INVITED REVIEW

Evolution of a biochemical model of steady-state photosynthesis

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Abstract
On the occasion of the 40th anniversary of the publication of the landmark model by Farquhar, von Caemmerer & Berry on steady-state C3 photosynthesis (known as the “FvCB model”), we review three major further developments of the model. These include: (1) limitation by triose phosphate utilization, (2) alternative electron transport pathways, and (3) photorespiration-associated nitrogen and C1 metabolisms. We discussed the relation of the third extension with the two other extensions, and some equivalent extensions to model C4 photosynthesis. In addition, the FvCB model has been coupled with CO2-diffusion models. We review how these extensions and integration have broadened the use of the FvCB model in understanding photosynthesis, especially with regard to bioenergetic stoichiometries associated with photosynthetic quantum yields. Based on the new insights, we present caveats in applying the FvCB model. Further research needs are highlighted.

KEYWORDS
(alternative) electron transport, mesophyll conductance, NADPH-ATP balance, nitrogen assimilation, photorespiration, quantum yield, re-assimilation, stoichiometry, triose phosphate utilization

1 | INTRODUCTION

The year of writing this paper marks the 40th anniversary of the widely used biochemical model of Farquhar, von Caemmerer, and Berry (1980) on C3 photosynthesis, known as the “FvCB model” (see Table 1 for all acronyms). The model is a mathematical representation of the biochemical processes in the chloroplast related to photosynthetic CO2 uptake of plants. The application of this model has gone far beyond the developers’ expectations even 20 years ago (see the reflections by Farquhar, von Caemmerer, & Berry, 2001) and continues to rapidly rise today. It has become one of the most widely used models in plant science and beyond. For understanding leaf physiology, the model has been used to analyse gas exchange (sometimes combined with chlorophyll fluorescence) data (e.g., Long & Bernacchi, 2003; Sharkey, Bernacchi, Farquhar, & Singsaas, 2007; von Caemmerer & Farquhar, 1981; Yin et al., 2009), to understand photosynthetic control of electron transport (e.g., Foyer, Neukermans, Queval, Noctor, & Harbinson, 2012), and to quantify photosynthetic limitations (e.g., Busch & Sage, 2017; Deans, Farquhar, & Busch, 2019). When coupled to models of stomatal control, it contributes to understanding how water is traded for CO2 (Farquhar & Wong, 1984; Leuning, 1990) and how photosynthetic gas-exchange and water-relation traits are coordinated (Deans, Brodribb, Busch, & Farquhar, 2020). The FvCB model forms the basis of our understanding of photosynthetic isotope discrimination (Busch, Holloway-Phillips, Stuart-Williams, & Farquhar, 2020; Farquhar, 1983; Farquhar, O’Leary, & Berry, 1982; Ubierna et al., 2019). It has also been used to scale photosynthetic processes from the chloroplast and leaf level to higher levels (Bagley et al., 2015; Yin & Struik, 2008), and for assessing the impact of genetic engineering for identified photosynthetic targets on...
Rubisco catalyses the reaction with one mol $O_2$ (see discussion later) such that $A$ is expressed as:

$$A = V_c - 0.5V_o - R_d = (1 - 0.5\phi)V_c - R_d \quad (1)$$

where $\phi$ is the oxygenation to carboxylation ratio. $R_d$ has also been called “mitochondrial respiration in the light”, but the term “day respiration” is preferred. This is to remain non-specific about where the respired $CO_2$ comes from, as $CO_2$ released is not necessarily mitochondrial in origin (Tcherkez et al., 2017). The model ignores any possible consumption of chloroplastic NADPH or ATP if $R_d$ does not originate in mitochondria.

The photosynthetic carbon-reduction cycle, the Calvin-Benson cycle, starts with the carboxylation of the $CO_2$ acceptor ribulose 1,5-bisphosphate (RuBP), a five-carbon molecule. The reaction is catalysed by Rubisco, yielding two mol of the three-carbon molecule 3-phosphoglycerate (3-PGA) for every mol RuBP carboxylated. When $CO_2$ supply is limiting (or when RuBP is saturating), $V_c$ is limited by RuBP-saturated Rubisco kinetics and can be described as $W_c$ by the Michaelis–Menten equation appropriate for the case where two substrates ($CO_2$ and $O_2$) compete for active sites of RuBP-bound Rubisco:

$$V_c = \frac{W_c}{C_c + K_{mc}(1 + O_c/K_{mo})} \quad (2a)$$

Likewise, $V_o$ can be expressed as:

$$V_o = \frac{O_cV_{omax}}{O_c + K_{om}(1 + C_c/K_{mc})} \quad (2b)$$

where $C_c$ and $O_c$ are the level of $CO_2$ and $O_2$ at the active sites of Rubisco, respectively; $V_{omax}$ and $V_{onax}$ are the maximum rate of carboxylation and oxygenation of Rubisco, respectively; and $K_{mc}$ and $K_{mo}$ are the Michaelis–Menten constants of Rubisco for $CO_2$ and $O_2$, respectively. One can derive the expression for the $V_o : V_c$ ratio from combining Equations (2a) and (2b) as: $[V_{onax}K_{mo}/(V_{onax}K_{mc})]C_c/O_c$, where the whole term within the [] has been defined as the relative $CO_2/O_2$ specificity of Rubisco, $S_{c/o}$ (Laing, Ogren, & Hageman, 1974). If we use $\Gamma$ to denote the $CO_2$ level at which the rate of $CO_2$ uptake by carboxylation is balanced by the rate of photorespiratory $CO_2$ release (i.e., $V_c = 0.5V_o$), also called the $CO_2$-compensation point in the absence of day respiration, $\Gamma$ can be expressed as $0.5O_c/S_{c/o}$ (Farquhar et al., 1980). Furthermore, the $V_o : V_c$ ratio, or $\phi$ can be expressed thereof as $2\Gamma/C_c$ (Farquhar et al., 1980). Therefore, Equation (1) can be written as:

$$A = (1 - \Gamma/C_c)V_c - R_d$$

Photosynthesis can also depend on the rate at which RuBP is regenerated. This usually occurs at high $CO_2$ concentration and/or low light. The model assumes RuBP regeneration-limited photosynthesis is controlled by electron transport (Farquhar et al., 1980). Photosynthetic linear electron transport (LET) produces both NADPH and ATP; so, RuBP regeneration-limited or electron transport-limited
**TABLE 2** List of model symbols

| Symbol | Definition | Unit |
|--------|------------|------|
| \(a\) | Fraction of oxaloacetate that is reduced in mesophyll cells to malate moving to drive bundle sheath mitochondrial electron transport to produce ATP | — |
| \(A\) | Rate of CO₂ assimilation | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(A_c\) | Rate of CO₂ assimilation limited by Rubisco activity | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(A_j\) | Rate of CO₂ assimilation limited by electron transport | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(A_p\) | Rate of CO₂ assimilation limited by triose phosphate utilization | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(C_c\) | CO₂ partial pressure at the carboxylating sites of Rubisco | \(\mu\)bar |
| \(C_i\) | CO₂ partial pressure at intercellular-air spaces | \(\mu\)bar |
| \(C_m\) | CO₂ partial pressure at mesophyll cytosol | \(\mu\)bar |
| \(f\) | Fraction of irradiance absorbed by photosynthetic pigments but unavailable for Calvin-Benson and photorespiratory cycles | — |
| \(F\) | Rate of photorespiratory CO₂ release | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(f_{cyc}\) | Fraction of Photosystem I electrons that follow cyclic electron transport | — |
| \(f_{NDH}\) | Fraction of cyclic electron transport that follow the NAD(P)H dehydrogenase-dependent pathway | — |
| \(f_{pseudo}\) | Fraction of the Photosystem I electrons that follow the pseudocyclic electron transport | — |
| \(f_{refix}\) | Fraction of respired and photorespired CO₂ that is refixed | — |
| \(f_{refix,cell}\) | Fraction of respired and photorespired CO₂ that is refixed within mesophyll cells | — |
| \(f_{refix,ias}\) | Fraction of respired and photorespired CO₂ that is refixed via the intercellular air spaces | — |
| \(f_Q\) | Fraction of electrons at plastoquinone that follow the Q cycle | — |
| \(g_{bs}\) | Bundle-sheath conductance | \(mol\) m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) |
| \(g_m\) | Mesophyll conductance (inverse of mesophyll resistance), \(=1/r_m\) | \(mol\) m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) |
| \(g_{ma}\) | Mesophyll conductance constant, applied to the constant mesophyll conductance mode | \(mol\) m\(^{-2}\) s\(^{-1}\) bar\(^{-1}\) |
| \(h\) | Protons required per ATP synthesis (i.e., the H\(^{+}\):ATP ratio) | \(mol\) mol\(^{-1}\) |
| \(I_{abs}\) | Irradiance absorbed by photosynthetic pigments | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(J\) | Potential electron transport rate | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(J_1\) | Potential electron transport rate through Photosystem I | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(J_2\) | Potential electron transport rate through Photosystem II | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(J_{atp}\) | Potential rate of chloroplastic ATP production | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(J_{max}\) | Light-saturated potential electron transport rate | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(J_{2max}\) | Light-saturated potential electron transport rate through Photosystem II | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(k\) | Factor allowing for the effect of chloroplast gaps and the cytosol resistance such that the term \(k_i\) defines as the fraction of (photo)respiratory CO₂ in the inner cytosol (0 ≤ \(k_i\) ≤ 1) | — |
| \(K_{mC}\) | Michaelis–Menten constant of Rubisco for CO₂ | \(\mu\)bar |
| \(K_{mO}\) | Michaelis–Menten constant of Rubisco for O₂ | mbar |
| \(K_p\) | Michaelis–Menten constant of PEPC for CO₂ | \(\mu\)bar |
| \(L\) | Rate of CO₂ leakage from bundle-sheath to mesophyll cells | \(\mu\)mol m\(^{-2}\) s\(^{-1}\) |
| \(m\) | Parameter lumping several mesophyll properties, \(= (1 – \alpha k_i) r_{ch}/r_m\) with 0 ≤ \(m\) ≤ 1 | — |
| \(n\) | ATP produced per NADH oxidation | \(mol\) mol\(^{-1}\) |
| \(O_c\) | O₂ partial pressure at the active sites of Rubisco | mbar |
| \(O_m\) | O₂ partial pressure at mesophyll cytosol | mbar |
| \(r_{ch}\) | Chloroplast envelope and stroma resistance | \(mol^{-1}\) m² s bar |
| \(r_{cx}\) | Carboxylation resistance | \(mol^{-1}\) m² s bar |
| \(r_m\) | Mesophyll resistance, \(=r_{wp} + r_{ch}\) | \(mol^{-1}\) m² s bar |
| \(r_{sc}\) | Stomatal resistance to CO₂ transfer | \(mol^{-1}\) m² s bar |
| \(r_{wp}\) | Cell-wall and plasma-membrane resistance | \(mol^{-1}\) m² s bar |
| \(S_{C/O}\) | Relative CO₂/O₂ specificity of Rubisco | \(\mu\)bar \(\mu\)bar\(^{-1}\) |

(Continues)
TABLE 2 (Continued)

| Symbol | Definition | Unit |
|--------|------------|------|
| \( T_p \) | Rate of triose phosphate utilization | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( u_{oc} \) | Coefficient that lumps diffusivities of \( \text{O}_2 \) and \( \text{CO}_2 \) in water and their respective Henry constants, \( = 0.047 \) at 25 °C | \( \mu \text{mol} \mu \text{bar} (\mu \text{mol mbar})^{-1} \) |
| \( V_c \) | RuBP carboxylation rate | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( V_{c,max} \) | CO\(_2\)-saturated maximum carboxylation rate of Rubisco | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( V_o \) | RuBP oxygenation rate | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( V_p \) | PEP carboxylation rate | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( V_{p,max} \) | Maximum carboxylation rate of PEPc | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( R_d \) | Day respiration (\( \text{CO}_2 \) release in the light by processes other than photorespiration) | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( R_m \) | Day respiration in the mesophyll cells | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( W_c \) | RuBP carboxylation rate limited by Rubisco activity | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( W_e \) | RuBP carboxylation rate limited by electron transport | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( W_p \) | RuBP carboxylation rate limited by triose phosphate utilization | \( \mu \text{mol m}^{-2} \text{s}^{-1} \) |
| \( \alpha \) | Fraction of glycolate carbon not returned to chloroplast | — |
| \( \alpha_{2(\text{LL})} \) | Quantum yield of Photosystem II electron transport (under limiting light) on the basis of light absorbed by both photosystems | mol mol\(^{-1}\) |
| \( \alpha_{BS} \) | Fraction of Photosystem II that is in the bundle-sheath cells | — |
| \( \alpha_C \) | Fraction of glycolate carbon taken out from the photosynthetic pathway as glycine | — |
| \( \alpha_S \) | Fraction of glycolate carbon taken out from the photosynthetic pathway as serine | — |
| \( \alpha_T \) | Fraction of glycolate carbon taken out from the photosynthetic pathway as CH\(_2\)-THF | — |
| \( \delta \) | Factor defining a variable mesophyll conductance mode | — |
| \( \phi \) | RuBP oxygenation : RuBP carboxylation ratio, \( = V_o/V_c \) | — |
| \( \phi_L \) | Leakiness, \( = L/V_p \) | — |
| \( \phi_{1(\text{LL})} \) | Quantum yield of Photosystem I electron transport (under limiting light) | mol mol\(^{-1}\) |
| \( \phi_{2(\text{LL})} \) | Quantum yield of Photosystem II electron transport (under limiting light) | mol mol\(^{-1}\) |
| \( \phi_{CO2(LL)} \) | Quantum yield of \( \text{CO}_2 \) uptake (under limiting light) | mol mol\(^{-1}\) |
| \( \phi_{O2(LL)} \) | Quantum yield of \( \text{O}_2 \) evolution (under limiting light) | mol mol\(^{-1}\) |
| \( \varphi \) | Chloroplastic ATP required per \( \text{C}_4 \) cycle, \( \delta \) for the \( \text{NADP-ME} \) and \( \text{NAD-ME} \) subtypes | mol ATP (mol \( \text{CO}_2 \))\(^{-1}\) |
| \( \gamma^* \) | Half the inverse of Rubisco specificity, \( = 0.5/\text{S}_{\text{c/o}} \) | \( \mu\text{bar mbar}^{-1} \) |
| \( \Gamma^* \) | CO\(_2\)-compensation point in the absence of day respiration, \( = 0.5\text{O}_2/\text{S}_{\text{c/o}} \) | \( \mu\text{bar} \) |
| \( \Gamma_{\text{GT}} \) | Modified \( \Gamma^* \) as a result of glycolate carbon exit in the form of glycine and CH\(_2\)-THF from the photosynthetic pathway, \( = (1 - \alpha_C + 2m)\Gamma^* \) | \( \mu\text{bar} \) |
| \( \lambda \) | Fraction of mitochondria that locate closely behind chloroplasts in the inner cytosol | — |
| \( \theta \) | Curvature factor of light response of electron transport | — |
| \( \rho_2 \) | Factor for excitation partitioning to Photosystem II, \( = \phi_{2(\text{LL})}/\phi_{2(\text{LL})} \) | — |

The carboxylation rate, \( \text{W}_c \), can be formulated in terms of either NADPH supply or ATP supply from LET:

\[
\text{NADPH supply: } \text{W}_c = \frac{(1/2)J}{2 + 2\phi} = \frac{CJ}{4C_C + 8\Gamma^*}, \tag{3a}
\]

\[
\text{ATP supply: } \text{W}_c = \frac{(2/3)J}{3 + 3.5\phi} = \frac{CJ}{4.5C_C + 10.5\Gamma^*}, \tag{3b}
\]

where \( J \) is the rate of potential LET.

Equation (3a) is based on the stoichiometry of NADPH or electron requirement by the Calvin–Benson cycle and the photosynthetic cycle. First, carboxylation of one mol RuBP results in two mol 3-PGA, reduction of each 3-PGA to triose phosphate (TP) requires one mol NADPH (Figure 1a), and production of one mol NADPH requires two mol electrons; so, four electrons are required per carboxylation. The whole term in the numerator, \( (1/2)J \), represents the rate of NADPH
FIGURE 1  Legend on next page.
production from LET. Secondly, although oxygenation of one mol RuBP initially results in only one mol 3-PGA, it also results in one mol of the two-carbon molecule, 2-phosphoglycolate, which is dephosphorylated to glycolate in the chloroplast (Figure 1a,b). The glycolate is transported from the chloroplast into the peroxisome, where it is converted to glyoxylate and further to glycine (two carbons). The glycine is exported to the mitochondrion, where 0.5 mol glycine and tetrahydrofolate (THF) are converted by glycine decarboxylase (GDC) to 5,10-methylene-tetrahydrofolate (CH₂-THF), releasing 0.5 mol ammonia and 0.5 mol CO₂ in the process. CH₂-THF reacts with the remaining 0.5 mol glycine to form 0.5 mol serine (three carbons). Serine moves to the peroxisome and is transformed to glycerate. The glycerate flows to the chloroplast and is converted to 0.5 mol 3-PGA. Its reduction before incorporation into the Calvin-Benson cycle consumes 0.5 mol NADPH. The 0.5 mol ammonia released by GDC is re-assimilated into glutamate requiring one mol reduced ferredoxin (equivalent to 0.5 mol NADPH). In sum, the photorespiratory cycle involving three organelles (chloroplast, peroxisome, and mitochondrion, Figure 1b) requires four electrons per oxygenation.

In Equation (3b), the coefficient 2/3 stems from understandings of that time (in 1980) about the stoichiometry that each mol electron in LET translocates two mol protons (H⁺) across the thylakoid membrane into the lumen, and synthesis of one mol ATP requires three mol H⁺; so, the whole term in the numerator, (2/3), represents the rate of ATP production from LET. The coefficient 3 in the denominator refers to the requirement of three mol ATP per mol RuBP carboxylated by the Calvin-Benson cycle, consisting of two mol ATP for the phosphorylation of two mol 3-PGA to two mol 1,3-bisphosphoglycerate (before the reduction step consuming NADPH) and one mol ATP for the subsequent phosphorylation of one mol ribulose 5-phosphate to one mol RuBP (Figure 1a). The coefficient 3.5 refers to the ATP requirement per oxygenation by the photorespiratory cycle. This consists of: (1) one mol ATP for the phosphorylation of one mol 3-PGA to one mol 1,3-bisphosphoglycerate before its reduction, (2) one mol ATP for the phosphorylation of ribulose 5-phosphate to RuBP, (3) 0.5 mol ATP for the phosphorylation of glycate to 3-PGA plus 0.5 mol ATP for the subsequent phosphorylation of this 0.5 mol 3-PGA, and (4) 0.5 mol ATP for the re-assimilation of 0.5 mol ammonia (Figure 1a).

There are several equation forms describing J in Equations (3a) and (3b) as a function of absorbed irradiance (I_ab), but a non-rectangular hyperbolic function as the smaller root to the quadratic equation of Farquhar and Wong (1984) is mostly used now:

\[
J = \frac{0.5(1-f)I_{ab} + J_{max} - \sqrt{\left(0.5(1-f)I_{ab} + J_{max}\right)^2 - 4\theta(0.5(1-f)I_{ab}J_{max})}}{2\theta}
\]

(4)

where J_{max} is the maximum rate of LET under the saturating irradiance, f is the fraction of I_{ab} unavailable for Calvin–Benson and photorespiratory cycles, 0.5 refers to the partitioning factor of the light energy between the two photosystems, and \theta is the curvature factor.

The carboxylation rate can be limited either by RuBP-saturated rate W_c or by RuBP-regeneration determined rate W_f; so, Equation (1) becomes:

\[
A = (1-0.5\phi)\min(W_c,W_f) - R_d = \left(1 - \frac{f}{C_c}\right)\min(W_c,W_f) - R_d
\]

Equation (5), combined with Equation (2a) for W_c, Equation (3a) or (3b) for W_f and Equation (4) for J, forms the basic FvCB model. We shall call it the canonical FvCB model.

Since its first publication, the model has been developed further several times for C_3 photosynthesis (Busch, 2020; Busch, Sage, & Farquhar, 2018; Harley & Sharkey, 1991; Sharkey, 1985a, 1985b; Yin, van Oijen, & Schaposnik, 2004) and extended for C_4 photosynthesis (von Caemmerer & Furbank, 1999). Also, this model has been integrated with models for mesophyll CO₂-diffusion for various applications. In this paper, we outline the major extensions and review how these extensions and integration have broadened the use of the model in exploring the underlying physiology of photosynthesis.

2 | EXTENSION 1: INTRODUCING THE THIRD LIMITATION SET BY TRIOSE PHOSPHATE UTILIZATION

2.1 | Accommodating photosynthetic insensitivity to CO₂ and O₂

The canonical FvCB model predicts that A will always increase with increasing CO₂ level, despite a lower increase in the W_f-limited range than in the W_c-limited range. However, many (e.g., Sharkey, 1985a)
showed that $A$ can be insensitive to changes in the CO$_2$ partial pressure within the high CO$_2$ range, in particular in combination with high irradiance, or low O$_2$ partial pressure, or at low temperature. Sharkey (1985b) hypothesized that this insensitivity was due to the limitation set by the rate at which TP is utilized in the synthesis of sucrose or starch. As the use of TP is stoichiometrically exchanged with the release of phosphate (P$_i$) during sucrose or starch synthesis, a limitation in TP utilization (TPU) could result in a limitation to photophosphorylation, and, thus, to RuBP regeneration. So, in addition to what has been assumed about the control of RuBP regeneration by electron transport in the canonical FvCB model, RuBP regeneration can be limited further by other components as in the Calvin-Benson cycle and beyond. If TPU limits, the equation for $A$, equivalent to Equation (5), in the FvCB model, should be extended as:

$$A = (1 - 0.5\phi)\min(W_c, W_f, W_p) - R_d = \left(1 - \frac{R_p}{C_C}\right)\min(W_c, W_f, W_p) - R_d$$  \hspace{1cm} (6a)$$

where $W_p$ is the rate of carboxylation set by TPU limitation.

The carboxylation of one mol RuBP results in two mol TP but the Calvin-Benson cycle stoichiometry suggests that only one-sixth of the TP is used for sucrose or starch synthesis, whereas the remaining five-sixths of the TP are drawn back into the cycle to contribute to the regeneration of RuBP (Taiz & Zeiger, 2002). Thus, the P$_i$ consumption by sucrose or starch synthesis is $2V_c/6 = V_c/3$. Considering the carbon loss in the photorespiratory cycle, the net P$_i$ consumption would be $(1 - 0.5\phi)V_c/3$, and this must be equal to the release of P$_i$ via TPU if P$_i$ is limiting. Let $T_p$ be the rate of TPU, then one can write:

$$V_c = W_p = \frac{T_p}{(1 - 0.5\phi)/3} = \frac{C_c(3T_p)}{C_c - R_d}$$  \hspace{1cm} (6b)$$

Substituting Equation (6b) into Equation (6a) gives the net CO$_2$-assimilation rate limited by TPU, $A_p$ as:

$$A_p = 3T_p - R_d$$  \hspace{1cm} (6c)$$

This is the simple equation given first by Sharkey (1985b), which suggests that if TPU is limiting, $A$ is no longer sensitive to changes in CO$_2$ or O$_2$ partial pressure, or in irradiance. It sets an upper limit to net assimilation rate.

2.2 | Accommodating the reversed sensitivity to CO$_2$ and O$_2$

It has been frequently observed that $A$ even declines with increasing CO$_2$ partial pressure within the high CO$_2$ range, particularly under low O$_2$ conditions (e.g., von Caemmerer & Farquhar, 1981). Similarly, increasing O$_2$ has been observed to stimulate CO$_2$ assimilation under high CO$_2$ conditions (Harley and Sharkey, 1991). These reversed sensitivities to CO$_2$ and O$_2$ cannot be explained by the simple model, Equation (6c).

Sharkey and Vassey (1989) proposed that the reverse sensitivity was caused by inhibition of starch synthesis capacity, and in turn caused reduced stromal phosphoglucomutase activity resulting from metabolites interfering with its activity. An alternative explanation was proposed by Harley and Sharkey (1991) that a fraction of the glycolate carbon, which leaves the chloroplast and is recycled to glycerate in the photorespiratory cycle, does not return to the chloroplast, but after converting to glycine, is diverted from the photorespiratory cycle and used elsewhere for amino acid synthesis. Thus, the P$_i$ normally used in converting glyceraldehyde to 3-PGA is made available for phosphorylation instead, thereby, stimulating RuBP regeneration. Based on this hypothesis, Harley and Sharkey (1991) used three values for the fraction (0.0, 0.5, and 1.0) to fit data and showed how the curvature of photosynthetic CO$_2$-response curves ($A$-$C_c$ curves) had varying extents of the reversed CO$_2$ and O$_2$ sensitivity.

Based on the analysis by Harley and Sharkey (1991), von Caemmerer (2000) formalized the model by using $\alpha$ as the fraction of the glycolate carbon that is not returned to the chloroplast. As one oxygenation produces 0.5 glyceraldehyde, which consumes one P$_i$, the rate of P$_i$ consumption, which usually is $(1 - 0.5\phi)V_c/3$, should be decreased by $\alpha V_c/2$, or $\alpha V_c/2$. Thus, the net P$_i$ consumption in this case would be $(1 - 0.5\phi)/3 - \alpha\phi/2)V_c$. In analogy to Equation (6b), $W_p$ as the rate of carboxylation set by TPU limitation becomes:

$$W_p = \frac{T_p}{(1 - 0.5\phi)/3 - \alpha\phi/2} = \frac{3T_p}{1 - 0.5\phi(1 + 3\alpha)} = \frac{C_c(3T_p)}{C_c - (1 + 3\alpha)R_d}$$  \hspace{1cm} (7a)$$

The model for the net CO$_2$-assimilation rate, $A_p$, becomes:

$$A_p = \frac{(C_c - R_d)(3T_p)}{C_c - (1 + 3\alpha)R_d} - R_d$$  \hspace{1cm} (7b)$$

where $0 \leq \alpha \leq 1$. If $\alpha = 0$, Equation (7b) becomes Equation (6c), representing the case that glycolate carbon maximally returns to the chloroplast (i.e., $\frac{1}{3}$ of the glycolate carbon is recycled as glyceraldehyde; the other $\frac{1}{3}$ is lost as CO$_2$ as the result of glycine decarboxylation). Harley and Sharkey (1991) showed that for the same value of $\alpha$, TPU starts to limit $A$ at a lowering CO$_2$ level with increasing irradiance, with decreasing O$_2$ level, and with decreasing temperature. The reverse sensitivity that can occur based on Equation (7b) is frequently observed but occasionally the reverse sensitivity is greater than what can be accounted for by Equation (7b). It is likely that both the incomplete photorespiratory cycle explanation and the starch inhibition explanation (Sharkey & Vassey, 1989) can be valid, although in our experience the incomplete photorespiratory cycle phenomenon is more common.

2.3 | Implications of TPU limitation in modelling leaf photosynthesis

Ellsworth, Crous, Lambers, and Cooke (2015) showed that TPU limitations to photosynthetic capacity are common in woody species grown...
in the field. However, TPU might not be the most important limitation under current climatic growth conditions, as evidenced by Kumarathunge, Medlyn, Drake, Rogers, and Tjoelker (2019), who reported that only ca 30% of A–C curves showed an obvious TPU limitation in a global data representing 141 species. Irrespective of its uncertain importance under field conditions, the inclusion of TPU limitation in models is important for elucidating the basic principles of photosynthetic mechanisms. In cases where TPU is actually limiting, the canonical, two-limitation FvCB model would underestimate \( \tilde{V}_{\text{max}} \) (when fitting to A–C curves) and \( V_{\text{max}} \) or \( J_{\text{max}} \) (when fitting to light response curves) because the maximum photosynthetic rate would be wrongly attributed to being limited by electron transport or by Rubisco activity.

It is important to note that TPU limitation is a form of very short-term sink–source disequilibrium (McClain and Sharkey, 2019). It concerns the ability to remove TP quickly from the Calvin–Benson cycle. The half-life time of the cycle intermediates can be shorter than 1 s, while some larger pools still have a half-life time of <1 min. This means that TPU limitation can build up and disappear quickly. As discussed by Sharkey (2019), when plants are put into TPU limited conditions for hours or days, the TPU limitation is observable at first; but then other components like electron transport are regulated to a level that TPU is no longer “apparently” limiting (e.g., Papenmeyer, Loreto, & Sharkey, 1993). Furthermore, over a longer time, a larger sink can remove short-term TPU limitation. Kaschuk et al. (2012) showed that nodulated soybean plants had 14%–31% higher rates of photosynthesis and accumulated less starch in the leaves than nitrogen-fertilized plants, supporting that rhizobial symbiosis could stimulate photosynthesis due to the removal of carbon sink limitation by nodule activities.

Conversely, a small sink, especially when combined with a large source, can cause TPU limitation. Fabre et al. (2019) reported the occurrence of TPU limitation in panicle-pruned rice plants, especially those grown under 800 \( \mu \text{mol mol}^{-1} \) CO\(_2\). This reduction was associated with sucrose accumulation in the flag leaf resulting from the sink limitation. The photosynthetic stimulation by the elevated CO\(_2\) was lower in pruned plants compared with control plants, and this response to CO\(_2\) in relation to sink size was also found when comparing various rice genotypes having contrasting leaf:panicle size ratios or source:sink ratios (Fabre et al., 2020). A recent review by Dingkuhn et al. (2020) even found the evidence from broader ranges of genotypes that stronger elevated-CO\(_2\) responsiveness in wild relatives and old cultivars of crops is related to sink strength as a result of adaptive plasticity involving branching. Perhaps, the most important result in recent work of Fabre et al. is that TPU, thus net CO\(_2\) assimilation rate, declines increasingly with time after the midday in a diurnal cycle. These findings suggest that not only TPU limitation in regulating photosynthesis should be considered, but also a shorter time-step would be needed to account for diurnal variations in sink feedback limitation to photosynthesis, in dynamic crop models for projecting the CO\(_2\)-fertilization effect on crop production.

3 | EXTENSION 2: INTRODUCING ALTERNATIVE ELECTRON TRANSPORT PATHWAYS

3.1 | Accommodating a balanced ATP:NADPH ratio

In the canonical FvCB model, there are two different equations, Equations (3a) and (3b), for the same electron transport-limited carboxylation rate, \( W_j \). By comparison of the two equations, one can immediately recognize that the value of \( W_j \) determined by the ATP supply is more limiting than that determined by the NADPH supply. The two equations were used largely in a random manner in the literature before 2000. To eliminate the “random” application of the FvCB model, Yin et al. (2004) developed a generalized model that covers, among others, the two forms of the FvCB model for the electron transport-limited rate.

It is apparent that, according to the stoichiometric coefficients accepted in 1980, the LET produces an ATP:NADPH ratio of 1.333 [resulting from \((2/3):(1/2)\)], see Equation (3a) vs. Equation (3b)], well below 1.5 as required by the Calvin–Benson cycle, with ATP in deficit relative to NADPH. Chloroplasts engage several mechanisms that could remove the disparity in terms of requirement for the correct ATP:NADPH ratio (Allen, 2003; Baker, Harbinson, & Kramer, 2007; Farquhar & von Caemmerer, 1982). First, instead of going to the end electron acceptor NADP\(^+\), a fraction of electrons passing PSI may follow a cyclic electron pathway (\( f_{\text{cyc}} \)) (Figure 2). The cyclic electron transport (CET) does not produce NADPH, but passes through the “coupling” sites of ATP synthase (Allen, 2003), thereby being able to increase the ATP:NADPH ratio. Second, part of the noncyclic electrons may be used to support processes like the Mehler ascorbate peroxidase reaction or nitrate reduction, where \( \text{O}_2 \) directly or nitrate indirectly act as the electron acceptors, respectively (Figure 2). Every one mol \( \text{O}_2 \) uptake in the Mehler ascorbate peroxidase reaction is accompanied by one mol \( \text{O}_2 \) production from the splitting of water at PSI; so this reaction consumes four electrons per \( \text{O}_2 \) but requires no ATP (Asada, 1999). The first step of the reduction of nitrite into nitrate takes place in the cytosol but may use reducing power generated in the chloroplast (e.g., via the malate shuttle) and the subsequent steps in converting nitrite to ammonia and to glutamate take place in the chloroplast stroma, using the reduced ferredoxin (Noctor & Foyer, 1998). One mol nitrate reduction requires 10 mol electrons and only one mol ATP (Noctor & Foyer, 1998). Thus, both the Mehler ascorbate peroxidase reaction and nitrate reduction can help to adjust the ATP:NADPH ratio as required by the Calvin–Benson and the photorespiratory cycles. There are some other minor processes like sulphur assimilation and fatty acid biosynthesis that might use chloroplastic electrons but these are quantitatively less significant. For the convenience of modelling, the noncyclic electron transport in support of the Mehler ascorbate peroxidase reaction, nitrate reduction and any other minor processes is collectively named as the pseudocyclic category, and this fraction is denoted as \( f_{\text{pseudo}} \) (Yin et al., 2004). Therefore, the fraction for LET (i.e., the fraction of
The scheme for pathways of linear, cyclic and pseudocyclic electron transport (blue arrows) as driven by light energy allocated to Photosystem II (PSII) and Photosystem I (PSI), in the light reactions (with light-blue background) of photosynthesis (redrawn with permission from Yin et al., 2004). Thick-curved arrows show O₂ reactions (with light-blue background) of photosynthesis (redrawn from von Caemmerer, 2000; also see Figure 2). Also, the H⁺/NADPH stoichiometry of the H⁺ATP synthase (Petersen, Förster, Turina, & Gräber, 2012; Steigmiller, Turina, & Gräber, 2008) or the c14 rotor ring of the H⁺-translocating chloroplast ATP synthase (Seeleert et al., 2000; Hahn, Vonck, Mills, Meier, & Kühilbrandt, 2018), instead of 3 used in the FvCB model. If \( f_q = 1 \), \( h = 4 \), and \( f_{cy} = 0 \), then the produced ATP:NADPH ratio from the noncyclic electron pathway is 1.5, exactly matching the ratio required by the Calvin–Benson cycle and Equation (9b) becomes Equation (2.22) in the book of von Caemmerer (2000) for this scenario, that is,

\[
W_i = \frac{C_J}{4C + 9.337}. \tag{9c}
\]

If \( f_q = 1 \), \( h = 4.67 \), and \( f_{cy} = 0 \), the ATP:NADPH ratio from the noncyclic electron pathway is 1.286 (even lower than 1.333 assumed in the canonical FvCB model), and the value has often been cited in the recent literature to stress the surplus of the reducing power which might be exported to cytosol (e.g., Lim et al., 2020). For such a case, Equation (9b) becomes:

\[
\text{NADPH supply : } W_j = \frac{(1 - f_{cy} - f_{pseudo}) C_J J_1}{4C + 87}. \tag{9a}
\]

Substituting Equation (8) to Equation (9a) gives:

\[
\text{ATP supply : } W_i = \frac{(2 + f_q - f_{cy}) C_J J_2}{h(1 - f_{cy})(3C + 7 + \Gamma_2)}. \tag{9b}
\]

The two forms of electron transport-limited part of the canonical FvCB model are special cases of this extended model. If \( f_{pseudo} = 0 \), Equation (9a) becomes Equation (3a); in such a case, the whole PSII electron flux equals the LET \( J_2 = J_1 \). If \( f_q = 0 \), \( h = 3 \), and \( f_{cy} = 0 \), Equation (9b) becomes Equation (3b). So, the canonical FvCB model implies no operation of the Q cycle and a requirement of three \( \text{H}^+ \) per ATP synthesis (the \( \text{H}^+:\text{ATP} \) ratio \( h = 3 \)).

However, the contemporary belief is that the Q cycle may operate obligatorily \( (f_q = 1; \text{e.g., Sacksteder, Kanazawa, Jacoby, & Kramer}, 2000) \), and this cycle will effectively double the stoichiometry of the \( \text{H}^+ \) translocation through the cytochrome b_{6f} complex from one \( \text{H}^+ \) to two \( \text{H}^+ \) per electron passed therein (Figure 2). So, plus one \( \text{H}^+ \) pumped from splitting the water molecule through the PSII complex, a total of three \( \text{H}^+ \) (instead of two) produced per electron are transferred along the whole-chain if the Q cycle operates (von Caemmerer, 2000; also see Figure 2). Also, the \( \text{H}^+:\text{ATP} \) ratio is probably either 4 based on thermodynamic experiments (Petersen, Förster, Turina, & Gräber, 2012; Steigmiller, Turina, & Gräber, 2008) or 4.67 (=14/3) from the structural data for the c14 rotor ring of the \( \text{H}^+ \) translocating chloroplast ATP synthase (Seeleert et al., 2000; Hahn, Vonck, Mills, Meier, & Kühilbrandt, 2018), instead of 3 used in the FvCB model. If \( f_q = 1 \), \( h = 4 \), and \( f_{cy} = 0 \), then the produced ATP:NADPH ratio from the noncyclic electron pathway is 1.5, exactly matching the ratio required by the Calvin–Benson cycle and Equation (9b) becomes Equation (2.22) in the book of von Caemmerer (2000) for this scenario, that is,

\[
W_i = \frac{C_J}{4C + 9.337}. \tag{9c}
\]

where \( h \) is the number of \( \text{H}^+ \) required per ATP synthesis and \( f_q \) is the fraction of electrons at the plastoquinone that follows the Q cycle (Figure 2).
\[W_l = \frac{C_l f}{4.67C_c + 10.89T_c}. \]  

(9d)

Clearly, the model of Extension 2 represents the generalized algorithm for various scenarios with regard to the H⁺:electron and the H⁺:ATP ratios. Equation (9b) actually contains the ATP production factor (z) per electron transferred through PSII when CET occurs simultaneously (also see Equation (B8b) in the Appendix B of Yin et al., 2004):

\[z = \frac{2 + f_q - f_{\text{cyc}}}{h(1 - f_{\text{cyc}})} \]

(9e)

This ATP:electron ratio factor z is 2/3 in Equation (3b) of the canonical FvCB model, 3/4 in the case of Equation (9c), and 9/14 in the case of Equation (9d). The z factor also predicts that for a given set of \(f_q\) and \(h\), the ATP:electron ratio increases expectedly with increasing \(f_{\text{cyc}}\) (see later for \(C_c\) photosynthesis). Given that the Q cycle may not necessarily switch absolutely on \(f_q = 1\) and off \(f_q = 0\) but run partially (Cornic, Bukhov, Wiese, Bligny, & Heber, 2000), the model allows such scenarios with \(0 \leq f_q \leq 1\). As noted by Yin et al. (2004), the model assumes that the Q cycle, either obligatorily or partially operated, is impartial to cyclic and noncyclic electrons (Allen, 2003).

### 3.2 | Quantum efficiency of electron transport when cyclic and noncyclic pathways co-occur

When CET and noncyclic (including linear and pseudocyclic) electron transport run simultaneously, a higher electron flux is expected in PSI than in PSII. This means that the fraction of light energy partitioned to PSI and PSII may not be 0.5 each as set by Equation (4) in the canonical FvCB model, but higher than 0.5 for PSI. On the other hand, the partitioning factor must also depend on the photochemical efficiency of the two photosystems, with partitioning in favour of the less efficient PSI in the absence of CET, given that the photochemical efficiency of PSII (\(\Phi_2\)) is lower than that of PSI (\(\Phi_1\)) (e.g., Hogewoning et al., 2004). Yin et al. (2004) developed an analytical equation for describing the parameter \(\alpha_{2LL}\), the quantum efficiency of PSII electron transport (under limiting light, LL) on the basis of absorbed photons by both photosystems:

\[\alpha_{2LL} = \frac{\Phi_{2LL}(1 - f_{\text{cyc}})}{\Phi_{2LL}(1 - f_{\text{cyc}}) + \Phi_{1LL}(1 - f_{\text{cyc}})} \]

(10a)

The fraction of absorbed light partitioned to PSII, \(\rho_2\), can be formulated as:

\[\rho_2 = \frac{\alpha_{2LL}}{\Phi_{2LL}(1 - f_{\text{cyc}}) + \Phi_{1LL}(1 - f_{\text{cyc}})} \]

(10b)

Equations (10a) and (10b) both suit for limiting light conditions, as well as for nonlimiting light conditions (if the subscript LL is removed) as long as A is limited by electron transport. The model for describing \(J_z\) as a function of the full range of absorbed irradiance \(I_{abs}\) can be formulated in analogy to Equation (4) as:

\[J_z = \frac{\alpha_{2LL}I_{abs} + J_{\text{max}} - \sqrt{(\alpha_{2LL}I_{abs} + J_{\text{max}})^2 - 4\alpha_{2LL}I_{abs} \cdot J_{\text{max}}}}{2} \]

(11)

where \(J_{\text{max}}\) is the maximum value of the potential \(J_z\) under saturating irradiance, to differentiate it from \(I_{\text{max}}\) in Equation (4) that stands for the maximum rate of the potential LET.

### 3.3 | Quantum yield of CO₂ uptake and of O₂ evolution

It is convenient to derive the expression for quantum yield of CO₂ uptake (\(\Phi_{\text{CO₂(LL)}}\)) from Equations (5), (9a), (10a) and (11) in terms of NADPH supply:

\[\Phi_{\text{CO₂(LL)}} = \frac{\rho_2 \Phi_{2LL}(1 - f_{\text{cyc}})}{\Phi_{2LL}(1 - f_{\text{cyc}}) + \Phi_{1LL}(1 - f_{\text{cyc}}) + \Phi_{p\text{pseudo}}} \]

\[\Phi_{\text{CO₂(LL)}} = \frac{\Phi_{2LL}(1 - f_{\text{cyc}})}{\Phi_{2LL}(1 - f_{\text{cyc}}) + \Phi_{1LL}(1 - f_{\text{cyc}}) + \Phi_{p\text{pseudo}}} \]

(12a)

Equivalent equations based on the canonical FvCB model are:

\[\Phi_{\text{CO₂(LL)}} = \frac{0.5(1 - f)(C_c - R_s)}{4C_c + 87^s} \]

(12c)

\[\Phi_{\text{CO₂(LL)}} = \frac{0.5(1 - f)(C_c - R_s)}{4C_c + 10.37^s} \]

(12d)

The FvCB model assumes that \(\rho_2 = 0.5\). Comparison of Equations (12a) and (12c) immediately identifies that the f factor in the FvCB model, representing the fraction of \(I_{abs}\) unavailable for Calvin–Benson and photorespiratory cycles, can be expressed as: \(f = 1 - \Phi_{2LL}[1 - \Phi_{\text{pseudo}}/(1 - f_{\text{cyc}})]\). In other words, the factor \(f\) actually lumps multiple components, including the non-photochemical loss of PSI (\(\Phi_{2LL}\) known not to be higher than 0.85, Björkman & Demmig, 1987), cyclic electron transport \(f_{\text{cyc}}\), and pseudocyclic electron components \(f_{\text{pseudo}}\) that support alternative metabolic processes. Much literature after Farquhar et al. (1980) often only refers to \(f\) to correct for spectral quality of the light (e.g., von Caemmerer, 2000). While this definition of \(f\) reflects the often-reported wavelength dependent photosystems’ photochemical efficiencies and absorption by carotenoids and nonphotosynthetic
pigments (e.g., Evans, 1987; Hogewoning et al., 2012), it is more difficult to reconcile well with the insights from the extended model.

Photosynthetic quantum yield can also be expressed in terms of O2 evolution ($\Phi_{O2,LL}$). The electron requirement in support of both Calvin–Benson and photorespiratory cycles leads to O2 evolution at PSII from the splitting of H2O; so, the total O2 evolved can be expressed as $(1 + 2f/L/C)W$. The O2 uptake by photorespiration consists of (i) one mol O2 consumed per mol RuBP oxygenation, and (ii) a further one mol O2 consumed in the conversion of one mol glycolate to one mol glycose by glycose oxidase in the peroxisome, producing one mol hydrogen peroxide (H2O2) which is immediately destroyed by the action of catalase into one mol H2O and 0.5 mol O2 (Figure 1b). So the total O2 uptake associated with the photorespiratory pathway is 1.5 mol O2 per mol RuBP oxygenated, which can be expressed as $1.5V_o = (3f/L/C)V_C$. Taking these together, the Rubisco-linked net O2 evolution is $(1 - f/L/C)V_C$, which is the same as for CO2 uptake (von Caemmerer, 2000).

The Mehler ascorbate peroxidase reaction consumes noncyclic electrons, but its stoichiometry is that for every mol O2 directly reduced in this reaction, 0.5 mol O2 is released by superoxide dismutase and 0.5 mol O2 is evolved through the splitting of H2O at PSII such that the reaction results in no net O2 exchange (Asada, 1999). In contrast, processes like nitrate reduction, also consuming noncyclic electrons, do result in O2 evolution. Thus, if photosynthetic quantum yield is expressed in terms of O2 evolution ($\Phi_{O2,LL}$), we can break down $f_{\text{pseudo}}$ in Equation (12a) into two components: one for the Mehler ascorbate peroxidase reaction and one for other basal components, and the latter is no longer needed in the equation for $\Phi_{O2,LL}$. As the Mehler ascorbate peroxidase reaction acts as a photosynthesis mechanism when absorbed light energy exceeds the enzymatic capacity of downstream metabolism (Ort & Baker, 2002), this reaction may be negligible under strictly limiting light conditions. Thus, quantum yield of O2 evolution for the limiting light conditions becomes:

$$\Phi_{O2,LL} = \frac{\Phi_{O2,LL}(1 - f_{\text{cyc}})(C_C - C_r)}{\Phi_{O2,LL} + (1 - f_{\text{cyc}})(4C_C + 8C_r)}$$  \hspace{1cm} (12e)

3.4 Using the quantum yield model to infer hard-to-measure parameters

The unique feature of Equation (12e) based on the extended model for $W_j$ is that $f_{\text{cyc}}$ is in the model for describing NADPH-dependent quantum yield, in contrast to the conventional belief that CET can generate additional ATP and must appear only in equations for the ATP-dependent quantum yield. Parameter $f_{\text{cyc}}$ does appear in Equation (12b) for the ATP-dependent quantum yield, but Equation (12b) includes uncertain parameters $f_Q$ and $h$ in addition to $f_{\text{cyc}}$. Relying on this unique feature and the generally conserved PSI:PSII efficiency ratio, Yin, Harbinson, and Struik (2006) showed that a hard-to-measure parameter $f_{\text{cyc}}$ can be calculated from Equation (12e), based on measurable parameters $\Phi_{O2,LL}$ and $\Phi_{O2,LL}$ under non-photorespiratory conditions:

$$f_{\text{cyc}} = \frac{\Phi_{O2,LL} - 4\Phi_{O2,LL}(1 + \Phi_{O2,LL}/\Phi_{LL,LL})}{\Phi_{O2,LL} - 4\Phi_{O2,LL}}$$  \hspace{1cm} (13a)

A typical $\Phi_{LL}$ based on chlorophyll fluorescence measurements is 0.8 and a typical $\Phi_{O2,LL}$ based on P700 absorption measurements is close to 1.0 or slightly lower (Genty & Harbinson, 1996); so, the $\Phi_{O2,LL}/\Phi_{LL,LL}$ ratio is ca 0.85. $\Phi_{O2,LL}$ of C3 photosynthesis in the absence of photorespiration is ca 0.105 (Björkman & Demmig, 1987). The solved $f_{\text{cyc}}$ from Equation (13a) is then ca 0.06. This cannot be considered as an absolute estimate, but suggests that very little CET is needed for C3 photosynthesis, in line with previous reports (e.g., Avenson et al., 2005).

Once $f_{\text{cyc}}$ is known, one can calculate another hard-to-measure light-partitioning parameter $f_Q$ from Equation (10b). The obtained $f_Q$ is ca 0.53, close to the assumed value 0.5 in the canonical FvCB model. This indicates that the requirement for a higher partitioning to the less efficient PSI is to some extent balanced by the requirement for a higher partitioning to PSI to run CET. Equation (10b) suggests that $f_Q$ equals exactly 0.5 only if the fraction for the noncyclic electron flow, $1 - f_{\text{cyc}}$, is equal to the $\Phi_{O2,LL}/\Phi_{LL,LL}$ ratio.

By dividing Equation (12a) by Equation (12e) that assumes no Mehler ascorbate peroxidase reaction for the limiting light condition, one can solve for basal $f_{\text{pseudo}}$ from the $\Phi_{O2,LL}/\Phi_{O2,LL}$ ratio:

$$f_{\text{pseudo}} = \left(1 - \frac{\Phi_{O2,LL}}{\Phi_{O2,LL}}(1 - f_{\text{cyc}})^{-1}\right)$$  \hspace{1cm} (13b)

Unlike Equation (13a), Equation (13b) applies to both non-photorespiratory and photorespiratory conditions. A typical value of $\Phi_{C3,LL}$ of C3 photosynthesis under limiting light in the absence of photorespiration is ca 0.093 (Long, Postl, & Bolhär-Nordenkampf, 1993). This gives an estimate of $f_{\text{pseudo}}$ being ca 0.10. The $\Phi_{C3,LL}/\Phi_{O2,LL}$ ratio is also known as the assimilatory quotient, and the value of its complement, $(1 - \text{ratio})$, indicates the extent to which electrons are used in support of the processes like nitrogen assimilation (Bloom, Caldwell, Finazzo, Warner, & Weissbart, 1989; Skillman, 2008).

Once $f_{\text{cyc}}$ and $f_{\text{pseudo}}$ are known, likely combinations of $f_Q$ and $h$ can be solved from Equation (8) for C3 photosynthesis. Using the above estimates of $f_{\text{cyc}}$ and $f_{\text{pseudo}}$ for the nonphotorespiratory conditions, the solved $h$ is ca 3.1 if $f_Q = 0$ and is ca 4.67 if $f_Q = 1$. The latter combination is very close to the contemporary belief that the operation of the Q cycle is obligatory (Sacksteder et al., 2000) and the structural data that chloroplast ATP synthase requires 4.67 c subunits or protons to produce one ATP (Seelert et al., 2000; Hahn et al., 2018). However, like the canonical FvCB model, Equation (8) does not account for small amounts of ATP required for starch synthesis and nitrogen assimilation. As ATP for these processes most likely come from chloroplasts (Noctor & Foyer, 1998), then the calculated $h$ would approach 4. Energy requirements for nitrogen assimilation will further be discussed next.
4  |  EXTENSION 3: INTRODUCING PHOTORESPIRATION-ASSOCIATED NITROGEN AND C1 METABOLISMS

Nitrogen (N) assimilation can be intrinsically linked to the photorespiratory pathway (Bloom, 2015). While the electron and ATP requirement associated with re-cycling of the ammonia released by photorespiration is already accounted for (see Section 1), the energy requirement for reduction and assimilation of new nitrogen that enters the leaf is not accounted for in the canonical FvCB model. De novo assimilation of nitrogen in leaves of C3 plants can arise via the photorespiratory pathway because, as discussed earlier, the photorespiratory intermediate glycine can be diverted from the photorespiratory pathway and used elsewhere for amino acid synthesis, which explains the reversed photosynthetic sensitivity to CO2 and O2 (Harley & Sharkey, 1991). In addition, serine, a product of glycine decarboxylation in the photorespiratory pathway, can act as a precursor of several other amino acids (Ros, Muñoz-Bertomeu, & Krueger, 2014). The nitrogen molecules of both glycine and serine, if exported from the photorespiratory pathway for other uses or accumulated...
temporarily, have to be replenished by de novo assimilation of nitrogen; otherwise the pathway cannot be continued. Busch et al. (2018) extended both \( W_o \) and \( W_l \)-limited rates of the FvCB model, by following the stoichiometry of energy requirement by both carbon and nitrogen assimilation as well as the stoichiometry for the amino-group balance. More recently, Busch (2020) further extended the model to account for the additional export of glycolate carbon as the photorespiratory pathway is also the main supply of the activated one-carbon units to the so-called C\(_1\) metabolism. This is because, as stated in Section 1, the glycine decarboxylation step can catalyse the conversion of the cofactor tetrahydrofolate (THF) to CH\(_2\)-THF that acts as the leaf’s currency for activated C\(_1\) units. Here, we collectively describe the extension involving both de novo nitrogen assimilation and C\(_1\) metabolisms (Figure 3).

### 4.1 The general model of extension 3 integrating nitrogen and C\(_1\) metabolisms

Busch et al. (2018) used \( \alpha_G \) and \( \alpha_S \) to denote the fractions of glycolate carbon taken out from the photorespiratory pathway as glycerine and serine, respectively. Likewise, Busch (2020) used \( \alpha_T \) to denote the fraction of glycolate carbon taken out from the photorespiratory pathway as CH\(_2\)-THF. As shown in Figure 3, the glycolate carbon exported in the form of the three-carbon molecule serine has to be less than or equal to the remaining carbon after the glycerine export, glycine decarboxylation, and CH\(_2\)-THF export: \( \frac{1}{2} \alpha_S \leq \frac{1}{2}(1 - \alpha_G) - \alpha_T \), where \( \frac{1}{2} \) refers to the glycerate: serine carbon ratio (Figure 1b), and \( \frac{1}{2} \) refers to half of the carbon lost during its decarboxylation. This relation can be converted into \( \alpha_G + 2\alpha_T + \frac{1}{2} \alpha_S \leq 1 \), thereby reflecting that the total proportion of glycolate carbon exports cannot exceed 1. Of course, none of \( \alpha_G \), \( \alpha_T \) and \( \alpha_S \) can be lower than 0. In analogy to the derivation of Equation (7a) by Harley and Sharkey (1991), the rate of Pi consumption, which usually is \( \left(1 - 0.5 \phi V_c / 3\right) \), should be decreased by \( \left(\alpha_G + 2\alpha_T + \frac{1}{2} \alpha_S \right) \phi V_c / 2 \), and the net Pi consumption would be \( \left(1 - 0.5 \phi \right) / \left(\alpha_G + 2\alpha_T + \frac{1}{2} \alpha_S \right) / 2 \). Thus, \( W_o \) as the rate of carboxylation set by TPU limitation in this case becomes:

\[
W_o = \frac{T_p}{(1 - 0.5 \phi) / 3 - (\alpha_G + 2\alpha_T + \frac{1}{2} \alpha_S) \phi / 2} = \frac{T_p}{1 - 0.5 \phi (1 + 3\alpha_G + 6\alpha_T + 4\alpha_S)}
\]  

Equation (14) becomes Equation (7a) if \( \alpha_S = 0 \) and \( \alpha_T = 0 \).

While \( W_o \) remains unchanged as Equation (2a), the rate of carboxylation as determined by electron transport, \( W_j \), will be affected as the potential electron transport rate \( J \) now has to support both carbon and nitrogen assimilation. Photorespiratory carbon entering the C\(_1\) metabolism, in contrast, causes a net release of electrons, as the reaction catalysed by GDC releases electrons and the exit of carbon from the photosynthetic pathway saves electrons downstream that would otherwise be consumed for converting serine to glycerate in the peroxisome and for reducing this glycerate-derived 3-PGA in the chloroplast (Figures 1b and 3). These together bring the equation for electron transport-determined carboxylation rate in terms of NADPH supply to:

\[
W_j = \frac{J}{4 + (4 + 8\alpha_G - 4\alpha_T + 4\alpha_S) \phi}
\]  

The denominator can be obtained by summing up all the electron requirements for individual steps, deducted by electron equivalents of the NADH release as a result of glycine decarboxylation, indicated in Figure 3. Likewise, photorespiratory carbon export via the C\(_1\) metabolism saves ATP that would otherwise be used for the phosphorylation of glycerate to 3-PGA and for the subsequent phosphorylation of this 3-PGA (Figures 1b and 3); thus, one can formulate the equation for \( W_j \) in terms of ATP supply:

\[
W_j = \frac{J_{\text{ATP}}}{3 + (3.5 - 0.5 \alpha_G - \alpha_T - \frac{3}{2} \alpha_S) \phi}
\]  

where \( J_{\text{ATP}} \), in the numerator is the total ATP production rate from chloroplastic electron transport (which is not expressed in \( J \) like Equation (15a), given the uncertainties discussed earlier in Extension 2). The
denominator in Equation (15b) can also be obtained by summing up all the ATP requirements indicated in Figure 3.

Traditionally, the proportion of glycolate carbon that does not return to chloroplasts (α) is relevant only for the TPU-limited carboxylation rate $W_p$ (see Equations (7a) and (7b)). Equations (15a) and (15b) suggest that the proportion parameters ($\alpha_C$, $\alpha_T$ and $\alpha_S$) affect not only $W_p$ but also $W_c$. The export of carbon as CH$_2$-THF always increases $W_c$. Glycine and serine export associated with de novo N assimilation decreases $W_p$ in terms of NADPH requirement whereas it increases $W_c$ in terms of ATP requirement. This suggests that photosynthesis-associated N assimilation can help alleviate the deficit of ATP relative to NADPH (see earlier discussions).

In the case of glycolate being diverted from the photorespiratory pathway, the amount of CO$_2$ released per oxygenation should be decreased by $\alpha_C$ (Busch et al., 2018). In contrast, as shown in Figure 3, every carbon exported as CH$_2$-THF from the pathway results in one carbon lost from glycine decarboxylation (Busch, 2020). Therefore, it is necessary to revise Equation (1) to:

$$A = V_c - [0.5(1 - \alpha_C) + \alpha_T]V_o - R_d \quad (16a)$$

And Equation (6a) becomes:

$$A = \left(1 - \frac{\Gamma_{\text{GT}}}{C_3}\right) \min(W_c, W_p, W_b) - R_d \quad (16b)$$

where $\Gamma_{\text{GT}} = [0.5(1 - \alpha_C + \alpha_T)O/S_{C\text{,o}}$, or $\Gamma_{\text{GT}} = (1 - \alpha_C + 2\alpha_T)\Gamma$.

It follows that the CO$_2$ compensation point in the absence of day respiration is no longer constant at given temperature and O$_2$ partial pressure, but decreases with increasing the fraction of glycine and increases with increasing the fraction of CH$_2$-THF diverted from the photosynthetic pathway. Therefore, equations for the net CO$_2$-assimilation rate corresponding to the three limitations become:

$$A_c = \frac{1 - [0.5(1 - \alpha_C) + \alpha_T]\phi}{C_3 + K_{nc}(1 + 0/K_{no})} - R_d$$

$$= \frac{C_c - G_{\phi}(1 - \alpha_C + 2\alpha_T)|V_{\text{cmax}}}{C_c + K_{nc}(1 + 0/K_{no})} \quad (16c)$$

$$A_g = \left\{1 - [0.5(1 - \alpha_C) + \alpha_T]\phi\right\}J$$

$$\frac{4 + (4 + 8\alpha_C - 4\alpha_T + 4\alpha_T)\phi}{C_c - G_{\phi}(1 - \alpha_C + 2\alpha_T)(J/4)} - R_d$$

$$= \frac{C_c}{C_c + (1 + 2\alpha_C - 2\alpha_T + \alpha_S)(2\Gamma)} \quad (16d)$$

$$A_p = \left\{1 - [0.5(1 - \alpha_C) + \alpha_T]\phi\right\}(3T_p)$$

$$\frac{1 - 0.5\phi(1 + 3\alpha_C + 6\alpha_T + 4\alpha_T)}{C_c - G_{\phi}(1 - \alpha_C + 2\alpha_T)(3T_p)} - R_d$$

$$= \left(1 + 3\alpha_C + 6\alpha_T + 4\alpha_T \Gamma\right) \quad (16e)$$

Applying quantitative isotopic techniques to sunflower leaves, Abadie, Boex-Fontvieille, Carroll, and Tcherkez (2016) showed that the stoichiometric ratio of O$_2$ fixation by Rubisco to CO$_2$ production by GDC increased from 2.0 (the theoretical value used in the canonical FvCB model) at very low-photorespiration gas mixtures, to 2.05 for the normal ambient condition, and to 2.09 to high-photorespiration gas mixtures. As the export of carbon in the form of CH$_2$-THF would make this ratio lower than 2.0, the observed ratio being ≥2.0 suggests that the export of carbon from the photorespiratory pathway via this form may be less important than the export via glycolate. If the value of ≥2.0 is due to glycolate export alone, then $\alpha_C$ can be estimated to be 0.0 for the conditions with little photorespiration, 0.024 for the ambient condition, and a maximum value of 0.043 for the conditions of high-photorespiration gas mixture. Using modelling to fit Equations (16c)-(16e) to A–C$_1$ curves, Busch et al. (2018) estimated $\alpha_C$ of the ambient condition to be 0.026 for plants fed with NH$_4^+$–N, 0.103 for plants fed with NO$_3^-$–N, and 0.077 for control plants. These all indicate that $\alpha_C$ is not zero as implicitly assumed in the canonical FvCB model. This means that even under Rubisco limitation where $W_c$ is not changed by any amino acid export, A could still be increased due to a slight decrease in the CO$_2$-compensation point if glycolate is removed from the photorespiratory pathway (see Equation (16c)). Under the TPU limitation where carbon uptake is limited by the rate at which carbohydrates can be metabolized, A could be further increased by short-circuiting carbon flux to glycine, serine, and CH$_2$-THF via the photosynthetic pathway. Only the NADPH-dependent electron transport-limited rate is decreased due to the electron consumption by the de novo nitrogen assimilation (if the potential electron transport rate $J$ remains the same; but see later discussion).

In addition to exploring the ratio of O$_2$ fixation by Rubisco to CO$_2$ production by GDC to estimate $\alpha_C$, Busch et al. (2018) showed that $\alpha_G$ and $\alpha_S$ could be roughly estimated from model fitting to A–C$_1$ curves. There is currently no information available about the possible value for the fraction of glycolate carbon diverted via the C$_3$ metabolism (Busch, 2020). Therefore, hereafter we mainly discuss the relations with regard to the amino-acid exports.

### 4.2 Relationships with the previous two extensions

It is clear, based on the model of Busch et al. (2018), that the parameter $\alpha$ in the model of Harley & Sharkey (1991) deals with the carbon side of the amino acid export but not the electron requirement for NO$_3^-$ assimilation. In addition, the energy associated with the changed RuBP regeneration and NH$_4^+$-recycling as a result of amino acid export was not considered in Harley & Sharkey’s model. Also, the decrease of CO$_2$-compensation point in the absence of $R_p$ as a result of the glycine exit is not explicitly included in the model although this was discussed by Harley and Sharkey (1991). Busch et al. (2018) treated amino acid exit from the photorespiratory pathway differently, depending on whether it is glycine or serine that is exited, whereas Harley and Sharkey (1991) only assumed the glycine exit. It is clear from Equation (16e) that if it is only glycine that exits, the model under a TPU-limitation is:

$$A_p = \frac{C_c - G_{\phi}(1 - \alpha_C)(3T_p)}{C_c - (1 + 3\alpha_C)\Gamma} - R_d \quad (17a)$$

If the CO$_2$-compensation point is to be maintained as in the canonical FvCB model, it would be internally consistent to assume that it is only
This fraction would decrease by 25% if Equation (17b) is used. In fact, actual fraction of glycolate carbon not returned to the chloroplast, partly being the artefact of ignoring the decrease of Equation (16e), the model for serine, instead of glycine, being exported. Then, based on Equation (7b) assuming only serine exit with \( \alpha_s \), reflecting \( \alpha_g \), and this positive impact on \( \alpha_G \) was much lower than \( \alpha_S \), causes a decrease in CO₂-compensation point, which is affected by \( \alpha_G \) but not by \( \alpha_S \).

It is also possible to connect the model of Busch et al. (2018) with the model of Yin et al. (2004). As stated earlier, parameter \( f_{\text{pseudo}} \) in the model of Yin et al. (2004) can largely reflect the proportion of electrons for supporting nitrogen assimilation, especially under electron transport-limited conditions. Thus, one can equate Equation (16d) without the \( \alpha_T \) terms to \( A_p \) formulated from Equation (9a):

\[
\frac{1 - 0.5\phi(1 - \alpha_G)}{4 + (4 + 8\alpha_G + 4\alpha_T)\phi} = \frac{1}{1 - f_{\text{pseudo}}} \frac{1 - 0.5\phi J_2}{1 - 4\phi} \tag{18a}
\]

Note that \( J \) on the left side of the equation must be equal to \( J_2 \) on the right side, as they both represent the rate of whole-chain electron transport in support of the Calvin-Benson cycle, the photosrespiratory pathway and nitrogen assimilation (in this context, \( J \) in the model of Busch et al., 2018 actually differs from \( J \) in the canonical FvCB model). Solving for \( f_{\text{pseudo}} \) gives:

\[
f_{\text{pseudo}} = \frac{\alpha_G 4 + (8\alpha_G + 4\alpha_T)\phi(1 - 0.5\phi) - 0.5\alpha_G(4 + 4\phi)}{4 + (4 + 8\alpha_G + 4\alpha_T)\phi((1 - 0.5\phi)(1 - f_{\text{cyc}}))} \tag{18b}
\]

As stated earlier, \( f_{\text{cyc}} \) for \( C_3 \) photosynthesis is negligible (set to nil here). The modelling by Busch et al. (2018) showed that for the ambient-air condition, \( \alpha_G \) was ca 0.10 and \( \alpha_S \) was ca 0.15 for plants fed with NO₃⁻ – N. Assuming \( \phi = 0.3 \) for the ambient condition, then 0.058 for \( f_{\text{pseudo}} \) can be calculated from Equation (18b). This value would become even lower if there are small amounts of CET. For non-photosynthetic conditions (\( \phi = 0 \)), Equation (18b) gives that \( f_{\text{pseudo}} = 0 \).

Equation (18b) also reveals that surprisingly \( f_{\text{pseudo}} \) does not increase monotonically with increasing \( \phi \) if \( \phi \) goes to a very high value (Figure 5a). The decline of \( f_{\text{pseudo}} \) beyond a threshold \( \phi \) occurs only in the presence of \( \alpha_G \); and the higher is \( \alpha_G \), the lower is the threshold \( \phi \). However, \( f_{\text{pseudo}} \) always increases monotonically with increasing \( \phi \) in the absence of \( \alpha_G \), regardless of values of \( \alpha_S \). All these responses are because \( \alpha_G \), not \( \alpha_S \), causes a decrease in CO₂-compensation point, and this positive impact on \( \alpha \) becomes increasingly important under high photosynthesis conditions (high \( \phi \) values) that mathematically require a lower \( f_{\text{pseudo}} \) to enable the left and right sides of Equation (18a) in balance. For the same reason, although \( f_{\text{pseudo}} \) generally increases with increasing \( \alpha_G \) or \( \alpha_S \), its response to \( \alpha_G \) is stronger than to \( \alpha_S \) at a low \( \phi \) (Figure 5b), is comparable at an intermediate \( \phi \) corresponding to ambient-air conditions (Figure 5c), and is weaker than to \( \alpha_S \) at a high \( \phi \) (Figure 5d).
It is noteworthy that \( f_{\text{pseudo}} \) calculated from Equation (18b) refers to the electron fraction responsible for supporting N assimilation only as a result of amino acid export from the photorespiratory pathway.

Therefore, the calculated \( f_{\text{pseudo}} \) depends on the amount of photorespiration as shown in Figure 5. In contrast, \( f_{\text{pseudo}} \) as one parameter in the model of Yin et al. (2004) for electron-transport-limited conditions lumps electron requirements for: (i) N assimilation of both via the photorespiratory pathway and not via this pathway and (ii) metabolic processes other than N assimilation that utilize chloroplastic electrons. As stated earlier, \( f_{\text{pseudo}} \) of 0.010 was estimated from the assimilatory quotient for nonphotorespiratory conditions. The higher \( f_{\text{pseudo}} \) estimated from the assimilatory quotient suggests that either not all nitrogen is assimilated via the photorespiratory pathway or/and processes other than N assimilation consumes chloroplastic electrons. Furthermore, the model of Busch et al. (2018) only applies to the case where it is NO\(_3\)-N that enters the leaf. However, it cannot be ruled out that nitrogen enters the leaf in the form of NH\(_4\)-N (Eichelmann, Oja, Peterson, & Laisk, 2011), and for such a case the stoichiometric coefficients of Equation (15a) has to be re-formulated whereas the model of Yin et al. (2004) remains the same but with a lower value of \( f_{\text{pseudo}}\).

5 | COUPLING WITH THE MESOPHYLL CO\(_2\)-DIFFUSION MODEL

While \( C_i \) (intercellular CO\(_2\) partial pressure) was used in the FvCB model at the time when this model was initially published, it is increasingly recognized that \( C_c \) should be used because the resistance of \( C_0 \) diffusion from intercellular-air spaces (IAS) to the chloroplast stroma of mesophyll cells cannot be ignored. This resistance is called mesophyll resistance \( r_m \), while its inverse is called mesophyll conductance \( g_m \), and has long been defined as such that the \( C_i \)-to-\( C_c \) gradient can be expressed (von Caemmerer & Evans, 1991):

\[
C_c = C_i - A r_m = C_i - A / g_m \tag{19a}
\]

Because \( A \) is the difference between carboxylation rate \( \left( V_c \right) \) and the rate of \( \text{CO}_2 \) release from photorespiration \( F = 0.5 V_o \) or \( 0.5(1 - \alpha_G) + \alpha_T V_o \) and respiration \( (V_R) \), Equation (19a) implicitly assumes that the \( \text{CO}_2 \) coming from IAS and the \( \text{CO}_2 \) released from (photo)respiration experience the same resistance \( r_m \). To diffuse to Rubisco, the \( \text{CO}_2 \) coming from IAS has to experience the resistance across mesophyll cell wall and plasma membrane \( r_\text{wp} \) as well as the resistance across the chloroplast envelope and inside the chloroplast stroma \( r_\text{c} \). In contrast, the (photo)respiratory \( \text{CO}_2 \) first enters the cytosol after being released by the mitochondria and therefore, if to be re-fixed by

**FIGURE 5** Equation (18b) calculated fraction of the total PSI electron flux as pseudocyclic electron transport \( f_{\text{pseudo}} \) for supporting nitrogen assimilation associated with the photorespiratory pathway (assuming a negligible cyclic electron transport), (a) as a function of the oxygenation to carboxylation ratio \( \phi \) when \( \alpha_G \) (fraction of glycolate carbon leaving the pathway as glycine) = 0.1 and \( \alpha_S \) (fraction of glycolate carbon leaving the pathway as serine) = 0.15, and (b–d) as a function of \( \alpha_G \) when \( \alpha_S \) is set to 0 (filled symbols) or of \( \alpha_S \) when \( \alpha_G \) is set to 0 (open symbols) when \( \phi \) is fixed at 0.05, 0.30 and 0.60, respectively.
Rubisco, may experience \( r_{ch} \) only. For this reason, Tholen, Ethier, Genty, Pepin, and Zhu (2012) presented a resistance model that explicitly differentiates the resistances faced by the two different sources of CO\(_2\):

\[
C_C = C_i - Ar_m - (F + R_d) r_{ch}
\]

(19b)

where \( r_m = r_{wp} + r_{ch} \). If the chloroplast resistance is negligible \((r_{ch} \rightarrow 0)\), then Equation (19b) becomes Equation (19a). Clearly, the earlier model, Equation (19a), also assumes that the chloroplast resistance is negligible so that only \( r_{wp} \) forms the mesophyll resistance as if RuBP carboxylation and (photo)respiratory CO\(_2\) production occur in the same compartment.

Equations (19a) and (19b) have been considered as two basic scenarios for CO\(_2\) diffusion path in \( C_3 \) leaves (von Caemmerer, 2013). However, the delivery of CO\(_2\) to Rubisco depends not only on simple physical resistance components but also on the intracellular arrangement of organelles that consume and produce CO\(_2\). Yin and Struik (2017b) considered six scenarios of the arrangement of mitochondria and chloroplasts, and came up with a generic model:

\[
C_C = C_i - Ar_m - (1 - k_i)(F + R_d) r_{ch}
\]

(19c)

where \( k \) is the fraction of mitochondria that locate closely behind chloroplasts in the inner cytosol (i.e., the area between chloroplasts and vacuole); then \( 1 - k \) is the fraction of mitochondria that locate in the outer cytosol, the area between the plasma membrane and chloroplasts, and \( k \) is a factor allowing the fraction of (photo)respiratory CO\(_2\) in the inner cytosol dependent not only on \( k \) but also on chloroplast gaps and the cytosol resistance. So, the term \( k_i \) can be regarded as the fraction of (photo)respiratory CO\(_2\) in the inner cytosol. If \( k_i = 1 \), Equation (19c) becomes Equation (19a), meaning that Equation (19a) also implicitly assumes that mitochondria exclusively lie behind chloroplasts that form a continuum without a gap as observed for rice (Sage & Sage, 2009). If \( k_i = 0 \), Equation (19c) becomes Equation (19b), meaning that Equation (19b) applies to the case where mitochondria exclusively lie in the outer cytosol \((k = 0)\) with chloroplasts that form a continuum without a gap \((k = 1)\) or to the case where there are chloroplast gaps but little cytosol resistance \((k = 0)\), and thus photosynthetic CO\(_2\) anywhere in the cytosol is completely mixed, independent of where the mitochondria are located. Equations (19a) and (19b) represent two extremes, and the reality should be somewhere in-between \((0 < k_i < 1)\). Equation (19c) can be further simplified to:

\[
C_C = C_i - [A + m(F + R_d)] r_m
\]

(19d)

where parameter \( m \) lumps several parameters: \( m = (1 - \lambda k) r_{wp} / r_m \) and \( 0 \leq m \leq 1 \) (also see Ubiena et al., 2019).

Combining the above forms of equations for \( r_m \) or \( g_m \), with the (extended) FvCB model and solving for \( A \) can lead to an expression that models \( A \) as a function of \( C_i \) (Ethier & Livingston, 2004; von Caemmerer, 2013; von Caemmerer, Evans, Hudson, & Andrews, 1994; Yin & Struik, 2017b). Here, based on the model of Yin, van der Putten, Belay, and Struik (2020), we present a form that covers all possibilities:

\[
A = \frac{-b' \pm \sqrt{b'^2 - 4a'c'}}{2a'}
\]

(20)

where

\[
d' = x_2 + \Gamma_{GT} (1 - m) - \delta (C_i + x_2)
\]

\[
b' = m(R_x x_2 + \Gamma_{GT} x_1) - [x_2 + \Gamma_{GT} (1 - m)][x_1 - R_d] - [C_i + x_2][g_m(x_2 + \Gamma_{GT}) + \delta (C_i - R_d)]
\]

\[
c' = -m(R_x x_2 + \Gamma_{GT} x_1) (x_1 - R_d) + [g_m(x_2 + \Gamma_{GT}) + \delta (C_i - R_d)][x_1 (C_i - \Gamma_{GT}) - R_d (C_i + x_2)]
\]

and

\[
x_1 = \left\{ \begin{array}{ll}
V_{\text{max}} & \text{for } W_C - \text{limited} \\
J/4 & \text{for } W_j - \text{limited} \\
3T_p & \text{for } W_p - \text{limited}
\end{array} \right.
\]

\[
x_2 = \left\{ \begin{array}{ll}
K_mC(1 + O/K_m) & \text{for } W_C - \text{limited} \\
(1 + 2\alpha_\Gamma - \alpha_\Gamma + \alpha x_2)(2\Gamma_\ast) & \text{for } W_j - \text{limited} \\
-(1 + 3\alpha_\Gamma + 6\alpha x_2 + 4\alpha_\ast)\Gamma_\ast & \text{for } W_p - \text{limited}
\end{array} \right.
\]

Whether or not \( g_m \) is variable is still under debate (Evans, 2021); in particular, Gu and Sun (2014) showed that the variable \( g_m \) pattern could be an artefactual response to uncertainties in measurements or in estimating parameters of the FvCB model. But Equation (20) suits for either a constant or a variable \( g_m \) mode. Setting \( \delta = 0 \) would make Equation (20) appropriate the constant \( g_m \) mode (= \( g_{m0} \) of Equation (20)). Setting \( g_{m0} = 0 \), then a positive value of \( \delta \), which defines the carboxylation resistance: mesophyll resistance ratio (Yin et al., 2020), allows the possibility that \( g_m \) is variable, responding to \( C_i \) irradiance, temperature, and \( O_2 \) as reported by, for example, Bernacchi, Portis, Nakano, von Caemmerer, and Long (2002), Flexas et al. (2007) and Yin et al. (2020). In Equation (20), \( \Gamma_{GT} \) is used in several places, instead of the usual \( \Gamma_\ast \), to account for the earlier discussed possible change in CO\(_2\) compensation point due to the carbon exit via glycine and CH\(_2\)-THF from the photosynthetic pathway. It is worthy to note that while the complete form of the equations for \( x_2 \) in case of the \( W_j \)-limitation is given, usually only \( x_2 = 2\Gamma_\ast \) is applied, especially if the model is used to estimate \( g_m \).

The solution to Equation (20) in case of \( W_C \) or \( W_j \) limitations is straightforward (the \( \sqrt{b'^2 - 4a'c'} \) term always taking the – sign). Gu, Pallardy, Tu, Law, and Wullschleger (2010) highlighted the mathematic complication arisen from a negative \( x_2 \) in the case that \( W_p \) limits if the fraction of glycolate carbon not returned to chloroplasts is >0 and suggested a solution to that.
the constant figure can be viewed at wileyonlinelibrary.com]

[Image 47x375 to 288x731]

The calculated difference in net photosynthesis A, using the coupled \( g_m \)-FvCB model, Equation (20), for two hypothetical leaves whose day respiration \( R_d \) is preset as 0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (\( R_d1 \)) and 3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (\( R_d2 \)), respectively. The difference in \( R_d \) of the two leaves is indicated by the horizontal line. The calculation used the algorithm assuming an electron transport limitation for the simplest situation of Equation (20), that is, \( \alpha_C = \alpha_S = \alpha_T = 0, m = 0, \delta = 0 \) (for the constant \( g_m \) scenario). The values used for \( g_m \) were 0.25 (filled symbols) or 0.15 (open symbols) \( \mu \text{mol m}^{-2} \text{s}^{-1} \) bar \(^{-1} \). The calculated fractions of refixation within the mesophyll cell (\( f_{\text{refx,cell}} \)) using Equation (21b) without the term \( r_{\text{sc}} \) (open symbols) or using the formula that \( f_{\text{refx,cell}} = 1 - [A_{R_d1} - A_{R_d2}] / [R_d2 - R_d1] \) (filled symbols). The calculation in (b) assumed that \( g_m = 0.25 \mu \text{mol m}^{-2} \text{s}^{-1} \) bar \(^{-1} \). Other parameter values used for both panels (a) and (b): \( J = 150 \mu \text{mol m}^{-2} \text{s}^{-1} \), and \( \Gamma = 40 \mu \text{bar} \) [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 6 (a) The calculated difference in net photosynthesis A, using the coupled \( g_m \)-FvCB model, Equation (20), for two hypothetical leaves whose day respiration \( R_d \) is preset as 0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (\( R_d1 \)) and 3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (\( R_d2 \)), respectively. The difference in \( R_d \) of the two leaves is indicated by the horizontal line. The calculation used the algorithm assuming an electron transport limitation for the simplest situation of Equation (20), that is, \( \alpha_C = \alpha_S = \alpha_T = 0, m = 0, \delta = 0 \) (for the constant \( g_m \) scenario). The values used for \( g_m \) were 0.25 (filled symbols) or 0.15 (open symbols) \( \mu \text{mol m}^{-2} \text{s}^{-1} \) bar \(^{-1} \). The calculated fractions of refixation within the mesophyll cell (\( f_{\text{refx,cell}} \)) using Equation (21b) without the term \( r_{\text{sc}} \) (open symbols) or using the formula that \( f_{\text{refx,cell}} = 1 - [A_{R_d1} - A_{R_d2}] / [R_d2 - R_d1] \) (filled symbols). The calculation in (b) assumed that \( g_m = 0.25 \mu \text{mol m}^{-2} \text{s}^{-1} \) bar \(^{-1} \). Other parameter values used for both panels (a) and (b): \( J = 150 \mu \text{mol m}^{-2} \text{s}^{-1} \), and \( \Gamma = 40 \mu \text{bar} \) [Colour figure can be viewed at wileyonlinelibrary.com]

The coupled \( g_m \)-FvCB model offers a method to estimate \( g_m \) (and other parameters) by fitting to gas exchange data only from exploring the curvature of A–C\(_i\) curves (Ether & Livingston, 2004). When the coupled model is fitted to combined gas exchange and chlorophyll fluorescence data (Yin & Struik, 2009), it can improve the reliability of the estimates compared with the value of \( g_m \) calculated from the conventional variable J method of Harley, Loreto, Di Marco, and Sharkey (1992). An alternative is using the stable \(^{13}\text{C}\)-isotope discrimination method (Farquhar et al., 1982), which was applied by Evans, Sharkey, Berry, and Farquhar (1986); Evans, von Caemmerer, Setchell, and Hudson (1994) to estimate \( g_m \) (see review by Pons et al., 2009, and the most current model by Busch et al., 2020). But the chlorophyll fluorescence-based methods are more widely used because of the wider availability of the required device, despite the limitations (Evans, 2021). To minimize the influence of these limitations and of basal alternative transport pathways on estimating \( g_m \), van der Putten, Yin, and Struik (2018) demonstrated the importance of calibration using the measurements under nonphotorespiratory conditions. Any calibration method assumes that the fractions for alternative electron pathways are constant between photorespiratory and non-photorespiratory conditions. However, recent reports by Abadie et al. (2016, 2018), Abadie and Tcherkez (2019) and Tcherkez and Limami (2019) suggest that the values of \( \alpha_C \) and \( \alpha_S \), as well as the percentage of phosphoenolpyruvate (PEP) carboxylation and malate production \( (\text{if any}) \), and N-assimilation relative to CO\(_2\)-assimilation may not be constant across various CO\(_2\)/O\(_2\) gas mixtures. Chlorophyll-fluorescence-based methods to estimate \( g_m \) require data that include the measurements under photorespiratory conditions such as at ambient CO\(_2\)/O\(_2\) levels (Yin et al., 2020), whereas the \(^{13}\text{C}\) isotopic method has no such a requirement. On the other hand, estimates of \( g_m \) by the \(^{13}\text{C}\) isotopic method are affected by assumptions made regarding the values of the fractionation factors (Busch et al., 2020; Gu & Sun, 2014; Pons et al., 2009). Thus, chlorophyll-fluorescence and \(^{13}\text{C}\) isotopic methods should be compared, whenever possible, for estimating \( g_m \).

As the chlorophyll-fluorescence-based method relies on the coupled \( g_m \)-FvCB model and the re-assimilation of photorespired CO\(_2\) to estimate \( g_m \), this coupled model should account for the amount of (photo)respired CO\(_2\) that are re-assimilated by Rubisco. For example, let us assume two hypothetical leaves where all parameters are the same except \( R_d \) which is nil for one leaf versus 3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) for the other. One would expect from the C\(_i\)-based model, for example, Equations (16c)–(16e), that A also differs by 3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) between the two leaves. However, the calculation using the coupled model shows that the difference in A was smaller than the difference in \( R_d \) of 3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Figure 6a) because part of CO\(_2\) released by day respiration in the second leaf is re-assimilated by Rubisco, demonstrating that the refixation is implicitly accounted for by the coupled model. The lower is \( g_m \), the harder it is for the (photo)respired CO\(_2\) to escape, and the higher is the proportion of refixation (Figure 6a). The calculated refixation proportion varies little with the assumed \( R_d \) values of the two leaves. In fact, the fraction of (photo)respired CO\(_2\) being refixed \( (f_{\text{refx}}) \) can be calculated directly using the resistance components (Tholen et al., 2012). They proposed an equation for the scenario which Equation (19b) represents. Yin and Struik (2017b) extended the approach to a general equation:

\[
f_{\text{refx}} = \frac{\frac{\delta k}{\Gamma} + \frac{1 - \delta k}{\Gamma}}{\frac{\delta k}{\Gamma} + \frac{1 - \delta k}{\Gamma} + \frac{\delta k}{\Gamma} + \frac{1 - \delta k}{\Gamma}}
\]  

(21a)

where \( r_{\text{sc}} \) is the stomatal resistance to CO\(_2\) diffusion, and \( r_{\text{cx}} \) is the resistance from the carboxylation reaction itself, which can be defined
as: \((C_c + x_2)/x_1\) (von Caemmerer, 2000, 2013) and was similarly as high as \(r_m (=r_{wp} + r_{ch})\) in rice leaves and \(ca 40\%\) higher than \(r_m\) in tomato leaves (Yin et al., 2020). If \(\lambda k = 1\), Equation (21a) is simplified to:

\[
\frac{f_{\text{refix}}}{f_{\text{refix}}} = \frac{r_{sc} + r_{wp} + r_{ch}}{r_{sc} + r_{wp} + r_{ch} + r_{cx}} \quad (21b)
\]

If \(\lambda k = 0\), Equation (21a) becomes Equation (14) of Tholen et al. (2012):

\[
\frac{f_{\text{refix}}}{f_{\text{refix}}} = \frac{r_{sc} + r_{wp}}{r_{sc} + r_{wp} + r_{ch} + r_{cx}} \quad (21c)
\]

It becomes obvious from Equations (21b) and (21c) that leaves having the anatomical structure close to what Equation (19a) describes have a higher \(f_{\text{refix}}\) than leaves having the structure that Equation (19b) describes, and this difference in \(f_{\text{refix}}\) leads to different CO2 compensation points (von Caemmerer, 2013; Yin & Struik, 2017b). As \(r_{sc}\) and \(r_{cx}\) vary in response to CO2, irradiance and other environmental conditions, it follows that the proportion of (photo)respired CO2 being refixed varies with these variables. For example, with an increase of CO2, \(r_{sc} = [C_c + x_2]/x_1\) will increase, and Equations (21b) and (21c) will predict a decrease of \(f_{\text{refix}}\), in line with the expectation that refixation contributes decreasingly to total assimilation with increasing CO2 (Busch, Sage, Cousins, & Sage, 2013). This appears to agree with the result in Figure 6a that with increasing \(C_c\), calculated differences in \(A\) approach the preset difference in \(R_d\).

Refixation can occur both within the mesophyll cell \(f_{\text{refix,cell}}\) and via the IAS \(f_{\text{refix,ias}}\), which together constitute the total refixation \(f_{\text{refix}} = f_{\text{refix,cell}} + f_{\text{refix,ias}}\) (Busch et al., 2013). In fact, the refixation of \(R_d\) illustrated in the above example using the coupled model with \(C_c\) as input (Figure 6a) actually refers to \(f_{\text{refix,cell}}\) and \(f_{\text{refix,ias}}\), and can also be directly calculated from resistance components and Yin et al. (2020) showed that if the term \(r_{sc}\) is removed, Equations (21a)–(21c) become equivalent equations to calculate \(f_{\text{refix,cell}}\). They showed that \(f_{\text{refix,cell}}\) generally dominates and leaves having the anatomical structure that Equation (19a) describes have a higher \(f_{\text{refix,cell}}\) and thus a higher \(f_{\text{refix}}\) than leaves having the structure that Equation (19b) describes despite the latter leaves having a higher \(f_{\text{refix,ias}}\). They quantitatively showed that for rice leaves where \(\lambda k = 1\), the estimated \(f_{\text{refix}}\) was often high (≥0.5). These ideas have been exploited by synthetic biology approaches that engineer photosynthetic bypasses to relocate the photosynthetic CO2 release from mitochondria to chloroplasts (Kebeish et al., 2007; Shen et al., 2019; South, Cavanagh, Liu, & Ort, 2019; Figure 1a). The bypasses may be effective in increasing CO2 assimilation for leaves described by Equation (19b) under low CO2 conditions. However, values calculated based on resistance components represent the gross refixation of (photo)respired CO2, which is higher than the refixation reflected by results of the coupled model (Figure 6b). This suggests (photo)respired CO2 or bypassed CO2 decrease the chance of CO2 coming from IAS being assimilated; so, the net benefit of refixation must be smaller than what Equations (21a)–(21c) predict. But the bypass-associated saving of electrons and ATP that otherwise are consumed by the ammonia recycling (Figure 1a) provides more advantages (von Caemmerer, 2013).

## 6 | THE C4 FORM OF THE MODEL

CO2 diffusion is also important for C4 photosynthesis because its CO2-concentrating mechanism (CCM) relies on the effective coordination of a series of diffusional processes and biochemical reactions. In the vast majority of terrestrial C4 species, this mechanism is achieved through the coordinated functioning via the Kranz structure involving mesophyll (M) and bundle-sheath (BS) cells (Hatch, 1987). CO2 initially diffuses to the M cytosol and is converted to HCO3−, which is fixed by PEP carboxylase (PEPc) into C4 acids. The C4 acids travel to the BS cells, where they are decarboxylated and the released CO2 is re-fixed by Rubisco exclusively localized in BS chloroplasts. The \(k_m\) of PEPc is lower, and its maximum carboxylation rate is generally higher, than that of Rubisco. This will elevate the CO2 partial pressure in the BS compartment, despite some leakage of CO2 from BS back to M cells, which effectively suppresses photorespiration. Because Rubisco is operated in high-CO2 compartments, kinetic constants of C4 Rubisco differ from those of C3 Rubisco (Boyd, Gandin, & Cousins, 2015; Cousins, Ghannoum, von Caemmerer, & Badger, 2010; Sharwood, Ghannoum, Kapralov, Gunn, & Whitney, 2016), which together with the CCM per se, underlies the high photosynthetic nitrogen use efficiency of C4 plants (Ghannoum et al., 2005). C4 species are traditionally classified into three subtypes according to the decarboxylation enzymes, thus also decarboxylation sites: NADP-malic enzyme (ME) in chloroplasts, NAD-ME in mitochondria, and PEP-carboxykinase (CK) in the cytosol (Hatch, 1987). However, more recent opinions (e.g., Furbank, 2011; Wang, Brautigam, Weber, & Zhu, 2014; Yin & Struik, 2018) suggest that C4 species often have a mixed decarboxylation pathway, where one enzyme acts as the main decarboxylating enzyme alongside the others.

### 6.1 The standard model for C4 photosynthesis

Berry and Farquhar (1978) presented a first model for C4 photosynthesis, which covered the CCM and the basis of high nitrogen use efficiency. The leakiness \(\phi_h\), as the ratio of the CO2 retro-leakage (L) to the rate of PEP carboxylation \(V_h\), was introduced in a model that included carbon isotope discrimination (Farquhar, 1983). Based on these earlier models, von Caemmerer and Furbank (1999) described a model, which is now considered as the standard C4 model that predicts net CO2-assimilation rate \(A\) as a function of mesophyll cytosol CO2 partial pressure \(C_m\). Several equations relevant to the C4 photosynthesis are:

\[
A = V_h - L - R_m \quad (22a)
\]
the rate of CO₂ leakage: \( L = g_{\text{bs}}(C_c - C_m) \) (22b)

the level of O₂ in the BS cell: \( Q_c = a_{\text{O}_2}A/(\omega_{\text{C}}g_{\text{bs}}) + O_m \) (22c)

the rate of PEP carboxylation: \( V_{\text{p}} = \frac{c_m V_{\text{max}}}{C_m + K_p} \phi = \frac{xJ_{\text{atp}}}{\phi} \) (22d)

where \( R_m \) is the respiration in the M cell (usually assumed to be 0.5\( R_{\text{a}} \)), \( g_{\text{bs}} \) is bundle-sheath conductance, \( C_c \) and \( O_c \) are the partial pressure of CO₂ and O₂ at the active sites of Rubisco, respectively, \( a_{\text{O}_2} \) is the fraction of O₂ evolution (or of PSII) in the BS cells, \( u_{\text{C}} \), is the coefficient that lumps diffusivities of O₂ and CO₂ in water and their respective Henry constants, \( O_m \) is the partial pressure of O₂ at the mesophyll cytosol, \( K_p \) and \( V_{\text{max}} \) are the Michaelis–Menten constant and the maximum carboxylation rate of PEPc, respectively, \( x \) is the fraction of ATP consumed by the CCM cycle, \( \phi \) is the mol chloroplastic ATP required for the CCM cycle, and \( J_{\text{atp}} \) is the rate of ATP production by chloroplastic electron transport. The original model of von Caemmerer and Furbank (1999) did not use \( J_{\text{atp}} \), but the rate of electron transport (\( J \)). Because it is ATP, not electrons, that are allocated between the CCM cycle and the Calvin–Benson cycle, according to the predefined stoichiometric fraction \( x \), it is more appropriate to use \( J_{\text{atp}} \) in Equation (22d) (Yin et al., 2011) and \( J_{\text{atp}} \) can be linked with electron transport rate via the ATP production factor \( z \) (see Equation (9e)). Equation (22d) for \( V_{\text{p}} \) contains either the PEPc activity-limited rate or the electron transport-limited rate, in analogy to the equations for \( V_c \).

The rate of CO₂-assimilation (\( A \)) based on \( V_c \) is the same for \( C_3 \) photosynthesis and can be collectively expressed as:

\[
A = \frac{(C_c - \gamma_i O_i) x_1}{C_c + O_c x_2 + x_3} - R_d
\]  

where \( \gamma_i = 0.5/S_{\text{LCO}_2} \), and \( x_1 = V_{\text{max}} x_2 = K_{\text{mg}}/K_{\text{mg}i} \), and \( x_3 = K_{\text{mg}} \) for the Rubisco-limited rate. For the RuBP regeneration-limited rate, \( x_1 = (1 - xJ_{\text{atp}}/3, x_2 = 7r/3, \) and \( x_3 = 0 \) if ATP supply is limiting, von Caemmerer and Furbank (1999) provided a solution to the combined Equations (22a)–(22e) that expresses \( A \) as a quadratic function of \( C_m \). As \( C_m \) is unknown generally, one may add an equation \( (C_m = C_i - A/ g_{\text{bs}}) \) in order to express \( A \) as a function of \( C_i \), Yin et al. (2011) provided the analytical solution to these, which became cubic if PEPc activity limits \( V_{\text{p}} \).

Unlike in \( C_3 \) leaves, the initial carbon fixation in \( C_4 \) leaves is catalysed by PEPc in the cytosol and therefore \( g_{\text{bs}} \) does not involve CO₂ diffusion from the cytosol to the chloroplast. Accordingly, estimates of \( g_{\text{bs}} \) in \( C_4 \) leaves are somewhat lower than those in \( C_3 \) leaves (e.g. Barbour, Evans, Simonin, & von Caemmerer, 2016), meaning \( g_{\text{bs}} \) appears to be less limiting to \( C_4 \) photosynthesis as it is to \( C_3 \) photosynthesis. However, \( g_{\text{bs}} \), which determines the amount of CO₂ leakage (see Equation (22b)), is fundamentally important for the CCM, and thus, for determining \( C_4 \) photosynthesis. So far there is no method that can directly estimate \( g_{\text{bs}} \). Its indirect estimate, mostly based on model fitting to gas exchange data (He & Edwards, 1996) and sometimes combined with chlorophyll fluorescence or \(^{13}\text{C} \) discrimination measurements, suggests a value between 1.0 and 10.0 mmol m\(^{-2} \) s\(^{-1} \) bar\(^{-1} \) (Yin et al., 2011), ca two- or three-order of magnitude smaller than \( g_{\text{bs}} \). Like \( g_{\text{bs}} \), \( g_{\text{bs}} \) varies with leaf age or N content (Yin et al., 2011), temperature (Alonso-Cantabrana et al., 2018; Kiirats, Lea, Franceschi, & Edwards, 2002; Yin, van der Putten, Driever, & Struik, 2016), and growth light conditions (Bellasio & Griffiths, 2014; Kromdijk, Griffiths, & Schepers, 2010; Ubierna, Sun, Kramer, & Cousins, 2013). Danila et al. (2021) showed that suberization of the BS lamellae is required for a low \( g_{\text{bs}} \) to minimize leakage. As \( g_{\text{bs}} \) is a lumped model parameter, its value may also depend on other anatomical characteristics (like BS cell wall thickness, plasmodemata density, bundle sheath surface-to-leaf area ratio, inter-vein spacing, sheath layers) as well as biochemical characteristics (like the location of decarboxylation). Further research is needed to clarify how these characteristics influence \( g_{\text{bs}} \).

### 6.2 Energetic aspects of \( C_4 \) photosynthesis

Although energy production or consumption can be cell-type specific (Yin & Struik, 2018), the model of von Caemmerer and Furbank (1999) for \( C_4 \) photosynthesis assumed that energy is shared between M and BS cells, and used \( x \) to allocate \( J_{\text{atp}} \) to the CCM cycle (see Equation (22d)) and thus, \( 1 - x \) to the Calvin–Benson cycle (see Equation (22e)). The default value for \( x \) is 0.4, arising from \( \phi/(\phi + 3) \), where \( \phi \) and 3 are ATP required for the CCM cycle and the Calvin–Benson cycle, respectively. For most \( C_4 \) species, \( \phi = 2 \); so \( x = 0.4 \) (von Caemmerer & Furbank, 1999; but see discussion later). Thus, the RuBP regeneration-limited form of Equation (22e) is expressed in terms of ATP supply. As with the \( C_3 \) model, it is metabolically important to keep ATP and NADPH in balance (Foyer et al., 2012; Kramer & Evans, 2011); so, one may argue that ATP and NADPH co-determine the RuBP regeneration. For Equation (22e) if NADPH supply is limiting, one can write, according to Equation (9a), that \( x_1 = (1 - f_{\text{psuedo}})/\left(1 - f_{\text{psuedo}}\right)J_{2/4} \), \( x_2 = 2r \), and \( x_3 = 0 \). Based on this NADPH-determined model, Yin and Struik (2012) stated that the photosynthetic quantum yield models for \( C_4 \) photosynthesis are the same as for \( C_3 \) photosynthesis, that is, Equation (12a) or Equation (12e), reflecting that there is no net NADPH requirement for the \( C_4 \) cycle (but again, see discussion later). Similarly, Equations (13a), (13b) and (10b) for calculating \( f_{\text{psuedo}} \), \( f_{\text{psuedo}} \), and \( p_2 \), respectively, also suit for \( C_4 \) photosynthesis.

As discussed earlier for \( C_3 \) photosynthesis, one can rely on the unique feature of the NADPH-dependent equation for quantum yield to infer possible values of \( f_{\text{psuedo}} \) from measurements on quantum yields. \( \phi_{\text{CELL}} \) of \( C_4 \) photosynthesis (virtually without photosupression) is ca 0.069 (Björkman & Demmig1987), considerably lower than its counterpart value of \( C_3 \) photosynthesis in the absence of photosupression. Using Equation (13a), Yin and Struik (2012) solved \( f_{\text{psuedo}} \), which was ca 0.45, considerably higher than the \( f_{\text{psuedo}} \) of \( C_4 \) photosynthesis. This suggests that CET is essential for \( C_4 \) photosynthesis, required for generating ATP required for the operation of the CCM cycle.
Once \( f_{\text{cyc}} \) is known, \( \rho_2 \) can be calculated from Equation (10b). The obtained \( \rho_2 \) is \( \rho_2 \approx 0.4 \) (Yin & Struik, 2012). This differs from Equation (4), where the energy partitioning factor of 0.5 is also used for \( C_4 \) photosynthesis (von Caemmerer, 2000, 2013; von Caemmerer & Furbank, 1999). When \( f_{\text{cyc}} \) is known, \( f_{\text{pseudo}} \) can also be estimated from the assimilatory quotient (see Equation (13b)) and is \( \rho \approx 0.07 \) (Yin & Struik, 2012).

The equation equivalent to Equation (8) for \( C_3 \) photosynthesis, for the fraction of \( \text{LET} \) that keeps \( \text{NADPH} \) and \( \text{ATP} \) balance as required by \( C_4 \) metabolism, can be formulated as (see Yin & Struik, 2012 for its derivation):

\[
1 - f_{\text{cyc}} - f_{\text{pseudo}} = \frac{(4C_c + 8\gamma_c, \gamma_c)(2 + f_Q - f_{\text{cyc}})(1-x)}{h(3C_c + 2\gamma_c, \gamma_c)(1 + x\phi_h)} \tag{23a}
\]

where \( \phi_h \) is leakiness (0 \( \leq \phi_h \leq 1 \)). Compared with Equation (8), Equation (23a) has an extra factor \((1 - x)/(1 + x\phi_h)\). This suggests that compared with \( C_4 \) photosynthesis, the \( \text{LET} \) of \( C_4 \) photosynthesis is decreased at least by this factor to accommodate the required increase in CET. One can solve Equation (23a) for leakiness:

\[
\phi_h = \frac{(4C_c + 8\gamma_c, \gamma_c)(2 + f_Q - f_{\text{cyc}})(1-x)}{h(3C_c + 2\gamma_c, \gamma_c)(1 - f_{\text{cyc}} - f_{\text{pseudo}})x} \cdot \frac{1}{x} \tag{23b}
\]

Given the above indicative values of \( f_{\text{cyc}} \) and \( f_{\text{pseudo}} \) based on quantum yield data, one can use Equation (23b) to explore likely values of uncertain parameters \( f_Q \) and \( h \) that can give a realistic estimate of leakiness. Using either obligatory or no operation of the \( Q \) cycle \((f_Q = 1 \) or 0) and three likely values of \( h \) (3, 4 and 4.67, see earlier), Yin and Struik (2012) showed that only the combination that \( f_Q = 1 \) and \( h = 4 \) can give a realistic value of \( \phi_h \) (Figure 7). The obligatory \( Q \) cycle has long been recognized for \( C_4 \) photosynthesis (Furbank, Jenkins, & Hatch, 1990). But whether the \( H^+:\text{ATP} \) ratio \((h) \) is 3, 4 or 4.67 is uncertain. The model results shown in Figure 7 support thermodynamic experiments (Petersen et al., 2012; Steimmler et al., 2008) showing that \( h \) is 4.

The model discussed so far, for both \( C_3 \) and \( C_4 \) photosynthesis, assumes that CET, when combined with the \( Q \) cycle, generates two \( H^+ \) per electron (Figure 2). However, CET may follow the NAD(P)H dehydrogenase (NDH)-dependent pathway (Ishikawa et al., 2016; Strand, Fisher, & Kramer, 2017; Yamar, Sakata, Suzuki, Shikani, & Makino, 2011). When this pathway is operating, CET generates four \( H^+ \) per electron and Kramer and Evans (2011) indicated that very likely this pathway is active in \( C_4 \) plants. Let \( f_{\text{NDH}} \) be the fraction of CET that follows the NDH-dependent pathway. Then, the ATP production factor \( z \) as in Equation (9e) for such a case is (Yin & Struik, 2021):

\[
z = \frac{2 + f_Q - f_{\text{cyc}}(1 - 2f_{\text{NDH}})}{n(1 - f_{\text{cyc}})} \tag{24a}
\]

Equation (24a) becomes Equation (9e) if \( f_{\text{NDH}} = 0 \). Again, the uncertainty with regard to the value of \( f_{\text{NDH}} \) has no impact on the model for the NADPH-dependent quantum yield, so the above estimation of \( f_{\text{cyc}} \) using the NADPH-dependent quantum yield model is still valid. Yin and Struik (2012) showed that this highly efficient \( H^+ \)-translocating pathway of CET cannot be obligatory as this would result in unrealistic high estimates of leakiness. Here we try to assess the extent to which CET should be this highly efficient pathway if \( h = 4.67 \) (14/3, Seelert et al., 2000; again recently, Hahn et al., 2018). This can be achieved by equating Equation (24a) with \( h = 14/3 \) to Equation (9e) with \( h = 4 \), and then solving for \( f_{\text{NDH}} \):

\[
f_{\text{NDH}} = \frac{2 + f_Q - f_{\text{cyc}}}{12f_{\text{cyc}}} \tag{24b}
\]

This gives that \( f_{\text{NDH}} \) is \( \approx 0.47 \) if \( f_Q = 1 \) and \( f_{\text{cyc}} = 0.45 \), meaning that about half of the total CET have to follow this highly efficient pathway in order to meet the high ATP requirement in \( C_4 \) photosynthesis, if the \( H^+ \) requirement per ATP synthesis is as high as 4.67. This suggests a method to estimate \( f_{\text{NDH}} \) as this parameter has been estimated only by trial and error (Bellasio & Farquhar, 2019).

Combining \( h = 4 \) and \( f_{\text{NDH}} = 0 \) or \( h = 4.67 \) and \( f_{\text{NDH}} = 0.47 \) suggests that the ATP production factor per PSII electron transport \((z)\) is \( \approx 1.16 \). This differs from the standard \( C_4 \) model of von Caemmerer and Furbank (1999), in which \( J_{\text{app}} \) is set to equal PSII electron transport rate. The standard model assumes: (i) the absence of CET and (ii) \( h = 3 \). Equation (9e) suggests that these assumptions combined with an obligatory \( Q \) cycle make \( z = 1 \).
6.3 Accommodating the C₄ species mixed with PEP-CK

It is important to point out that the above results of energetics are valid only for NADP-ME or NAD-ME subtypes of C₄ photosynthesis, although the standard model has been wrongly applied in some reports to the PEP-CK subtype. As stated earlier, the value of 0.4 for $x$ stems from that the parameter $\varphi$ in Equation (22d) is 2, referring to two mol ATP required per CCM cycle for regenerating PEP by pyruvate phosphate dikinase (PPDK) in the M cell (Hatch, 1987; Kanai & Edwards, 1999). This high ATP requirement is reflected in measured quantum yields in species of the malic-enzyme subtypes, from which the model derived $f_{\text{cyc}}$ was high (ca 0.45). In the PEP-CK subtype, however, part of the oxaloacetates produced by the initial PEP carboxylation step move to and are decarboxylated in the BS cytosol by PEP-CK (Hatch, 1987). This decarboxylation reaction also generates PEP (requiring only one molecule of ATP per reaction), thereby partly bypassing the expensive step of PEP regeneration by PPDK. The remaining oxaloacetates are reduced to malate in the M cells, which move to and are decarboxylated in BS mitochondria. This decarboxylation also releases NADH, which drives mitochondrial electron transport to provide ATP for fuelling PEP-CK possibly (Kanai & Edwards, 1999), thereby further decreasing the chloroplastic ATP requirement. Given that the pure PEP-CK type hardly exists in nature and species having PEP-CK are often mixed with other decarboxylation types (e.g., Kumarathunge et al., 2019). The model extensions reviewed here are hardly meant to replace the canonical FvCB model for that, but more to provide tools for analysing uncertainties and better understanding underlying physiology of photosynthesis. From our review in this context, we can make the following summary points:

1. Relative to the ATP-determined form, the extended NADPH-determined form for electron transport-limited rate has fewer uncertain parameters and is yet related to the fraction for CET ($f_{\text{cyc}}$). This singular feature of the model allows $f_{\text{cyc}}$ to be first estimated from easily measured quantum yield for photosynthesis and quantum yield for photosystem electron transport. The estimated $f_{\text{cyc}}$ is negligible (ca 0.06) for C₃ photosynthesis vs ca 0.45–0.50 for malic-enzyme subtypes of C₄ photosynthesis. The NADPH-determined form also has an advantage in modelling C₄ photosynthesis involving decarboxylation by PEP-CK, which requires additional NADPH, a lower ATP:NADPH ratio and probably a lower $f_{\text{cyc}}$, than the malic-enzyme subtypes.

2. Because of such a difference in $f_{\text{cyc}}$, the factor for excitation partitioning to PSII ($\psi_Q$) was ca 0.5 or slightly higher for C₃ photosynthesis, but ca 0.4 for malic-enzyme subtypes of C₄ photosynthesis. This differs from the canonical FvCB model, where 0.5 is always set for both C₃ and C₄ photosynthesis models.

3. If $f_{\text{cyc}}$ is known, one can also estimate $f_{\text{pseudo}}$, based on the assimilatory quotient (see Equation (13b)), and further infer values for uncertain parameters $I_Q$ and $h$ in view of the ATP:NADPH ratio as required by metabolism. The most likely values are: $I_Q = 1$ combined with $h = 4$ for C₄ plants, and with $h = 4.00$ or 4.67 for C₃ plants. If $h$ is 4.67 for C₄ plants, then ca 50% of CET must follow the NDH-dependent pathway in the malic-enzyme subtypes of C₄ plants. The stoichiometric coefficients ($I_Q = 0$ and $h = 3$) assumed in the ATP-limited form of the canonical C₃ model (Equation (3b)) and of the standard C₄ model are obsolete.

4. The TPU limitation is commonly ignored in modelling C₄ photosynthesis probably because it is hard to identify this limitation from its

These equations have taken into account the required balance of NH₂-groups between M and BS cells. The analysis of Yin and Struijk (2021) suggested that $0 \leq a \leq 0.36 \sim 0.40$, and if $a = 0$, the model returns to the equations discussed earlier for the malic-enzyme subtypes. The model predicts that the additional cost with a mol NADPH requirement per mol CO₂ assimilated is overcompensated by the decreased chloroplastic ATP requirement for the CCM cycle, thereby predicting a higher $\varphi_{\text{CO₂}}$ in species involving the PEP-CK activity. However, the observed little advantage in $\varphi_{\text{CO₂}}$ of the PEP-CK over the NADP-ME species (Ehleringer & Pearcy, 1983) suggests the need of more studies to understand whether the energetic advantages are cancelled out by leakiness in the PEP-CK types.

7 Conclusions and Remarks

The FvCB model has been proven successful in most cases in fitting response curves for predicting photosynthetic rates (e.g., Kumarathunge et al., 2019). The model extensions reviewed here are hardly meant to replace the canonical FvCB model for that, but more to provide tools for analysing uncertainties and better understanding underlying physiology of photosynthesis. From our review in this context, we can make the following summary points:

1. Relative to the ATP-determined form, the extended NADPH-determined form for electron transport-limited rate has fewer uncertain parameters and is yet related to the fraction for CET ($f_{\text{cyc}}$). This singular feature of the model allows $f_{\text{cyc}}$ to be first estimated from easily measured quantum yield for photosynthesis and quantum yield for photosystem electron transport. The estimated $f_{\text{cyc}}$ is negligible (ca 0.06) for C₃ photosynthesis vs ca 0.45–0.50 for malic-enzyme subtypes of C₄ photosynthesis. The NADPH-determined form also has an advantage in modelling C₄ photosynthesis involving decarboxylation by PEP-CK, which requires additional NADPH, a lower ATP:NADPH ratio and probably a lower $f_{\text{cyc}}$, than the malic-enzyme subtypes.

2. Because of such a difference in $f_{\text{cyc}}$, the factor for excitation partitioning to PSII ($\psi_Q$) was ca 0.5 or slightly higher for C₃ photosynthesis, but ca 0.4 for malic-enzyme subtypes of C₄ photosynthesis. This differs from the canonical FvCB model, where 0.5 is always set for both C₃ and C₄ photosynthesis models.

3. If $f_{\text{cyc}}$ is known, one can also estimate $f_{\text{pseudo}}$, based on the assimilatory quotient (see Equation (13b)), and further infer values for uncertain parameters $I_Q$ and $h$ in view of the ATP:NADPH ratio as required by metabolism. The most likely values are: $I_Q = 1$ combined with $h = 4$ for C₄ plants, and with $h = 4.00$ or 4.67 for C₃ plants. If $h$ is 4.67 for C₄ plants, then ca 50% of CET must follow the NDH-dependent pathway in the malic-enzyme subtypes of C₄ plants. The stoichiometric coefficients ($I_Q = 0$ and $h = 3$) assumed in the ATP-limited form of the canonical C₃ model (Equation (3b)) and of the standard C₄ model are obsolete.

4. The TPU limitation is commonly ignored in modelling C₄ photosynthesis probably because it is hard to identify this limitation from its
A–C1 curves. While the extension of the canonical FvCB model to account for this limitation to C3 photosynthesis in relation to the glycine export from the photorespiratory pathway has long been made, it appears now that assuming serine (rather than glycine) to exit from the pathway is more likely and internally consistent with regard to the CO2 compensation point. However, this notion may change as we find out more about the nature of carbon export as CH2-THF.

5. Under TPU limited conditions plants can increase CO2 uptake, by serine, glycine, or CH2-THF exit from the photosynthetic pathway and associated de novo nitrogen assimilation or C4 metabolism in leaves of C3 plants. However, there exists nitrogen assimilation not associated with the photosynthetic pathway, especially for low-photorespiration situations as occurring in C4 plants or in C3 plants under high CO2/low O2 conditions.

6. Loss as a result of photorespiration in C3 plants is lower than the commonly suggested value, owing to: (i) glycine, serine and CH2-THF exports, and (ii) significant refixation of (photo)respired CO2 both within mesophyll cells and via IAS. On the other hand, (photo)respired CO2 release decreases the chance of CO2 coming from IAS being assimilated. It is this net refixation of the (photo)respired CO2 that is taken into account by the coupled CO2-diffusion and FvCB model.

This review did not discuss the C3–C4 intermediate photosynthesis, for which von Caemmerer (2000) outlined a modelling framework. We also hardly discussed modelling photosynthetic temperature response (see Bernacchi et al., 2013), but focused on photosynthetic CO2- and light-responses. One may be surprised to notice that Equations (4) and (11) for modelling the light-response of electron transport are still empirical. However, Farquhar and von Caemmerer (1981) presented some mechanistic basis for using these simple equations. Harbinson and Yin (2017) reported a mechanistic but more complex equation for the irradiance response of PSI electron transport rate. The essence of the FvCB model is its simplicity while capturing the most important contributing mechanisms of photosynthesis (Farquhar et al., 2001). This feature is maintained in the extended models as all the equations we reviewed are analytical, and users can easily implement them for thought experiments to explore changes of photosynthetic pathways. The simplicity means that the models are for steady-state photosynthesis. Excellent, more detailed models for photosynthesis under either steady-state or fluctuating conditions and for the photosynthetic acclimation to growth environment are all omitted in this review, despite their high relevance for photosynthesis in field environments.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable as no datasets were generated during writing this review article.

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REFERENCES
Abadie, C., Bathellier, C., & Tcherkez, G. (2018). Carbon allocation to major metabolites in illuminated leaves is not just proportional to photosynthesis when gaseous conditions (CO2 and O2) vary. New Phytologist, 218, 94–106.
Abadie, C., Boex-Fontvieille, E. R. A., Carroll, A. J., & Tcherkez, G. (2016). In vivo stoichiometry of photorespiratory metabolism. Nature Plants, 2, 15220. https://doi.org/10.1038/NPLANTS.2015.220
Abadie, C., & Tcherkez, G. (2019). In vivo phosphoenolpyruvate carboxylase activity is controlled by CO2 and O2 mole fractions and represents a major flux at high photorespiration rates. New Phytologist, 221, 1843–1852.
Allen, J. F. (2003). Cyclic, pseudocyclic and noncyclic photosophorylation: New links in the chain. Trends in Plant Science, 8, 15–19.
Alonso-Cantabrana, H., Cousins, A. B., Danila, F., Ryan, T., Shawood, R. E., von Caemmerer, S., & Furnarb, R. T. (2018). Diffusion of CO2 across the mesophyll-bundle sheath cell interface in a C4 plant with genetically reduced PEP carboxylase activity. Plant Physiology, 178, 72–81.
Asada, K. (1999). The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. Annual Review of Plant Physiology and Plant Molecular Biology, 50, 601–639.
Avenson, T. J., Kanazawa, A., Cruz, J. A., Takizawa, K., Ettinger, W. E., & Kramer, D. M. (2005). Integrating the proton circuit into photosynthesis: Progress and challenges. Plant, Cell & Environment, 28, 97–109.
Bagley, J., Rosenthal, D. M., Ruiz-Vera, U. M., Siebers, M. H., Kumar, P., Ort, D. R., & Bernacchi, C. J. (2015). The influence of photosynthetic acclimation to rising CO2 and warmer temperatures on leaf and canopy photosynthesis models. Global Biogeochemical Cycles, 29(2), 194–206.
Baker, N. R., Harbinson, J., & Kramer, D. M. (2007). Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant, Cell & Environment, 30, 1107–1125.
Barbour, M. M., Evans, J. R., Simonin, K. A., & von Caemmerer, S. (2016). Online CO2 and H2O oxygen isotope fractionation allows estimation of mesophyll conductance in C4 plants, and reveals that mesophyll conductance decreases as leaves age in both C4 and C3 plants. New Phytologist, 210, 875–889.
Bellasio, C., & Farquhar, G. D. (2019). A leaf-level biochemical model simulating the introduction of C3 and C4 photosynthesis in C3 rice: Gains, losses and metabolite fluxes. New Phytologist, 223, 150–166.
Bellasio, C., & Griffiths, H. (2014). Acclimation to low light by C4 maize: Implications for bundle sheath leakiness. Plant, Cell & Environment, 37, 1046–1058.
Bernacchi, C. J., Bagley, J. E., Serbin, S. P., Ruiz-Vera, U. M., Rosenthal, D. M., & Vanlloocke, A. (2013). Modelling C3 photosynthesis from the chloroplast to the ecosystem. Plant, Cell & Environment, 36, 1641–1657.
Bernacchi, C. J., Portis, A. R., Nakano, H., von Caemmerer, S., & Long, S. P. (2002). Temperature response of mesophyll conductance. Implication for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiology, 130, 1992–1998.
Berry, J. A., & Farquhar, G. D. (1978). The CO2-concentrating function of C4 photosynthesis. A biochemical model. Paper presented at D. O. Hall, J. Coombs & T. W. Goodwin (Eds.) Proceedings of the 4th International Congress on Photosynthesis; London, UK: Biochemical Society. pp. 119–131.
Björkman, O., & Demmig, B. (1987). Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Plant, 170*, 489–504.

Bloom, A. J. (2015). Photorespiration and nitrate assimilation: A major intersection between plant carbon and nitrogen. *Photosynthesis Research, 123*, 117–128.

Bloom, A. J., Caldwell, R. M., Finazzo, J., Warner, R. L., & Weißbart, J. (1989). Oxygen and carbon dioxide fluxes from barley shoots depend on nitrate assimilation. *Plant Physiology, 91*, 352–356.

Boyd, R. A., Gandin, A., & Cousins, A. B. (2015). Temperature response of C4 plants in the context of Rubisco biochemistry. *Phytochemistry, 100*, 31–36.

Busch, F. A. (2020). Photorespiration in the context of Rubisco biochemistry, C4 CO2 diffusion and metabolism. *The Plant Journal, 101*, 919–939.

Busch, F. A., Holloway-Phillips, M., Stuart-Williams, H., & Farquhar, G. D. (2020). Revisiting carbon isotope discrimination in C3 plants shows respiration rules when photosynthesis is low. *Nature Plants, 6*, 245–258. https://doi.org/10.1038/s41477-020-0606-6

Busch, F. A., & Sage, R. F. (2017). The sensitivity of photosynthesis to O2 and CO2 concentration identifies strong Rubiscos control above the thermal optimum. *New Phytologist, 213*, 1036–1051.

Busch, F. A., Sage, R. F., & Farquhar, G. D. (2018). Plants increases CO2 uptake by assimilating nitrogen via the photorespiratory pathway. *Nature Plants, 4*, 46–54.

Busch, F. A., Sage, T. L., Cousins, A. B., & Sage, R. F. (2013). C3 plants enhance rates of photosynthesis by re-assimilating photorespired and respired CO2. *Plant, Cell & Environment, 36*, 200–212.

Comin, G., Bukhov, N. G., Wiese, C., Bligny, R., & Heber, U. (2000). Flexible coupling between light-dependent electron and vectorial proton transport in illuminated leaves of C3 plants. Role of photosystem I-dependent proton pumping. *Planta, 210*, 466–477.

Cousins, A. B., Ghannoum, O., von Caemmerer, S., & Badger, M. R. (2010). Simultaneous determination of Rubisco carboxylase and oxygenase kinetic parameters in *Tritium aestivum* and Zea mays using membrane inlet mass spectrometry. *Plant, Cell & Environment, 33*, 444–452.

Danila, F. R., Thakur, V., Chatterjee, J., Bala, S., Coe, R. A., Acebron, K., ... Quick, W. P. (2021). Bundle sheath suberisation is required for C4 photosynthesis in *Setaria viridis* mutant. *Communication Biology, 4*, 254. https://doi.org/10.1038/s42003-020-02177-4

Deans, R. M., Brodribb, T. J., Busch, F. A., & Farquhar, G. D. (2020). Optimization can provide the fundamental link between leaf photosynthesis, gas exchange and water relations. *Nature Plants, 6*, 1116–1125.

Deans, R. M., Farquhar, G. D., & Busch, F. A. (2019). Estimating stomatal and biochemical limitations during photosynthetic induction. *Plant, Cell & Environment, 42*, 3227–3240.

Dingkuhn, M., Luquet, D., Fabre, D., Muller, M., Yin, X., & Paul, M. (2020). The case for improving crop carbon sink strength or plasticity for a CO2-rich future. Current Opinion in Plant Biology, 56, 259–272.

Ehleringer, J., & Peary, R. W. (1983). Variation in quantum yield for CO2 uptake among C3 and C4 plants. *Plant Physiology, 73*, 555–559.

Eichelmann, H., Oja, V., Peterson, R. B., & Laisk, A. (2011). The rate of nitrite reduction in leaves as indicated by O2 and CO2 exchange during photosynthesis. *Journal of Experimental Botany, 62*, 2205–2215.

Ellsworth, D. S., Crous, K. Y., Lambers, H., & Cooke, J. (2015). Phosphorus recycling in photorespiration maintains high photosynthetic capacity in woody species. *Plant, Cell & Environment, 38*, 1142–1156.

Ethier, G. J., & Livingston, N. J. (2004). On the need to incorporate sensitivity to CO2 transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. *Plant, Cell & Environment, 27*, 137–153.

Evans, J. R. (1987). The dependence of quantum yield on wavelength and growth irradiance. *Australian Journal of Plant Physiology, 14*, 69–79.

Evans, J. R. (2021). Mesophyll conductance: Walls, membranes and spatial complexity. *New Phytologist, 229*, 1864–1876.

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.

Evans, J. R., von Caemmerer, S., Setchell, B. A., & Hudson, G. S. (1994). The relationship between CO2 transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of Rubisco. *Australian Journal of Plant Physiology, 21*, 475–495.

Farquhar, G. D., & von Caemmerer, S. (1982). Modelling of photosynthetic response to environmental conditions. In O. L. Lange, P. S. Nobel, C. B. Osmond, & H. Ziegler (Eds.), *Physiological plant ecology II, water relations and carbon assimilation*. Encyclopedia of plant physiology, New series (Vol. 12 B, pp. 549–588). Berlin, Germany: Springer Verlag.

Farquhar, G. D., von Caemmerer, S., & Berry, J. A. (1980). A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. *Planta, 149*, 78–90.

Farquhar, G. D., von Caemmerer, S., & Berry, J. A. (2001). Models of photosynthesis. *Plant Physiology, 125*, 42–45.

Farquhar, G. D., & Wong, S. C. (1984). An empirical model of stomatal conductance. *Australian Journal of Plant Physiology, 11*, 191–210.

Fengel, D., Langer, E. A., Gomes, J., Kaldenhoff, R., Medrano, H., & Ribas-Carbó, M. (2007). Rapid variation of mesophyll conductance in response to changes in CO2 concentration around leaves. *Plant, Cell & Environment, 30*, 1284–1298.

Foy, C. H., Neukermans, J., Queval, G., Noctor, G., & Harbinson, J. (2012). Photosynthetic control of electron transport and the regulation of gene expression. *Journal of Experimental Botany, 63*, 1637–1661.

Furbank, R. T. (2011). Evolution of the C4 photosynthetic mechanism: Are there really three C4 acid decarboxylation types? *Journal of Experimental Botany, 62*, 3203–3208.

Furbank, R. T., Jenkins, C. L. D., & Hatch, M. D. (1990). C4 photosynthesis: Quantum requirement, C3 acid overcycling and Q-cycle involvement. *Australian Journal of Plant Physiology, 17*, 1–7.

Genty, B., & Harbinson, J. (1996). Regulation of light utilization for photosynthetic electron transport. In N. R. Baker (Ed.), *Photosynthesis and the environment*, Vol 5 book series *Advances in photosynthesis and respiration* (pp. 67–99). The Netherlands: Kluwer Academic Publishers.

Ghannoum, O., Evans, J. R., Chow, W. S., Andrews, J., Conroy, J., & von Caemmerer, S. (2005). Faster Rubisco is the key to superior nitrogen-use efficiency in NADP-malic enzyme relative to NAD-malic enzyme C4 grasses. *Plant Physiology, 137*, 638–650.

Gu, L., Pallardy, S. D., 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 & Environment, 33*, 1852–1874.
Gu, L., & Sun, Y. (2014). Artefactual responses of mesophyll conductance to CO₂ and irradiance estimated with the variable J and online isotope discrimination methods. Plant, Cell & Environment, 37, 1221–1249.

Hahn, A., Vonck, J., Mills, D. J., Meier, T., & Kühlbrandt, W. (2018). Structure, mechanism, and regulation of the chloroplast ATP synthase. Science, 360, eaat4318. https://doi.org/10.1126/science.aat4318

Harbinson, J., & Yin, X. (2017). A model for the irradiance responses of photosynthesis. Physiologia Plantarum, 161, 109–123.

Harley, P. C., Loreto, F., Di Marco, G., & Sharkey, T. D. (1992). Theoretical considerations when estimating the mesophyll conductance to CO₂ flux by analysis of the response of photosynthesis to CO₂. Plant Physiology, 98, 1429–1436.

Harley, P. C., & Sharkey, T. D. (1991). An improved model of C₃ photosynthesis at high CO₂: Reversed O₂ sensitivity explained by lack of glycerate reentry into the chloroplast. Photosynthesis Research, 27, 169–178.

Hatch, M. D. (1987). C₄ photosynthesis: A unique blend of modified biochemistry, anatomy and ultrastructure. Biochimica et Biophysica Acta, 895, 81–106.

He, D., & Edwards, G. E. (1996). Estimation of diffusive resistance of bundle sheath cells to CO₂ from modeling of C₄ photosynthesis. Photosynthesis Research, 49, 195–208.

Hogewoning, S. W., Wientjes, E., Douwstra, P., Trouwborst, G., van Ieperen, W., Croce, R., & Harbinson, J. (2012). Photosynthetic quantum yield dynamics: From photosystems to leaves. The Plant Cell, 24, 1921–1935.

Ishikawa, N., Takabayashi, A., Noguchi, K., Tazoe, Y., Yamamoto, H., von Kebeish, R., Niessen, M., Thirshnaveni, K., Bari, R., Hirsch, H.-J., Harley, P. C., & Sharkey, T. D. (1991). An improved model of C₃ photosynthesis. Plant Physiology, 98, 1429–1436.

Kanai, R., & Edwards, G. E. (1999). The biochemistry of the C₄ photosynthesis. In R. F. Sage & R. K. Manson (Eds.), C₄ plant biology (pp. 49–87). Toronto, ON, Canada: Academic Press.

Kaschuk, G., Yin, X., Hungria, M., Leffelaar, P. A., Giller, K. E., & Kuyper, T. W. (2012). Photosynthetic adaptation of soybean due to varying effectiveness of N₂ fixation by two distinct Bradyrhizobium japonicum strains. Environmental and Experimental Botany, 76, 1–6.

Kebeish, R., Niessen, M., Thirshnaveni, K., Bari, R., Hirsch, H.-J., Rosenkranz, R., & Peterhansel, C. (2007). Chloroplast photorespiratory bypass increases photosynthesis and biomass production in Arabidopsis thaliana. Nature Biotechnology, 25, 593–599.

Kiráts, O., Lea, P. J., Franceschi, V. R., & Edwards, G. E. (2002). Bundle sheath diffusive resistance to CO₂ and effectiveness of C₄ photosynthesis and reoxidation of photorespired CO₂ in a C₄ cycle mutant and wild-type Amaranthus edulis. Plant Physiology, 130, 964–976 (with Corrections in Plant Physiology, v. 132, p. 400).

Kramer, D. M., & Evans, J. R. (2011). The importance of energy balance in improving photosynthetic productivity. Plant Physiology, 155, 70–78.

Kromdijk, J., Griffiths, H., & Schepers, H. E. (2010). Can the progressive increase of CO₂ bundle sheath leakiness at low PFD be explained by incomplete suppression of photorespiration? Plant, Cell & Environment, 33, 1935–1948.

Kumarathunge, D. P., Medlyn, B. E., Drake, J. E., Rogers, A., & Tjoelker, M. G. (2019). No evidence for triose phosphate limitation of photosynthesis in Earth System Models. Plant & Cell Physiology, 50, 756–772.

Seelert, H., Poetsch, A., Dencher, N. A., Engel, A., Stahlberg, H., & Müller, D. J. (2000). Proton-powered turbine of a plant motor. Nature, 405, 418–419.

Sellers, P. J., Randall, D. A., Collatz, G. J., Berry, J. A., Field, C. B., Dazlich, D. A., & Bounoua, L. (1996). A revised land surface parameterization (SiB2) for atmospheric GCMS. Part I: Model formulation. Journal of Climate, 9(4), 676–705.

Sharkey, T. D. (1985a). O₂-insensitive photosynthesis in C₃ plants: Its occurrence and a possible explanation. Plant Physiology, 78, 71–75.

Sharkey, T. D. (1985b). Photosynthesis in intact leaves of C₃ plants: Physics, physiology and rate limitations. The Botanical Review, 51, 53–105.

Sharkey, T. D. (2019). Is triose phosphate utilization important for understanding photosynthesis? Journal of Experimental Botany, 70, 5521–5525.
Sharkey, T. D., Bernacchi, C. J., Farquhar, G. D., & Singsaas, E. L. (2007). Fitting photosynthetic carbon dioxide response curves for C3 leaves. Plant, Cell & Environment, 30, 1033–1040.

Sharkey, T. D., & Vasse, T. L. (1989). Low oxygen inhibition of photosynthesis is caused by inhibition of starch synthesis. Plant Physiology, 90, 385–387.

Darwood, R., Ghannoum, O., Kapralov, M. V., Gunn, L. H., & Whitney, S. M. (2016). Temperature responses of rubisco from Panicae grasses provide opportunities for improving C3 photosynthesis. Nature Plants, 2, 16186. https://doi.org/10.1038/nplants.2016.186

Shen, B., Wang, L., Lin, X., Yao, Z., Xu, H., Zhu, C., ... Peng, X. (2019). Engineering a new chloroplastic photosynthetic bypass to increase photosynthetic efficiency and productivity in rice. Molecular Plant, 12, 199–214.

Skillman, J. B. (2008). Quantum yield variation across the three pathways of photosynthesis: Not yet out of the dark. Journal of Experimental Botany, 59, 1647–1661.

South, P. F., Cavanagh, A. P., Liu, H. W., & Ort, D. R. (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science, 363(6422), eaat9077.

Steigmiller, S., Turina, P., & Gräber, P. (2008). The thermodynamic H+/ATP ratios of the H+-ATPsynthases from the chloroplasts and Echerichia coli. Proceedings of the National Academy of Sciences of the United States of America, 105, 3745–3750.

Strand, D. D., Fisher, N., & Kramer, D. M. (2017). The higher plant plastid NAD(P)H dehydrogenase-like complex (NDH) is a high efficiency proton pump that increases ATP production by cyclic electron flow. Journal of Biological Chemistry, 292, 11850–11860.

Taiz, L., & Zeiger, E. (2002). Plant physiology (3rd ed.). Sunderland, UK: Sinauer Associates 690pp.

Tcherkez, G., Gauthier, P., Buckley, T. N., Busch, F. A., Barbour, M. M., Bruhn, D., ... Cornic, G. (2017). Leaf day respiration: Low CO2 flux but high significance for metabolism and carbon balance. New Phytologist, 216, 986–1001.

Tcherkez, G., & Limani, A.N. (2019). Net photosynthetic CO2 assimilation: more than just CO2 and O2 reduction cycles. New Phytologist, 223, 520–529.

Tholen, D., Ethier, G., Genty, B., Pepin, S., & Zhu, X.-G. (2012). Variable mesophyll conductance revisited: Theoretical background and experimental implications. Plant, Cell & Environment, 35, 2087–2102.

Ubierna, N., Chemusak, L. A., Holloway-Phillips, M., Busch, F. A., Cousins, A. B., & Farquhar, G. D. (2019). Critical review: Incorporating the arrangement of mitochondria and chloroplasts into models of photosynthesis and carbon isotope discrimination. Photosynthesis Research, 141, 5–31.

Ubierna, N., Sun, W., Kramer, D. M., & Cousins, A. B. (2013). The efficiency of C4 photosynthesis under low light conditions in Zea mays, Miscanthus × giganteus and Flaveria bidentis. Plant, Cell & Environment, 36, 365–381.

van der Putten, P. E. L., Yin, X., & Struik, P. C. (2018). Calibration matters: On the procedure of using the chlorophyll fluorescence method to estimate mesophyll conductance. Journal of Plant Physiology, 220, 167–172.

von Caemmerer, S. (2000). Biochemical models of leaf photosynthesis. Collingwood, VIC, Australia: CSIRO Publishing.

von Caemmerer, S. (2013). Steady-state models of photosynthesis. Plant, Cell & Environment, 36, 1617–1630.

von Caemmerer, S., & Evans, J. R. (1991). Determination of the average partial pressure of CO2 in chloroplasts from leaves of several C3 plants. Australian Journal of Plant Physiology, 18, 287–305.

von Caemmerer, S., Evans, J. R., Hudson, G. S., & Andrews, T. J. (1994). The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. Planta, 195, 88–97.

von Caemmerer, S., & Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta, 153, 376–387.

von Caemmerer, S., & Furbank, R. T. (1999). Modeling C4 photosynthesis. In R. F. Sage & R. K. Monson (Eds.), C4 plant biology (pp. 173–211). Toronto, ON, Canada: Academic Press.

Wang, Y., Brautigam, A., Weber, A. P. M., & Zhu, X.-G. (2014). Three distinct biochemical subtypes of C4 photosynthesis? A modelling analysis. Journal of Experimental Botany, 65, 3567–3578.

Wu, A., Hammer, G. L., Doherty, A., von Caemmerer, S., & Farquhar, G. D. (2019). Quantifying impacts of enhancing photosynthesis on crop yield. Nature Plants, 5(4), 380–388.

Yamori, W., Sakata, N., Suzuki, Y., Shikanai, T., & Makino, A. (2011). Cyclic electron transport around photosystem I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. The Plant Journal, 68, 966–976.

Yin, X., Harbinson, J., & Struik, P. C. (2006). Mathematical review of literature to assess alternative electron transports and interphotosystem excitation partitioning of steady-state C3 photosynthesis under limiting light. Plant, Cell & Environment, 29, 1771–1782.

Yin, X., & Struik, P. C. (2008). Applying modelling experiences from the past to shape crop systems biology: The need to converge crop physiology and functional genomics. New Phytologist, 179, 629–642.

Yin, X., & Struik, P. C. (2009). Theoretical reconsiderations when estimating the mesophyll conductance to CO2 diffusion in leaves of C3 plants by analysis of combined gas exchange and chlorophyll fluorescence measurements. Plant, Cell & Environment, 32, 1513–1524 (with corrigendum in PC&E, v. 33, p. 1595).

Yin, X., & Struik, P. C. (2012). Mathematical review of the energy transduction stoichiometries of C4 leaf photosynthesis under limiting light. Plant, Cell & Environment, 35, 1299–1312.

Yin, X., & Struik, P. C. (2017a). Can increased leaf photosynthesis be converted into higher crop mass production? A simulation study for rice using the crop model GECROS. Journal of Experimental Botany, 68, 2345–2360.

Yin, X., & Struik, P. C. (2017b). Simple generalisation of a mesophyll resistance model for various intracellular arrangements of chloroplasts and mitochondria in C3 leaves. Photosynthesis Research, 132, 211–220.

Yin, X., & Struik, P. C. (2018). The energy budget in C4 photosynthesis: Insights from a cell-type-specific electron transport model. New Phytologist, 218, 986–998.

Yin, X., & Struik, P. C. (2021). Exploiting differences in the energy budget among C4 subtypes to improve crop productivity. New Phytologist, 229, 2400–2409.

Yin, X., Struik, P. C., Romero, P., Harbinson, J., Evers, J. B., van der Putten, P. E. L., & Vos, J. (2009). Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C3 photosynthesis model: A critical appraisal and a new integrated approach applied to leaves in a wheat (Triticum aestivum) canopy. Plant, Cell & Environment, 32, 448–464.

Yin, X., Sun, Z., Struik, P. C., van der Putten, P. E. L., van Ieperen, W., & Harbinson, J. (2011). Using a biochemical C4-photosynthesis model and combined gas exchange and chlorophyll fluorescence measurements to estimate bundle-sheath conductance of maize leaves differing in age and nitrogen content. Plant, Cell & Environment, 34, 2183–2199.

Yin, X., van der Putten, P. E. L., Belay, D., & Struik, P. C. (2020). Using photosynthetic oxygen response to analyse leaf mesophyll resistance. Photosynthesis Research, 144, 85–99.

Yin, X., van der Putten, P. E. L., Driever, S. M., & Struik, P. C. (2016). Temperature response of bundle-sheath conductance in maize leaves. Journal of Experimental Botany, 67, 2699–2714.

Yin, X., van Oijen, M., & Schapendonk, A. H. C. M. (2004). Extension of a biochemical model for the generalized stoichiometry of electron
transport limited $\text{C}_3$ photosynthesis. *Plant, Cell & Environment*, 27, 1211–1222.

Zhu, X.-G., Portis, A. R., Jr., & Long, S. P. (2004). Would transformation of $\text{C}_3$ crop plants with foreign rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. *Plant, Cell & Environment*, 27, 155–165.

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