Updating the steady state model of $C_4$ photosynthesis

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Running title: Steady state model of $C_4$ photosynthesis
Highlight

The C₄ photosynthesis model by von Caemmerer and Furbank (1999) has been updated. It now includes temperature dependencies and equations to calculate electron transport rate from measured CO₂ assimilation rates.

Abstract

C₄ plants play a key role in world agriculture. For example, C₄ crops such as maize and sorghum are major contributors to both first and third world food production and the C₄ grasses sugarcane; miscanthus and switchgrass are major plant sources of bioenergy. In the challenge to manipulate and enhance C₄ photosynthesis, steady state models of leaf photosynthesis provide an important tool for gas exchange analysis and thought experiments that can explore photosynthetic pathway changes. Here the C₄ photosynthetic model by von Caemmerer and Furbank (1999) has been updated with new kinetic parameterisation and temperature dependencies added. The parameterisation was derived from experiments on the C₄ monocot, Setaria viridis, which for the first time provides a cohesive parameterisation. Mesophyll conductance and its temperature dependence have also been included, as this is an important step in the quantitative correlation between the initial slope of the CO₂ response curve of CO₂ assimilation and in vitro PEP carboxylase activity. Furthermore, the equations for chloroplast electron transport have been updated to include cyclic electron transport flow and equations have been added to calculate electron transport rate from measured CO₂ assimilation rates.

Key words:
A-ci curves, C₄ photosynthesis, chloroplast electron transport, CO₂ diffusion, leaf temperature, PEP carboxylase
Introduction

To meet the challenge of increasing crop yield for a growing world population, it has become apparent that photosynthetic efficiency and capacity must be increased per unit leaf area to improve yield potential (Long et al., 2015). High yields from C₄ crops have stimulated considerable interest in the C₄ photosynthetic pathway which is characterised by high photosynthetic rate and high nitrogen and water use efficiency relative to plants with the C₃ photosynthetic pathway (Mitchell and Sheehy, 2006). In the challenge to increase photosynthetic rate per leaf area steady state models of leaf photosynthesis provide an important tool for gas exchange analysis and thought experiments that can explore photosynthetic pathway changes (Long et al., 2015; Price et al., 2011; von Caemmerer and Evans, 2010; von Caemmerer and Furbank, 2016; von Caemmerer et al., 2003). The mathematical simplicity of these leaf level models has facilitated incorporation into higher order canopy, crop and earth system models (Rogers et al., 2017; Wu et al., 2018; Wu et al., 2019; Yin and Struik, 2009).

C₄ photosynthesis requires the coordinated functioning of mesophyll and bundle-sheath cells of leaves and is characterised by a CO₂ concentrating mechanism which allows Rubisco, located in the bundle sheath cells, to operate at high CO₂ partial pressures. This overcomes the low affinity Rubisco has for CO₂ and largely inhibits its oxygenation reaction, reducing photorespiration rates. In the mesophyll, CO₂ is initially fixed by phosphoenolpyruvate (PEP) carboxylase into C₄ acids, which are then decarboxylated in the bundle sheath to supply CO₂ for Rubisco. Both the structure of the bundle-sheath wall (which has a low permeability to CO₂) and the relative biochemical capacities of the C₃ cycle in the bundle sheath and C₄ acid cycle (which operates across the mesophyll bundle-sheath interface) contribute to the high CO₂ partial pressure in the bundle sheath. The biochemistry of the C₄ photosynthetic pathway is not unique and three main biochemical subtypes are recognised on a basis of the predominant decarboxylating enzyme: NADP-ME (NADP-dependent malic enzyme), NAD-ME (NAD-dependent malic enzyme) and PEPCK (PEP carboxykinase) (Hatch, 1987).

The first models to capture the C₄ photosynthetic biochemistry were designed by Berry and Farquhar (1978) and (Peisker, 1979). The Berry and Farquhar model did not provide analytical solutions but was able to predict high bundle sheath CO₂ partial pressures and its dependence on bundle sheath conductance. Many of the gas exchange characteristics of C₄
photosynthesis observed with intact leaves could be predicted by these models. Collatz et al. (1992) and von Caemmerer and Furbank (1999) have revised and expanded these original models with analytical solutions.

C₄ models have not been used as frequently as the C₃ models so less data relating leaf biochemistry with gas exchange is available in the literature. Massad et al. (2007) have parameterised the model by von Caemmerer (2000) for Zea mays and developed the first temperature dependencies for key parameters. Fitting routines have also been developed (Bellasio et al., 2016; Bellasio et al., 2017; Zhou et al., 2019).

Here an update of the C₄ photosynthetic model by von Caemmerer and Furbank (1999) and von Caemmerer (2000) is provided with new parameterisation and temperature dependencies derived from experiments on the C₄ monocot species Setaria viridis (green foxtail millet), a NADP-malic enzyme type which is closely related to agronomically important C₄ crops. It has become a popular model species due to its rapid generation time, small stature, high seed production, diploid status and small sequenced and publicly available genome and it can be readily transformed (Alonso-Cantabrana et al., 2018; Brutnell et al., 2010; Doust, 2007; Ermakova et al., 2019; Li and Brutnell, 2011; Osborn et al., 2016).

The basic model equations

Figure 1 shows a schematic representation of the proposed carbon fluxes in C₄ photosynthesis. After diffusion of CO₂ across the mesophyll cell interface CO₂ is converted to HCO₃⁻ by carbonic anhydrase, CA, which is fixed by PEP carboxylase, PEPC into C₄ acids, which diffuse to and are decarboxylated in the bundle sheath. Rubisco and the complete C₃ photosynthetic pathway are located in the bundle-sheath cells, bounded by a relatively gas tight cell wall such that the C₃ cycle relies almost entirely on C₄ acid decarboxylation as its source of CO₂.

The net rate of CO₂ fixation for C₄ photosynthesis can be given by two equations. The first describes Rubisco carboxylation in the bundle sheath. Since all carbon fixed into sugars ultimately must be fixed by Rubisco, overall CO₂ assimilation, A, can be given by
\[ A = V_c - 0.5V_o - R_d \]  

where \( V_c \) and \( V_o \) are the rates of Rubisco carboxylation and oxygenation and \( R_d \) is the rate of mitochondrial respiration not associated with photorespiration.

Mitochondrial respiration may occur in the mesophyll as well as in the bundle sheath. As Rubisco may more readily refix \( \text{CO}_2 \) released in the bundle sheath, \( R_d \) is described by its mesophyll and bundle-sheath components

\[ R_d = R_m + R_s. \]  

(2)

\( \text{CO}_2 \) assimilation rate, \( A \), can also be written in terms of the mesophyll reactions as

\[ A = V_p - L - R_m, \]  

(3)

where \( V_p \) is the rate of PEP carboxylation, \( R_m \) is the mitochondrial respiration occurring in the mesophyll and \( L \) is the rate of \( \text{CO}_2 \) leakage from the bundle sheath to the mesophyll (Figure 1). This assumes that in the steady state the rate of PEP carboxylation and the rate of \( \text{C}_4 \) acid decarboxylation are equal.

The leak rate, \( L \), is given by

\[ L = g_{bs}(C_s - C_m) \]  

(4)

where \( g_{bs} \) is the conductance to \( \text{CO}_2 \) leakage and is determined by the properties of the bundle-sheath cell wall; \( C_s \) and \( C_m \) are the bundle sheath and mesophyll \( \text{CO}_2 \) partial pressures.

It is assumed that there is a negligible amount of \( \text{HCO}_3^- \) leakage from the bundle sheath since the \( \text{HCO}_3^- \) pool should be small due to the absence of carbonic anhydrase activity in the cytosol of these cells (Farquhar, 1983; Jenkins et al., 1989; Ludwig et al., 1998).

The \( \text{C}_4 \) cycle consumes additional energy during the regeneration of PEP and leakage of \( \text{CO}_2 \) from the bundle sheath is an energy cost to the leaf. This represents a compromise between retaining \( \text{CO}_2 \), allowing efflux of \( \text{O}_2 \) and permitting metabolites to diffuse in and out at rates fast enough to support the rate of \( \text{CO}_2 \) fixation (Hatch and Osmond, 1976; Raven, 1977). The \( \text{CO}_2 \) leak rate depends upon the balance between the rates of PEP carboxylation and Rubisco activity and the conductance of the bundle sheath to \( \text{CO}_2 \).
Leakiness ($\phi$), a term coined by Farquhar (1983), defines leakage as a fraction of the rate of PEP carboxylation and thus describes the efficiency of the C4 cycle

$$\phi = L/V_p.$$ (5)

A related term “overcycling” has also been used (Furbank et al., 1990; Jenkins, 1989). Overcycling defines the leak rate as a fraction of CO2 assimilation rate and gives the fraction by which the flux through the C4 acid cycle has to exceed net CO2 assimilation rate

$$\text{Overcycling} = \frac{L}{A} = \frac{V_p - (A + R_m)}{A}.$$ (6)

C4 photosynthesis can be either limited by the enzymatic rates of PEP carboxylase and Rubisco or by the irradiance and the capacity of chloroplast electron transport which supports the regeneration of PEP and RuBP.

Enzyme limited rate equations

Many important features of the C4-model can be examined with the enzyme-limited rates, which are presumed to be appropriate under conditions of high irradiance. As is the case in C3 models of photosynthesis (Farquhar and von Caemmerer, 1982; Farquhar et al., 1980; von Caemmerer, 2000), Rubisco carboxylation at high irradiance can be described by its RuBP saturated rate

$$V_c = \frac{c_s V_{c,max}}{c_s + K_c(1+O_s/K_o)}$$ (7)

where $O_s$ is the O2 partial pressure in the bundle sheath. Following the oxygenation of one mol of RuBP, 0.5 mol of CO2 is evolved in the photorespiratory pathway and the ratio of oxygenation to carboxylation can be expressed as

$$V_o/V_c = 2\Gamma^*/c_s$$ (8)

where $\Gamma^*$ is the CO2 compensation point in a C3 plant in the absence of other mitochondrial respiration, and

$$\Gamma^* = 0.5[V_{c,max}K_c/V_{c,max}K_o]O_s = \gamma^*O_s,$$ (9)
where the term in the bracket is the reciprocal of Rubisco specificity, $S_{c/o}$ (Farquhar et al., 1980). In what follows the $O_2$ dependence of $I^*$ is highlighted since the $O_2$ partial pressure in the bundle sheath may vary.

The Rubisco limited rate of CO$_2$ assimilation can be derived from equations 1, 7, 8 and 9.

$$A_c = \frac{(C_s - \gamma O_s)V_{cmax}}{C_s + K_c(1 + O_s/K_o)} - R_d.$$  \hspace{1cm} (10)

To derive an overall expression for CO$_2$ assimilation rate as a function of mesophyll CO$_2$ and $O_2$ partial pressure, $C_m$ and $O_m$, one needs to derive an expression for $C_s$ and $O_s$. Equation 10 can be used to derive an expression for $C_s$:

$$C_s = \frac{\gamma O_s + K_c(1 + O_s/K_o)(V_{cmax})}{1 - (A + R_d)/V_{cmax}}.$$  \hspace{1cm} (11)

If $V_{cmax}$ could be estimated accurately from biochemical measurements together with $A$ it would provide a means of estimating bundle sheath CO$_2$ partial pressure. One can also obtain an expression for $C_s$ from equation 3 and 4:

$$C_s = C_m + \frac{V_p - A - R_m}{g_{bs}}.$$  \hspace{1cm} (12)

Photosystem II activity and $O_2$ evolution in the bundle sheath varies widely amongst the C$_4$ species. Some NADP-ME species such as *Zea mays* and *Sorghum bicolor* have little or none, whereas NADP-ME dicots and NAD and PCK species can have high PSII activity (Chapman et al., 1980; Hatch, 1987; Pfundel and Pfeffer, 1997). In *S. viridis* the amount of PSII activity depends on growth light environment (Ermakova et al., 2021). Because the bundle sheath is a fairly gas tight compartment this has implications for the steady state $O_2$ partial pressure of the bundle sheath (Berry and Farquhar, 1978; Raven, 1977). Following Berry and Farquhar (1978), we assume that the net $O_2$ evolution, $E_o$, in the bundle sheath cells equals its leakage, $L_o$, out of the bundle sheath, that is

$$E_o = L_o = g_o(O_s - O_m).$$  \hspace{1cm} (13)

The conductance to leakage of $O_2$ across the bundle sheath, $g_o$, can be related to the conductance to CO$_2$ by way of the ratio of diffusivities and solubilities by

$$g_o = g_{bs}(D_{O2}S_{O2}/D_{CO2}S_{CO2}),$$  \hspace{1cm} (14)
where \( D_{O_2} \) and \( D_{CO_2} \) are the diffusivities for \( O_2 \) and \( CO_2 \) in water, respectively and \( S_{O_2} \) and \( S_{CO_2} \) are the respective Henry constants such that

\[
g_o = 0.047g_{bs} \quad \text{(15)}
\]

at 25° C (Berry and Farquhar, 1978; Farquhar, 1983). If \( E_o = \alpha A \), where \( \alpha \) (0<\( \alpha \)<1) denotes the fraction of \( O_2 \) evolution occurring in the bundle sheath, then \( O_s \):

\[
O_s = \frac{\alpha A}{0.0047g_{bs}} + O_m. \quad \text{(16)}
\]

Like Berry and Farquhar (1978), it is assumed that a steady state balance exists between the rate of PEP carboxylation and the release of \( C_4 \) acids in the bundle sheath. Furthermore, it is assumed that PEP carboxylation provides the rate limiting step and not, for example, the rate of hydration of \( CO_2 \) by carbonic anhydrase. As PEP carboxylase utilises \( HCO_3^- \) rather than \( CO_2 \), hydration of \( CO_2 \) is really the first step in carbon fixation in \( C_4 \) species (Hatch and Burnell, 1990).

When \( CO_2 \) is limiting the rate of PEP carboxylation is given by a Michaelis Menten equation

\[
V_p = \frac{c_m v_{pmax}}{c_m + K_p} \quad \text{(17)}
\]

where \( v_{pmax} \) is the maximum PEP carboxylation rate, \( K_p \) is the Michaelis Menten constant for \( CO_2 \). This assumes that the substrate PEP is saturating under these conditions. When the rate of PEP regeneration is limiting, for example by the capacity of pyruvate orthophosphate dikinase (PPDK) then

\[
V_p = v_{pr} \quad \text{(18)}
\]

where \( v_{pr} \) is a constant (Peisker, 1986; Peisker and Henderson, 1992) and

\[
V_p = \min\left(\frac{c_m v_{pmax}}{c_m + K_p}, v_{pr}\right) \quad \text{(19)}
\]

To obtain an overall rate equation for \( CO_2 \) assimilation as a function of the mesophyll \( CO_2 \) and \( O_2 \) partial pressures (\( c_m \) and \( O_m \)) one combines equations (10), (12) and (16). The resulting expression is a quadratic of the form
\[ aA_c^2 + bA_c + c = 0, \]  
where

\[ A_c = \left( -b + \sqrt{b^2 - 4ac} \right) / (2a) \]  

\[ a = \frac{\alpha K_c}{0.047 K_o} \]  

\[ b = -\left\{ \left( V_p - R_m + g_{bs}C_m \right) + \left( V_{cmax} - R_d \right) + g_{bs}(K_c(1 + O_m/K_o)) \right\} \]  

\[ c = \left( V_{cmax} - R_d \right)\left( V_p - R_m + g_{bs}C_m \right) - \left( V_{cmax}g_{bs}\gamma O_m + R_d g_{bs}K_c(1 + O_m/K_o) \right) \]  

Equation (21) can be approximated by:

\[ A_c = \min\left\{ \left( V_{cmax} - R_d \right), \left( V_p - R_m + g_{bs}C_m \right) \right\} \]  

where \( \min \{ \} \) stands for minimum of.

At low CO\(_2\) partial pressures, CO\(_2\) assimilation rate can be approximated by

\[ A_c = \frac{c_m V_{pmax}}{c_m + K_p} - R_m + g_{bs}C_m. \]  

Under these conditions \( A_c \) is linearly related to the maximum PEP carboxylase activity, \( V_{pmax} \).

The product \( g_{bs}C_m \) is the inward diffusion of CO\(_2\) into the bundle sheath and because \( g_{bs} \) is low (0.003 mol m\(^{-2}\) s\(^{-1}\)) the flux is only 0.3 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) at \( C_m \) of a 100 \( \mu \)bar and can thus be ignored.

At high CO\(_2\) partial pressures CO\(_2\) assimilation rate is given by either the maximal Rubisco activity, \( V_{cmax} \) or the rate of PEP regeneration (\( V_{pr} \)).

Light and electron transport limited rate equations

The energy requirements for the regeneration of RuBP in the bundle sheath are the same as in a C\(_3\) leaf (Farquhar et al., 1980; von Caemmerer, 2000). There is however the additional cost of 2 mol ATP for the regeneration of one mol of PEP from pyruvate in the mesophyll such that:
Rate of ATP consumption = \(2V_p + (3 + 7\gamma_* O_s/C_s)V_c\) \hspace{1cm} (27)

where \((7\gamma_* O_s/C_s)V_c\) is the energy requirement due to photorespiration (since \(V_o/V_c = 2\gamma_* O_s/C_s\)) (Berry and Farquhar, 1978). In the PCK type \(C_4\) species some of the ATP for PEP regeneration may come from the mitochondria such that the photosynthetic requirement may be less (for review see Furbank, 2011).

There is no net NADPH requirement by the \(C_4\) cycle itself, although for example in NADP-ME species NADPH consumed in the production of malate from OAA in the mesophyll is released in the bundle sheath during decarboxylation (Hatch and Osmond, 1976). This may have implications on the behaviour of \(C_4\) photosynthesis under fluctuating light environments (Krall and Pearcy, 1993; Kubásek \textit{et al.}, 2013). The rate of NADP consumption is given by the requirement of the \(C_3\) cycle:

Rate of NADP consumption = \((2 + 4\gamma_* O_s/C_s)V_c\). \hspace{1cm} (28)

It is important to note that under most situations \(C_4\) is probably sufficiently large that the photorespiratory term in equations 27 and 28 can be ignored, but it does become relevant at low mesophyll \(CO_2\) partial pressures, or at very low light (Siebke \textit{et al.}, 1997).

NADPH and ATP are produced by chloroplast electron transport. The reduction of NADP\(^+\) to NADPH + H\(^+\) requires the transfer of 2 electrons through the whole chain electron transport which in turn requires 2 photons each at photosystem II (PSII) and Photosystem I (PSI). The generation of ATP can be coupled to the proton production via whole chain electron transport, or ATP can be generated via cyclic electron transport around PSI.

Photosystem II activity in the bundle sheath varies amongst \(C_4\) species with different \(C_4\) decarboxylation types. Presumably, when PSII is deficient or absent from the bundle sheath chloroplasts, some ATP is generated via cyclic photophosphorylation and 50% of the NADPH required for the reduction of PGA is derived from NADPH generated by NADP\(^+\) malic enzyme (Chapman \textit{et al.}, 1980). The remainder of the PGA must be exported to the mesophyll chloroplast where it is reduced and then returned to the bundle sheath (Hatch and Osmond, 1976). Measurements of metabolite pools of \textit{Amaranthus edulis}, a NAD\(^+\) malic enzyme species, having PSII activity in the bundle sheath, suggest that it may also export a part of the
PGA to the mesophyll for reduction (Leegood and von Caemmerer, 1988). It appears therefore that energy production and consumption is shared between mesophyll and bundle sheath cells more generally across decarboxylation types (von Caemmerer and Furbank, 2016).

A very simple approach was taken in the basic photosynthesis model. Electron transport is modelled as a whole, allocating a different fraction of it to the C₄ and C₃ cycle rather than compartmenting it to mesophyll and bundle sheath chloroplasts (von Caemmerer, 2000; von Caemmerer and Furbank, 1999).

That is whole chain linear electron transport

\[ J = J_m + J_s \]  

and \( J_m = xJ \) and \( J_s = (1-x)J \) where 0<\( x > 1 \). Because at most 2 out of 5 ATP are required in the mesophyll, \( x \approx 0.4 \) (equation 27). This partitioning approach of electron transport has been adopted by subsequent users of the model (Kromdijk et al., 2010; Massad et al., 2007; Ubierna et al., 2011; Yin and Struik, 2009; Yin et al., 2011; Zhou et al., 2017). Peisker (1988) has modelled the optimisation of \( x \) at low light in some detail. See also Figure 4.22 in von Caemmerer (2000).

New information exists for the calculation of the ATP requirement. Following Furbank et al. (1990), von Caemmerer and Furbank assumed a stoichiometry of 3 H⁺ per ATP produced and the operation of a Q-cycle (von Caemmerer, 2000; von Caemmerer and Furbank, 1999). Current models of rotational catalysis predict that the H⁺/ATP ratio is identical to the stoichiometric ratio of c-subunits to β-subunits which is \( c/β = 4.7 \) for spinach chloroplasts (Vollmar et al., 2009). However measured values are closer to 4 for the chloroplast enzyme (Petersen et al., 2012). If 4 H⁺ are required per ATP generated, it seems necessary to also have a functional Q-cycle which yields 3 H⁺ per linear electron flow. The proton production during cyclic electron flow is only 2 H⁺ per electron so that the overall proton production per electron is dependent on the balance of linear to cyclic electron flow (Yin and Struijk, 2012).

Following the derivations by Yin and Struijk (2012), the rate of proton production from linear and cyclic electron flow is

\[ J_{H^+} = 3J + 2J_{cyc} = \frac{3-f_{cyc}}{1-f_{cyc}}J \]  

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Since $J_1 = J + J_{cyc} = J/(1 - f_{cyc})$ where $J_1$ is the electron flow out of photosystem 1 and
$f_{cyc}$ is the fraction of $J_1$ that precedes via cyclic electron flow. For details see Figure 2. The
rate of ATP production is given by

$$J_{ATP} = J_{H^+}/h = \frac{3-f_{cyc}}{h(1-f_{cyc})}J = zJ$$

Where $h$ is the number of protons required per ATP generated, which here is assumed to be
4 and $z$ relates linear electron flow $J$ to the rate of ATP production (Yin and Struik, 2012). It
follows that

$$J_m = \frac{2}{z}V_p$$

Where $z=0.75$ when there is no cyclic electron flow and $z=1.25$ when $f_{cyc}=0.5$.

$$J_s = \frac{3}{z}(1 + 7\gamma_3 O_s/3C_s)W_c.$$  

The relationship between the electron transport, $J$, and the absorbed irradiance that is used
here is the same as that used previously where

$$\theta J^2 - J(I_2 + J_{max}) + I_2J_{max}$$

$I_2$ is the photosynthetically useful light absorbed by PSII, $J_{max}$ is the maximum electron
transport and $\theta$ is an empirical curvature factor. 0.7 is a good average value for C₃ species
(Evans, 1989) but has not been explored for C₄ species. $I_2$ is related to incident irradiance by

$$I_2 = I \times absorptance (1 - f)/2$$

In sunlight the absorptance of leaves is commonly about 0.85 and $f$ is to correct for spectral
quality of the light (~0.15 (Evans, 1987)). Ögren and Evans (1993) give a detailed discussion
of the parameters of equation 16. The 2 in the denominator is because we assume half the
light absorbed needs to reach each photosystem. This assumption may need to be
considered as with an increase in cyclic electron flow commonly observed in C₄ species less
than half of the light may be absorbed by PSII.
Putting it all together gives a light and electron transport limited quadratic expression. From equations (3) and (1) one can derive two equations for an electron transport limited CO₂ assimilation rate

\[ A_j = \frac{z}{2} x J - g_{bs} (C_s - C_m) - R_m \]  

(36)

and

\[ A_j = \frac{(1 - \gamma_s O_s/C_s) z (1-x) J}{(1 + 7 \gamma_s O_s/3 C_s)} - R_d \]  

(37)

Equation (37) can be solved for the bundle sheath CO₂ partial pressure and

\[ C_s = \frac{(\gamma_s O_s/3) (A_j + R_d) + z (1-x) J/3}{z (1-x) J/3 - (A_j + R_d)}. \]  

(38)

Combining equations (16), (36) and (36) then yields a quadratic expression of the form

\[ aA_j^2 + bA_j + c = 0, \]  

(39)

where

\[ A_j = (-b + \sqrt{b^2 - 4ac})/(2a) \]  

(40)

and

\[ a = 1 - \frac{7 \gamma_s \alpha}{3 \times 0.047} \]  

(41)

\[ b = -\left\{\left(\frac{z}{2} x J - R_m + g_{bs} C_m\right) + \left(\frac{x}{3} (1-x) J - R_d\right) + g_{bs} \left(\frac{7 \gamma_s O_m}{3}\right) + \frac{\alpha \gamma_s}{0.047} \left(\frac{x}{3} (1-x) J + \frac{\gamma_s}{3} R_d\right)\right\} \]  

(42)
Equation (4.40) can be approximated by

$$A_j = \min \left\{ \left( \frac{x}{2} J - R_m + g_b s C_m \right), \left( \frac{x}{3} (1 - x) J - R_d \right) \right\} \quad (44)$$

Where \( \min \{ \} \) stands for minimum of. Sometimes, when the equations are used to fit gas exchange measurements it is sufficient to use equation (44).

Summary of equations

Equations (21) and (40) are the two basic equations of the C₄ model and

$$A = \min\{A_c, A_j\}. \quad (45)$$

Peisker and Henderson (1992) pointed out that either the enzyme activity or the substrate regeneration rate can limit both Rubisco and PEP carboxylase reactions and that in theory four types of combinations of rate limitations are possible. In the way the electron transport limited equations are presented here, it is assumed that light or the electron transport capacity limit both PEP and RuBP regeneration rates simultaneously. In the model of C₃ photosynthesis by Farquhar et al. (1980) and von Caemmerer and Farquhar (1981) it was assumed that the limitation of RuBP regeneration could be adequately modelled by an electron transport limitation without consideration of limitations by other PCR cycle enzymes. This is probably the case in most instances however in transgenic studies care needs to be taken. Transgenic tobacco with reduced sedoheptulose 1,7 bisphosphatase (SBPase) regeneration of RuBP has been shown to be the more limiting step (Harrison et al., 2001; Harrison et al., 1998). In the case of C₄ photosynthesis the possibility that PEP regeneration may also be limited by the enzyme activity of enzymes such as PPDK and malic enzyme at high irradiance has also been found in transgenic studies with Flaveria bidentis a C₄ dicot (Furbank et al., 2001; Pengelly et al., 2012; Trevanion et al., 1997). C₄ transgenic plants with altered RuPB regeneration capacity have not yet been reported on.
An update on the parameterisation of the C₄ photosynthesis model

This model is built on the same principal as the Farquhar et al. (1980) model and many of the model’s parameters can be assigned a priori and this is indicated in Table 1, leaving only key variables like Vₐₘₐₓ Vₚₙₐₓ, Vₚₑ, and Jₘₐₓ to be assigned. It is however important to note that the kinetic constants of Rubisco from C₄ species differ from those of C₃ species and vary amongst the different C₄ decarboxylation types (Badger et al., 1974; Ghannoum et al., 2005; Jordan and Ogren, 1981, 1983; Sharwood et al., 2016).

The C₄ monocot species Setaria viridis (green foxtail millet), a NADP-malic enzyme type which is closely related to agronomically important C₄ crops including Setaria italica (foxtail millet), Z. mays (maize), Sorghum bicolor (sorghum) and Saccharum officinarum (sugarcane) has been suggested as a new model species (Brutnell et al., 2010). It has become a popular model species due to its rapid generation time, small stature, high seed production, diploid status and small sequenced and publicly available genome and it can be readily transformed (Alonso-Cantabrana et al., 2018; Brutnell et al., 2010; Doust, 2007; Li and Brutnell, 2011; Osborn et al., 2016). This has led to excellent biochemical characterisation of S. viridis PEPC and Rubisco (Boyd et al., 2015; DiMario and Cousins, 2019). Most parameters were taken from Boyd et al. (2015), but the Michaelis Menten constant for CO₂, Kₚ, was updated with more recent measurements by Di Mario and Cousins (2019). It is important to note that PEPC fixes bicarbonate rather than CO₂ and Kₚ is converted from measured values of Kₘ for HCO₃⁻. Here a cytosolic pH of 7.2 and pKa =6.12 was assumed (Hatch and Burnell, 1990). The precise value of cytosolic pH is unknown and if a pH of 7.4 is assumed Kₚ decreases from 82 µbar to 50 µbar. PEPC S. viridis RNAi line has been used to characterise bundle-sheath conductance to CO₂ diffusion (gₑₛ) and its temperature dependence (Alonso-Cantabrana et al., 2018). This has for the first time provided a cohesive parameter set and the associated temperature functions (Table 1).

It is best to estimate the temperature response of Jₘₐₓ from a series of light response curves made at high CO₂ and different temperatures as was done by Massad et al (2007) for Zea mays. They used an Arrhenius function for their parameterisation. The simpler temperature function suggested by June et al. (2004) is used here to parametrise the temperature dependence of electron transport (Table 1) and the parameterisation for tobacco has been used (Yamori et al., 2010) since these experiments still need to be done for S. viridis.
Sonawane et al. (2017), who characterised the temperature response of CO₂ assimilation rate in a number of C₄ grasses used this function to fit the saturated rate of CO₂ assimilation measured at high irradiance. The tobacco values chosen here fit within the range values reported for these C₄ grasses (Sonawane et al., 2017).

Calculating the electron transport required to sustain CO₂ assimilation

Von Caemmerer and Farquhar (1981) suggested that measurements of CO₂ assimilation rate can be used to calculate the electron transport rate needed to support the CO₂ assimilation rate. Equation 37 can be used in the same way. Using equation 39 to solve for \( J_t \) this results in the following quadratic equation:

\[
J = \left( -b + \sqrt{b^2 - 4ac} \right) / (2a) \tag{46}
\]

\[
a = z^2 x \frac{1-x}{3} \tag{47}
\]

\[
b = \frac{z(1-x)}{3} \left[ g_{sb} (C_m - \gamma, O_m) - R_m - A(1 - \gamma, \alpha) \right] - \frac{zx}{2} (A + R_d) \tag{48}
\]

\[
c = (A + R_d) \left[ R_m - g_{sb} (C_m + \gamma, O_m 7/3) + A(1 - \gamma, \alpha 7/3) \right] \tag{49}
\]

These equations were introduced by Ubierna et al. (2013) for linear electron flow only (\( z=0.75 \)) and the assumption of 3 H⁺/ATP.

Model evaluation

Modelled CO₂ response of CO₂ assimilation

In C₃ species CO₂ response curves are widely used to assess photosynthetic capacity (Ainsworth and Rogers, 2007; Sharkey et al., 2007; von Caemmerer, 2000; von Caemmerer and Farquhar, 1981). Figure 3 compares the model output of the Farquhar, von Caemmerer and Berry model of C₃ photosynthesis (Farquhar et al., 1980) with the current C₄ model presented here. In the C₃ model the enzyme limited rate is dominated by Rubisco and its kinetic parameters at low CO₂ and the electron transport capacity limits at high CO₂ (Fig. 3a).

In the C₄ model it is also possible to distinguish an enzyme limited CO₂ assimilation rate at high light (equations 20-25) and an electron transport limited rate (equations 37-44).

However, the enzyme limited rate is determined by PEPC at low CO₂ and Rubisco at high...
CO₂. The electron transport limited rate can also determine the CO₂ assimilation rate at high CO₂ (Fig. 3b). Thus, it is more difficult to identify biochemical limitations to C₄ photosynthesis.

Usually good correlations are found between in vitro Rubisco activity and the CO₂ saturated rate of CO₂ assimilation rate at high CO₂ (Sonawane et al., 2017; Usuda, 1984; Usuda et al., 1984). The relationship should be almost one to one as Rubisco operates close to its saturated rate in vivo. In the study of *Flaveria bidentis* transgenics with varying reductions in Rubisco content show a slight curvilinear relationship between Rubisco content and CO₂ assimilation rate hinting at a possible electron transport limitation in wild type plants (Furbank et al., 1996; von Caemmerer et al., 1997). These studies have also provided evidence that Rubisco limits CO₂ assimilation at high CO₂. Transgenic plants with reduced Rubisco content showing a clear decline in CO₂ assimilation rate at high CO₂ (Pengelly et al., 2012; von Caemmerer et al., 1997). Recent photosynthetic engineering that increased Rubisco content in maize leaves resulted in an increase in CO₂ saturated CO₂ assimilation rate (Salesse-Smith et al., 2018).

Here the model has been tuned in such a way that at 25 °C electron transport rate is limiting CO₂ assimilation at high CO₂ and high irradiance. This balance can of course vary with growth conditions or species, but there is no straightforward technique to determine the limitation. Furthermore, the assumption has been made the electron transport capacity and PEP and RuBP regeneration generally co-limit. In transgenic studies where regeneration of the C₄ cycle has been curtailed by molecular manipulation, it is clear that this also limits CO₂ assimilation at high CO₂ (Pengelly et al., 2012; Trevanion et al., 1997). In a study, transgenic *S. viridis* with overexpression of the Rieske iron sulphur protein in the cytochrome b₅f complex had increased CO₂ assimilation rates at ambient and high CO₂ confirming that electron transport capacity can limit CO₂ assimilation rate (Ermakova et al., 2019). The fact that electron transport rate limits CO₂ assimilation rate at high CO₂ means that a reduction in irradiance is also predicted to primarily affects the CO₂ saturated rate of CO₂ assimilation rather than the initial slope of the CO₂ response curve except at low irradiance (Leegood and von Caemmerer, 1989; Pfeffer and Peisker, 1998).
There are three possible limitations to the initial slope of the CO2 response curve; the mesophyll conductance to CO2 diffusion from intercellular airspace to the mesophyll cytosol, $g_m$, the rate of CO2 hydration by carbonic anhydrase, CA, and the rate of PEP carboxylation. It is thought that most C4 leaves have sufficient CA for it not to be rate limiting (Cousins et al., 2008; Hatch and Burnell, 1990). However, studies with transgenic or mutant plants in \textit{Flaveria bidentis}, \textit{Zea mays} and \textit{Setaria viridis} have shown that when CA activity is greatly reduced a reduction in initial slope of the CO2 response is observed (Osborn et al., 2016; Studer et al., 2014; von Caemmerer et al., 2004).

The initial C4 photosynthesis models did not consider a diffusion limitation between the intercellular airspace and the mesophyll cytosol. In C4 species mesophyll conductance, $g_m$, is likely to be proportional to mesophyll surface area exposed to intercellular airspace (Evans and von Caemmerer, 1996). The standard techniques used to quantify mesophyll conductance in C3 species such as combined measurements of gas exchange and chlorophyll fluorescence, or measurements of $^{13}$C isotope discrimination cannot be used in C4 species, however a new technique has been developed to measure mesophyll conductance in C4 species using C$^{18}$O$^{16}$O isotope discrimination (Barbour et al., 2016; Gillon and Yakir, 2000; Ogée et al., 2018; Osborn et al., 2016) and here the temperature dependence of $g_m$ measured for \textit{Setaria viridis} has been used for parameterisation of the model (Table 1, Ubierna et al., 2017).

The drop in CO2 partial pressure from intercellular airspace, $C_i$ to that of the mesophyll, $C_m$ are related in the following equation

$$A = g_m(C_i - C_m). \quad (50)$$

Incorporating equation (50) into equation (21) results in a cubic expression which is not easily solved. It can be incorporated into equation (40) giving a slightly more complex quadratic. In case of the initial slope of the CO2 response curve one can use equation (26) and ignoring the term $g_sC_m$ and combining it with equation (50) a quadratic similar to the one given for C3 leaves is obtained (von Caemmerer, 2000; von Caemmerer and Evans, 1991).

$$A^2 - A(g_m(C_i + K_p) + V_{pmax} - R_m) + g_m \left( V_{pmax}C_i - R_m(C_i + K_p) \right) = (51)$$
The first derivative with respect to $C_i$ at $C_i=0$ is given by

$$\frac{dA}{dc_i} = \frac{g_m V_{pmax}}{g_m k_p + V_{pmax}}$$ (52)

Pfeffer and Peisker (1988 and 1998) used this equation together with measurements of PEP carboxylase activity and initial slope ($dA/dC_i$) to estimate $g_m$ in plants grown under different light intensities. Figure 4 shows the effect inclusion of mesophyll conductance has on the initial slope. Equation 52 was used by Ubierna et al. (2016) to estimate $g_m$ from in vitro measurements of PEP carboxylase activity, $V_{pmax}$, and they found good agreement with estimates of $g_m$ from measurements of $C^{18}O^{16}O$ isotope discrimination, however the uncertainties surrounding estimates of $K_p$ discussed above need to be considered.

Strong correlations between leaf nitrogen, CO$_2$ assimilation rate and PEP carboxylase activity have been observed in several studies (Meinzer and Zhu, 1998; Sage and Pearcy, 1987; Usuda, 1984; Wong et al., 1985) however it has been more difficult to provide quantitative correlations. Without the inclusion of a mesophyll conductance estimates of $V_{pmax}$ form the initial slope are often less than what is measured in vitro. For example if the initial slope of the lower curve in Figure 4a is used to estimate $V_{pmax}$ with an infinitely large $g_m$ the predicted $V_{pmax}$ is 58 µmol m$^{-2}$ s$^{-1}$ whereas it has here been modelled with a $V_{pmax}$ of 200 µmol m$^{-2}$ s$^{-1}$ and $g_m = 1$ mol m$^{-2}$ s$^{-1}$ bar$^{-1}$. Hence mesophyll conductance is an important parameter in linking C$_4$ biochemistry with gas exchange.

**Modelled light response of CO$_2$ assimilation**

It is well recognised that the light response of C$_4$ photosynthesis does frequently not saturate (Cousins et al., 2006; Leakey et al., 2006). Figure 5 shows typical modelled light response curves of CO$_2$ assimilation rate at several mesophyll CO$_2$ partial pressures. In the current parametrisation CO$_2$ assimilation rate is electron transport limited at all irradiances above $C_m=150$ µbar at 25 °C. The shapes of the curves are determined by equation (34) which as for the C$_3$ photosynthetic model remains empirical and the partition partitioning of electron transport between C$_4$ and C$_3$ cycle has been set at x=0.4 (equation 29). Furbank and von Caemmerer gave a detailed discussion about the optimal partitioning of electron transport capacity between C$_3$ and C$_4$ cycle (Peisker, 1988; von Caemmerer, 2000; von Caemmerer and Furbank, 1999). It is noteworthy that the fraction of electron transport
allocated to the C₄ cycle, x, equals 0.4 over a wide range of irradiances but drops at very low irradiance. Under low light the bundle-sheath CO₂ partial pressures are close to the mesophyll CO₂ partial pressure and electron transport is required for recycling of photorespiratory CO₂. The optimal partitioning increases from 0.404 to 0.417 if oxygen is evolved in the bundle sheath (α=1). It also declines slightly with increasing temperature as Rubisco specificity for CO₂ decreases (Jordan and Ogren, 1984; Sharwood et al., 2016).

In C₃ species there a close link has been established between chloroplast electron transport capacity and electron transport chain intermediates such as cytochrome f (Yamori et al., 2010). In C₄ photosynthesis this quantitative link between cytochrome f content and electron transport capacity needs also to be investigated.

**Modelled temperature response of CO₂ assimilation rate**

C₄ plants have higher CO₂ assimilation rates at high temperatures and higher photosynthetic temperature optima than their C₃ counterparts largely because of the elimination of photorespiratory CO₂ losses. (Berry and Björkman, 1980; Long, 1999). The temperature response of electron transport is not well characterised in C₄ species. With the parameterisation used here, CO₂ assimilation rate is electron transport limited above 25°C, and enzyme limited below a Ci=150 µbar at height light, which corresponds to the operating Ci of many C₄ species at ambient CO₂ (Figure 6). There is some evidence that this is not unreasonable. Figure 7 shows a comparison of a temperature response of CO₂ assimilation rate of *Flaveria bidentis* wildtype and transgenic Flaveria with reduced Rubisco content (Kubien et al., 2003). In Figure 7b CO₂ assimilation rate is expressed on a Rubisco site basis (in vivo kcat) and compared the temperature response of Rubisco in vitro activity. For Flaveria with reduced amount of Rubisco there is a match between in vivo and in vitro kcat up to approximately 30°C whereas for the wild type in vivo kcat is less than the in vitro Rubisco kcat around 20°C indicating other limitations to CO₂ assimilation rate such as electron transport capacity. Temperature optima of CO₂ assimilation rate are dependent on growth environment (Berry and Björkman, 1980; Dwyer et al., 2007). The modelling suggests that the temperature optimum is most likely determined by the properties of the electron transport capacity (Figure 6).
In Figure 8a the CO2 response curves have been modelled for different leaf temperatures. Figure 8a, shows that at low temperature the CO2 response is enzyme limited at all Ci, as temperature is increased the transition from enzyme limited CO2 assimilation rate to electron transport limited rate occurs at progressively lower Ci. There is an increase in initial slope with increasing temperature which is caused by the temperature response of the mesophyll conductance and the different temperature dependencies of maximal PEPC carboxylation and is Michaelis Menten constant ($V_{p_{\text{max}}}$ and $K_p$) (Figure 8 a and b, Table 1). These model predictions fit well with experimental observations by Sonawane et al.(2017).

A note on leakiness

The bundle sheath resistance or its inverse the bundle sheath conductance to CO2 diffusion are key parameters that together with relative capacities for the C4 cycle and Rubisco and electron transport capacity determine the effectiveness of the CO2 concentration mechanism. This is often quantified by a term called leakiness ($\phi$), which is defined as the ratio of the rates of CO2 leakage out of the bundle sheath over the rate of CO2 supply to the bundle sheath (equation 4 and 5). Carbon isotope discrimination can be used to determine leakiness (Farquhar, 1983). Combined measurements of gas exchange and carbon isotope discrimination have been used to assess leakiness under different environmental conditions (Henderson et al., 1992; King et al., 2012; Kromdijk et al., 2010; Pengelly et al., 2010; Sun et al., 2012; Ubierna et al., 2011; von Caemmerer and Furbank, 2003). It is tempting to predict leakiness from the C4 photosynthesis model, but because it is a flux model little can be said about the rate of the component that is not limiting and leakiness estimates are not realistic as non-rate limiting steps are likely to be down regulated. However, when CO2 assimilation rates and leakiness are known from combined measurements of gas exchange and carbon isotope discrimination the model can be used to calculated the rate of the C4 cycle and the leak rate from the following equations and equation 4

$$V_p = \frac{A+R_m}{1-\phi}.$$  (53)

With the assumption of a bundle sheath conductance bundle sheath CO2 can also be estimated (Pengelly et al., 2012).
Conclusion

The steady state C\(_4\) photosynthesis model has been updated and parameterized with the \textit{in vitro} kinetic constants for Rubisco and PEP carboxylase and values for mesophyll and bundle sheath conductance and their temperature dependencies. Furthermore, electron transport rate equations have been updated to include cyclic electron transport flow. Now it is important to compare gas exchange measurements and biochemical measurements to confirm the quantitative relationships predicted by the model. In particular a parameterization of the temperature response of electron transport rate is needed and information of how it relates to thylakoid electron transport components such as the b\(_{6f}\) complex which has shown to be a good correlator of C\(_3\) photosynthetic electron transport.

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Table 1. Photosynthetic parameters used in the model. When available values for *Setaria viridis* have been chosen.

| Parameter | Value at 25°C | Definition | Activation energy E (kJmol⁻¹) | Reference |
|-----------|---------------|------------|------------------------------|-----------|
| \(V_{c_{\text{max}}}\) | 40 µmol m⁻² s⁻¹ or variable | maximum Rubisco activity | 78 | (Boyd *et al.*, 2015) |
| \(K_c\) | 1210 µbar | Michaelis constant of Rubisco for CO₂ | 64.2 | (Boyd *et al.*, 2015) |
| \(K_o\) | 292 mbar | Michaelis constant of Rubisco for O₂ | 10.5 | (Boyd *et al.*, 2015) |
| \(\gamma_\ast\) | ~0.0003817 (0.5/2619) | 0.5/(S_{\text{c/o}}) half the reciprocal of Rubisco specificity | 31.1 | (Boyd *et al.*, 2015) |
| \(V_{p_{\text{max}}}\) | 300 µmol m⁻² s⁻¹ or variable | maximum PEP carboxylase activity | 94.8 | (Boyd *et al.*, 2015) |
| \(V_{p_{r}}\) | 80 µmol m⁻² s⁻¹ or variable | PEP regeneration rate | | |
| \(K_p\) | 82 µbar | Michaelis constant of PEP carboxylase for CO₂ | 36.3 | (DiMario and Cousins, 2019) |
| \(g_{\text{bs}}\) | 0.003 mol m⁻² s⁻¹ bar⁻¹ | bundle sheath conductance to CO₂ | Constant with temperature | (Alonso-Cantabrana *et al.*, 2018) |
| \(g_{\text{o}}\) | 0.047 g_{\text{s}} | bundle sheath conductance to O₂ | | (Farquhar, 1983) |
| \(R_d\) | 0.01*\(V_{c_{\text{max}}}\) | Leaf mitochondrial respiration | 66.4 | (Farquhar *et al.*, 1980) |
| \(R_m\) | 0.5 \(R_d\) | mesophyll mitochondrial respiration | | |
| \(\alpha\) | 0<\alpha<1, | fraction of PSII activity in the bundle sheath | | |
| \(x\) | 0.4 | partitioning factor of electron transport rate | | |
| \(J_{\text{max}}\) | 170 µmol electrons m⁻² s⁻¹ or variable | maximal linear electron transport rate | | |
| \(T_o\) | 43°C | T optimum | \(J_{\text{max}}(T_L) = J_{\text{max}}(T_o)e^{-\left(\frac{T_L-T_o}{\Omega}\right)^2}\) | (June *et al.*, 2004; Yamori *et al.*, 2010) |
| \(\Omega\) | 26 | \(\Omega\) is the difference in temperature from \(T_o\) at which \(J\) falls to e⁻¹ (0.37) | | (Yamori *et al.*, 2010) |
| \(J_{\text{max}@T_o}\) | 300 µmol electrons m⁻² s⁻¹ or variable | | | |
| \(h\) | 4 | Number of protons per ATP | | |
| \(f_{\text{cyc}}\) | 0.5 or variable | Fraction of cyclic electron transport | | (Yin and Struik, 2012) |
| \(z\) | | Ratio of the rate of ATP production to linear electron transport (\(J_{\text{ATP}}/J\)) | | (Yin and Struik, 2012) |
| \(g_{m}\) | 1 mol m⁻² s⁻¹ bar⁻¹ | Mesophyll conductance to CO₂ | 40.6 | (Ubierna *et al.*, 2017) |
Values of parameters where activation energies have been given can be calculated at any temperature from the following equation: \( P_{\text{activation}}(T) = P_{\text{activation}}(25)^\circ C \exp \left[ \frac{(T - 298)E_{298}}{298RT} \right] \), where \( R (8.314\text{JK}^{-1}\text{mol}^{-1}) \) is the universal gas constant and \( T \) is temperature in degrees Kelvin (K).
Figure legends

Figure 1
Schematic representing the main features of the C₄ photosynthetic pathway. CO₂ diffuses into the mesophyll where it is converted to HCO₃⁻ and fixed by PEP carboxylase at the rate \( V_p \). In the steady state C₄ acid decarboxylation occurs at the same rate. CO₂ released in the bundle sheath either leaks out of the bundle sheath at (\( L \)) or is fixed by Rubisco (\( V_c \)). In the photosynthetic carbon oxidation cycle CO₂ is released at the half the oxygenation rate (\( V_o \)). CO₂ is also released by respiration (\( R_m, R_s \)) in mesophyll and bundle sheath respectively. Electron transport components are not shown.

Figure 2
Scheme for linear and cyclic electron transport in the light reactions of photosynthesis. The arrows indicate electron transport. Thick curved arrows show the number of protons produced per electron transported. \( J \) denotes linear electron transport through photosystem II, \( J_1 \) is electron transport through photosystem I and \( J_{cyc} \) the rate of cyclic electron transport. The symbol \( f_{cyc} \) denotes the fraction of \( J_1 \) that flows via the cyclic mode. The diagram has been adapted from Yin et al. (2004).

Figure 3
A comparison of modelled rates of CO₂ assimilation rate as functions of partial pressures of CO₂ for C₃ (a) and C₄ photosynthesis (b).

a) Modelled rate of CO₂ assimilation as a function of chloroplast CO₂ partial pressure for the C₃ photosynthetic pathway at 25 °C. The Rubisco limited (RuBP saturated rate) of CO₂ assimilation has a dashed line extension at high CO₂. The electron transport (RuBP regeneration) limited rate of CO₂ assimilation has a dotted line extension at low CO₂. The solid curve represents the minimum rate that is the actual rate of CO₂ assimilation. A possible triose phosphate limitation at high CO₂ is not shown. (Redrawn from von Caemmerer 2000).

b) Modelled rate of CO₂ assimilation as a function of mesophyll cytosolic CO₂ partial pressure for the C₄ photosynthetic pathway at 25 °C and an irradiance of 1500 µmol m⁻² s⁻¹. The enzyme limited CO₂ assimilation rate (PEPC limitation at low CO₂ and Rubisco limitation at high CO₂) shows the Rubisco limited rate as a dashes line extension. The electron transport (RuBP and PEP regeneration) limited rate of CO₂ assimilation has a dotted line extension at low CO₂. The solid curve represents the minimum rate that is the actual rate of CO₂ assimilation. Parameters used are given in Table 1.

Figure 4
The effect of mesophyll conductance, \( g_m \) on the initial slope of the CO₂ response curve.

a) Modelled rate of CO₂ assimilation as a function of intercellular CO₂ partial pressure for the C₄ photosynthetic pathway at 25 °C and an irradiance of 2000 µmol m⁻² s⁻¹ modelled with \( g_m = 1 \) mol m⁻² s⁻¹ bar⁻¹ or an infinite \( g_m \).

b) Initial slope (equation x) as a function of mesophyll conductance. Model parameters are those given in Table 1.
Figure 5

The effect of irradiance on modelled rate of CO₂ assimilation.

a) Light response of CO₂ assimilation rate at mesophyll cytosolic CO₂ partial pressures, Cₘ, in
 dictated in the figure. The C₄ photosynthesis model predicts electron transport limitations at
 all irradiance at Cₘ values above 150 µbar. At lower Cₘ, CO₂ assimilation rates are enzyme
 limited at high irradiance. The model was parameterised at 25 °C with values given in Table
 1 and x=0.4.

b) CO₂ assimilation rate as a function of intercellular CO₂ at irradiances indicated. The model
 was parameterised at 25 °C with values given in Table 1 and x=0.4.

Figure 6

Modelled CO₂ assimilation rate as a function of leaf temperature. The dotted line and its
 extension line show the enzyme limited rate and the dashed line and its extension line show the
 electron transport limited rate. CO₂ assimilation rate was modelled at an irradiance of 2000
 µmol m⁻² s⁻¹ and mesophyll CO₂, Cₘ of 150 µbar. Other parameters are as given in Table 1.

Figure 7

a) Temperature responses CO₂ assimilation rate in Faveria bidentis wild type and anti-Rubisco plants
 Photosynthesis was measured at different leaf temperatures and ambient CO₂ of 370 µbar and 200
 mbar O₂ and an irradiance of 1500 µmol m⁻² s⁻¹. Each point represents the mean (± SE) of
 measurements on five different leaves.

b) Temperature dependence of the in vitro and in vivo kₗₐₜ for Rubisco in wildtype and anti-Rubisco F.
 bidentis. The in vitro data reflect the activity of the fully carbamylated enzyme; in vivo kₗₐₜ is esti-
mated as gross photosynthesis divided by the number of Rubisco catalytic sites. Each value
 represents the mean (± SE) of four measurements.

The data is redrawn from Figures 1 and 4 (Kubien et al., 2003)

Figure 8

a) Modelled rate of CO₂ assimilation as a function of intercellular CO₂ partial pressure, Cᵢ, for the C₄
 photosynthetic pathway at three leaf temperatures of 15, 25 and 35°C and an irradiance of 2000
 µmol m⁻² s⁻¹. At 15°C CO₂ assimilation rate is enzyme limited at all Cᵢ. For 25 and 35°C the arrow
 indicates the transition from enzyme limitation at low Cᵢ to electron transport limitation at high Cᵢ.
 Parameters used are given in Table 1.

b) Initial slope (dA/dCᵢ) calculated from equation 52 as a function of leaf temperature. Parameters
 used are given in Table 1.
Figure 1

Schematic representing the main features of the C₄ photosynthetic pathway. CO₂ diffuses into the mesophyll where it is converted to HCO₃⁻ and fixed by PEP carboxylase at the rate $V_p$. In the steady state C₄ acid decarboxylation occurs at the same rate. CO₂ released in the bundle sheath either leaks out of the bundle sheath at ($L$) or is fixed by Rubisco ($V_c$). In the photosynthetic carbon oxidation cycle CO₂ is released at the half the oxygenation rate ($0.5V_o$). CO₂ is also released by respiration ($R_m, R_s$) in mesophyll and bundle sheath respectively. Electron transport components are not shown.
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Scheme for linear and cyclic electron transport in the light reactions of photosynthesis. The arrows indicate electron transport. Thick curved arrows show the number of protons produced per electron transported. $J$ denotes linear electron transport through photosystem II, $J_1$ is electron transport through photosystem I and $J_{cyc}$ the rate of cyclic electron transport. The symbol $f_{cyc}$ denotes the fraction of $J_1$ that flows via the cyclic mode. The diagram has been adapted from Yin et al. (2004).
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**c)** Modelled rate of CO₂ assimilation as a function of chloroplast CO₂ partial pressure for the C₃ photosynthetic pathway at 25 °C. The Rubisco limited (RuBP saturated rate) of CO₂ assimilation has a dashed line extension at high CO₂. The electron transport (RuBP regeneration) limited rate of CO₂ assimilation has a dotted line extension at low CO₂. The solid curve represents the minimum rate that is the actual rate of CO₂ assimilation. A possible triose phosphate limitation at high CO₂ is not shown. (Redrawn from von Caemmerer 2000).

**d)** Modelled rate of CO₂ assimilation as a function of mesophyll cytosolic CO₂ partial pressure for the C₄ photosynthetic pathway at 25 °C and an irradiance of 1500 µmol m⁻² s⁻¹. The enzyme limited CO₂ assimilation rate (PEPC limitation at low CO₂ and Rubisco limitation at high CO₂) shows the Rubisco limited rate as a dashes line extension. The electron transport (RuBP and PEP regeneration) limited rate of CO₂ assimilation has a dotted line extension at low CO₂. The solid curve represents the minimum rate that is the actual rate of CO₂ assimilation. Parameters used are given in Table 1.
Figure 4

The effect of mesophyll conductance, $g_m$ on the initial slope of the CO$_2$ response curve.

a) Modelled rate of CO$_2$ assimilation as a function of intercellular CO$_2$ partial pressure for the C$_4$ photosynthetic pathway at 25 °C and an irradiance of 2000 µmol m$^{-2}$ s$^{-1}$ modelled with $g_m = 1$ mol m$^{-2}$ s$^{-1}$ bar$^{-1}$ or an infinite $g_m$.

b) Initial slope (equation x) as a function of mesophyll conductance. Model parameters are those given in Table 1.
Figure 5
The effect of irradiance on modelled rate of CO₂ assimilation.

**c)** Light response of CO₂ assimilation rate at mesophyll cytosolic CO₂ partial pressures, Cₘ, in dictated in the figure. The C₄ photosynthesis model predicts electron transport limitations at all irradiance at Cₘ values above 150 µbar. At lower Cₘ, CO₂ assimilation rates are enzyme limited at high irradiance. The model was parameterised at 25 °C with values given in Table 1 and x=0.4.

**d)** CO₂ assimilation rate as a function of intercellular CO₂ at irradiances indicated. The model was parameterised at 25 °C with values given in Table 1 and x=0.4.
Figure 6

Modelled CO₂ assimilation rate as a function of leaf temperature. The dotted line and its extension line show the enzyme limited rate and the dashed line and its extension line show the electron transport limited rate. CO₂ assimilation rate was modelled at an irradiance of 2000 µmol m⁻² s⁻¹ and mesophyll CO₂, Cₘ of 150 µbar. Other parameters are as given in Table 1.
Figure 7

a) Temperature responses CO₂ assimilation rate in *Flaveria bidentis* wild type and anti-Rubisco plants. Photosynthesis was measured at different leaf temperatures and ambient CO₂ of 370 µbar and 200 mbar O₂ and an irradiance of 1500 µmol m⁻² s⁻¹. Each point represents the mean (± SE) of measurements on five different leaves.

b) Temperature dependence of the in vitro and in vivo k_{cat} for Rubisco in wildtype and anti-Rubisco *F. bidentis*. The in vitro data reflect the activity of the fully carbamylated enzyme; in vivo k_{cat} is estimated as gross photosynthesis divided by the number of Rubisco catalytic sites. Each value represents the mean (± SE) of four measurements.

The data is redrawn from Figures 1 and 4 (Kubien *et al*., 2003)
Figure 8

a) Modelled rate of CO₂ assimilation as a function of intercellular CO₂ partial pressure, Cᵢ, for the C₄ photosynthetic pathway at three leaf temperatures of 15, 25 and 35°C and an irradiance of 2000 µmol m⁻² s⁻¹. At 15°C CO₂ assimilation rate is enzyme limited at all Cᵢ. For 25 and 35°C the arrow indicates the transition from enzyme limitation at low Cᵢ to electron transport limitation at high Cᵢ. Parameters used are given in Table 1.

b) Initial slope (dA/dCᵢ) calculated from equation 52 as a function of leaf temperature. Parameters used are given in Table 1.
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