Phosphorus recycling in photorespiration maintains high photosynthetic capacity in woody species

DAVID S. ELLSWORTH1,*, KRISTINE Y. CROUS1, HANS LAMBERS2, JULIA COOKE1,3

1 Hawkesbury Institute for the Environment, Locked Bag 1797, University of Western Sydney, Penrith, NSW, 2751, Australia
2 School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley (Perth), WA 6009, Australia
3 Department of Biological Sciences, Faculty of Science, Macquarie University, North Ryde, NSW 2109, Australia

*Corresponding author (D.Ellsworth@uws.edu.au)

Received: 18th September 2014

Running Head: PHOSPHORUS RECYCLING IN PHOTORESPARATION
ABSTRACT

Leaf photosynthetic CO₂ responses can provide insight into how major nutrients such as phosphorus (P) constrain leaf CO₂-assimilation rates ($A_{net}$). However, triose-phosphate limitations are rarely employed in the classic photosynthesis model and it is uncertain to what extent these limitations occur in field situations. In contrast to predictions from biochemical theory of photosynthesis, we found consistent evidence in the field of lower $A_{net}$ in high [CO₂] and low [O₂] than at ambient [O₂]. For ten species of trees and shrubs across a range of soil P availability in Australia, none of them showed a positive response of $A_{net}$ at saturating [CO₂] (i.e. $A_{max}$) to 2 kPa O₂. Three species showed >20% reductions in $A_{max}$ in low [O₂], a phenomenon explained by orthophosphate (Pi) savings during photorespiration. These species, with largest photosynthetic capacity and Pi > 2 mmol P m⁻², rely the most on additional Pi made available from photorespiration rather than species growing in P-impoverished soils. The results suggest that rarely-used adjustments to a biochemical photosynthesis model are useful for predicting $A_{max}$, and give insight into the biochemical limitations of photosynthesis rates at a range of leaf P concentrations. Phosphate limitations to photosynthetic capacity are likely more common in the field than previously considered.
INTRODUCTION

The net CO₂-assimilation rate ($A_{net}$) of C₃ leaves in sunlight comprises three principal processes occurring at the same time: photosynthesis, photorespiration and mitochondrial respiration in the light. A major theoretical advance in the ability to understand and model leaf and canopy CO₂ exchange incorporating elements of all three processes was afforded by the biochemical model of photosynthesis of Farquhar et al. (1980), further described in von Caemmerer (2000). This model, originally formulated by Farquhar et al. (1980) (here termed the FvCB model) allows for inferences to be made about biochemical limitations to leaf and canopy functioning, overlain by environmental constraints (Long & Bernacchi 2003). The original FvCB model (Farquhar et al. 1980) and its subsequent modifications (Sharkey 1985; Sharkey et al. 2007; von Caemmerer 2000) successfully predicts photosynthesis under a very wide range of conditions, and has been applied to scales ranging from the chloroplast (von Caemmerer 2013) to forest canopies (Groenendijk et al. 2011) and biomes (Bonan et al. 2011; Kattge et al. 2009). A unique element of the FvCB model is the ability to estimate photorespiratory CO₂ efflux concurrent with photosynthetic CO₂ influx as component processes contributing to the net CO₂-assimilation rate of leaves (Busch 2013; Sage & Sharkey 1987). Both component processes need to be considered to predict net CO₂ assimilation as they occur at the same time, and together have implications for predicting the response of leaf CO₂ assimilation to rising atmospheric CO₂ concentrations, given that elevated [CO₂] both stimulates photosynthesis and suppresses photorespiration (Sharkey 1988; von Caemmerer 2000).

The FvCB biochemical model of photosynthesis has provided a useful context for interpreting many mechanistic aspects of plant function, including how the availability of major nutrients to plant canopies can restrict photosynthetic capacity and net primary productivity (Kattge et al. 2009). Analyses of nitrogen (N) limitations to photosynthetic capacity have been based on the fact that a major fraction of leaf N is allocated to the Rubisco enzyme (Evans 1989). The large proportion of leaf N invested in Rubisco and related photosynthetic proteins means that two major parameters of the FvCB model, the maximum carboxylation rate ($V_{cmax}$) and the capacity for electron transport to support RuBP regeneration ($J_{max}$), tend to scale linearly with leaf [N] in herbaceous crop species (Archontoulis et al. 2012; Evans 1989) and in woody species (Ellsworth et al. 2004; Rogers 2014). Such relationships are now used in a number of
ecosystem and global-scale models to assess ecosystem productivity of N-limited
However, P limitation of plant productivity is also widespread, with up to one-third of
the world’s soil orders demonstrating low P availability (Yang & Post 2011). In contrast
to N, it is less clear how low leaf P concentration in P-limited systems may affect
photosynthetic biochemistry. There are suggestions of both direct and indirect roles of
P regulating $A_{\text{net}}$ (Domingues et al. 2010; Pieters et al. 2001; Thomas et al. 2006). Hence,
a mechanistic representation of P limitations to leaf CO$_2$ assimilation is rarely
implemented in either leaf-to-canopy (Bernacchi et al. 2013; Long & Bernacchi 2003;
Manter & Kerrigan 2004) or large-scale models (Wang et al. 2010), despite the
importance of P as a major limiting element across tropical, subtropical and some
temperate ecosystems (Aerts & Chapin 2000; Lambers et al. 2010; Vitousek et al. 2010).

Low P supply from soils can affect bulk leaf P concentration and decrease leaf
orthophosphate (P$_1$) pools as well as reduce leaf net CO$_2$-assimilation rate and other
components of photosynthetic biochemistry (Hammond & White 2011; Veneklaas et al.
2012). Since P-containing molecules such as ATP, NADPH, and sugar-phosphates
including ribulose-1,5-bisphosphate (RuBP) have key roles in the Calvin-Benson cycle,
lack of sufficient P and P$_1$ would be expected to limit the maximum light- and CO$_2$-
saturated $A_{\text{net}}$ ($A_{\text{max}}$) that can be achieved in leaves (Brooks 1986; Loustau et al. 1999).
Such a decrease in the concentration of these metabolites upon P starvation is typical
for plants that are not adapted to P-impoverished soils. Conversely, Proteaceae from
severely P-impoverished soils in Australia do not operate at lower leaf P metabolite
concentrations at very low soil P availability, but rather replace phospholipids by
galactolipids and sulfolipids (Lambers et al. 2012) and operate at very low levels of
ribosomal RNA and proteins (Sulpice et al. 2014). Still, P-limitation might be manifest in
limiting RuBP regeneration as the underlying control over $A_{\text{max}}$ in leaves (Campbell &
Sage 2006; Jacob & Lawlor 1993). Whilst evidence for RuBP regeneration limitation by
low P$_1$ exists in laboratory studies (Jacob & Lawlor 1993), the mechanism by which low
leaf [P] decreases photosynthetic capacity is not well defined and field evidence of such
limitations is still lacking.

One approach for quantifying P-limitations to the biochemistry of net CO$_2$
assimilation is by estimating triose-P limitations following theory proposed by Sharkey
(1985). The basis of this theory is that RuBP regeneration is adenylate-limited, and a
release from this limitation is achieved by the release of Pi associated with precursors for sucrose synthesis in the cytosol. Exchange of each released Pi for triose-P produced in the chloroplast allows continued triose-P export to the cytosol (Paul & Foyer 2001; Stitt et al. 2010; see A in Fig. 1). An alternative hypothesis is that triose-P limitation is related to how 'closed' the photorespiratory cycle is with regard to the return of glycerate to the chloroplast via photorespiratory glycolate metabolism in the peroxisomes and mitochondria (Harley & Sharkey 1991; Fig. 1, highlighted as B). Short-term low-photorespiratory conditions using low O₂ partial pressure in air (pO₂) can be used as a probe of these biochemical limitation mechanisms to Aₙₑ𝐭. However, whilst triose-P limitations are often mentioned in publications describing the FBvC photosynthesis model, they are rarely parameterised (Bernacchi et al. 2013; Manter & Kerrigan 2004), except in very few studies where plants are grown at very low P supply (Bown et al. 2009; Domingues et al. 2010; Loustau et al. 1999).

To investigate P limitations to photosynthetic capacity in the field, we sought to determine if these limitations have a role in regulating the biochemical processes underlying leaf Aₙₑᵗ in the field. We specifically asked i) if the standard two-limitation version of the FvCB model (Farquhar et al. 1980; Farquhar et al. 2001) is adequate to characterise the major parameters controlling photosynthetic capacity for species growing at a range of leaf P and Pi concentrations, ii) is there evidence of triose-P limitations to Aₙₑᵗ using non-photorespiratory gas exchange analysis, and iii) are triose-P-utilisation limitations to Aₙₑᵗ associated with concentrations of bulk leaf P or Pi? Our null hypothesis was that leaf photosynthetic capacity at both normal (ambient pO₂ of 21 kPa) and low pO₂ could be described adequately by the two-limitation version of the FvCB model. In this study we used the tool of providing nearly non-photorespiratory conditions by measurements under low pO₂ to gain insight into the processes regulating leaf Aₙₑᵗ. This was done for Australian sclerophyll plants at a range of leaf P levels in both eastern and south-western Australia, including locations characterised by some of the lowest soil P availabilities on earth (Lambers et al. 2010) as well as sites with moderate P availability. In so doing, we sought to resolve whether plants with low leaf P were more likely to show triose-P limitations than those at higher leaf P levels, an idea that is occasionally cited (see Domingues et al. 2010; Loustau et al. 1999). We chose a set of native species that included species of Eucalyptus and Acacia and species in the
Proteaceae as three groups dominating the Australian continent, and *Liquidambar styraciflua* L. which is not native to Australia or similarly low-P soils.

**METHODS AND THEORIES**

*Theory from the Farquhar et al. (1980) photosynthesis model*

Analysis of the instantaneous response of leaf net photosynthesis to brief changes in the CO₂ concentration surrounding leaves underpins the FBvC model parameterisation (Long & Bernacchi 2003; von Caemmerer 2000). According to the standard FvCB model based on the stoichiometry of carbon in photosynthesis and photorespiration (Farquhar et al. 1980; von Caemmerer 2000), the net CO₂-assimilation rate of a leaf (*A*ₙₑₙ) can be expressed as

\[
A_{\text{net}} = v_c - 0.5v_o - R_d = v_c \left(1 - \frac{\Gamma^*}{C_i}\right) - R_d
\]

with *v*ₙₙ denoting the carboxylation and oxygenation rates of the Rubisco enzyme, *R*ₜₜ representing the rate of mitochondrial respiration in the light, *Γ* representing the CO₂ concentration at which the photorespiratory efflux of CO₂ equals the rate of photosynthetic CO₂ uptake, and *C*ᵢ indicating the internal CO₂ concentration in the substomatal cavity. As there is also a liquid-phase resistance between the intercellular surfaces and the sites of carboxylation in the thylakoids, this equation is best expressed using *C*ₜₜ, the chloroplastic CO₂ concentration, rather than *C*ᵢ thus incorporating mesophyll conductance to CO₂ transfer in the liquid phase (Pons et al. 2009). Thus, carboxylation rate and hence *A*ₙₑₙ is limited by one of two rates, *W*ₙₜ and *W*ₙₜ (Farquhar et al. 1980), later revised to include a third rate-limiting process *W*ₙₜ (Sharkey 1985):

\[
v_c = \min\left\{W_c, W_j, W_t\right\}
\]

*W*ₙₜ is the carboxylation-limited rate of net CO₂ assimilation when chloroplastic RuBP is saturating, *W*ₙₜ is the energy transduction for ATP synthesis leading to the subsequent regeneration of ribulose 1,5-bisphosphate (RuBP) in the photosynthetic carbon-reduction cycle, and *W*ₙₜ is the net CO₂-assimilation rate when triose-P pools tie up the available orthophosphate (Pᵣ) for synthesising ATP needed in the photosynthetic carbon reduction or Calvin-Benson cycle (Bernacchi et al. 2013; Sharkey et al. 2007).

When Rubisco activity limits photosynthetic CO₂ assimilation (*W*ₙₜ), *A*ₙₑₙ is given by
\[ A_{\text{net}} = \frac{V_{\text{cmax}}}{4} \frac{C_c - \Gamma^*}{(C_c + K') - R_d} \]  

(3)

where the half-saturation constant \( K' = k_c \left( 1 + \frac{O_i}{k_o} \right) \). Here \( V_{\text{cmax}} \) is the maximum catalytic activity of Rubisco with saturating RuBP, \( C_c \) and \( O_i \) are the chloroplastic \( CO_2 \) and intercellular \( O_2 \) gas partial pressures, respectively, and \( k_c \) and \( k_o \) are the Michaelis-Menten coefficients of Rubisco for \( CO_2 \) and \( O_2 \) (see Bernacchi et al. 2013; Sharkey et al. 2007). The photosynthetic \( CO_2 \)-compensation point (\( \Gamma^* \)) is the \( CO_2 \) concentration at which the photorespiratory efflux of \( CO_2 \) equals the rate of photosynthetic \( CO_2 \) assimilation. Given that the Rubisco enzyme is characterised by relatively conservative kinetic properties among different lineages of higher \( C_3 \) plant species, \( k_c \), \( k_o \) and \( \Gamma^* \) can be assumed as relatively invariant among species (Bernacchi et al. 2001; but see Galmés et al. 2005; Walker et al. 2013). In the classic version of the FvCB model, when \( C_c \) is close to saturation for photosynthesis such that RuBP regeneration limits photosynthesis (\( W_l \) is limiting), \( A_{\text{net}} \) is given by

\[ A_{\text{net}} = J_{\text{max}} \frac{C_c - \Gamma^*}{(4C_c + 8\Gamma^*)} - R_d \]  

(4)

where \( J_{\text{max}} \) is the maximum rate of electron transport at saturating quantum flux density to provide energy for RuBP regeneration in the PCR cycle. Most frequently, the parameters \( V_{\text{cmax}} \) and \( J_{\text{max}} \) are investigated as the major components of the photosynthesis model (Cernusak et al. 2011; Kattge et al. 2009; Rogers 2014; Walker et al. 2014) assuming two major biochemical limitations to \( A_{\text{net}} \). However, as originally stated, the FvCB photosynthesis model has no explicit dependence of \( J_{\text{max}} \) on \( O_2 \) partial pressure except \( \Gamma^* \) in Eqn 4. The \( \Gamma^* \) term is a function of the in vivo substrate specificity factor for the Rubisco enzyme (\( S_{c/o} \)), given as:

\[ \Gamma^* = \frac{0.5 \cdot O}{S_{c/o}} \]  

(5)

Where \( S_{c/o} \) is here considered \( \approx 92 \text{ mol mol}^{-1} \) at 25°C, within the range reported for \( C_3 \) woody species (Galmés et al. 2005). The original version of the FvCB photosynthesis model produces predictions of the \( A_{\text{net}} - C_c \) response at normal air \( pO_2 \) (21 kPa, hereafter referred to as normal \( pO_2 \)) and low-photorespiratory \( pO_2 \) that are illustrated in Fig. 2 (see also von Caemmerer 2000).
FvCB photosynthesis model with triose-phosphate limitation included

Two modifications of the original FvCB model were subsequently proposed to account for the behaviour of $A_{\text{net}}$ measured at high CO$_2$ partial pressures and with suppression of photorespiration at experimentally reduced O$_2$ partial pressures. These changes to the model accounted for two physiological states that have been observed both at high CO$_2$ partial pressures: 

1) O$_2$ insensitivity of $A_{\text{net}}$ at high pCO$_2$, and 

2) reverse O$_2$ sensitivity of $A_{\text{net}}$. In the first version, synthesis of sucrose from triose-phosphates was thought to make a contribution to Pi recycling for photophosphorylation since the triose-P transporter exchanges triose-P for Pi. For the situation when the rate at which triose phosphates are utilised ($T_p$) in the synthesis of carbohydrates limits $A_{\text{net}}$ ($W_t$ in Eqn 2), Sharkey (1985) proposed that

$$A_{\text{net}} = 3 \cdot T_p - R_d$$  \hspace{1cm} (6)

As there is no term dependent on pO$_2$ in Eqn 6, there is no explicit sensitivity to low pO$_2$ in this variant of the model. It was found that this model version might not always account for leaf gas exchange behaviour in low pO$_2$ (Harley & Sharkey 1991; Sage & Sharkey 1987), promoting an updated version of the model formulation.

In this updated version, Harley & Sharkey (1991) further proposed consideration of the pO$_2$ sensitivity of light- and CO$_2$-saturated net CO$_2$-assimilation capacity ($A_{\text{max}}$) through an 'open' photorespiratory C cycle. This version of the FvCB model has a pO$_2$ sensitivity that originates indirectly from ATP consumed with metabolism of the photorespiratory product, glycolate, in the chloroplast ($\alpha_g$) (Fig. 1, B) as given by

$$A_p = \frac{(C_c - \Gamma') \cdot 3 T_p}{C_c - (1 + 3 \cdot \alpha_g) \cdot \Gamma'} - R_d$$  \hspace{1cm} (7)

The parameter $\alpha_g$ is multiplied by three to reflect the stoichiometry of P$_i$ consumption in oxygenation (von Caemmerer 2000; note the correct version of the equation here), and varies as a fraction between 0 and 1 depending on whether all glycolate returns to the chloroplast (a 'closed' photorespiratory cycle where C is maximally conserved, in which case Eqn 7 simplifies to Eqn 6), the return is partial, or glycolate is entirely diverted to amino acid synthesis leaving none to return (Harley & Sharkey 1991).

More than 20 years after it was proposed, this third term of the model (Eqns 6 and 7) is rarely considered in photosynthesis model fits to data (von Caemmerer 2013) and most often ignored (Kattge et al. 2009; Manter & Kerrigan 2004; Walker et al.)
This is due in part to a lack of appropriate measurements (Long & Bernacchi 2003; von Caemmerer 2000) and because the evidence supporting its importance in leaves with low P concentration has been equivocal (Domingues et al. 2010). Moreover, $T_p$ has almost never been parameterised in field situations, so it remains unclear if this term needs to be considered in modelling limitations to photosynthetic CO$_2$ assimilation (Bernacchi et al. 2013). If $T_p$ can largely be ignored, we expect a stimulation of $A_{net}$ by low pO$_2$ in all parts of the CO$_2$-response curve as per Figure 2. Our field measurements of plants at high and low leaf P status aimed to understand if the mechanistic hypotheses of triose-P limitations to photosynthesis portrayed in Eqns 6 and 7 are consistent with field data, and if these revisions can reflect the role of P availability for regulating $A_{net}$. If there is an association between plant P status and $T_p$, then incorporation of this parameter into models may improve the predictability of $A_{net}$, especially where rising atmospheric CO$_2$ concentration and low soil P availability are concerned.

Research sites and plant material

The research was conducted on trees and shrubs growing at five different sites in eastern and south-western Australia (Table 1), with different soil substrates and parent materials resulting in different leaf P content in their characteristic species. Sites were chosen based on known aspects of their mineralogy and previous studies on leaf nutrients (e.g., Lambers et al. 2012) so that they would provide a range in leaf total P and P$_i$ fraction and thus presumably represent a range in P$_i$ limitations to $A_{net}$. Four of the five sites were infertile and low in P availability, with the fifth site on a richer soil. The Davies Park site is located at 390 m above sea level (a.s.l.) in the Blue Mountains in eastern Australia on thin soils overlaying Hawkesbury sandstone, a Triassic sedimentary quartzose sandstone formed over 200 Mya. The soils derived from the Hawkesbury sandstone in the Blue Mountains are shallow (5-20 cm depth) and very infertile with low P availability. The Hawkesbury Forest Experiment and adjacent Hawkesbury campus and EucFACE sites are all located at 30 m a.s.l. within 1 km of one another on Clarendon loamy sand, a deep, alluvial soil formed in the late Pleistocene by meanders of the Hawkesbury river around 1.5 Mya. The soil is a low-fertility loamy sand, with soil surface total P concentrations of 60 mg kg$^{-1}$ soil in the upper 15 cm (Ellsworth et al., unpubl. data), but a large fraction of this P is sorbed onto
aluminosilicates and ferro-manganese silicates (Holford 1997). One of the plantations at this site (**Liquidambar styraciflua** L.) was horticulturally managed and had periodically-amended soil P. The Lesueur National Park site is described in detail in (Lambers et al. 2012). This site is located near Jurien Bay, WA and occurs at 80 m a.s.l. on shallow colluvial sand and lateritic gravel over weathered sandstone from the late Jurassic Yarragadee Formation (150-185 million years old; Griffin & Burbidge 1990). The sandy soil at this site is extremely low in P, with a total P of 9.5 mg kg\(^{-1}\) soil in the upper 30 cm (Lambers et al. 2012). The fifth site, Illawarra Fly in Robertson NSW, is a fertile site on young soils. This site occurs at 710 m a.s.l. elevation on soils of the Illawarra escarpment that are brown clay loams underlain by Paleocene/Pliocene basalt. These basaltic soils in the area are relatively fertile with total P of 1010 mg kg\(^{-1}\) soil and frequently managed for farming, though this particular site was in a never-farmed parcel of mature remnant wet sclerophyll forest. Since sites differed in elevation, amounts of gases such as CO\(_2\) and O\(_2\) are reported as partial pressures (e.g., pO\(_2\)) rather than mole fractions.

Whilst the focus was on measuring species of **Eucalyptus** as native dominants in the study regions, non-Myrtaceous species were also included (**Banksia** spp. and **Persoonia levis**, all Proteaceae, and **Acacia oblongifolia**). An exotic deciduous plantation tree, **Liquidambar styraciflua**, was also included in the study so that inferences would not be strictly limited to native Australian sclerophyll species, which are considered to be well-adapted to low soil P (Beadle 1966).

**CO\(_2\)-exchange measurements**

In this study, photosynthetic CO\(_2\)-response curves (**A\(_{net}\) - C\(_i\) response curves**) were made **in situ** on ten species of trees and shrubs at five sites in Australia (Table 1) using a portable photosynthesis system (LiCor 6400XT, Licor Inc., Nebraska USA) with 6 cm\(^2\) chamber. All measurements were made on attached, intact leaves at the top of the crown or the outer shell of the crown when open-grown which meant accessing leaves from 1 m up to 25 m high (Table 1). For tall species, access to the upper parts of the tree crowns was achieved by three different means: an articulated boom lift (Snorkel MHP13/35 Trailer Mounted Lift, Snorkel Ltd., Meadowbrook, Qld, Australia) used at the Hawkesbury site in Richmond NSW, a set of 36 m tall construction cranes (Jaso crane J-4010, Jaso S.L., Idiazabal, Spain) at the nearby EucFACE site in Richmond NSW, and a
custom-built steel-alloy canopy walkway going up from ground level to 30 m height
('Illawarra fly') at Robertson, NSW. Canopy access was not necessary at the Lesueur
National Park site or at Davies Park, as trees and shrubs were open-grown in each of the
sclerophyll woodlands, and unshaded leaves at the outside of the crown could be
readily measured.

We made field measurements of the instantaneous response of leaf net CO₂
assimilation to changes in the external CO₂ concentration according to Ellsworth et al.
(2004), using standard coefficients recommended in Sharkey et al. (2007) when fitting
the FvCB model (see below). \( A_{\text{net}}-C_{\text{c}} \) response measurements on all species were made
during the growing season in summer and autumn at seasonal temperatures and during
periods of recent rainfall to reduce complications due to drought. Previous–year’s
leaves were measured rather than newly-emerged leaves to ensure that leaves were
operating at their full photosynthetic capacity (see Denton et al. 2007; Lambers et al.
2012). The \( A_{\text{net}} \) measurements were made in morning hours on sunny days so as to
avoid stomatal closure and mid-day depression of \( A_{\text{net}} \).

The \( A_{\text{net}}-C_{\text{c}} \) response curves were started by maintaining the CO₂ concentration
\((C_a)\) in the gas exchange chamber at ambient CO₂ partial pressure (~38-39 Pa in this
study) until gas-exchange rates were stable, then recording measurements. Steps for the
curves were generated by decreasing \( C_a \) to near the compensation point (5 Pa), and then
increasing \( C_a \) stepwise across 8-9 steps (Ellsworth et al. 2012) at a constant
photosynthetic photon flux density of 1800 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), 50-70% relative humidity, and
a controlled leaf temperature (between 26 and 28°C, depending on species). The mean
leaf-air vapour pressure deficit of the measurements was 1.5 ± 0.1 kPa. At each \( C_a \) step,
we recorded \( A_{\text{net}}, g_s, C_i \) and associated variables when stability was reached. Upon
completion of measurements, leaves were placed on ice or liquid nitrogen until ready
for further analysis. In the lab, leaf thickness was measured at five points on the leaf
lamina using digital callipers (Mitutoyo Corp, Kawasaki, Kanagawa, Japan).

In the process of these \( A_{\text{net}}-C_{\text{c}} \) response measurements, at four or five of the \( C_a \)
steps, we ensured that parallel measurements at ambient oxygen (21 kPa) and low-
photorespiratory oxygen (2 kPa) were made. Low pO₂ inside the gas exchange chamber
was generated by routing a low-O₂ tank gas (Air Liquide Australia Ltd., Melbourne,
Australia) to the leaf chamber, supplied at the same slight over-pressure as for ambient
air as described by Li-Cor (Li-Cor 2008) and with the excess flow to the Li-6400 pump
monitored with a rotameter. A Teflon T-valve was toggled between ambient air with 21 kPa pO2 and 2 kPa tank gas at the appropriate Cₐ steps (up to five Cₐ steps including at saturation). These steps were chosen in order to minimally define the initial rise to a maximum and the maximum asymptote for the Aₙet-Cₐ curve at low pO₂, given that the shape of these curves has long been known (Laing et al. 1974; von Caemmerer 2000). The flow excess was maintained around 0.3 L min⁻¹. Measurements of Aₙet in 2 kPa pO₂ were completely reversible as described in Laing et al. (1974) (see Supporting Information, Fig. S1).

Calculations of O₂ corrections and mesophyll conductance to CO₂

We used three corrections for changes in pO₂ in the carrier-gas in the LI-6400XT photosynthesis system that originated from the change in density due to different gas concentrations. The corrections employed were: i) increased air-flow rate through the CO₂-injector system due to reduced air viscosity with decreased pO₂, ii) band broadening of CO₂ infrared absorption (Burch et al. 1962) incorporated into the standard Li-6400 software, and iii) band broadening of water vapour infrared absorption (Bunce 2002).

Given theoretical issues raised by Gu & Sun (2014) concerning the dependence of mesophyll conductance to CO₂ (gₘ) on Cᵢ, we assumed a constant gₘ for different Cᵢ steps in the response-curve data. Mesophyll conductance was either measured or estimated for each species for calculations of Cᵢ. For three species amongst those in Table 1 ranging in Aₙet from highest and lowest, we measured instantaneous gₘ with online carbon-isotope discrimination using tunable diode laser absorption spectroscopy (TDLAS; Campbell Scientific TGA100A, Logan, UT, USA). Our gₘ calculations follow Tazoe et al. (2011) with further description in Crous et al. (2013). We then estimated mean gₘ of all the species using a relationship for gₘ as a function of gₛ from our measurements (gₘ = -0.04 + 1.34 * gₛ, r² = 0.54; Supporting Information Fig. S2). In a review of available data, gₘ usually scaled with gₛ especially amongst well-watered plants (Flexas et al. 2012). After incorporating gₘ, we derived biochemical model parameters using the Aₙet-Cₐ data.

Photosynthetic parameter fits were done in R (Team 2014) using kinetic coefficients in Sharkey et al. (2007) to standardise the fits across species, but using Γ* and its temperature dependence specifically measured for Eucalyptus (Crous et al.)
We fit $V_{cmax}, J_{max}$ and $T_p$ piece-wise using specified ranges of conditions where each parameter was judged to limit $A_{net}$ following guidelines in Sharkey et al. (2007) with the nonlinear solutions generated using the 'optim' package in R. $T_p$ was fit for $A_{net}$ when $C_c > 40$ Pa and pO$_2$ of 2 kPa. As a more robust fitting approach with fewer assumptions, we also pooled data for all leaves within a species and simultaneously solved for species-level $V_{cmax}, J_{max}$ and $T_p$ at both pO$_2$ levels using the 'nls' package in R. Across species, the two sets of solutions agreed well with one another, since slopes for each parameter were close to unity (slopes of 0.981, 0.966 and 0.840 for $V_{cmax}, J_{max}$ and $T_p$, respectively, estimated for piecewise compared with simultaneously-solved). $V_{omax}$, the maximum velocity of oxygenase activity, was fit to the data from both pO$_2$ levels for low $C_c$ where oxygenase activity of Rubisco is considered limiting, following equations in Farquhar et al. (1980).

**Leaf chemical analyses**

After measurements, leaves were immediately placed on ice and transported to the laboratory, where thickness and area were measured on a subsample, whilst the remainder was frozen and subsequently dried to a constant mass at 70°C. The leaf lamina dry mass per unit area ($M_a$) was calculated from the ratio of dry mass to fresh area. The dried sample was ground finely in a ball mill, and used for analyses of total N concentration, total P concentration, inorganic P (Pi) concentration, and starch and soluble sugar concentrations. Leaf N concentration was analysed by elemental analysis after combustion using a CHN elemental analyser (TruSpec micro, LECO Corp., St. Joseph, MI, USA; or FLASH EA 1112 Series CHN analyser, Thermo-Finnigan, Waltham, MA USA). Leaf total P concentrations were measured after digesting dried leaf tissue with concentrated sulfuric acid (H$_2$SO$_4$) and hydrogen peroxide (H$_2$O$_2$) in a microwave digester apparatus (Berghof speedwave four, Berghof Products GmbH, Eningen, Germany). The solutions containing total P or the Pi fraction were analysed colourimetrically at 880 nm (AQ2, SEAL Analytical, Ltd., Milwaukee, WI USA) after a standard molybdate reaction (Close & Beadle 2004). Analyses of N and P concentrations used international standards run blind alongside the samples, and are expressed as N and P content (mmol m$^{-2}$) in this manuscript due to differences in leaf thickness amongst the species (Table 1). Bulk leaf Pi was determined by extracting samples in 0.3 M TCA at 4°C before cold centrifuging at 9224 × g (10,000 rpm) for 5 min and collecting
The filtrate (Close & Beadle 2004). The P\textsubscript{i} concentrations in the samples were determined against standards made with KH\textsubscript{2}PO\textsubscript{4} in serial dilution.

RESULTS

The set of species used in this study ranged two-fold in their leaf thickness, and nearly ten-fold in their leaf P content (Table 1). \(A_{\text{net}}\) varied more than two-fold, between 10 and 26 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) among the species when measured at \(C_i\) between 27-28 Pa. As a stoichiometric index of P versus N limitation, six of the ten species studied had N:P ratios > 20, while \(E.\ fastigata\) and \(L.\ styraciflua\), both from moderately-fertile conditions, had N:P of 10-13 (see Supporting Information, Table S1).

Biochemical modelling from \(A_{\text{net}} - C_c\) response curves at both 21 and 2 kPa pO\textsubscript{2} using the classic FvCB model would suggest a slightly higher \(A_{\text{net}}\) asymptote at high \(C_c\) and low pO\textsubscript{2} (i.e. similar \(A_{\text{max}}\) at ambient and low pO\textsubscript{2}) due to the lower \(\Gamma^*\) as per Eqn 5 above (Fig. 2). Thus, a stimulation of \(A_{\text{net}}\) by low pO\textsubscript{2} was expected both in the carboxylation-limited region of the CO\textsubscript{2}-response curve and, though smaller, also in the RuBP-regeneration-limited region or where \(A_{\text{net}}\) is saturated with respect to \(C_a\).

Consistent with this, there was an average of 23\% stimulation in \(A_{\text{net}}\) at ambient CO\textsubscript{2} under low-photorespiratory conditions using 2 kPa pO\textsubscript{2} in the carboxylation-limited region of the \(A_{\text{net}} - C_c\) response curve (Fig. 3 and data not shown). However, in contrast to theoretical expectations, none of the ten species measured showed the expected small \(A_{\text{net}}\) stimulation in the RuBP-regeneration-limited region in 2 kPa pO\textsubscript{2} compared with \(A_{\text{net}}\) in normal pO\textsubscript{2}. Rather, species either showed similar \(A_{\text{max}}\) values as asymptotes to the \(A_{\text{net}} - C_c\) response in 2 kPa pO\textsubscript{2} compared with 21 kPa pO\textsubscript{2} (Fig. 3A,B), or a dramatic reverse response for the \(A_{\text{max}}\) in 2 versus 21 kPa pO\textsubscript{2} (Fig. 3C,D), with about a 20\% reduction in \(A_{\text{max}}\) at 2 kPa pO\textsubscript{2} compared with normal pO\textsubscript{2}. The cross-over between curves at normal and low pO\textsubscript{2} occurred at \(C_i\) values as low as 28 Pa, up to 40 Pa depending on species. In low pO\textsubscript{2} there was also a sharp transition between Rubisco-limited photosynthesis at low \(C_c\) and RuBP-regeneration and \(T_p\) limited photosynthesis compared to normal pO\textsubscript{2} (Fig. 3). For our study species, the difference in \(A_{\text{max}}\) between normal and low pO\textsubscript{2} was between 2 and 14 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) (average of 5.8 \(\mu\text{mol m}^{-2}\text{s}^{-1}\)), with low pO\textsubscript{2} values consistently lower. This was significantly different from zero for all species, even for \(B.\ attenuata\) (\(P=0.017\) in a one-tailed t-test) and \(P.\ levis\) (\(P = 0.01\)), both
of which had rather small mean differences in asymptotic $A_{\text{net}}$ between normal and low pO$_2$ of about 2 µmol m$^{-2}$ s$^{-1}$ (Fig. 3A,B). This result establishes that there was a reverse sensitivity of $A_{\text{net}}$ to the reduction in pO$_2$ at high $C_c$ across the range of species measured in the field. Given that this reverse sensitivity in low pO$_2$ conditions was significant in all species, we considered it valid to use the model of Harley & Sharkey (1991) to estimate the P limitation component of the biochemical model, rather than the simpler model of Sharkey (1985) that has been recommended to standardise model fitting.

Estimates of $V_{c,max}$ from independent gas-exchange measurements at either pO$_2$ level were similar (Fig. 4A). However, we could not recover the same $A_{\text{max}}$ in different pO$_2$ levels using the traditional two-parameter FvCB photosynthesis model (Fig. 4B). The $A_{\text{max}}$ estimated in low pO$_2$ was lower than expected based on $A_{\text{max}}$ in normal pO$_2$ (under the 1:1 line in Fig. 4B). The largest $A_{\text{max}}$ reductions in low pO$_2$ were in species with high $A_{\text{max}}$ at normal pO$_2$, such as *E. fastigata* and *L. styraciflua* (average of 8 and 12 µmol m$^{-2}$ s$^{-1}$ lower, respectively). This was further evidence that an additional parameter to the FvCB photosynthesis model was needed to fit photosynthesis to our field measurements. The difference in modelled $A_{\text{max}}$ using the traditional FvCB model to the $A_{\text{max}}$ predicted by the model revision proposed by Harley & Sharkey (1991) was largest for species with high $A_{\text{max}}$ in normal air (21 kPa pO$_2$; Fig. 4C). Fitting the $T_p$ parameter using Eqn 7 proposed by Harley & Sharkey (1991) to the data, we were able to recover the $A_{\text{max}}$ that we had measured in low pO$_2$ (Fig. 4D). Taken together, all species showed a reduced $A_{\text{max}}$ at low pO$_2$, with the largest reductions occurring in species with the highest $A_{\text{max}}$. These reductions were recovered once the $T_p$ parameter (Eqn 7) was employed in the model fits.

The difference in $A_{\text{max}}$ for the model without $T_p$ considered versus the model with $T_p$ considered was positively correlated with leaf P$_i$ content up to a threshold of about 2 mmol P$_i$ m$^{-2}$ ($r^2 = 0.4$, P < 0.0001), beyond which there was no apparent relationship (Fig. 5). There was a similar but weaker relationship ($r^2 = 0.2$) for total $T_p$ below a threshold of ~10 mmol P m$^{-2}$ (not shown). $T_p$ was itself only very weakly correlated with leaf P$_i$ content up to a threshold of 2 mmol P$_i$ m$^{-2}$ ($r^2 = 0.10$, P < 0.01; data not shown). Six species (*A. oblongifolia*, *B. attenuata*, *B. serrata*, *E. todtiana*, *E. tereticornis* and *P. levis*) all had leaf P$_i$ contents in the linear region, where the magnitude of suppression of CO$_2$-saturated photosynthesis by low pO$_2$ varied strongly with P$_i$. *E. fastigata* and *L. styraciflua* both had high leaf P$_i$ contents and high $A_{\text{max}}$ differences,
falling in the saturating region of Fig. 5. The amount of total leaf P present as P_i averaged 30 ± 2% (mean ± s.e.) among the species in our study. *Liquidambar styraciflua* had the highest free P_i in leaves, at 46 ± 4% of total leaf P concentration. *B. attenuata, B. serrata* and *P. levis* had the lowest total leaf P concentrations (around 0.35 mg P g^-1; Table S1), but similar P_i fractions as the species average above.

For the set of ten species across a range in soils and P supply levels, the individual photosynthetic model components *V*_cmax, *J*_max and *T*_p were all correlated with leaf chemical traits, though correlations with total leaf P_area were strongest (Table 2, Fig. 6). The three species from Davies Park in the Blue Mountains of NSW had the lowest leaf P_area closely followed by those from Lesueur National Park in Western Australia. The strongest relationship between the biochemical components of leaf photosynthetic capacity and leaf chemistry was between *J*_max and leaf P_area (*R^2* = 0.66, Fig. 6c). Bivariate relationships between photosynthetic model components and leaf N_area were not significant (*P* > 0.10, Table 2), nor was *A*_max associated with leaf N_area across the set of species. There were no significant relationships between any of these traits and *M_a*. *V*_omax was not significantly correlated with N_area and was only marginally significantly correlated with P_area (*P* = 0.052; Table 2 and Fig. 6b). *V*_omax fit to data at both measurement pO_2 levels was significantly correlated with *V*_cmax fit to measurements at both pO_2 (*P* = 0.0052; not shown) with a slope of 0.17 and a significant y-intercept.

**DISCUSSION**

Reductions in *A*_max during exposure to low pO_2 have been documented for over 50 years (Joliffe & Tregunna 1968), but have rarely been measured in the field. Despite suppression of photorespiration by low pO_2 at the current atmospheric C_a (Fig. 2), we have shown that *A*_net at high C_i and low pO_2 is reduced, rather than higher as would be expected from theory based on the biochemical regulation of photosynthesis (Farquhar *et al.* 1980; Laing *et al.* 1974; von Caemmerer & Farquhar 1981). All ten tree and shrub species studied at a range of Australian sites showed this response to varying degrees, at moderate summertime temperatures (Fig. 4). According to the Harley & Sharkey (1991) theoretical model, when leaves operate at near-saturating C_b, photorespiratory glycerate may not completely re-enter the PCR cycle, so that P_i released by phosphoglycolate phosphatase in the chloroplast, that would normally have been used by the
glycerate kinase reaction upon photorespiratory C return to the chloroplast, is instead available in the stroma for RuBP regeneration (Fig. 1, B). Under low-photorespiratory conditions, this additional source of P\textsubscript{i} becomes unavailable, resulting in slower RuBP regeneration and lower $A_{\text{max}}$ at low pO\textsubscript{2} than at normal pO\textsubscript{2}. Modelling using the equation for $T_p$ in Harley & Sharkey (1991) gives $A_{\text{max}}$ results that are broadly consistent with our data (Fig. 4). While limitations to photosynthesis by triose-P utilisation are considered to be uncommon and are often ignored in photosynthetic model-fitting, our field measurements under low-photorespiratory conditions show that $T_p$ can be limiting $A_{\text{max}}$ in a wide range of woody species.

An alternative hypothesis for $T_p$ limitations to $A_{\text{max}}$ suggests that excessive synthesis of triose-P to be exported from the chloroplast increases recycling of P\textsubscript{i} entering chloroplasts, with higher stromal P\textsubscript{i} leading to the accumulation of 3PGA and decreasing phosphoglucoisomerase activity and suppressing starch synthesis (Sharkey 1985; Stitt et al. 2010; Fig. 1, A). While simpler in concept and in formulation (Eqn 6), the $T_p$ limitation emerging from conservative C cycling back to the chloroplast cannot explain what we found here, because it describes pO\textsubscript{2}-insensitive photosynthesis, whilst we found a strong reverse sensitivity of $A_{\text{max}}$ to low pO\textsubscript{2} which is only predicted by the Harley & Sharkey (1991) model of $T_p$ limitation. Previous treatments using the Sharkey (1985) formulation did not conduct measurements at low pO\textsubscript{2} at a range of $C_a$ levels, and thus have not been able to distinguish between pO\textsubscript{2}-insensitive and reverse-sensitive photosynthesis.

On the basis of the Harley & Sharkey (1991) model, our data provide strong evidence that not only is photorespiration a source of amino acids through NH\textsubscript{3} release in glycine metabolism (Wingler et al. 2000), but also that glycolate diversion from re-entry into the chloroplast during photorespiration simultaneously frees stromal P\textsubscript{i} to permit enhanced photophosphorylation and RuBP regeneration, thus permitting high $A_{\text{max}}$. Measurements of this phenomenon on a much broader set of C\textsubscript{3} plant species is needed to understand the generality of this phenomenon, but the set of species we studied represents a range of phylogenies and includes species with different affinities for growing on low-P sites. All these species showed significant decreases in $A_{\text{max}}$ measured during transient non-photorespiratory conditions. The decreases in $A_{\text{max}}$ in low pO\textsubscript{2} for *L. styraciflua*, *E. fastigata* and *E. dunnii* were all greater than 5 µmol m\textsuperscript{-2} s\textsuperscript{-1} and as high as 12 µmol m\textsuperscript{-2} s\textsuperscript{-1} (in *E. fastigata*), and thus were much larger than those of
the order of 2 µmol m⁻² s⁻¹ shown for soybean in Harley & Sharkey (1991). Therefore, we suggest that this phenomenon may be common amongst a number of plant genera, and potentially across a significant geographic expanse. There is a need for broader consideration of this mechanism among species, as currently $T_p$ limitations to $A_{\text{max}}$ are ascribed to the parameter $J_{\text{max}}$ in a large number of studies (for example, Kattge et al. 2009; Manter & Kerrigan 2004; Walker et al. 2014). We also suggest that the mechanism proposed by Harley & Sharkey (1991) is more properly called phosphate limitation rather than triose-P limitation, since triose-P is not necessarily integral to the proposed mechanism (see Fig. 1, B). Nevertheless, we have retained the terminology of Harley & Sharkey (1991) in fitting Eqn 7, but suggest that $T_p$ can be more broadly considered as phosphate limitation to $A_{\text{net}}$

Internal recycling of Pi in cells is important for the balanced production of ATP and regeneration of RuBP as essential requirements for high CO₂-assimilation rates. While a source for Pi for photophosphorylation to regenerate RuBP as depicted in Fig. 1 (see B in Fig. 1) could be a valuable mechanism for sustaining $A_{\text{net}}$ at high $C_i$ in plants in conditions with limiting soil P, our measurements do not suggest this occurs at the extremely low P levels characterising both the Lesueur National Park and Davies Park sites. Among the ten woody species we measured including some on infertile sites with low soil P-availabilities, plants with low leaf P concentrations (total leaf P < 400 µg g⁻¹, for instance) also had slow rates of photorespiration and an apparent high fractional return of photorespiratory glycerate to the chloroplast, resulting in a relatively small inhibition of $A_{\text{max}}$ in low pO₂ and high $C_i$ (Fig. 3a,b). However, our findings are consistent with the previously-overlooked mechanism of glycerate sequestration during photorespiration may in fact be common in a number of woody species. This mechanism operates at high $C_i$ (but to $C_i$ as low as 28 Pa depending on species; Fig. 3) which means that it is relevant for a substantial fraction of canopy leaves maintained in shade where RuBP regeneration and triose-P supplies may limit $A_{\text{net}}$. It may also be relevant in elevated atmospheric CO₂ concentrations (Campbell & Sage 2006) with a role in increasing the degree of cellular P-deficiency with decreased photorespiration, expected as $C_a$ increases in the future. The mechanism hypothesised by Harley & Sharkey (1991) and supported by our data is not yet considered in physiologically-based models used to project plant CO₂ assimilation behaviour into the future (Wang et al. 2010). Our identification of this mechanism in the field opens an important new area
of research relevant to expected future conditions including elevated [CO₂], and further
field measurements of this sort are crucial to help resolve the range of ecological
contexts where Pᵢ regulation over Aₘₐₓ may be most important.

The hypothesised mechanism for net Pᵢ release in the chloroplast described by
Eqn 7 and shown in Fig. 1 requires glycolate exported from the chloroplast to be
sequestered, metabolised or exported from the cell, rather than being converted into
glycerate for re-entry into the chloroplast. What are the possible mechanisms for this C
"diversion" rather than conservation by chloroplast re-entry? Harley & Sharkey (1991)
cited ¹⁴C labelling evidence to suggest photorespiratory C export by the vascular system
to other parts of the plant (Wingler et al. 2000), and at least 12% of the amino acid
composition of phloem in Eucalyptus comprises serine and glycine (Merchant et al.
2010), demonstrating that this export is plausible. There are other plausible fates for
this C that may also be important (Reumann & Weber 2006). Glycolate and glyoxylate
products of photorespiration (Fig. 1; Wingler et al. 2000) can be oxidised by glycolate
oxidase in the peroxisome to form oxalic acid, which is stored in vacuoles or
metabolised to calcium oxalate crystals, common in a wide range of plants (Franceschi
& Nakata 2005) and documented for both Eucalyptus and Acacia (Brown et al. 2013).
Alternatively, oxalate might be metabolised again (Havrir 1984) and allow glycerate re-
entry into the chloroplast when the requirement for Pᵢ is less. Glycine participates in the
early steps of porphyrin synthesis in the mitochondria as part of chlorophyll assembly
(Beale 1978) as well as in the synthesis of glutathione, which is involved in stress
protection (Wingler et al. 2000). Whilst the ultimate fate of photorespiratory glycolate
may vary amongst different plant species, evidence of multiple mechanisms driving a
lack of C return to the chloroplast after photorespiratory metabolism provides support
for the sequestration of glycolate or its products after photorespiration, a key part of the

Some implications of the incomplete photorespiratory glycerate re-entry and
subsequent extra available Pᵢ (see B in Fig. 1) are that species with low photorespiration
such as Proteaceae (B. attenuata, B. serrata, P. levis; see Supporting Information, Table
S1) would have a low flux rate of chloroplastic Pᵢ made available by this mechanism
compared with species with higher photorespiration. Some Proteaceae species also
allocate more P to their mesophyll cells rather than their epidermal cells (Lambers et al.
2015), compared with other dicots that have relatively high P levels in epidermal cells
Indeed, five species in our study (B. attenuata, B. serrata, E. todtiana, E. tereticornis and P. levis) all have a leaf Pi content where the magnitude of suppression of $A_{\text{max}}$ by low pO$_2$ varies strongly with Pi (P$_i$ < 2 mmol m$^{-2}$, Fig. 5). This suggests that at low leaf P contents, these species must rely on existing stromal Pi pools, rather than those saved by the lack of glycerate re-entry during photorespiration at high $C_i$. Lambers et al. (2012) showed that photosynthetic cells of mature Banksia leaves extensively replace phospholipids by lipids that do not contain P, i.e. galactolipids and sulfolipids, which reduces their demand for Pi for lipid synthesis and hence increases Pi available for participation in photosynthetic carbon metabolism. Moreover, these species also operate at very low levels of ribosomal RNA (Sulpice et al. 2014), which is a major fraction of leaf P (Veneklaas et al. 2012). Mechanisms for internal P conservation such as these may obviate the need for P contributed from the lack of photorespiratory glycerate re-entry mechanism in P-impoverished ecosystems.

Amongst the species we measured, L. styraciflua, E. saligna and E. fastigata showed the fastest RuBP regeneration rates (i.e. high $J_{\text{max}}$), the highest leaf Pi contents, and also showed the largest decreases in $A_{\text{max}}$ at low pO$_2$. Why do these fast-metabolism plants show an apparently large $T_p$ limitation of $A_{\text{max}}$, when they also have high P$_i$? The bulk leaf Pi measurements are indicative but inconclusive as only the chloroplastic Pi fraction is relevant to the hypothesised mechanism. The reverse sensitivity to pO$_2$ at high $C_i$ can occur in species with high photosynthetic activity where the requirement for P$_i$ for ATP synthesis is balanced against the need to maintain low P$_i$ for starch and sucrose synthesis (Sharkey & Vassey 1989). With rapid triose-P production in photosynthesis exceeding the capacity to use triose-P in such species, low pO$_2$ would decrease photorespiration and reduce P$_i$ from dephosphorylation of phosphoglycolate as well as greatly reduce carbon leaving the Calvin-Benson cycle by serine and/or glycine export. It is not clear yet if these two mechanisms are mutually exclusive, but they are consistent with the data in Figure 5.

There is an additional possibility that the $T_p$ limitation of species with high $A_{\text{max}}$ may occur due to the high P requirements in such species for ribosomal RNA (rRNA), which is needed to support rapid rates of protein synthesis and growth (Matzek & Vitousek 2009; Niklas et al. 2005). The P contained in RNA, particularly rRNA, is a significant fraction of the total non-vacuolar P in leaves (Raven 2012). Hence, if high P costs of rRNA for protein turnover are necessary to support rapid photosynthesis in
mature leaves as suggested by Veneklaas et al. (2012) and others (Matzek & Vitousek 2009), then this protein synthesis may be achieved from two concurrent photorespiratory products. Amino acids are generated from photorespiratory ammonia \((\text{NH}_3)\) release in glycine decarboxylation (Wingler et al. 2000), and the lack of photorespiratory glycerate re-entering the chloroplast frees chloroplastic ATP for enhancing RuBP regeneration and increasing \(A_{\text{max}}\), while also freeing \(P_i\) for P-rich ribosomes to generate proteins in the stroma (Fig. 1). How much glycine or serine is directed away from the photorespiratory pathway and chloroplast re-entry and rather used for protein biosynthesis is unclear. However, the release of N from photorespiration may be as large as from nitrate reduction (Wingler et al. 2000), and hence the release of ATP for RuBP regeneration may also be large (Fig. 3B,D). There is supporting evidence as one of the slow-growing species in our study, Banksia attenuata, with low photorespiration (Fig. 3C) was recently demonstrated to have low rRNA concentrations in mature leaves at the Lesueur site (Sulpice et al. 2014). We believe that the hypothesis of both N and P release in photorespiration establishes new significance for what has previously been considered to be a “wasteful” process (Busch 2013; Ogren 1984; Wingler et al. 2000), but it also requires further investigation. There has been considerable interest in the role of P in limiting photosynthesis and whether P can directly influence leaf photosynthetic capacity (Reich et al. 2009). The relationships between biochemical parameters underlying photosynthetic capacity and leaf P content in Fig. 6 across a range in P supply argue for a stronger and more direct role for P in regulating \(A_{\text{max}}\) in this set of species than for N. Our data have provided evidence of a direct role of P in leaf photosynthetic capacity that is likely not currently realised much since current \(C_i\) is often lower than ~ 28 Pa, but could become important with rising \(C_a\). Though the nature and biochemistry of \(T_p\) limitations to \(A_{\text{max}}\) are not fully elucidated, when leaf P concentrations are moderate it appears that the extra photorespiratory source of \(P_i\) derived from a net C export from the chloroplast can help sustain rapid rates of \(A_{\text{max}}\).

**CONCLUSIONS**

While triose-P utilisation \((T_p)\) limitations to photosynthesis are considered to be uncommon and are often ignored in photosynthetic model-fitting, we have shown that \(T_p\) can be limiting in a wide range of species from across soil P gradients in the field,
with short-term high $C_i$. Hence, what are actually $T_p$ limitations judged from
measurements at low pO$_2$, are currently attributed to $J_{\text{max}}$ limitations in the two-phase
FvCB model that is frequently fit to measurements at normal pO$_2$. The results suggest
that pO$_2$ manipulation in measurements of $A_{\text{net}}$ can lead to insights into P$_i$ limitations to
$A_{\text{net}}$ both in the present and in a future with elevated atmospheric CO$_2$ leading to
reduced photorespiration. Intracellular P$_i$ release from photorespiration is inhibited at
low pO$_2$, reducing $A_{\text{max}}$ in all species, but to varying extent depending on their available
P$_i$ pools. Species with largest photosynthetic capacity and highest P$_i$ contents apparently
rely most on ATP made available from photorespiration. Hence, this mechanism is most
important in fast-growing species at moderate P levels and with high photosynthetic
capacity, rather than species growing in P-impoverished soils. The mechanism we have
identified should be further explored, but is expected to contribute to the economy of P
for plants in tropical or subtropical rainforest vegetation as well as in Mediterranean
vegetation on soils with moderate to low P availability, but not in those species that
deploy alternative mechanisms to function at very low leaf [P]. Phosphate limitations to
photosynthetic capacity are likely more common in the field than previously thought,
and likely contribute to improving the predictability of CO$_2$-assimilation rates in such
instances. It is recommended that those interested in modelling how biochemistry
regulates $A_{\text{net}}$ should consider the role of photorespiration and employ three limitations
in the biochemical model of photosynthesis with the possibility of glycerate not re-
entering the Calvin-Benson cycle.

**ACKNOWLEDGEMENTS**

This research was supported by the Australian Research Council (ARC Discovery grant
DP110105102 to DSE and DP110101120 to HL). Data from this study is stored at
Research Data Australia (doi: tobedetermined). We are grateful to O.K. Atkin (ANU), K.
Bloomfield (Leeds University), and P. Milham (NSW government) for advice concerning
the chemical analysis of P$_i$ and total P in leaves. S. Wohl expertly operated the cranes for
canopy access at EucFACE. We thank the Blue Mountains City Council (BMCC) and
particularly Michael Hensen, for permission to sample at Davies Park in the Blue
Mountains, NSW, the staff at the Illawarra Fly for providing access to the canopy
walkway in Robertson, NSW, and the Western Australia Department of Parks and
Wildlife for sampling access to Lesueur National Park, WA. Further, we thank Prof. Tom
Sharkey and two anonymous referees for their very useful comments on an earlier draft. A portion of this work was conducted at the EucFACE facility, an initiative of the Australian Government’s economic stimulus package and part of Australia’s national collaborative research infrastructure (NCRIS).

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Table 1. Description of species and sites included in the study along with number of individuals measured (N), the mean height that measurements were taken at, and the mean leaf thickness, leaf dry mass-to-area ratio, and total leaf P concentration per unit leaf area (Pa). Data are means ± s.e. The species name abbreviation is used to denote the different species in the figures.

<table>
<thead>
<tr>
<th>Species name and abbrev.</th>
<th>Type</th>
<th>Site</th>
<th>Location</th>
<th>N</th>
<th>Height (m)</th>
<th>Leaf thickness (µm)</th>
<th>Mₐ (g m⁻²)</th>
<th>Leaf Pₐ (mmol P m⁻²)</th>
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<tbody>
<tr>
<td><em>Acacia oblongifolia</em> (A. obl)</td>
<td>Native shrub</td>
<td>Davies Park, Springwood, NSW</td>
<td>33° 42' 28&quot; S, 150° 32' 51&quot; E</td>
<td>4</td>
<td>1-2</td>
<td>315</td>
<td>192.5 ± 11.8</td>
<td>2.5 ± 0.5</td>
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<tr>
<td><em>Banksia attenuata</em> (B. att)</td>
<td>Native shrub</td>
<td>Lesueur National Park, Jurien Bay, WA</td>
<td>30° 11' 02&quot; S, 115° 09' 27&quot; E</td>
<td>3</td>
<td>1</td>
<td>430</td>
<td>271.5 ± 19.5</td>
<td>2.7 ± 0.4</td>
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<td><em>B. serrata</em> (B. ser)</td>
<td>Native shrub</td>
<td>Davies Park, Springwood, NSW</td>
<td>33° 42' 28&quot; S, 150° 32' 51&quot; E</td>
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<td>1-2</td>
<td>540</td>
<td>207.2 ± 3.7</td>
<td>1.7 ± 0.2</td>
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<td>Hawkesbury Forest Experiment, Robertson, Hawkesbury, Richmond, NSW</td>
<td>33° 36' 40&quot; S, 150° 44' 27&quot; E</td>
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<td>10</td>
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<td><em>E. fastigata</em> (E. fas)</td>
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<td>Illawarra escarpment, Robertson, NSW</td>
<td>34° 37' 06&quot; S, 150° 42' 48&quot; E</td>
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<td>25</td>
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<td>Plantation tree</td>
<td>Hawkesbury campus, Richmond, NSW</td>
<td>33° 36' 57&quot; S, 150° 45' 06&quot; E</td>
<td>4</td>
<td>4</td>
<td>237</td>
<td>111.9 ± 2.0</td>
<td>6.6 ± 1.5</td>
</tr>
<tr>
<td>Persoonia levis (P. lev)</td>
<td>Native shrub</td>
<td>Davies Park, Springwood, NSW</td>
<td>33° 42’ 28” S, 150° 32’ 51” E</td>
<td>4</td>
<td>2</td>
<td>420</td>
<td>167.0 ± 18.8</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>
Table 2. Summary of relationships between biochemical parameters of leaf photosynthetic capacity and leaf chemistry for ten species in this study. Leaf $N_{area}$ and $P_{area}$ are expressed in mmol m$^{-2}$.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Equation</th>
<th>Coefficient of determination ($R^2$)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{cmax}$ by $N_{area}$</td>
<td>N.S.</td>
<td>-</td>
<td>0.874</td>
</tr>
<tr>
<td>$V_{cmax}$ by $P_{area}$</td>
<td>N.S.</td>
<td>0.371</td>
<td>0.062</td>
</tr>
<tr>
<td>$V_{omax}$ by $N_{area}$</td>
<td>N.S.</td>
<td>-</td>
<td>0.361</td>
</tr>
<tr>
<td>$V_{omax}$ by $P_{area}$</td>
<td>$V_{omax} = 27.66 + 3.54*P_{area}$</td>
<td>0.394</td>
<td>0.052</td>
</tr>
<tr>
<td>$J_{max}$ by $N_{area}$</td>
<td>N.S.</td>
<td>-</td>
<td>0.322</td>
</tr>
<tr>
<td>$J_{max}$ by $P_{area}$</td>
<td>$J_{max} = 88.56 + 400.99*P_{area}$</td>
<td>0.656</td>
<td>0.045</td>
</tr>
<tr>
<td>$T_p$ by $N_{area}$</td>
<td>N.S.</td>
<td>-</td>
<td>0.201</td>
</tr>
<tr>
<td>$T_p$ by $P_{area}$</td>
<td>$T_p = 6.51 + 14.64*P_{area}$</td>
<td>0.446</td>
<td>0.035</td>
</tr>
</tbody>
</table>
Figure 1. Diagram of the biochemical models hypothesised to account for the $O_2$ sensitivity of photosynthesis, with emphasis placed on areas indicated by the encircled as A) showing limitations by end-product synthesis (depicted in Eqn 6), and the circle B) showing the hypothesised mechanism from Harley & Sharkey (1991) with emphasis on $P_i$ release by P-glycolate phosphatase and subsequent incomplete photorespiratory glycerate re-entry to the chloroplast (depicted in Eqn 7), resulting in a net increase in stromal $P_i$ for photophosphorylation and RuBP regeneration. The small boxes are membrane-bound transporters.
Figure 2. A theoretical depiction of the prediction of $A_{\text{net}}$ as a function of the CO$_2$ partial pressure inside the chloroplast ($C_c$) (a) for normal O$_2$ and (b) for low pO$_2$ predicted by the biochemical model of FvCB, showing the component limitations to $A_{\text{net}}$ in colour. Blue dashes show the theoretical $A_{\text{net}}$ limited by the maximum capacity for carboxylation, orange dashes shows the theoretical $A_{\text{net}}$ limited by electron transport for the regeneration of RuBP in the Calvin-Benson cycle, and green dashes show the theoretical $A_{\text{net}}$ limited by triose-phosphate utilisation as per Eqn 6. The dark black line shows the overall relationship between $A_{\text{net}}$ and $C_c$ predicted by the minimum of the three limitations. The grey line in (b) represents the black curve in (a) for direct comparison with predictions for low pO$_2$. 
Figure 3. $A_{\text{net}}$ as a function of chloroplastic pCO$_2$ partial pressure ($C_c$) measured in the field for four woody species examples ($P. \text{levis}$, $B. \text{attenuata}$, $L. \text{stryraciflua}$, and $E. \text{fastigata}$, panels a-d, respectively) at both normal (21 kPa, filled light blue symbols) and low pO$_2$ (2 kPa, open red symbols), with ensemble response curve fits for each species and pO$_2$ level. Data are for three to four leaves from different trees on which measurements at both pO$_2$ values had been made (see Table 1). The lines shown represent separate fits of the standard model of Farquhar et al. (1980) to all the data at each respective pO$_2$ for a given species.
Figure 4. Results of the independent fits of the standard Farquhar et al. (1980) model to data at normal versus at low pO₂ for ten species for a) carboxylation capacity ($V_{\text{cmax}}$), b) light- and CO₂-saturated photosynthetic capacity ($A_{\text{max}}$), and c) the $A_{\text{max}}$ difference in low pO₂ modelled including $T_p$ limitation versus not including $T_p$ limitation as a function of $A_{\text{max}}$ in normal pO₂. Panel (d) shows $A_{\text{max}}$ in low pO₂ modelled including $T_p$ limitation as a function of $A_{\text{max}}$ measured in normal pO₂. The 1:1 line in panels a,b,d is shown as a dashed line across each panel and the solid line in panel c represents the line delineated by $Y = 0.50x - 6.83$, with $r^2 = 0.79$. Species are abbreviated to four letters representing the genus and species names (see Table 1).
Figure 5. The difference in $A_{\text{max}}$ in low pO$_2$ when modelled including $T_p$ limitation from Eqn 7 versus $A_{\text{max}}$ in low pO$_2$ without $T_p$ limitation considered is shown as a function of bulk leaf inorganic P, $P_i$. Species abbreviations are given in Table 1.
Figure 6. The relationships between four modelled biochemical components of photosynthetic or photorespiratory capacity ($V_{cmax}$, panel a; $V_{omax}$, panel b; $J_{max}$, panel c; $T_p$, panel d) and leaf P concentration for the ten species studied (abbreviation in Table 1). Each point represents the mean of individuals of a species (see Table 1), fit ensemble. $V_{cmax}$ and $V_{omax}$ species means across different individuals are reported in Supporting Information, Table S1. Dashed lines are shown where the relationships are significant. The data point for *Acacia obtusifolia* is obscured by that of *Banksia serrata* in panel C, as they had very similar $J_{max}$ values. Equations for these relationships are shown in Table 2.
Supporting Information

Table S1. Summary of species means for $V_{cmax}$, fit using both pO2 levels up to a $C_c$ threshold of 18 Pa, $V_{omax}$ as a measure of photorespiratory capacity, also fit from data from both pO2 up to a $C_c$ threshold of 18 Pa, and leaf N and P concentrations and N to P ratios for the ten species in this study. Data are means ± s.e.

<table>
<thead>
<tr>
<th>Species name</th>
<th>$V_{cmax}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>$V_{omax}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>Leaf N (mg N g$^{-1}$)</th>
<th>Leaf P (mg P g$^{-1}$)</th>
<th>N:P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia oblongifolia</em></td>
<td>109.8 ± 14.9</td>
<td>40.1 ± 3.9</td>
<td>17.8 ± 2.0</td>
<td>0.31 ± 0.10</td>
<td>67</td>
</tr>
<tr>
<td><em>Banksia attenuata</em></td>
<td>63.8 ± 11.5</td>
<td>38.4 ± 5.1</td>
<td>11.3 ± 1.4</td>
<td>0.31 ± 0.6</td>
<td>39</td>
</tr>
<tr>
<td><em>B. serrata</em></td>
<td>129.2 ± 23.5</td>
<td>35.9 ± 5.9</td>
<td>6.5 ± 0.5</td>
<td>0.25 ± 0.03</td>
<td>27</td>
</tr>
<tr>
<td><em>Eucalyptus dunnii</em></td>
<td>163.5 ± 6.1</td>
<td>32.7 ± 5.8</td>
<td>19.2 ± 1.1</td>
<td>1.18 ± 0.04</td>
<td>16</td>
</tr>
<tr>
<td><em>E. fastigata</em></td>
<td>204.6 ± 12.6</td>
<td>33.2 ± 5.7</td>
<td>18.0 ± 0.5</td>
<td>1.50 ± 0.28</td>
<td>13</td>
</tr>
<tr>
<td><em>E. saligna</em></td>
<td>144.7 ± 3.5</td>
<td>34.3 ± 8.0</td>
<td>21.3 ± 1.2</td>
<td>1.35 ± 0.39</td>
<td>19</td>
</tr>
<tr>
<td><em>E. tereticornis</em></td>
<td>100.7 ± 18.0</td>
<td>39.1 ± 5.0</td>
<td>16.3 ± 0.7</td>
<td>0.78 ± 0.09</td>
<td>21</td>
</tr>
<tr>
<td><em>E. todtiana</em></td>
<td>118.4 ± 10.3</td>
<td>27.0 ± 7.3</td>
<td>11.8 ± 1.1</td>
<td>0.38 ± 0.05</td>
<td>32</td>
</tr>
<tr>
<td><em>Liquidambar styraciflua</em></td>
<td>138.4 ± 22.3</td>
<td>20.0 ± 8.0</td>
<td>17.7 ± 1.4</td>
<td>1.83 ± 0.42</td>
<td>11</td>
</tr>
<tr>
<td><em>Persoonia levis</em></td>
<td>100.1 ± 13.9</td>
<td>12.7 ± 3.5</td>
<td>7.8 ± 0.3</td>
<td>0.30 ± 0.04</td>
<td>24</td>
</tr>
</tbody>
</table>
**Figure S1.** Time series of measurements of $A_{\text{net}}$ before, during and after a pulse of low pO$_2$ was delivered to the leaf chamber for two *Eucalyptus* species (a, b). At the flow rate used, 65 sec was the time constant for mixing. Data shown are examples from (a) *Eucalyptus todtiana* measured at Lesueur National Park in Western Australia, and (b) *E. tereticornis* measures at EucFACE in NSW, Australia, for measurements at a $C_a$ of 40 Pa. The final measurement in low pO$_2$ before switching O$_2$ back to normal pO$_2$ was considered at equilibriium and was used in the calculations.
$g_m$ (mol CO$_2$ m$^{-2}$ s$^{-1}$ bar$^{-1}$)

$g_s$ (mol m$^{-2}$ s$^{-1}$)

$R^2 = 0.54$

$E. ter$

$E. glo$

$E. sal$

$P. lev$