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

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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 C₃ plant canopies. I then present a case study of water-use efficiency in northern
13 Australian tree species. In general, C₃ plants face a trade-off whereby increasing stomatal
14 conductance for a given set of conditions will result in a higher CO₂ 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 C₃ 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 refixation of respired
25 CO₂ 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 CO₂. In coming decades, increases in plant water-use efficiency caused by rising CO₂
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 CO₂ 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⁻¹ (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 CO₂ back to the atmosphere. In the steady state, the same quantity of CO₂ 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 CO₂ 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 H₂O yr⁻¹ (Hetherington and Woodward, 2003; Jasechko et al., 2013). Plant water-use efficiency describes the amount of CO₂ taken up by photosynthesis for a given amount water vapour lost to the atmosphere. Assuming intermediate global estimates for photosynthesis (130×10^{15} g C yr⁻¹) and transpiration (40×10^{18} g H₂O yr⁻¹) results in an average leaf-level water-use efficiency for terrestrial plants, expressed in molar units, of about 5 mmol CO₂ mol⁻¹ H₂O. 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 (¹³C) to major (¹²C) 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 ¹³C contains one neutron more than the much more abundant ¹²C, and comprises about 1% of carbon in terrestrial ecosystems (Craig, 1953). The ratio ¹³C/¹²C 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 ¹³C 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 (δ_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 δ 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 δ 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}C during photosynthetic 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 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 CO_2 and R_p is that of plant biomass. Analytical determinations of carbon isotope ratios will typically be reported as δ values of the form given by Eqn. 1. These δ 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 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 CO_2 AND WATER VAPOUR

Perhaps the simplest mathematical description of photosynthesis is one based on diffusion of CO_2 into the leaf. The abbreviation typically applied to photosynthesis is A , for assimilation of CO_2 . In order to calculate the diffusive flux of CO_2 into the leaf, one must take account of both the concentration gradient for 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 CO₂ 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 μmol CO₂ m⁻² s⁻¹. The term g_c represents the conductance to CO₂ 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 mol m⁻² s⁻¹. The terms c_a and c_i represent CO₂ concentrations in the atmosphere and intercellular air spaces, respectively, expressed here as mole fractions, with units of μmol CO₂ 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: CO₂, water vapour and air. As CO₂ 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 CO₂ 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 CO₂ 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 mol H₂O 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 CO₂ 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 H₂O 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 H₂O and g_b is the boundary layer conductance to H₂O. 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 CO₂, and because the diffusion pathways for CO₂ 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 CO₂, g_{sc}, can be calculated as g_s/1.6; the boundary layer conductance to CO₂, 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 CO₂, to calculate the total conductance to CO₂, g_c. Once this is obtained, it can be combined with the measured CO₂ uptake rate and equation 4 to solve for c_i, the intercellular CO₂ 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 CO₂ 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 CO₂ 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 CO₂ concentration from the

atmosphere to the leaf interior. Thus, all else equal, a lower intercellular CO₂ 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 CO₂ mole fractions:

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

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:

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

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 C₃ 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 ¹³CO₂ and ¹²CO₂ 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 CO₂ 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 CO₂ (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, ϕ_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 ϕ_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: C₃, C₄, 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 C₃, because reduction of CO₂ by the Calvin cycle results initially in the production of three-carbon sugar phosphate molecules. Globally, C₃ photosynthesis accounts for about 80% of terrestrial GPP, (Still et al., 2003). Nearly all woody plants employ the C₃ photosynthetic pathway, although there are notable exceptions (Pearcy and Troughton, 1975; Lüttege, 2006; Sage, 2016). As a result, more than 99% of terrestrial plant biomass resides in C₃ plants (Still et al., 2003). Plants that employ the C₄ photosynthetic pathway typically have a higher water-use efficiency than those that employ the C₃ photosynthetic pathway. The C₄ photosynthetic pathway suppresses photorespiration and uses PEP-carboxylase for CO₂ 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 CO₂ 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 C₃ photosynthetic pathway.

THE BIOCHEMICAL MODEL OF C₃ PHOTOSYNTHESIS

A second approach to mathematically describing photosynthesis is through the biochemical processes that result in CO₂ assimilation. The assimilation of CO₂ during photosynthesis in C₃ 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 CO₂ assimilation rate in C₃ 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, Γ_* is the CO₂ compensation point in the absence of day respiration, c_c is the CO₂ partial pressure in the chloroplast, and R_d is the day respiration rate. Equation 14 holds for situations when c_c is larger than Γ_* . The Rubisco-limited carboxylation rate can be defined as

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

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 CO₂ 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 CO₂ 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_{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 $w_l - w_a$ 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 CO₂ 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 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 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 $\delta^{13}\text{C}_p$ and instantaneous gas exchange rates. The relationship between leaf $\delta^{13}\text{C}_p$ 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 $\delta^{13}\text{C}_p$ among species was driven by variation in the maximum g_s achieved by the different species under well-watered conditions. Although $\delta^{13}\text{C}_p$ was linearly related to the rate of CO₂ assimilation, the slope of the relationship is opposite to that which would be expected if photosynthetic capacity were driving variation in $\delta^{13}\text{C}_p$ (Fig. 4A). If this were the case, a higher A would be expected to be associated with a less negative $\delta^{13}\text{C}_p$, rather than a more negative $\delta^{13}\text{C}_p$.

I observed a strong correlation between leaf $\delta^{13}\text{C}_p$ 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 CO₂ 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 CO₂ 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 CO₂ exchange in sunlight will trend from net CO₂ efflux, whereby leaf respiration is larger than gross photosynthesis, to net CO₂ 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 CO₂ exchange necessarily means that the intercellular CO₂ 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 CO₂ 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 CO₂ assimilation, stomatal conductance, and intercellular CO₂ 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 CO₂ 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 CO₂ assimilation to intercellular CO₂ concentration (A - c_i curves) in the leaves shown in Fig. 7. The biochemical model of C₃ photosynthesis was fitted to the A - c_i curves by submitting the data to the website www.leafweb.org (Gu et al., 2010) to estimate the parameters V_{cmax} and J_{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_{cmax} and J_{max} , and very high R_d which causes low net CO₂ 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 CO₂ 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, CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ for CO₂ assimilation (Cernusak et al., 2001).

Refixation of respired CO₂ by photosynthetic bark increases plant water-use efficiency (Cernusak and Cheesman, 2015; Vandegehuchte et al., 2015). This is because refixation relies upon internally produced CO₂ 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 CO₂. 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 ϕ_c . Refixation reduces the loss of respiratory CO₂ from the plant, thereby decreasing ϕ_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 Huty, 2011). Because there are at least two possible fates for respired CO₂ in this case, diffusion to the atmosphere or assimilation by bark chloroplasts, there is opportunity for discrimination against ¹³C. The main mechanism is discrimination by Rubisco. A third possible fate for stem-respired CO₂ 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 CO₂ into this third pathway does not result in detectable fractionation of $\delta^{13}\text{C}$ of CO₂ (Ubierna et al., 2009).

The extent to which Rubisco fractionation is manifested in the $\delta^{13}\text{C}$ of CO₂ efflux and refixed photosynthate depends on the extent of refixation, but the expected trend differs for refixed photosynthate versus CO₂ efflux (Fig. 9). If the proportional refixation rate is high, then there will be little opportunity for discrimination by Rubisco because nearly all available CO₂ will effectively be captured. In this case, refixed photosynthate will show little depletion in $\delta^{13}\text{C}$ compared to dark respiration CO₂. 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 $\delta^{13}\text{C}$. However, the pattern for the $\delta^{13}\text{C}$ of CO₂ 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 CO₂ will be removed from the efflux and so the overall impact will be small.

A useful application of the refixation signal in the $\delta^{13}\text{C}$ of CO₂ 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 $\delta^{13}\text{C}$ of net CO₂ efflux:

$$\frac{P}{D} = \frac{\delta^{13}\text{C}_n - \delta^{13}\text{C}_D}{b - a - (\delta^{13}\text{C}_a - \delta^{13}\text{C}_n - a) \frac{c_a}{c_i}}, \quad (\text{Eqn 19})$$

where P is the gross photosynthesis rate in the bark, D is the day respiration rate of the woody tissue, $\delta^{13}\text{C}_n$ is the $\delta^{13}\text{C}$ of net CO₂ efflux, $\delta^{13}\text{C}_D$ is that of day respiration, $\delta^{13}\text{C}_a$ is that of atmospheric CO₂, b is discrimination by Rubisco, a is diffusional fractionation, c_i is the CO₂ 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 $\delta^{13}\text{C}$ 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 CO_2 , some stems have stomata and are capable of net photosynthetic uptake of 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 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 (Ehlernerger et al., 1987; Comstock and Ehlernerger, 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 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 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 CO₂ 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 CO₂ 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 C₃ and C₄ 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 (Nesotto 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 CO₂ 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 Pg C yr⁻¹ (Le Quere et al., 2018). This terrestrial sink is slowing the rate of increase in atmospheric CO₂ 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 CO₂, 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 CO₂ 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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REFERENCES

- Aalto T., Hari P., Vesala T. (2002) Comparison of an optimal stomatal regulation model and a biochemical model in explaining CO₂ exchange in field conditions. *Silva Fennica*, **36**, 615-623.
- Ahlstrom A., Raupach M.R., Schurgers G., Smith B., Arneth A., Jung M., Reichstein M., Canadell J.G., Friedlingstein P., Jain A.K., Kato E., Poulter B., Sitch S., Stocker B.D., Viovy N., Wang Y.P., Wiltshire A., Zaehle S., Zeng N. (2015) The dominant role of semi-arid ecosystems in the trend and variability of the land CO₂ sink. *Science*, **348**, 895-899.
- Amthor J.S. (2000) The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. *Annals of Botany*, **86**, 1-20.
- Anderson J.E., Williams J., Kriedemann P.E., Austin M.P., Farquhar G.D. (1996) Correlations between carbon isotope discrimination and climate of native habitats for diverse eucalypt taxa growing in a common garden. *Australian Journal of Plant Physiology*, **23**, 311-320.
- Ávila-Lovera E., Tezara W. (2018) Water-use efficiency is higher in green stems than in leaves of a tropical tree species. *Trees*, **In press**.
- Ávila E., Herrera A., Tezara W. (2014) Contribution of stem CO₂ fixation to whole-plant carbon balance in nonsucculent species. *Photosynthetica*, **52**, 3-15.
- Beer C., Reichstein M., Tomelleri E., Ciais P., Jung M., Carvalhais N., Rodenbeck C., Arain M.A., Baldocchi D., Bonan G.B., Bondeau A., Cescatti A., Lasslop G., Lindroth A., Lomas M., Luyssaert S., Margolis H., Oleson K.W., Roupsard O., Veenendaal E., Viovy N., Williams C., Woodward F.I., Papale D. (2010) Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. *Science*, **329**, 834-838.
- Beringer J., Hutley L.B., Abramson D., Arndt S.K., Briggs P., Bristow M., Canadell J.G., Cernusak L.A., Eamus D., Edwards A.C., Evans B.J., Fest B., Goergen K., Grover S.P., Hacker J., Haverd V., Kanniah K., Livesley S.J., Lynch A., Maier S., Moore C., Raupach M., Russell-Smith J., Scheiter S., Tapper N.J., Uotila P. (2015) Fire in Australian savannas: from leaf to landscape. *Global Change Biology*, **21**, 62-81.
- Beringer J., Hutley L.B., McHugh I., Arndt S.K., Campbell D., Cleugh H.A., Cleverly J., de Dios V.R., Eamus D., Evans B., Ewenz C., Grace P., Griebel A., Haverd V., Hinko-Najera N., Huete A., Isaac P., Kanniah K., Leuning R., Liddell M.J., Macfarlane C., Meyer W., Moore C., Pendall E., Phillips A., Phillips R.L., Prober S.M., Restrepo-Coupe N., Rutledge S., Schroder I., Silberstein R., Southall P., Yee M.S., Tapper N.J., van Gorsel E., Vote C., Walker J., Wardlaw T. (2016) An introduction to the Australian and New Zealand flux tower network - OzFlux. *Biogeosciences*, **13**, 5895-5916.

- Beringer J., Hutley L.B., Tapper N.J., Cernusak L.A. (2007) Savanna fires and their impact on net ecosystem productivity in North Australia. *Global Change Biology*, **13**, 990-1004.
- Boyer J.S., Kawamitsu Y. (2011) Photosynthesis gas exchange system with internal CO₂ directly measured. *Environment Control Biology*, **49**, 193-207.
- Briggs L.J., Shantz H.L. (1914) Relative water requirement of plants. *Journal of Agricultural Research*, **3**, 1-64.
- Brock J. (1993) *Native plants of Northern Australia*. New Holland Publishers, Sydney: 355 pp.
- Brodrribb T., Hill R.S. (1998) The photosynthetic drought physiology of a diverse group of southern hemisphere conifer species is correlated with minimum seasonal rainfall. *Functional Ecology*, **12**, 465-471.
- Brooker M.I.H., Kleinig D.A. (2004) *Field guide to eucalypts: Northern Australia*. Blooming Books, Melbourne: 383 pp.
- Cernusak L.A., Aranda J., Marshall J.D., Winter K. (2007a) Large variation in whole-plant water-use efficiency among tropical tree species. *New Phytologist*, **173**, 294-305.
- Cernusak L.A., Cheesman A.W. (2015) The benefits of recycling: how photosynthetic bark can increase drought tolerance. *New Phytologist*, **208**, 995-997.
- Cernusak L.A., Hutley L., Beringer J., Tapper N.J. (2006) Stem and leaf gas exchange and their responses to fire in a north Australian tropical savanna. *Plant Cell and Environment*, **29**, 632-646.
- Cernusak L.A., Hutley L.B. (2011) Stable isotopes reveal the contribution of corticular photosynthesis to growth in branches of *Eucalyptus miniata*. *Plant Physiology*, **155**, 515-523.
- Cernusak L.A., Hutley L.B., Beringer J., Holtum J.A.M., Turner B.L. (2011a) Photosynthetic physiology of eucalypts along a sub-continental rainfall gradient in northern Australia. *Agricultural and Forest Meteorology*, **151**, 1462-1470.
- Cernusak L.A., Marshall J.D. (2000) Photosynthetic refixation in branches of Western White Pine. *Functional Ecology*, **14**, 300-311.
- Cernusak L.A., Marshall J.D., Comstock J.P., Balster N.J. (2001) Carbon isotope discrimination in photosynthetic bark. *Oecologia*, **128**, 24-35.
- Cernusak L.A., Tcherkez G., Keitel C., Cornwell W.K., Santiago L.S., Knohl A., Barbour M.M., Williams D.G., Reich P.B., Ellsworth D.S., Dawson T.E., Griffiths H., Farquhar G.D., Wright I.J. (2009) Why are non-photosynthetic tissues generally ¹³C enriched compared to leaves in C₃ plants? Review and synthesis of current hypotheses. *Functional Plant Biology*, **36**, 199-213.

- Cernusak L.A., Ubierna N., Winter K., Holtum J.A.M., Marshall J.D., Farquhar G.D. (2013a) Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist*, **200**, 950-965.
- Cernusak L.A., Winter K., Aranda J., Turner B.L., Marshall J.D. (2007b) Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *Journal of Experimental Botany*, **58**, 3549-3566.
- Cernusak L.A., Winter K., Dalling J.W., Holtum J.A.M., Jaramillo C., Körner C., Leakey A.D.B., Norby R.J., Poulter B., Turner B.L., Wright S.J. (2013b) Tropical forest responses to increasing atmospheric CO₂: current knowledge and opportunities for future research. *Functional Plant Biology*, **40**, 531-551.
- Cernusak L.A., Winter K., Martinez C., Correa E., Aranda J., Garcia M., Jaramillo C., Turner B.L. (2011b) Responses of legume versus nonlegume tropical tree seedlings to elevated CO₂ concentration. *Plant Physiology*, **157**, 372-385.
- Choinski J.S., Ralph P., Eamus D. (2003) Changes in photosynthesis during leaf expansion in *Corymbia gummifera*. *Australian Journal of Botany*, **51**, 111-118.
- Comstock J.P., Ehleringer J.R. (1988) Contrasting photosynthetic behaviour in leaves and twigs of *Hymenoclea salsola*, a green-twiggled warm desert shrub. *American Journal of Botany*, **75**, 1360-1370.
- Comstock J.P., Ehleringer J.R. (1992) Correlating genetic variation in carbon isotopic composition with complex climatic gradients. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 7747-7751.
- Coplen T.B. (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, **25**, 2538-2560.
- Cornwell W.K., Wright I.J., Turner J., Maire V., Barbour M.M., Cernusak L.A., Dawson T.E., Ellsworth D., Farquhar G.D., Griffiths H., Keitel C., Knohl A., Reich P.B., Williams D.G., Bhaskar R., Cornelissen J.H.C., Richards A., Schmidt S., Valladares F., Körner C., Schulze E.-D., Buchmann N., Santiago L.S. (2018) Climate and soils together regulate photosynthetic carbon isotope discrimination within C₃ plants worldwide. *Global Ecology and Biogeography*, **In press**.
- Cowan I.R. (1978) Stomatal behaviour and environment. In: Preston R.D., Woolhouse H.W. (Eds), *Advances in Botanical Research*. Academic Press: 117-228.
- Cowan I.R., Farquhar G.D. (1977) Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society of Experimental Biology*, **31**, 471-505.

- Craig H. (1953) The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, **3**, 53-92.
- Craig H. (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta*, **12**, 133-149.
- Cramer M.D., Hawkins H.J., Verboom G.A. (2009) The importance of nutritional regulation of plant water flux. *Oecologia*, **161**, 15-24.
- Cramer M.D., Hoffmann V., Verboom G.A. (2008) Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist*, **179**, 1048-1057.
- Dalling J.W., Cernusak L.A., Winter K., Aranda J., Garcia M., Virgo A., Cheesman A.W., Baresch A., Jaramillo C., Turner B.L. (2016) Two tropical conifers show strong growth and water-use efficiency responses to altered CO₂ concentration. *Annals of Botany*, **118**, 1113-1125.
- Damesin C., Ceschia E., Le Goff N., Ottorini J.M., Dufrene E. (2002) Stem and branch respiration of beech: from tree measurements to estimations at the stand level. *New Phytologist*, **153**, 159-172.
- Dawson T.E., Mambelli S., Plamboeck A.H., Templer P.H., Tu K.P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**, 507-559.
- Dekker S.C., Groenendijk M., Booth B.B.B., Huntingford C., Cox P.M. (2016) Spatial and temporal variations in plant water-use efficiency inferred from tree-ring, eddy covariance and atmospheric observations. *Earth System Dynamics*, **7**, 525-533.
- Diefendorf A.F., Mueller K.E., Wing S.L., Koch P.L., Freeman K.H. (2010) Global patterns in leaf ¹³C discrimination and implications for studies of past and future climate. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 5738-5743.
- Donohue R.J., Roderick M.L., McVicar T.R., Farquhar G.D. (2013) Impact of CO₂ fertilization on maximum foliage cover across the globe's warm, arid environments. *Geophysical Research Letters*, **40**, 3031-3035.
- Eamus D. (1999) Ecophysiological traits of deciduous and evergreen woody species in the seasonally dry tropics. *Trends in Ecology & Evolution*, **14**, 11-16.
- Ehleringer J.R. (1993) Carbon and water relations in desert plants: an isotopic perspective. In: Ehleringer J.R., Hall A.E., Farquhar G.D. (Eds), *Stable isotopes and plant carbon/water relations*. Academic Press, San Diego: 155-172.
- Ehleringer J.R., Comstock J.P., Cooper T.A. (1987) Leaf-twig carbon isotope ratio differences in photosynthetic-twig desert shrubs. *Oecologia*, **71**, 318-320.
- Evans J.R., Sharkey T.D., Berry J.A., Farquhar G.D. (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO₂ diffusion in leaves of higher plants. *Australian Journal of Plant Physiology*, **13**, 281-292.

- Farquhar G.D., Ball M.C., von Caemmerer S., Roksandic Z. (1982a) Effect of salinity and humidity on $\delta^{13}\text{C}$ value of halophytes- evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. *Oecologia*, **52**, 121-124.
- Farquhar G.D., Cernusak L.A. (2012) Ternary effects on the gas exchange of isotopologues of carbon dioxide. *Plant Cell and Environment*, **35**, 1221-1231.
- Farquhar G.D., Hubick K.T., Condon A.G., Richards R.A. (1989) Carbon isotope fractionation and plant water-use efficiency. In: Rundel P.W., Ehleringer J.R., Nagy K.A. (Eds), *Stable isotopes in ecological research*. Springer-Verlag, New York: 21-46.
- Farquhar G.D., O'Leary M.H., Berry J.A. (1982b) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, **9**, 121-137.
- Farquhar G.D., Richards R.A. (1984) Isotopic composition of plant carbon correlates with water-use efficiency in wheat genotypes. *Australian Journal of Plant Physiology*, **11**, 539-552.
- Farquhar G.D., von Caemmerer S., Berry J.A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta*, **149**, 78-90.
- Flexas J., Barbour M.M., Brendel O., Cabrera H.M., Carriqui M., Diaz-Espejo A., Douthe C., Dreyer E., Ferrio J.P., Gago J., Galle A., Galmes J., Kodama N., Medrano H., Niinemets U., Peguero-Pina J.J., Pou A., Ribas-Carbo M., Tomas M., Tosens T., Warren C.R. (2012) Mesophyll diffusion conductance to CO_2 : an unappreciated central player in photosynthesis. *Plant Science*, **193**, 70-84.
- Francey R.J., Gifford R.M., Sharkey T.D., Weir B. (1985) Physiological influences on carbon isotope discrimination in huon pine (*Lagarostrobos franklinii*). *Oecologia*, **66**, 211-218.
- Frank D.C., Poulter B., Saurer M., Esper J., Huntingford C., Helle G., Treydte K., Zimmermann N.E., Schleser G.H., Ahlstrom A., Ciais P., Friedlingstein P., Levis S., Lomas M., Sitch S., Viovy N., Andreu-Hayles L., Bednarz Z., Berninger F., Boettger T., D'Alessandro C.M., Daux V., Filot M., Grabner M., Gutierrez E., Haupt M., Hilasvuori E., Jungner H., Kalela-Brundin M., Krapiec M., Leuenberger M., Loader N.J., Marah H., Masson-Delmotte V., Pazdur A., Pawelczyk S., Pierre M., Planells O., Pukiene R., Reynolds-Henne C.E., Rinne K.T., Saracino A., Sonninen E., Stievenard M., Switsur V.R., Szczepanek M., Szychowska-Krapiec E., Todaro L., Waterhouse J.S., Weigl M. (2015) Water-use efficiency and transpiration across European forests during the Anthropocene. *Nature Climate Change*, **5**, 579-583.

- Gaastra P. (1959) Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. *Mededelingen van de Landbouwhogeschool te Wageningen, Nederland*, **59**, 1-68.
- Garrish V., Cernusak L.A., Winter K., Turner B.L. (2010) Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. *Journal of Experimental Botany*, **61**, 3735-3748.
- Givnish T.J., Wong S.C., Stuart-Williams H., Holloway-Phillips M., Farquhar G.D. (2014) Determinants of maximum tree height in *Eucalyptus* species along a rainfall gradient in Victoria, Australia. *Ecology*, **95**, 2991-3007.
- Goedhart C.M., Pataki D.E. (2012) Do arid species use less water than mesic species in an irrigated common garden? *Urban Ecosystems*, **15**, 215-232.
- Gu L.H., Pallardy S.G., Tu K., Law B.E., Wullschleger S.D. (2010) Reliable estimation of biochemical parameters from C₃ leaf photosynthesis-intercellular carbon dioxide response curves. *Plant Cell and Environment*, **33**, 1852-1874.
- Guy R.D., Holowachuk D.L. (2001) Population differences in stable carbon isotope ratio of *Pinus contorta* Dougl. ex Loud.: relationship to environment, climate of origin, and growth potential. *Canadian Journal of Botany*, **79**, 274-283.
- Hanba Y.T., Miyazawa S.I., Kogami H., Terashima I. (2001) Effects of leaf age on internal CO₂ transfer conductance and photosynthesis in tree species having different types of shoot phenology. *Australian Journal of Plant Physiology*, **28**, 1075-1084.
- Hetherington A.M., Woodward F.I. (2003) The role of stomata in sensing and driving environmental change. *Nature*, **424**, 901-908.
- Huang M.T., Piao S.L., Sun Y., Ciais P., Cheng L., Mao J.F., Poulter B., Shi X.Y., Zeng Z.Z., Wang Y.P. (2015) Change in terrestrial ecosystem water-use efficiency over the last three decades. *Global Change Biology*, **21**, 2366-2378.
- Hubick K.T., Gibson A. (1993) Diversity in the relationship between carbon isotope discrimination and transpiration efficiency when water is limited. In: Ehleringer J.R., Hall A.E., Farquhar G.D. (Eds), *Stable Isotopes and Carbon-Water Relations*. Academic Press, San Diego: 311-325.
- Huntingford C., Zelazowski P., Galbraith D., Mercado L.M., Sitch S., Fisher R., Lomas M., Walker A.P., Jones C.D., Booth B.B.B., Malhi Y., Hemming D., Kay G., Good P., Lewis S.L., Phillips O.L., Atkin O.K., Lloyd J., Gloor E., Zaragoza-Castells J., Meir P., Betts R., Harris P.P., Nobre C., Marengo J., Cox P.M. (2013) Simulated resilience of tropical rainforests to CO₂-induced climate change. *Nature Geoscience*, **6**, 268-273.

- Hutley L.B., Beringer J., Isaac P., Hacker J. (2011) A continental-scale living laboratory: observations of environmental and vegetation change across the north Australian savannas. *Agricultural and Forest Meteorology*, **151**, 1417-1428.
- Jarman P.D. (1974) The diffusion of carbon dioxide and water vapour through stomata. *Journal of Experimental Botany*, **25**, 927-936.
- Jasechko S., Sharp Z.D., Gibson J.J., Birks S.J., Yi Y., Fawcett P.J. (2013) Terrestrial water fluxes dominated by transpiration. *Nature*, **496**, 347-350.
- Keeling R.F., Graven H.D., Welp L.R., Resplandy L., Bi J., Piper S.C., Sun Y., Bollenbacher A., Meijer H.A.J. (2017) Atmospheric evidence for a global secular increase in carbon isotopic discrimination of land photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 10361-10366.
- Keenan T.F., Hollinger D.Y., Bohrer G., Dragoni D., Munger J.W., Schmid H.P., Richardson A.D. (2013) Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature*, **499**, 324-327.
- Keith H., van Gorsel E., Jacobsen K.L., Cleugh H.A. (2012) Dynamics of carbon exchange in a *Eucalyptus* forest in response to interacting disturbance factors. *Agricultural and Forest Meteorology*, **153**, 67-81.
- Koch G.W., Vitousek P.M., Steffen W.L., Walker B.H. (1995) Terrestrial transects for global change research. *Vegetatio*, **121**, 53-65.
- Kohn M.J. (2010) Carbon isotope compositions of terrestrial C₃ plants as indicators of (paleo)ecology and (paleo)climate. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 19691-19695.
- Lauteri M., Scartazza A., Guido M.C., Brugnoli E. (1997) Genetic variation in photosynthetic capacity, carbon isotope discrimination and mesophyll conductance in provenances of *Castanea sativa* adapted to different environments. *Functional Ecology*, **11**, 675-683.
- Law B.E., Ryan M.G., Anthoni P.M. (1999) Seasonal and annual respiration of a ponderosa pine ecosystem. *Global Change Biology*, **5**, 169-182.
- Le Quere C., Andres R.J., Boden T., Conway T., Houghton R.A., House J.I., Marland G., Peters G.P., van der Werf G.R., Ahlstrom A., Andrew R.M., Bopp L., Canadell J.G., Ciais P., Doney S.C., Enright C., Friedlingstein P., Huntingford C., Jain A.K., Jourdain C., Kato E., Keeling R.F., Goldewijk K.K., Levis S., Levy P., Lomas M., Poulter B., Raupach M.R., Schwinger J., Sitch S., Stocker B.D., Viovy N., Zaehle S., Zeng N. (2013) The global carbon budget 1959-2011. *Earth System Science Data*, **5**, 165-185.
- Le Quere C., Andrew R.M., Friedlingstein P., Sitch S., Pongratz J., Manning A.C., Korsbakken J.I., Peters G.P., Canadell J.G., Jackson R.B., Boden T.A., Tans P.P., Andrews O.D., Arora

- V.K., Bakker D.C.E., Barbero L., Becker M., Betts R.A., Bopp L., Chevallier F., Chini L.P., Ciais P., Cosca C.E., Cross J., Currie K., Gasser T., Harris I., Hauck J., Haverd V., Houghton R.A., Hunt C.W., Hurt G., Ilyina T., Jain A.K., Kato E., Kautz M., Keeling R.F., Goldewijk K.K., Kortzinger A., Landschutzer P., Lefevre N., Lenton A., Liener S., Lima I., Lombardozzi D., Metzl N., Millero F., Monteiro P.M.S., Munro D.R., Nabel J., Nakaoka S., Nojiri Y., Padin X.A., Peregon A., Pfeil B., Pierrot D., Poulter B., Rehder G., Reimer J., Rodenbeck C., Schwinger J., Seferian R., Skjelvan I., Stocker B.D., Tian H.Q., Tilbrook B., Tubiello F.N., van der Laan-Luijkx I.T., van der Werf G.R., van Heuven S., Viovy N., Vuichard N., Walker A.P., Watson A.J., Wiltshire A.J., Zaehle S., Zhu D. (2018) Global Carbon Budget 2017. *Earth System Science Data*, **10**, 405-448.
- Le Quere C., Raupach M.R., Canadell J.G., Marland G., Bopp L., Ciais P., Conway T.J., Doney S.C., Feely R.A., Foster P., Friedlingstein P., Gurney K., Houghton R.A., House J.I., Huntingford C., Levy P.E., Lomas M.R., Majkut J., Metzl N., Ometto J.P., Peters G.P., Prentice I.C., Randerson J.T., Running S.W., Sarmiento J.L., Schuster U., Sitch S., Takahashi T., Viovy N., van der Werf G.R., Woodward F.I. (2009) Trends in the sources and sinks of carbon dioxide. *Nature Geoscience*, **2**, 831-836.
- LI-COR Biosciences. (2008) *Using the LI-6400 Portable Photosynthesis System*. LI-COR Biosciences, Inc., Lincoln, Nebraska, USA: 1280 pp.
- Li C.Y., Berninger F., Koskela J., Sonninen E. (2000) Drought responses of *Eucalyptus microtheca* provenances depend on seasonality of rainfall in their place of origin. *Australian Journal of Plant Physiology*, **27**, 231-238.
- Lopes A.P., Nelson B.W., Wu J., Graca P., Tavares J.V., Prohaska N., Martins G.A., Saleska S.R. (2016) Leaf flush drives dry season green-up of the Central Amazon. *Remote Sensing of Environment*, **182**, 90-98.
- Lütge U. (2006) Photosynthetic flexibility and ecophysiological plasticity: questions and lessons from *Clusia*, the only CAM tree, in the neotropics. *New Phytologist*, **171**, 7-25.
- Maguas C., Griffiths H. (2003) Applications of stable isotopes in plant ecology. *Progress in Botany*, **64**, 472-505.
- Malhi Y., Baldocchi D.D., Jarvis P.G. (1999) The carbon balance of tropical, temperate and boreal forests. *Plant Cell and Environment*, **22**, 715-740.
- Marchi S., Tognetti R., Minnocci A., Borghi M., Sebastiani L. (2008) Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (*Prunus persica*) and an evergreen sclerophyllous Mediterranean shrub (*Olea europaea*). *Trees-Structure and Function*, **22**, 559-571.

- McCree K.J. (1970) An equation for the rate of respiration of white clover plants grown under controlled conditions. In: Setlik I. (Ed), *Prediction and Measurement of Photosynthetic Productivity (Proc. IBP/PP Technical Meet., Trebon)*. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands: 221-229.
- Medlyn B.E., De Kauwe M.G., Lin Y.S., Knauer J., Duursma R.A., Williams C.A., Arneth A., Clement R., Isaac P., Limousin J.M., Linderson M.L., Meir P., Martin-StPaul N., Wingate L. (2017) How do leaf and ecosystem measures of water-use efficiency compare? *New Phytologist*, **216**, 758-770.
- Meinzer F.C., Andrade J.L., Goldstein G., Holbrook N.M., Cavelier J., Jackson P. (1997) Control of transpiration from the upper canopy of a tropical forest: the role of stomatal, boundary layer and hydraulic architecture components. *Plant Cell and Environment*, **20**, 1242-1252.
- Midgley G.F., Aranibar J.N., Mantlana K.B., Macko S. (2004) Photosynthetic and gas exchange characteristics of dominant woody plants on a moisture gradient in an African savanna. *Global Change Biology*, **10**, 309-317.
- Miller J.M., Williams R.J., Farquhar G.D. (2001) Carbon isotope discrimination by a sequence of *Eucalyptus* species along a subcontinental rainfall gradient in Australia. *Functional Ecology*, **15**, 222-232.
- Miyazawa S.I., Terashima I. (2001) Slow development of leaf photosynthesis in an evergreen broad-leaved tree, *Castanopsis sieboldii*: relationships between leaf anatomical characteristics and photosynthetic rate. *Plant Cell and Environment*, **24**, 279-291.
- Morris H., Plavcova L., Cvecko P., Fichtler E., Gillingham M.A.F., Martinez-Cabrera H.I., McGlinn D.J., Wheeler E., Zheng J.M., Ziemska K., Jansen S. (2016) A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. *New Phytologist*, **209**, 1553-1565.
- Moss D.N., Rawlins S.L. (1963) Concentration of carbon dioxide inside leaves. *Nature*, **197**, 1320-1321.
- Nilsen E.T. (1995) Stem photosynthesis: extent, patterns, and role in plant carbon economy. In: Gartner B. (Ed), *Plant Stems: Physiology and Functional Morphology*. Academic Press, San Diego: 223-240.
- Nilsen E.T., Sharifi M.R. (1997) Carbon isotopic composition of legumes with photosynthetic stems from mediterranean and desert habitats. *American Journal of Botany*, **84**, 1707-1713.
- Nosetto M.D., Jobbagy E.G., Paruelo J.M. (2005) Land-use change and water losses: the case of grassland afforestation across a soil textural gradient in central Argentina. *Global Change Biology*, **11**, 1101-1117.

- Pearcy R.W., Troughton J. (1975) C₄ photosynthesis in tree form *Euphorbia* species from Hawaiian rainforest sites. *Plant Physiology*, **55**, 1054-1056.
- Pfanz H., Aschan G., Langenfeld-Heyser R., Wittmann C., Loose M. (2002) Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. *Naturwissenschaften*, **89**, 147-162.
- Poulter B., Frank D., Ciais P., Myneni R.B., Andela N., Bi J., Broquet G., Canadell J.G., Chevallier F., Liu Y.Y., Running S.W., Sitch S., van der Werf G.R. (2014) Contribution of semi-arid ecosystems to interannual variability of the global carbon cycle. *Nature*, **509**, 600-+.
- PP Systems. (2006) *TPS-2 Portable Photosynthesis System: Operator's Manual*. PP Systems Inc., Hitchin, UK: 56 pp.
- Prentice I.C., Meng T.T., Wang H., Harrison S.P., Ni J., Wang G.H. (2011) Evidence of a universal scaling relationship for leaf CO₂ drawdown along an aridity gradient. *New Phytologist*, **190**, 169-180.
- Read J., Farquhar G.D. (1991) Comparative studies in Nothofagus (Fagaceae). 1. Leaf carbon isotope discrimination. *Functional Ecology*, **5**, 684-695.
- Roderick M.L., Greve P., Farquhar G.D. (2015) On the assessment of aridity with changes in atmospheric CO₂. *Water Resources Research*, **51**, 5450-5463.
- Ryan M.G. (1990) Growth and maintenance respiration in stems of *Pinus contorta* and *Picea engelmannii*. *Canadian Journal of Forest Research*, **20**, 48-57.
- Sage R.F. (2016) A portrait of the C₄ photosynthetic family on the 50th anniversary of its discovery: species number, evolutionary lineages, and Hall of Fame. *Journal of Experimental Botany*, **67**, 4039-4056.
- Saveyn A., Steppe K., Ubierna N., Dawson T.E. (2010) Woody tissue photosynthesis and its contribution to trunk growth and bud development in young plants. *Plant Cell and Environment*, **33**, 1949-1958.
- Schulze E.-D., Turner N.C., Nicolle D., Schumacher J. (2006) Leaf and wood carbon isotope ratios, specific leaf areas and wood growth of *Eucalyptus* species across a rainfall gradient in Australia. *Tree Physiology*, **26**, 479-492.
- Schulze E.-D., Williams R.J., Farquhar G.D., Schulze W., Langridge J., Miller J.M., Walker B.H. (1998) Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Australian Journal of Plant Physiology*, **25**, 413-425.
- Schulze E.D., Buschbom U., Evenari M., Lange O.L., Kappen L. (1972) Stomatal responses to changes in humidity in plants growing in the desert. *Planta*, **108**, 259-270.

- Schulze E.D., Lange O.L., Evenari M., Kappen L., Buschbom U. (1974) Role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. 1. Simulation of daily course of stomatal resistance. *Oecologia*, **17**, 159-170.
- Searson M.J., Thomas D.S., Montagu K.D., Conroy J.P. (2004) Leaf water use efficiency differs between Eucalyptus seedlings from contrasting rainfall environments. *Functional Plant Biology*, **31**, 441-450.
- Seibt U., Rajabi A., Griffiths H., Berry J.A. (2008) Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia*, **155**, 441-454.
- Simbo D.J., Van den Bilcke N., Samson R. (2013) Contribution of cortical photosynthesis to bud development in African baobab (*Adansonia digitata* L.) and Castor bean (*Ricinus communis* L.) seedlings. *Environmental and Experimental Botany*, **95**, 1-5.
- Sprugel D.G., Benecke U. (1991) Measuring woody-tissue respiration and photosynthesis. In: Lassoie J.P., Hinckley T.M. (Eds), *Techniques and Approaches in Forest Tree Ecophysiology*. CRC Press, Boca Raton, FL: 329-355.
- Stewart G.R., Turnbull M.H., Schmidt S., Erskine P.D. (1995) ^{13}C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. *Australian Journal of Plant Physiology*, **22**, 51-55.
- Still C.J., Berry J.A., Collatz G.J., DeFries R.S. (2003) Global distribution of C₃ and C₄ vegetation: carbon cycle implications. *Global Biogeochemical Cycles*, **17**, 1006.
- Swann A.L.S., Hoffman F.M., Koven C.D., Randerson J.T. (2016) Plant responses to increasing CO₂ reduce estimates of climate impacts on drought severity. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 10019-10024.
- Terwilliger V.J. (1997) Changes in the $\delta^{13}\text{C}$ values of trees during a tropical rainy season: some effects in addition to diffusion and carboxylation by Rubisco? *American Journal of Botany*, **84**, 1693-1700.
- Terwilliger V.J., Kitajima K., Le Roux-Swarthout D.J., Mulkey S., Wright S.J. (2001) Intrinsic water-use efficiency and heterotrophic investment in tropical leaf growth of two Neotropical pioneer tree species as estimated from $\delta^{13}\text{C}$ values. *New Phytologist*, **152**, 267-281.
- Teskey R.O., McGuire M.A. (2002) Carbon dioxide transport in xylem causes errors in estimation of rates of respiration in stems and branches of trees. *Plant Cell and Environment*, **25**, 1571-1577.
- Teskey R.O., Saveyn A., Steppe K., McGuire M.A. (2008) Origin, fate and significance of CO₂ in tree stems. *New Phytologist*, **177**, 17-32.
- Thornley J.H.M. (1970) Respiration, growth, and maintenance in plants. *Nature*, **227**, 304-305.

- Tosens T., Niinemets U., Vislap V., Eichelmann H., Diez P.C. (2012) Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in *Populus tremula*: how structure constrains function. *Plant Cell and Environment*, **35**, 839-856.
- Ubierna N., Farquhar G.D. (2014) Advances in measurements and models of photosynthetic carbon isotope discrimination in C₃ plants. *Plant Cell and Environment*, **37**, 1494-1498.
- Ubierna N., Kumar A.S., Cernusak L.A., Pangle R.E., Gag P.J., Marshall J.D. (2009) Storage and transpiration have negligible effects on $\delta^{13}\text{C}$ of stem CO₂ efflux in large conifer trees. *Tree Physiology*, **29**, 1563-1574.
- van der Sleen P., Groenendijk P., Vlam M., Anten N.P.R., Boom A., Bongers F., Pons T.L., Terburg G., Zuidema P.A. (2015) No growth stimulation of tropical trees by 150 years of CO₂ fertilization but water-use efficiency increased. *Nature Geoscience*, **8**, 24-28.
- Vandegehuchte M.W., Bloemen J., Vergeynst L.L., Steppe K. (2015) Woody tissue photosynthesis in trees: salve on the wounds of drought? *New Phytologist*, **208**, 998-1002.
- VanLoocke A., Twine T.E., Zeri M., Bernacchi C.J. (2012) A regional comparison of water use efficiency for miscanthus, switchgrass and maize. *Agricultural and Forest Meteorology*, **164**, 82-95.
- von Caemmerer S., Farquhar G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**, 376-387.
- Walz H. (2005) *Portable Gas Exchange Fluorescence System GFS-3000: Handbook of Operation*. Heinz Walz GmbH, Effeltrich, Germany: 145 pp.
- Wang H., Prentice I.C., Keenan T.F., Davis T.W., Wright I.J., Cornwell W.K., Evans B.J., Peng C.H. (2017) Towards a universal model for carbon dioxide uptake by plants. *Nature Plants*, **3**, 734-741.
- Waring R.H., Landsberg J.J., Williams M. (1998) Net primary production of forests: a constant fraction of gross primary production. *Tree Physiology*, **18**, 129-134.
- Welp L.R., Keeling R.F., Meijer H.A.J., Bollenbacher A.F., Piper S.C., Yoshimura K., Francey R.J., Allison C.E., Wahlen M. (2011) Interannual variability in the oxygen isotopes of atmospheric CO₂ driven by El Nino. *Nature*, **477**, 579-582.
- Wenzel S., Cox P.M., Eyring V., Friedlingstein P. (2016) Projected land photosynthesis constrained by changes in the seasonal cycle of atmospheric CO₂. *Nature*, **538**, 499-501.
- Williams R.J., Duff G.A., Bowman D.M.J.S., Cook G.D. (1996) Variation in the composition and structure of tropical savannas as a function of rainfall and soil texture along a large-scale climatic gradient in the Northern Territory, Australia. *Journal of Biogeography*, **23**, 747-756.

- Winter K., Aranda J., Holtum J.A.M. (2005) Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. *Functional Plant Biology*, **32**, 381-388.
- Wu J., Albert L.P., Lopes A.P., Restrepo-Coupe N., Hayek M., Wiedemann K.T., Guan K., Stark S.C., Christoffersen B., Prohaska N., Tavares J.V., Marostica S., Kobayashi H., Ferreira M.L., Campos K.S., Dda Silva R., Brando P.M., Dye D.G., Huxman T.E., Huete A.R., Nelson B.W., Saleska S.R. (2016) Leaf development and demography explain photosynthetic seasonality in Amazon evergreen forests. *Science*, **351**, 972-976.
- Wu J., Serbin S.P., Xu X., Albert L.P., Chen M., Meng R., Saleska S.R., Rogers A. (2017) The phenology of leaf quality and its within-canopy variation is essential for accurate modeling of photosynthesis in tropical evergreen forests. *Global Change Biology*, **23**, 4814-4827.
- Yakir D., Sternberg L.d.L. (2000) The use of stable isotopes to study ecosystem gas exchange. *Oecologia*, **123**, 297-311.
- Yang J.Y., He Y.J., Aubrey D.P., Zhuang Q.L., Teskey R.O. (2016) Global patterns and predictors of stem CO₂ efflux in forest ecosystems. *Global Change Biology*, **22**, 1433-1444.
- Zhou S., Yu B.F., Schwalm C.R., Ciais P., Zhang Y., Fisher J.B., Michalak A.M., Wang W.L., Poulter B., Huntzinger D.N., Niu S.L., Mao J.F., Jain A., Ricciuto D.M., Shi X.Y., Ito A., Wei Y.X., Huang Y.F., Wang G.Q. (2017) Response of water use efficiency to global environmental change based on output from terrestrial biosphere models. *Global Biogeochemical Cycles*, **31**, 1639-1655.
- Zhu Z.C., Piao S.L., Myneni R.B., Huang M.T., Zeng Z.Z., Canadell J.G., Ciais P., Sitch S., Friedlingstein P., Arneth A., Cao C.X., Cheng L., Kato E., Koven C., Li Y., Lian X., Liu Y.W., Liu R.G., Mao J.F., Pan Y.Z., Peng S.S., Penuelas J., Poulter B., Pugh T.A.M., Stocker B.D., Viovy N., Wang X.H., Wang Y.P., Xiao Z.Q., Yang H., Zaehle S., Zeng N. (2016) Greening of the Earth and its drivers. *Nature Climate Change*, **6**, 791-795.
- Zotz G., Harris G., Koniger M., Winter K. (1995) High rates of photosynthesis in the tropical pioneer tree, *Ficus insipida* Willd. *Flora*, **190**, 265-272.

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 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 CO_2 assimilation rate (A), transpiration rate (B), the ratio of CO_2 assimilation to transpiration (C), and the ratio of intercellular to ambient 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 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 CO₂ 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 CO₂ 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 CO₂ assimilation rates (A) and stomatal conductance (B) were accompanied by higher intercellular CO₂ 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_{cmax} , J_{max} and R_d were estimated by fitting the biochemical model of C₃ photosynthesis to curves of CO₂ assimilation rate versus intercellular CO₂ 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 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 CO_2 efflux rate (A) and the $\delta^{13}\text{C}$ of 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).

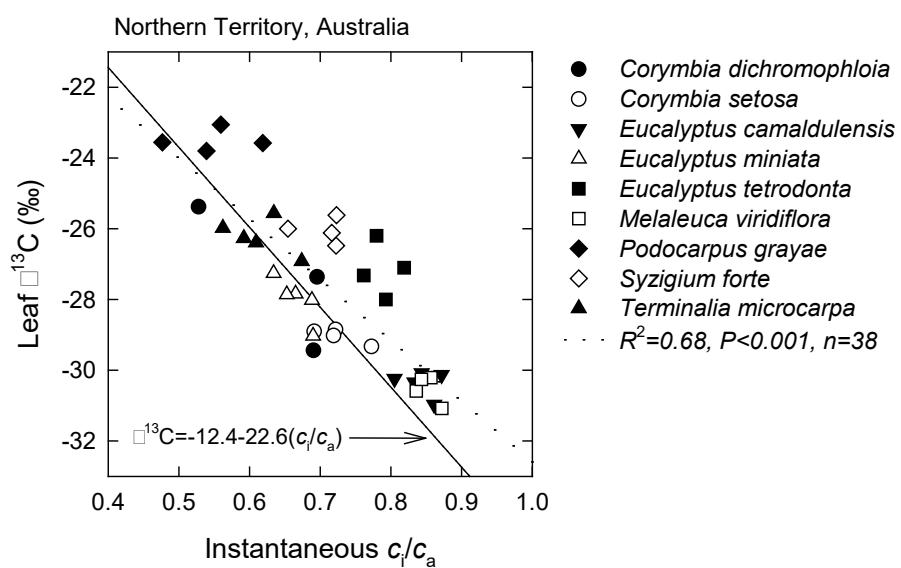
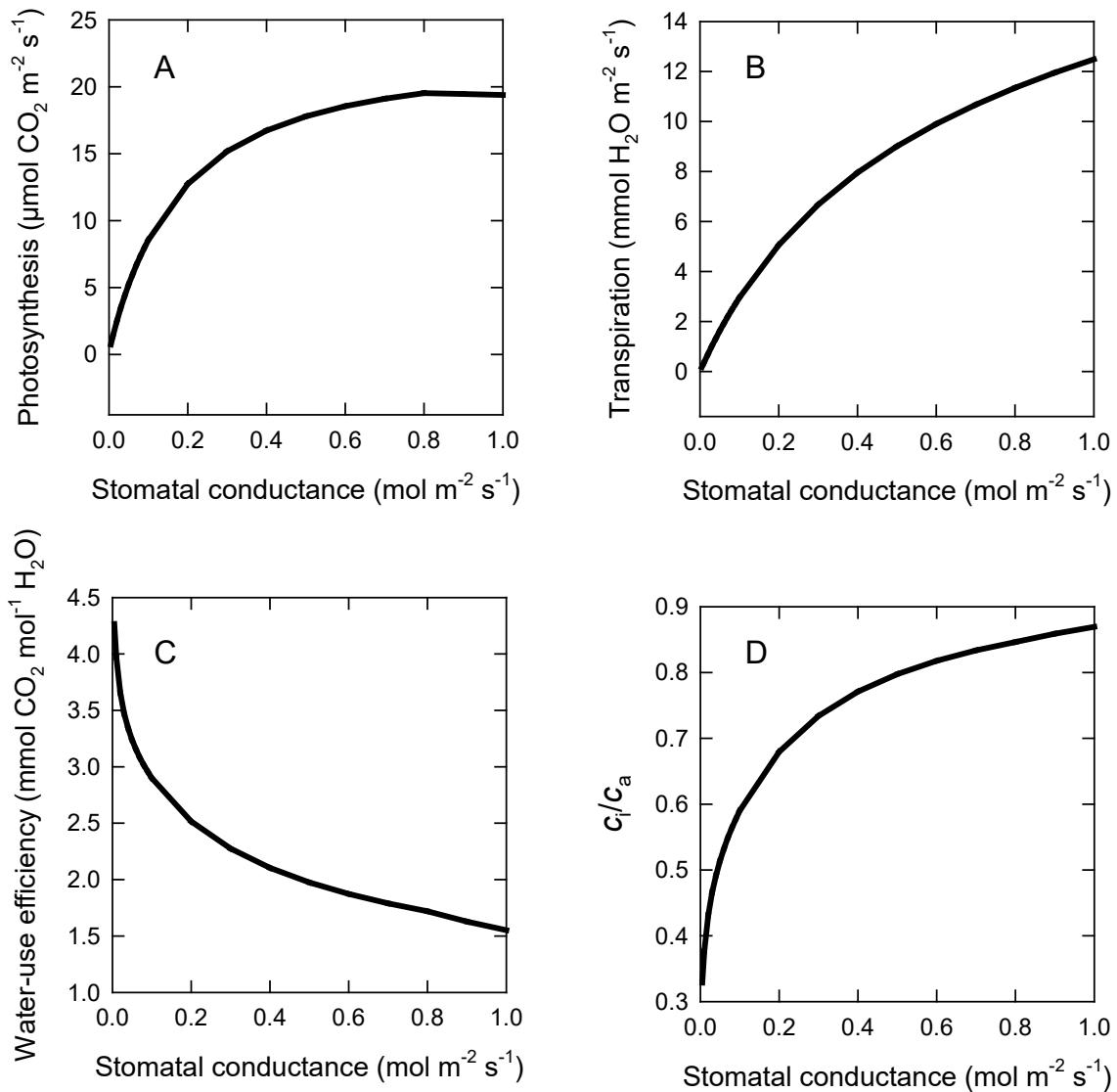


Fig. 1.

**Fig. 2.**

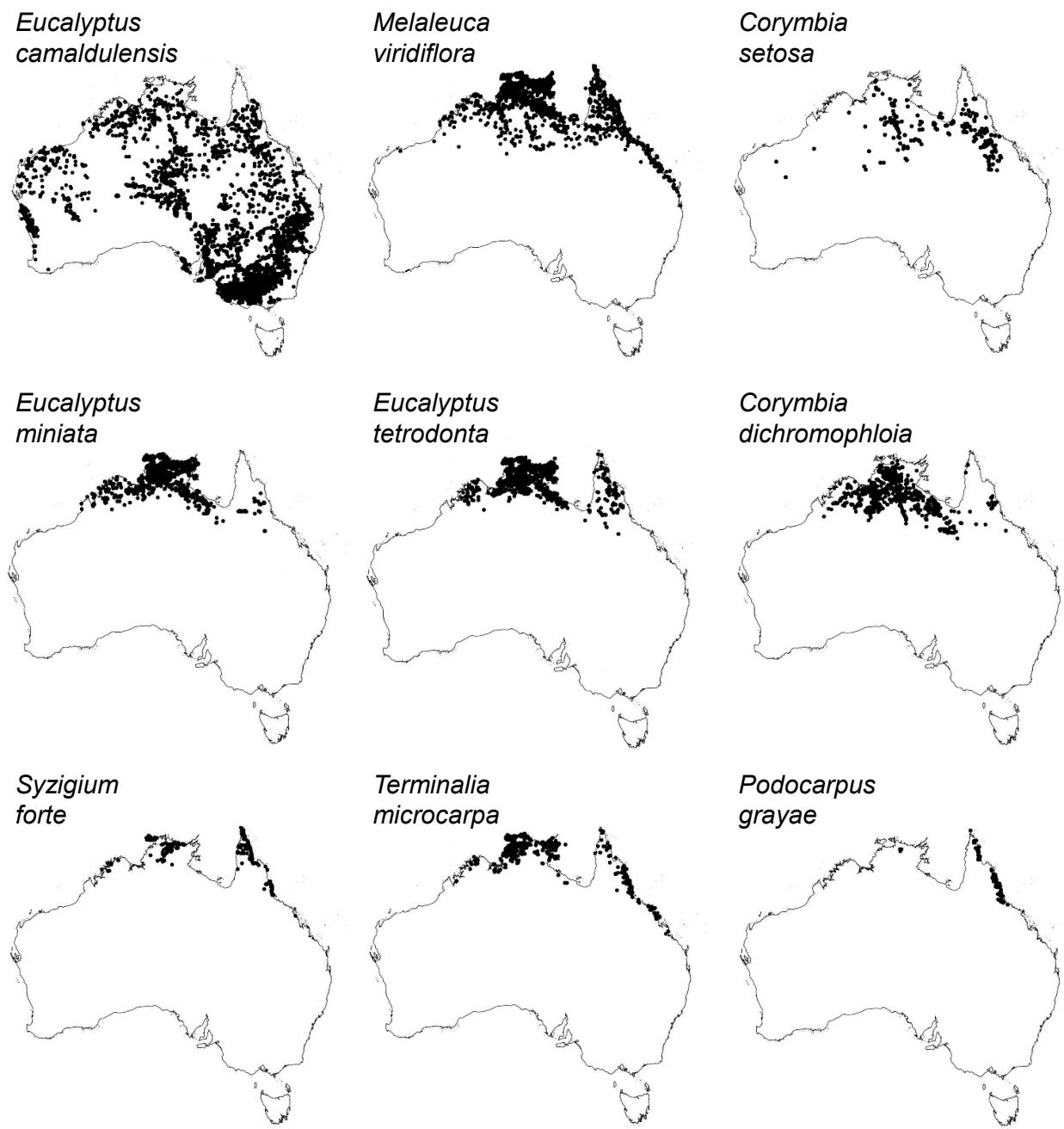


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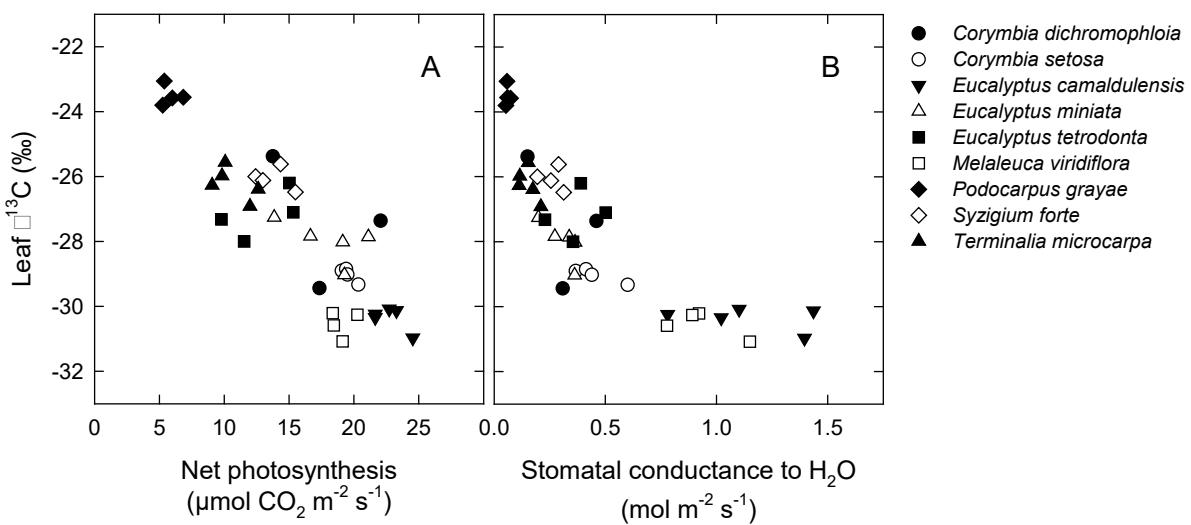
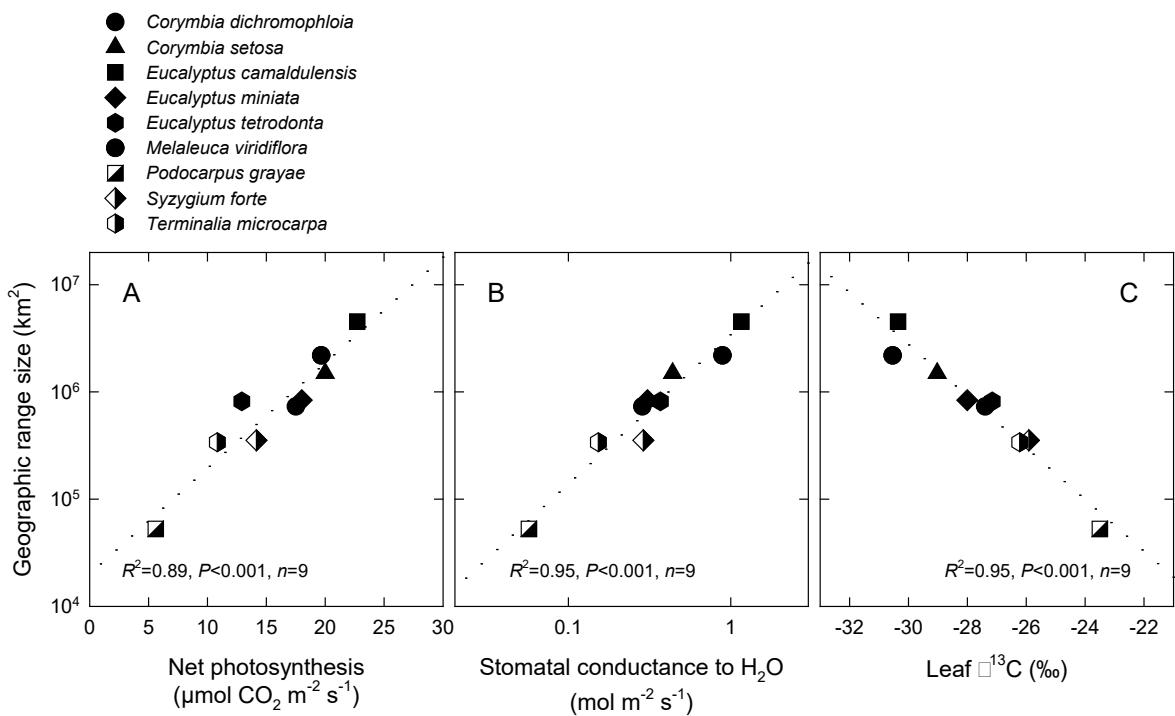


Fig. 4.

**Fig. 5.**

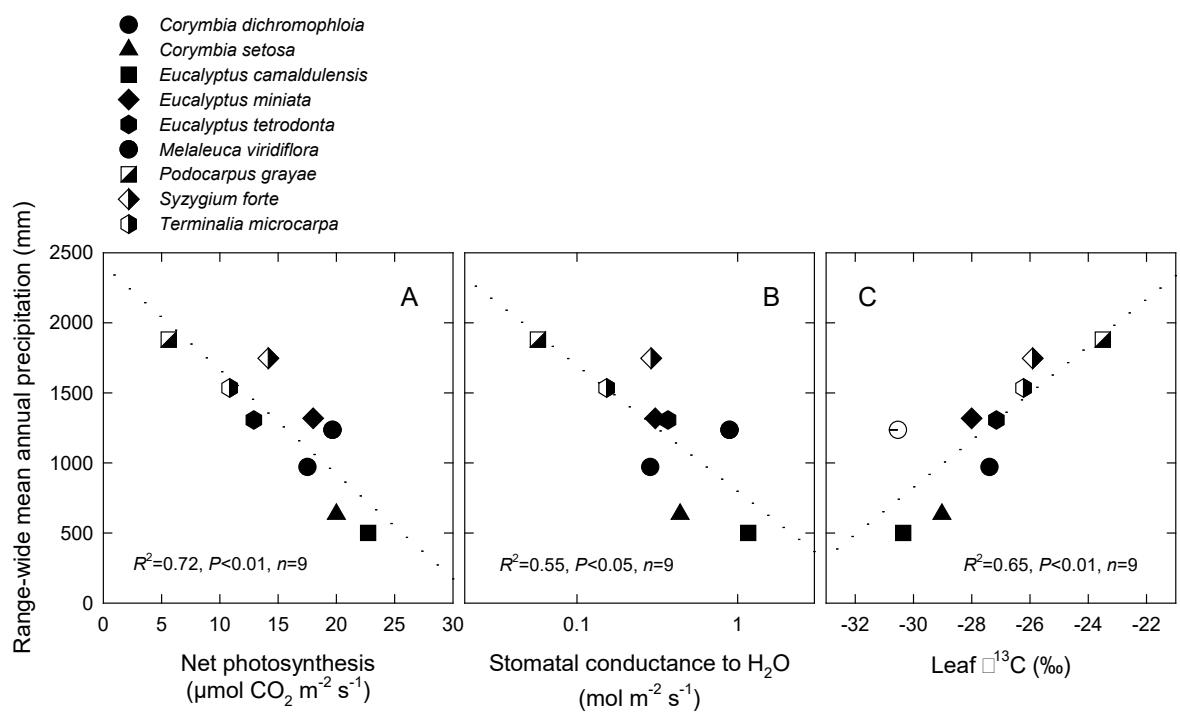
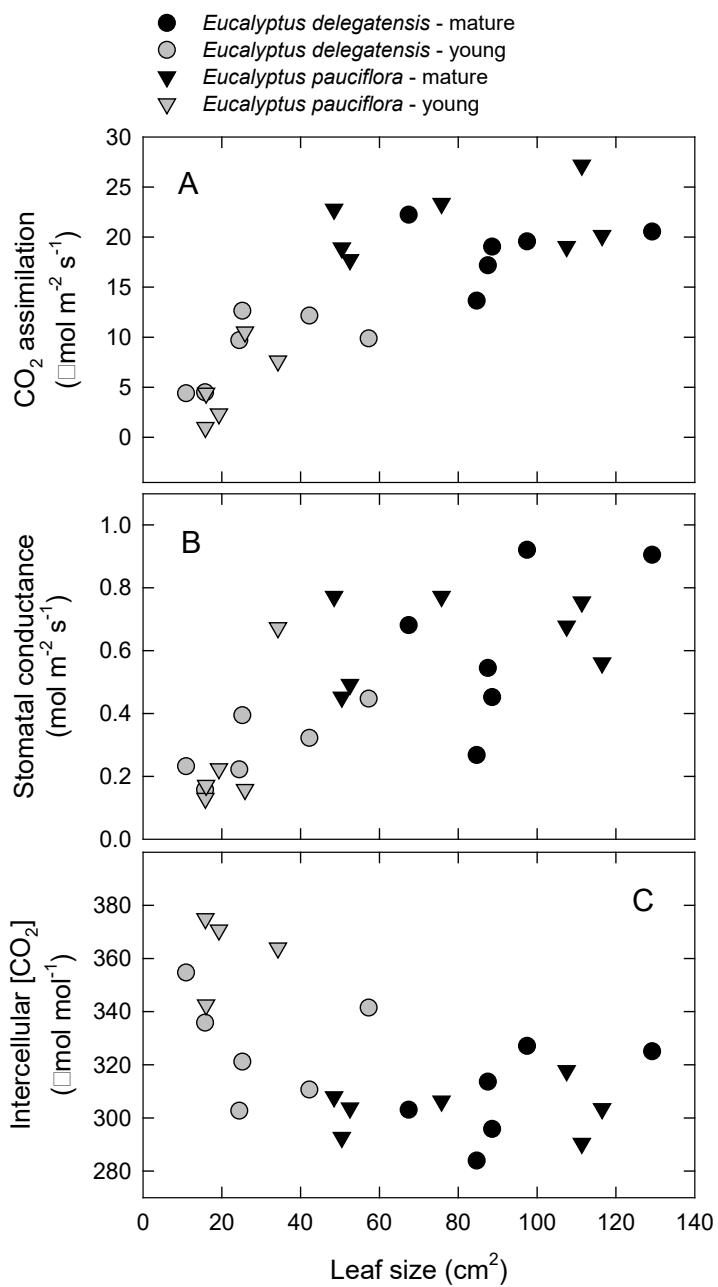
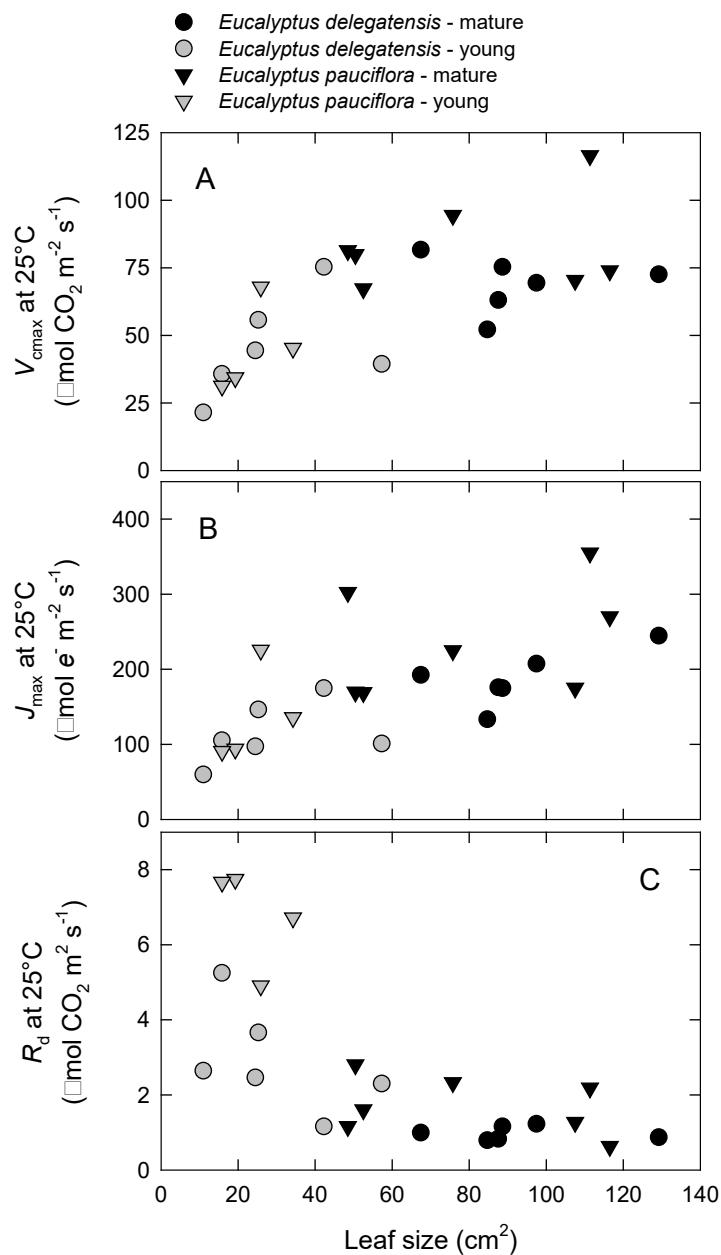


Fig. 6.

**Fig. 7.**

**Fig. 8.**

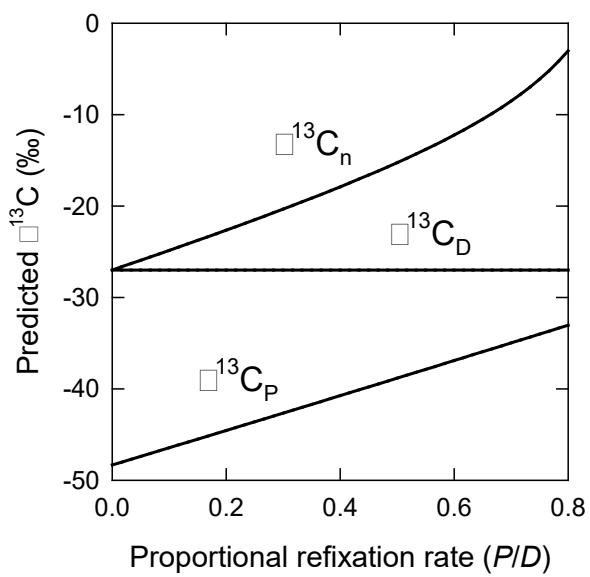


Fig. 9.

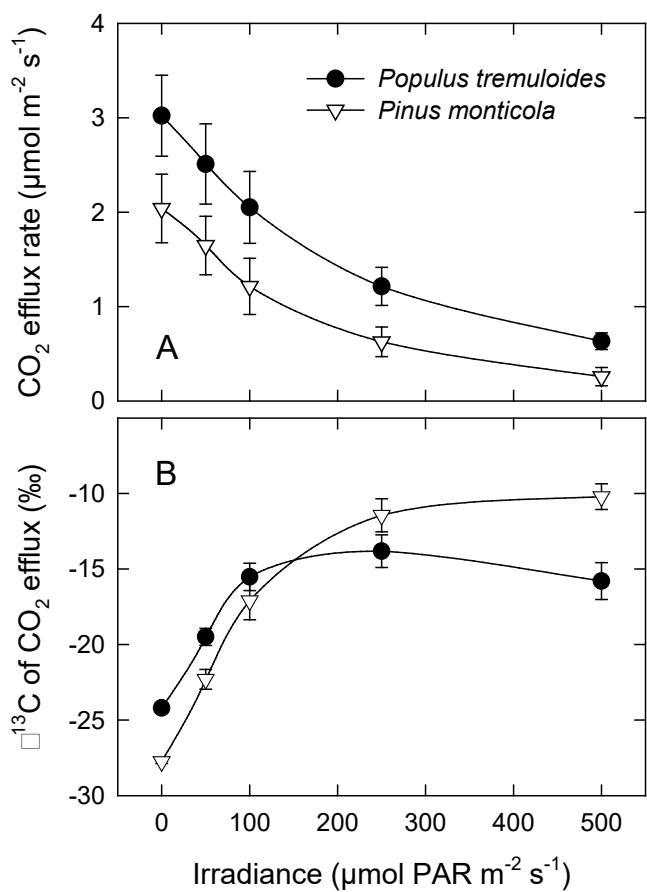


Fig. 10.