomatal conductance are displayed on a logarithmic scale. The figure shows that variation in geographic range size is strongly correlated with physiological characteristics expressed in seedlings when grown under common garden conditions. Species with larger geographic range sizes have distributions that extend into more arid conditions.

**Fig. 6.** Mean annual precipitation averaged for all individual occurrence records in Atlas of Living Australia for each of nine northern Australian tree species plotted against instantaneous measurements of CO<sub>2</sub> assimilation rate (A) and stomatal conductance (B), and  $\delta^{13}\text{C}$  of leaf dry matter (C) for seedlings grown in a common garden at Charles Darwin University, Darwin, Northern Territory. Species with more arid distributions have higher  $A$  and  $g_s$  and lower  $\delta^{13}\text{C}_p$  in the common garden than species with more mesic distributions.

**Fig. 7.** Instantaneous gas exchange parameters measured with a Li-Cor 6400 portable photosynthesis system on young and mature leaves of two eucalypt species. Plants were sapling sized and were growing naturally in gaps in the understory of a tall eucalypt forest near Tumbarumba, New South Wales. Light intensity was set at 2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Young leaves were still expanding and therefore had smaller leaf areas. Lower CO<sub>2</sub> assimilation rates (A) and stomatal conductance (B) were accompanied by higher intercellular CO<sub>2</sub> concentrations (C) in young compared to mature leaves, and therefore lower intrinsic water-use efficiency. Measurements were made between 9am and 5pm local time.

**Fig. 8.** The maximum Rubisco carboxylation rate (A), maximum electron transport rate (B), and day respiration rate (C) plotted against leaf size for the same leaves and measurements conditions as shown in Figure 7. The  $V_{\text{cmax}}$ ,  $J_{\text{max}}$  and  $R_d$  were estimated by fitting the biochemical model of C<sub>3</sub> photosynthesis to curves of CO<sub>2</sub> assimilation rate versus intercellular CO<sub>2</sub> concentration. The analysis shows that young, expanding leaves have lower photosynthetic capacity and higher day respiration rates than mature leaves, contributing to their higher  $c_i$  and therefore lower intrinsic water-use efficiency.

**Fig. 9.** Predicted  $\delta^{13}\text{C}$  of  $\text{CO}_2$  efflux ( $\delta^{13}\text{C}_n$ ) and refixed photosynthate ( $\delta^{13}\text{C}_p$ ) as a function of the proportional refixation rate in a woody tissue with photosynthetic bark. Predictions are made according to the model of Cernusak et al. (2001). The  $\delta^{13}\text{C}$  of dark respiration ( $\delta^{13}\text{C}_D$ ) is assumed to remain constant as the refixation rate varies.

**Fig. 10.** Measurements of  $\text{CO}_2$  efflux rate (A) and the  $\delta^{13}\text{C}$  of  $\text{CO}_2$  efflux (B) as a function of increasing photosynthetically active radiation and therefore increasing refixation rate by photosynthetic bark in two North American tree species. Data are from Cernusak et al. (2001).

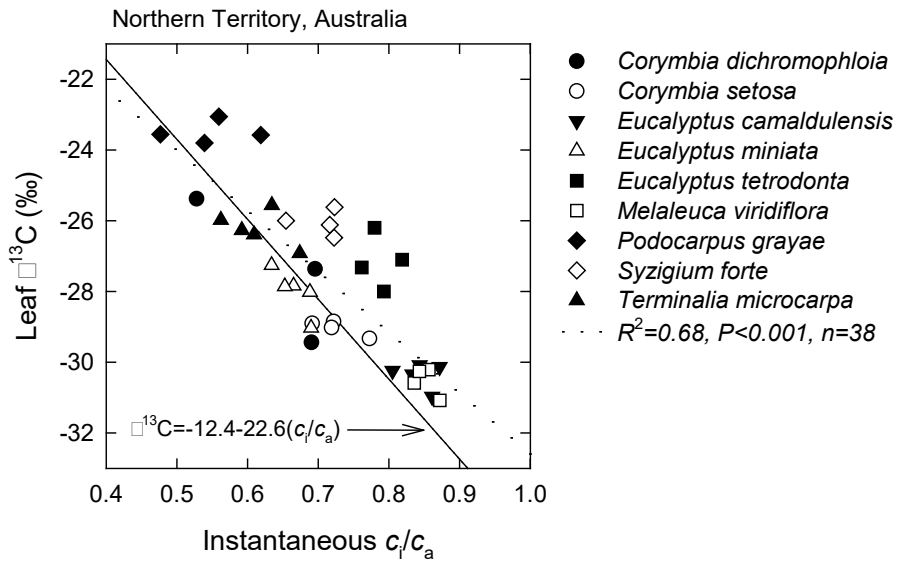
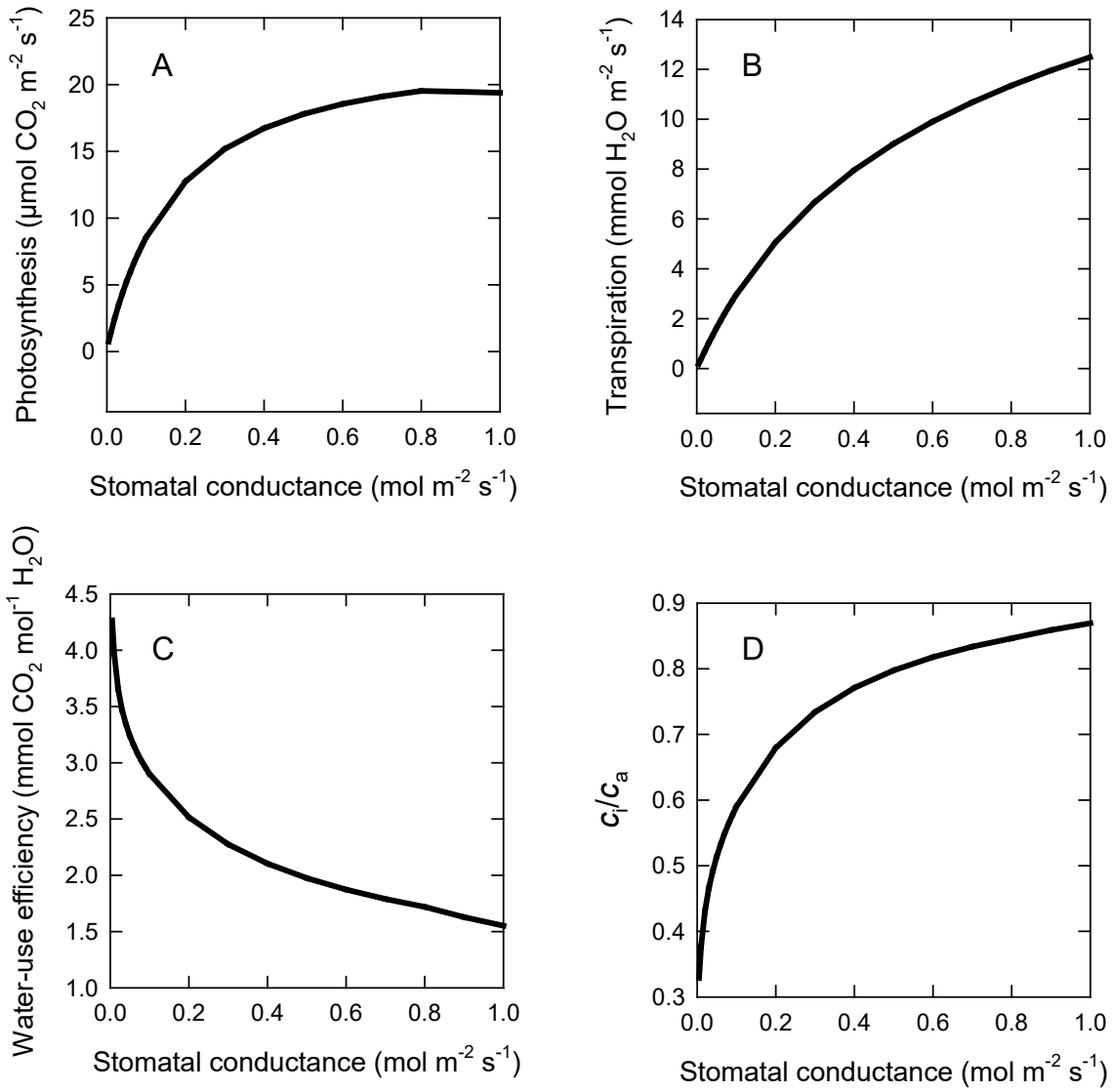
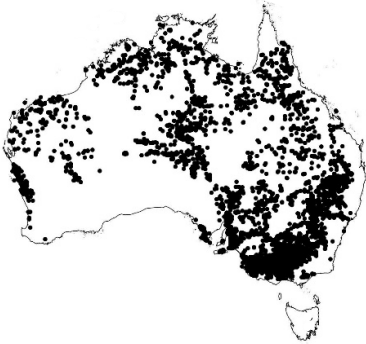


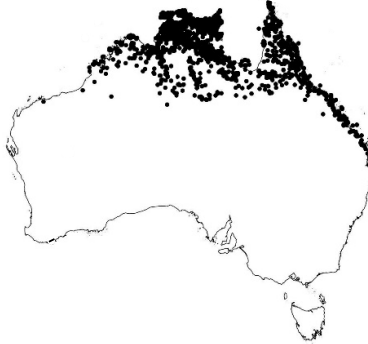
Fig. 1.

**Fig. 2.**

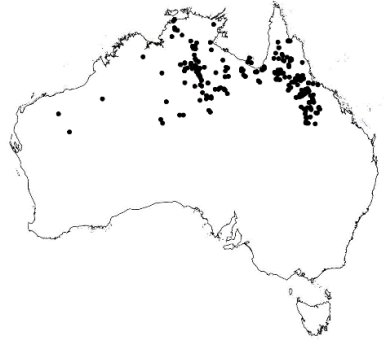
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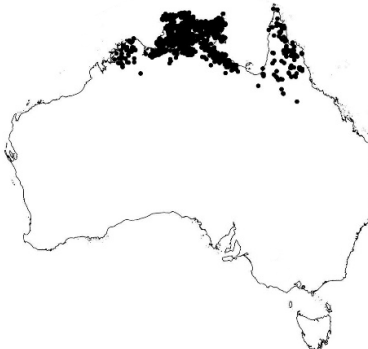
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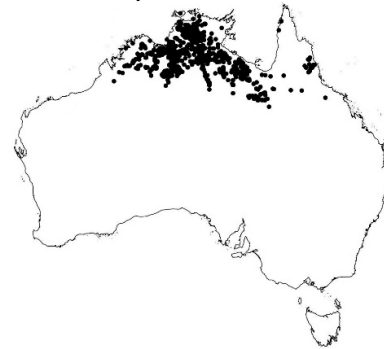
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*Eucalyptus  
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dichromophloia*



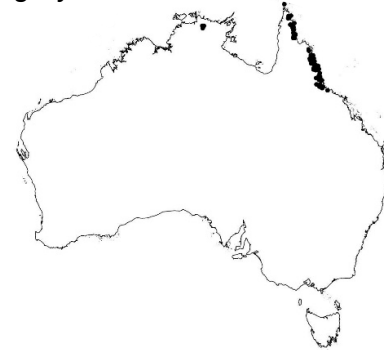
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*Podocarpus  
grayae*



**Fig. 3.**

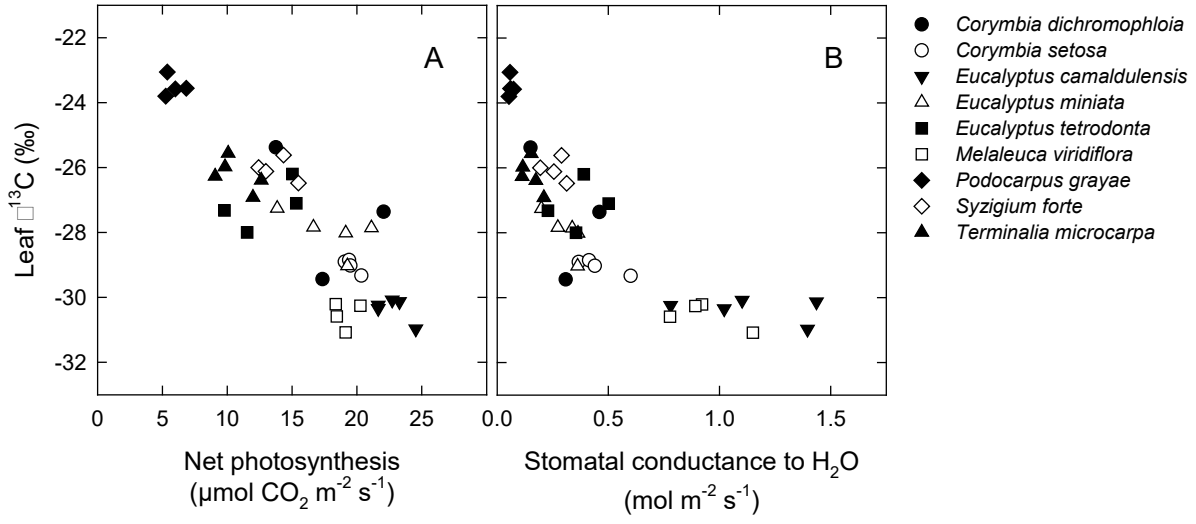
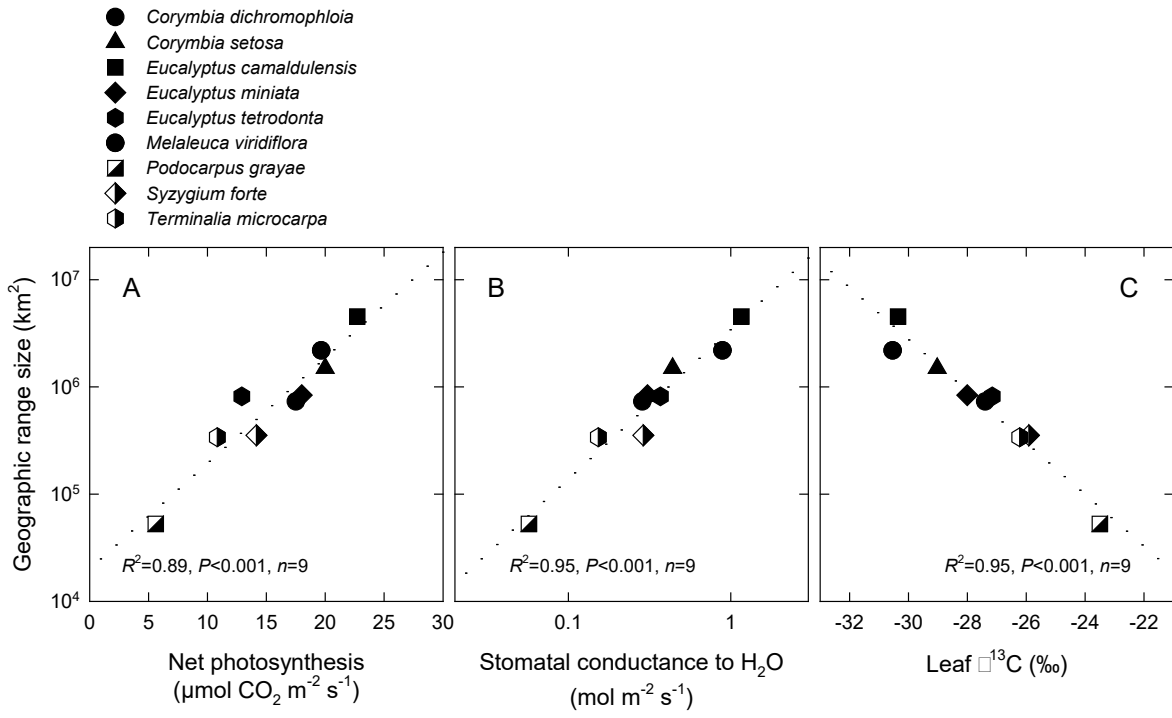


Fig. 4.



**Fig. 5.**



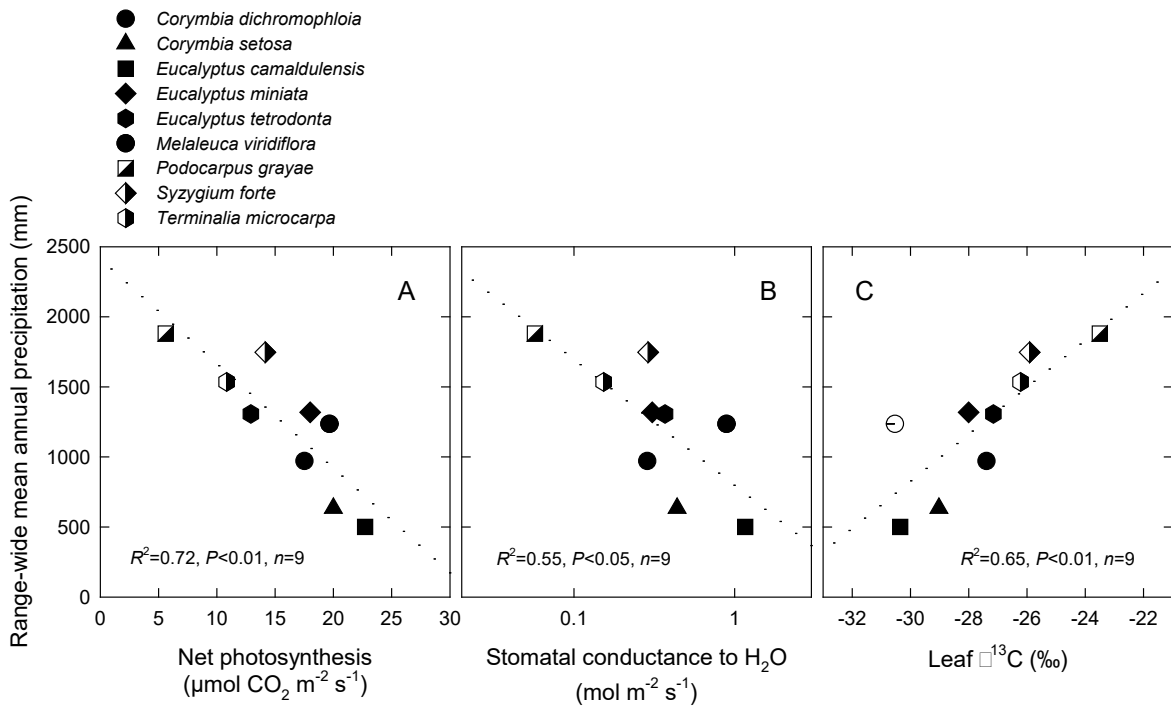


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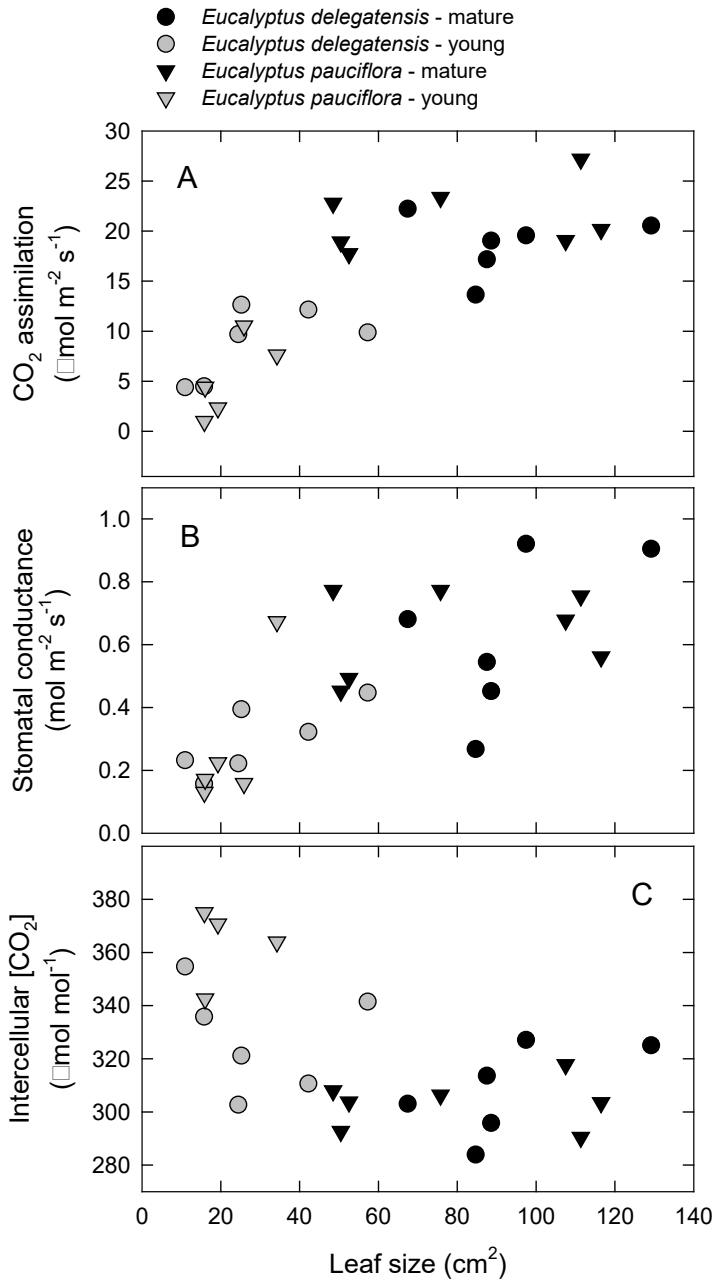


Fig. 7.

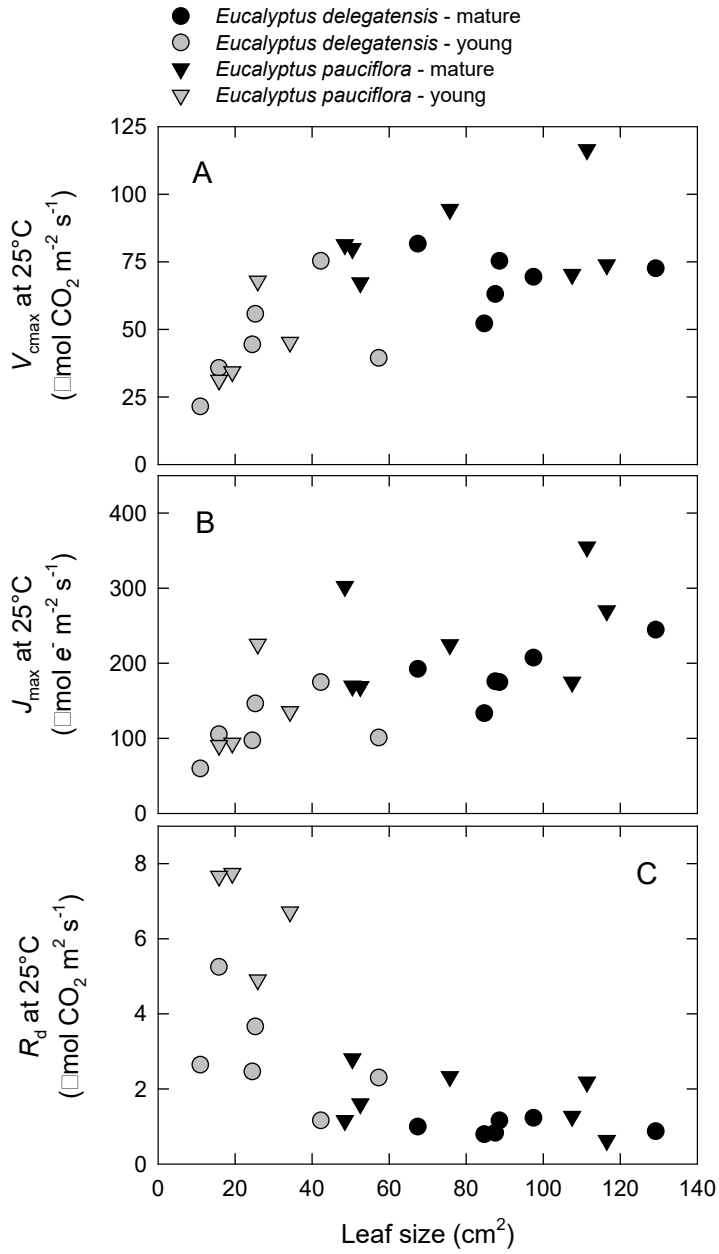
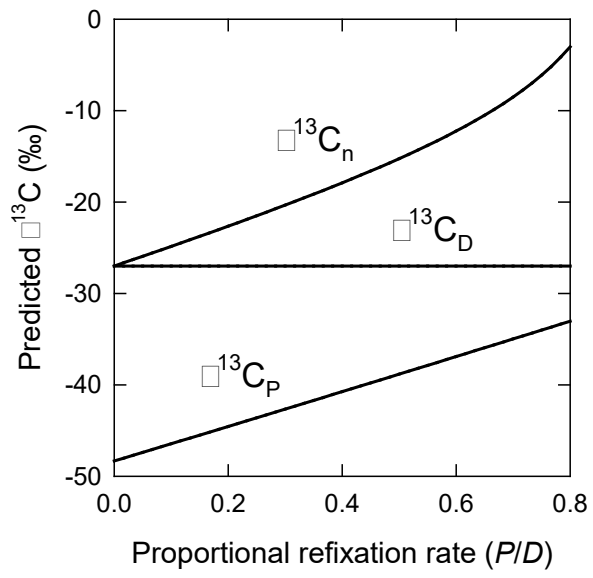
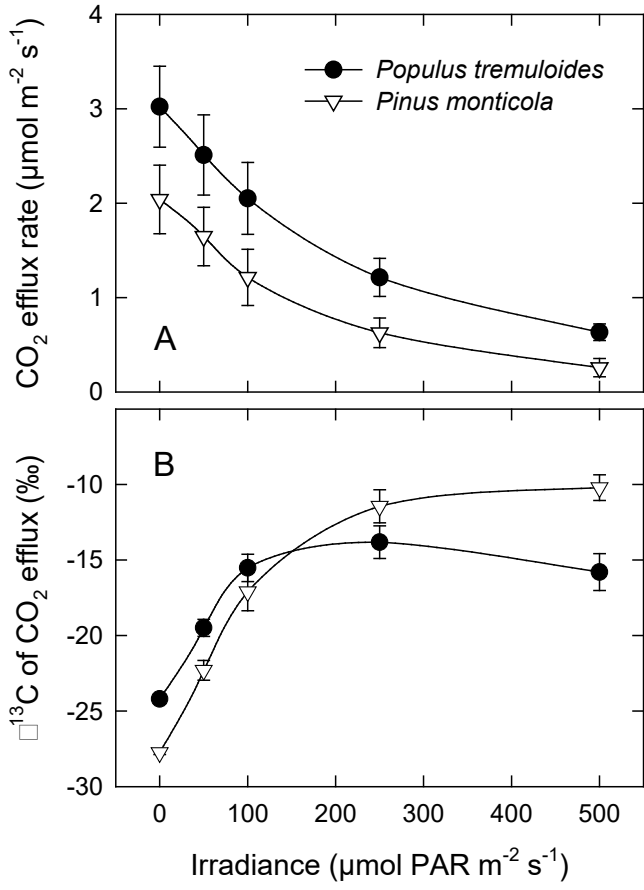


Fig. 8.

**Fig. 9.**

**Fig. 10.**