A generalised dynamic model of leaf-level C₃ photosynthesis combining light and dark reactions with stomatal behaviour

Chandra Bellasio

Research School of Biology, Australian National University, Acton, ACT, 2601 Australia
University of the Balearic Islands 07122 Palma, Illes Balears, Spain
Trees and Timber institute, National Research Council of Italy, 50019 Sesto Fiorentino (Florence)

chandra.bellasio@anu.edu.au    ORCiD 0000-0002-3865-7521

Running title

Dynamic model of C₃ leaf assimilation

Keywords

Mechanistic model, Microsoft® Excel®, stomatal model, time, transients, stomatal conductance, assimilation, photorespiration, light fleck.

Acknowledgments

I am deeply grateful to the Editor of this special issue, Nerea Ubierna Lopez, for editing that improved the clarity and readability, to Joe Quirk for a substantial contribution to writing the first version, I thank Ross Deans (Australian National University, ANU) for unpublished spinach leaf gas exchange data, and Florian Busch (ANU) for help, review, and critical discussion. I am funded through a H2020 Marie Sklodowska-Curie individual fellowship (DILIPHO, ID: 702755).
I have no conflict of interest.
Abstract

Global food demand is rising, impelling us to develop strategies for improving the efficiency of photosynthesis. Classical photosynthesis models based on steady state assumptions are inherently unsuitable for assessing biochemical and stomatal responses to rapid variations in environmental drivers. To identify strategies to increase photosynthetic efficiency, we need models that account for the timing of CO2 assimilation responses to dynamic environmental stimuli. Herein, I present a dynamic process-based photosynthetic model for C3 leaves. The model incorporates both light and dark reactions, coupled with a hydro-mechanical model of stomatal behaviour. The model achieved a stable and realistic rate of light-saturated CO2 assimilation and stomatal conductance. Additionally, it replicated complete typical assimilatory response curves (stepwise change in CO2 and light intensity at different oxygen levels) featuring both short lag times and full photosynthetic acclimation. The model also successfully replicated transient responses to changes in light intensity (light flecks), CO2 concentration, and atmospheric oxygen concentration. This dynamic model is suitable for detailed ecophysiological studies and has potential for superseding the long-dominant steady-state approach to photosynthesis modelling. The model runs as a stand-alone workbook in Microsoft® Excel® and is freely available to download along with a video tutorial.

Introduction

The pace of increases in crop yields has stalled over recent decades, urging researchers to develop innovative solutions to safeguard the productivity necessary to sustain expected future global demand for food and feed (Ray et al. 2012; Ray et al. 2013). The photosynthetic efficiency of C3 crop plants falls short of theoretical potentials and is little or negatively affected by selective breeding (Long et al. 2015), making efficiency gains a key aim for improving yields from existing agricultural land (Taylor and Long 2017). Photosynthetic responses to dynamic environmental drivers are increasingly recognised as an area where photosynthetic efficiency can be improved by minimising the assimilatory and, or stomatal lag response(s) to environmental fluctuations, particularly light intensity (Kaiser et al. 2014; Lawson and Blatt 2014).

Leaves may experience large transient variations in light intensity (measured as photosynthetic photon flux density, PPFD) as they move into the shade of leaves higher in the canopy and clouds move overhead to create light- and shade-flecks of varying intensity and spectral quality (Bellasio and Griffiths 2014; Pearcy et al. 1985; Pearcy 1990; Valladares et al. 1997). Shaded leaves can contribute up to 50% of canopy photosynthesis (Long 1993; Long et al. 1996) and accurate quantification of CO2 assimilation (A) requires modelling of leaf responses to fluctuations in the canopy light environment (Allen and Richardson 1968; Song et al. 2013). In addition, atmospheric CO2 concentration (Ca) can vary locally under natural field conditions, but variability in Ca is more frequent and pronounced when [CO2] is experimentally enriched (Hendrey et al. 1997).
Stomata and photosynthesis respond continuously to environmental changes, but stomatal adjustments, which regulate the diffusion of CO₂ into the leaf and the conductance of water vapour to the atmosphere (gs), can be an order of magnitude slower than assimilatory responses (McAusland et al. 2016). This lack of coordination between carbon gains (A) and water losses (E) often results in suboptimal water-use efficiency (WUE = A/E) and photosynthetic shortfalls (Lawson and Blatt 2014; Bellasio et al. 2017). Further, A may be biochemically limited due to a lag time in the induction of biochemical activity following environmental fluctuations (Naumburg and Ellsworth 2002; Taylor and Long 2017). By improving the speed at which the photosynthetic machinery responds and adjusts to fluctuating environmental conditions, substantial accrual of marginal gains in A and water savings over time are possible (Bellasio et al. 2017; Lawson and Blatt 2014; McAusland et al. 2016; Way and Pearcy 2012).

Most photosynthesis models, used at leaf level and broader scales, are based on steady state principles [for review (Bellasio et al. 2016b; Bellasio et al. 2016a)]. Assimilation is often predicted using steady-state sub-models rooted in the Farquhar et al. (1980) framework, which have since been updated (Busch et al. 2017; Yin et al. 2014). Steady-state photosynthesis models tend to overestimate integrated A under fluctuating PPFD (Kaiser et al. 2014), but also under variable Cₐ (Hendrey et al. 1997). This results, for instance, in poor understanding of plant growth and acclimation responses in CO₂ enrichment experiments, particularly under free air CO₂ enrichment (FACE) conditions (Long et al. 2006). This confounds the interpretation of experimental findings and hinders prediction of vegetation responses to rising CO₂ levels in the future. Moreover, incorporation of the latest developments in plant manipulation, including the effect of a modified reductive pentose phosphate pathway [RPP, (Driever et al. 2017)] and light reaction processes (Kromdijk et al. 2016), require further biochemical complexity than that of traditional models. In broader scale vegetation modelling, photosynthesis models are coupled with models characterising stomatal behaviour (Berry et al. 2010; Beerling 2015; Bonan et al. 2014; Ostle et al. 2009; Sato et al. 2015). The stomatal sub-models generally estimate gs empirically from environmental or internal variables rather than from process-based mechanistic principles (Damour et al. 2010).

Empirical models may lose accuracy as simulated conditions deviate further from those under which the models were calibrated (Way et al. 2011) and then cannot provide insight into underlying physiological mechanisms (Buckley 2017).

Dynamic models characterise photosynthesis and stomatal behaviour under non steady-state conditions. Although dynamic models of photosynthesis and gs exist [e.g. (Kirschbaum et al. 1997; Laisk and Eichelmann 1989)], their application has been limited by the accessibility of the code or because their treatment of photosynthetic processes is either phenomenological (Vialet-Chabrand et al. 2016; McAusland et al. 2016), elementary (Pearcy et al. 1997; Gross et al. 1991), or so complex as to require dedicated software and high-capability computing (Laisk et al. 2009; Wang et al.)
2014a; Wang et al. 2014b; Zhu et al. 2013; Zhu et al. 2007). Consequently, most studies, including those simulating dynamic conditions, have used steady-state models [e.g. (Taylor and Long 2017)].

Here I developed a biochemical, process-based framework for modelling photosynthetic dark reactions that is incorporated with light reactions and coupled to a mechanistic hydro-mechanical model of stomatal behaviour. I demonstrate its applicability using a range of examples including classical $A-PPFD$ and $A-C_i$ response curves, a mid-term acclimation to variable $C_a$ and $PPFD$, and response to rapid transitions in light intensity and oxygen concentration. To maximise the potential user base of the model, I coded and developed it in a Microsoft® Excel® workbook, which is openly available from the Supplementary Information along with a video user guide.

**Model development**

*Overview*

A process-based, stock-and-flow model of leaf-level C$_3$ photosynthesis that runs in Excel® was developed incorporating leaf-level diffusion with a comprehensive treatment of assimilatory biochemistry and stomatal behaviour (Figure 1, equations are detailed in the Appendix). The modelled leaf consists of three compartments: the atmosphere, intercellular space and mesophyll. The processes of CO$_2$ diffusion through stomata, and CO$_2$ dissolution and hydration are described mechanistically. To reduce computational requirements, intercellular space and mesophyll are assumed uniform with no internal concentration gradients. Consequently, limitations imposed by the diffusion of metabolites are not considered. This is justified by a number of studies showing minimal reduction in $A$ by heterogeneous distribution of metabolites (Wang et al. 2017; Retta et al. 2016; Tholen et al. 2012; Ho et al. 2015).

A light reactions submodel, modified from Yin et al. (2004), was used to estimate the potential rates of ATP and NADPH production for any $PPFD$. In the original Yin et al. (2004) formulation, the ratio of ATP to NADPH production rates could be adjusted by varying the cyclic electron flow rate (CEF, although this is close to zero for C$_3$ types). However, up–regulating CEF required additional light to be absorbed by photosystem I (PSI) because a constant electron flow through PSII ($J_2$) was assumed to facilitate implementation with fluorescence measurements. Here, a constant level of total light absorbed by PSI and PSII was used and was partitioned between photosystems using Yin et al. (2004) equations but modified (see Figure 2) to account for the presence of the nicotinamide adenine dinucleotide (NADH) dehydrogenase–like (NDH) complex (Ishikawa et al. 2016; Yamori and Shikanai 2016).

After passing through PSI, electrons are either cycled to plastoquinone, used by alternative sinks ($J_{Pseudocy}$ includes all sinks that are not assimilatory dark reactions, such as O$_2$ and NO$_3^-$), or used to reduce NADP$^+$ ($J_{NADPH}$ is the NADPH used in assimilatory dark reactions). In this way, the power requirements for nitrogen reduction (Busch et al. 2017) are explicitly accounted for as a fraction of
pseudo–cyclic electron flow ($f_{\text{Pseudocyc NR}}$), in line with Yin and Struik (2012). The remainder is consumed by the water–water cycle, also modelled explicitly. Although $f_{\text{Pseudocyc}}$ has a small value (~0.1, (Yin et al. 2004; Yin and Struik 2012)), its inclusion is important, as it influences the ATP/NADP ratio. The total ATP production rate ($J_{\text{ATP}}$) was obtained by summing the proton flow to the lumen and dividing by $h$, the number of protons required by ATP synthase. The potential rates of ATP and NADPH production are used by ATP and NADPH synthesis, which were modelled through a Michaelis–Menten kinetics function after Wang et al. (2014a). The proportion of actual to potential ATP and NADPH synthesis continuously feeds back to dark reactions by adjusting PSII yield [$Y(II)$] and the level of CEF. Time–delay functions allow simulation of photosynthetic acclimation of the potential rate of ATP ($J_{\text{ATP}}$) and NADPH ($J_{\text{NADPH}}$) synthesis to changes in PPFD.

A dynamic submodel of dark reactions, including key reactions involved in the RPP cycle, photorespiration pathway and carbohydrate synthesis, was developed by synthesis of the model of Zhu et al. (2007). This model related enzyme activity to the concentration of substrates, including ATP and NADPH, and enzyme kinetic properties. Equations were simplified where possible with modifications according to the theoretical work of Bellasio (2017). Metabolite flows were calculated using a set of differential equations derived from the stoichiometry of Bellasio (2017) by removing the assumption of steady–state. Time delay functions are used for Rubisco activation state ($R_{\text{act}}$) and carbohydrate synthesis, $CS$.

The model also includes a stomatal component based on the hydro–mechanical formulation of Bellasio et al. (2017), developed after Buckley et al. (2003) and Rodriguez-Dominguez et al. (2016). Hydro–mechanical forcing links guard cell responses to leaf water status and turgor, which are in turn related to soil water status and plant hydraulic conductance. Leaf turgor varies from a maximum value (corresponding to negative osmotic potential, $\pi_e$) to zero as a function of the equilibrium between water demand (determined by the leaf–to–boundary layer water mole–fraction gradient [$D_S$] and $g_S$) and water supply (determined by soil water potential, $\Psi_{\text{Soil}}$, and soil–to–leaf hydraulic conductance [$K_h$]). The influence of biochemical factors relative to hydro–mechanical forcing is determined by the parameter $\beta$ (defined as hydromechanical/biochemical response parameter), while stomatal morphology is described by $\chi$ (defined as turgor to conductance scaling factor). The strength of biochemical forcing (accounting for factors such as light intensity and CO$_2$ concentration) is represented by $\tau$. In this formulation, $\tau$ was set to equal $f_{\text{RuBP}}$, a function describing the degree of ribulose 1,5–bisphosphate (RuBP) saturation of RuBP carboxylase/oxygenase (Rubisco) active sites, thus, $\tau$ is a measure of the balance between the light and dark reactions of photosynthesis, in sensu Farquhar and Wong (1984). Consistent behaviour of $\tau$ is supported via evidence suggesting that stomata respond to the supply and demand for energy carriers in photosynthesis (Wong 1979; Busch 2014; Mott et al. 2014; Messinger et al. 2006) – i.e., increasing with PPFD and decreasing with $C_i$. The use of $\tau$ as a predictor of stomatal behaviour is
empirically based. This is justified by its capacity to predict parallel events occurring in chloroplasts and guard cells, but I make no claim about whether τ offers a faithful mechanistic description of stomatal behaviour [for discussions see Farquhar and Wong (1984); Bellasio et al. (2017); and Buckley (2017)].

Stomata respond to any perturbation with a delay due to the kinetics of adjustment of guard cell osmotic pressure. The time constant for that delay is species-specific and typically differs between opening and closing movements (Lawson and Blatt 2014). With the delay functions included, the stomatal sub-model can be used for simulating the gs dynamic response to fast changes in humidity, hydraulic conductance and ΨSoil [but see considerations on the ‘wrong way response’ made in Bellasio et al. (2017)]. Yet, because changes in these inputs typically occur on timescales of hours to weeks, they will be approximated by steady state behaviour, not addressed here [but see Bellasio et al. (2017)]. The model should also be suitable for calculating fast dynamics of gs in response to light flecks [e.g. Pearcy et al. (1997)], though gs responses shorter than one minute have not yet been calibrated.

**Parameterisation**

Literature values for the different parameters were averaged because the aim was to simulate realistic, general behaviour, not behaviour specific to a particular species or environmental conditions. Values for the parameters are reported in Supplementary Tables S1 and S2. Biochemical constants were primarily derived from Zhu et al. (2007) and Wang et al. (2014a and 2014b). Some biochemical and electron transport parameters were taken from Bellasio et al. (2016b), or from von Caemmerer (2000). Stomatal parameters were taken from, or assigned values similar to, Bellasio et al. (2017). For parameterisation of combined or simplified processes, I either derived parameters from the original equations or assigned plausible, physiologically realistic values. Parameters defining the PPFD dependence of Rubisco activation (Eqn 19) were initially set at values from Seemann et al. (1988) and adjusted by fitting the steady state PGA concentration in light curves shown in Figure 4. Parameters defining the dependence of Rubisco activation on CO₂ concentration at the M carboxylating sites, C₈M (Eqn 20) were derived empirically following these considerations: 1) by comparing measurements and model outputs (Figure 3, Figure 4) and considering data from Sage et al. (2002) I established that Rubisco is fully activated for C₈M above 200 µmol mol⁻¹; 2) Tangible inactivation occurs for C₈M below 100 µmol mol⁻¹ (Sage et al. 2002); and 3) Activity decreases to zero for [CO₂] approaching zero (Portis et al. 1986), but yet a substantial residual activity exists for CO₂ concentration around the CO₂ compensation point. The values were then adjusted by fitting the steady state PGA concentration in A/Ci curves shown in Figure 4 and the final values proposed are shown in Table S2. Additional parameter tuning may be required before the model is applied to specific species or growth conditions.
Outputs

At each time step, the model calculates nine metabolite stocks (expressed both as mol per metre squared of leaf or concentration, mM): \( C_i \), mesophyll \([\text{CO}_2] \), bicarbonate, RuBP, PGA, dihydroxyacetone phosphate (DHAP), ATP, NADPH and Ribulose 5–Phosphate (Ru5P). The concentrations of inorganic phosphorus (Pi), adenosine diphosphate (ADP) and NADP are calculated by subtraction from a total pool (Figure 1). From this, 12 flow rates are calculated (expressed in mmol m\(^{-2}\) s\(^{-1}\) and plotted in the figures in units of \( \mu\text{mol m}^{-2}\text{s}^{-1} \)): actual ATP and NADPH synthesis (\( v_{\text{ATP}} \) and \( v_{\text{NADPH}} \)), Rubisco carboxylation and oxygenation (\( V_C \), and \( V_O \)), rates of glycine decarboxylase (GDC), phosphoribulokinase (\( \text{RuP} \text{Phosp} \)), PGA reduction (PR), carbohydrate synthesis (CS), \( \text{CO}_2 \) stomatal diffusion, \( \text{CO}_2 \) dissolution, carbonic anhydrase hydration (CA), and the reactions through the RPP cycle (RPP).

Simulations

A typical dynamic simulation involves first clearing any previous results, defining the initial state of the leaf, including metabolite concentrations (see Supplementary Table S2), and then iteratively calculating the ‘flows’ and subsequent variation in ‘stocks’. Over time, the stocks reach steady state, where they depend solely on flows, but not on their initial value. A dynamic simulation may involve perturbing steady state conditions, and observing how a new steady state is reached. Figure S1 shows a typical trace of an output quantity (\( \text{CO}_2 \) stomatal diffusion) plotted over time while \( C_a \) and PPFD were varied to simulate a typical gas exchange experiment.

1. \( A–\text{PPFD} \) and \( A–C_i \) response curves

Figure 3 shows the modelled response of \( A \), \( g_s \) and \( v_{\text{NADPH}} \) to variation in PPFD and \( C_a \). Model output corresponding to the values calculated for the last second of each PPFD or \( C_a \) step is compared with Bellasio et al. (2016b) \( A–\text{PPFD} \) and \( A–C_i \) response curves measured in tobacco. Overall, the agreement between observations and model output was high, even if the model was parameterized with literature values not explicitly fitted to the data. For the \( A–\text{PPFD} \) curves, there was no noteworthy deviation between modelled and observed values of \( A \), \( g_s \) or \( v_{\text{NADPH}} \) (Figure 3, left–hand panels). There was also overall good agreement between modelled and measured \( A–C_i \) curves, though both \( A \) (Figure 3b) and \( v_{\text{NADPH}} \) (Figure 3f) were underestimated at ambient O\(_2\) and \( C_i > 300 \mu\text{mol mol}^{-1} \), and \( g_s \) was slightly overestimated at low \( C_a \) (Figure 3d). Further analysis revealed that under these conditions, modelled \( A \) was relatively unresponsive to individual increases in relevant flow rates including \( J_{\text{ATP}} \) and \( J_{\text{NADPH}} \) synthesis, RuP phosphorylation and \( V_C \). This suggests complex, concurrent regulation of light and dark reactions that require further exploration.

2. Steady state concentration of photosynthetic metabolites
Figure 4 compares model output for different metabolite pools with data measured by von Caemmerer and Edmondson (1986) on radish leaves. In that experiment, photosynthesis was allowed to reach full induction before measurements were taken, therefore 1800 s were allowed between each model step to ensure quasi steady state. RuBP concentration was underestimated in the $A – PPFD$ curve (Figure 4a), but correctly estimated it in the $A – C_i$ curve at ambient CO$_2$ concentration (Figure 4b), suggesting that the model properly averaged data. At 2% O$_2$, the model replicated the measured RuBP concentration at low $C_i$, but it underestimated it at high $C_i$ (Figure 4b). The variation in PGA concentration as a function of $C_i$, both under low and ambient O$_2$ (Figure 4d), was well captured by the model. However, the PGA pool was overestimated when $PPFD < 500 \mu$mol m$^{-2}$ s$^{-1}$ (Figure 4c).

The model also captured the overall trends in relative Rubisco activity in response to $PPFD$ and $C_i$ at different O$_2$ concentrations, though absolute values were underestimated (Figure 4e and 4f). At low $C_i$, Rubisco deactivation is due to the decarbamylation of the active site, captured by $f([CO_2])$. At low $PPFD$, the decrease in Rubisco activity is due to the redox regulation of Rubisco activase, captured by $f(PPFD)$. The model calculates the relative activity of Rubisco as the product of $f$(RuBP) and Rubisco activation state ($R_{act}$). In contrast, measured data come from comparing the in vitro Rubisco activity under physiological conditions with the in vitro activity of Rubisco after full induction of enzymatic activity. The discrepancy between model and observed absolute values is plausibly because the modelled activity also accounts for the effect of partial RuBP saturation, while in vitro data were taken under full RuBP saturation.

At steady state, under a $PPFD$ of 500 $\mu$mol m$^{-2}$ s$^{-1}$, the ratio [DHAP]/[PGA] was 0.2 between the values of 0.1 (in the stroma) and 0.35 (in the cytosol) measured in the light (400 $\mu$mol m$^{-2}$ s$^{-1}$) by Heineke et al. (1991). Modelled values for the ratio [ATP]/[ADP] under a $PPFD$ of 500 or 1000 $\mu$mol m$^{-2}$ s$^{-1}$ were ~1.5 and 7, respectively, which compare well with the ratio of 3 measured in the light by Heineke et al. (1991). Finally, the model predicted a [NADPH]/[NADP] ratio of 0.16 and 0.5 under the $PPFD$ of 50 and 1000 $\mu$mol m$^{-2}$ s$^{-1}$, respectively, that is similar to the values of 0.2 and 0.5 measured by Heineke et al. (1991) for dark and light conditions, respectively.

Notably, this output was obtained with identical parameterisation to the previous simulations with tobacco.

3. Dynamic responses to an increase and decrease in $PPFD$

A simulation was run to replicate the response of a spinach leaf to a steep increase and decrease in $PPFD$ at 21% O$_2$ (unpublished data courtesy of Ross Deans, Farquhar Lab, Australian National University). The leaf was acclimated under a $PPFD$ of 50 $\mu$mol m$^{-2}$ s$^{-1}$ until steady state was reached, and subsequently $PPFD$ was increased to 1500 $\mu$mol m$^{-2}$ s$^{-1}$. Model parametrization was the same as in preceding simulations with tobacco and radish, except for maximum rate of Rubisco
carboxylation ($V_{\text{C MAX}}$) and the speed of stomatal opening. Simulated dynamic responses of $A$ and $g_s$ corresponded closely with the measured data (Figure 5a and 5c). After the steep increase in PPFD, ATP and NADPH production rates (Figure 5a), the Rubisco activation state (Figure 5b), and ATP and DHAP concentrations (plotted as relative to the total pool of adenosilates in Figure 5g) followed a hyperbolic increase. ATP production increased faster than Rubisco activation state, which resulted in an initial decrease in [PGA], a fast increase in [RuBP] and a subsequently sharp decrease in [Pi] (Figure 5e). After ~150s, there was a continuous smooth decrease in $f(RuBP)$, resulting from the combination of increasing [PGA] and decreasing [RuBP] (Figure 5c).

Decreasing PPFD from 1500 µmol m$^{-2}$ s$^{-1}$ to 50 µmol m$^{-2}$ s$^{-1}$ resulted in a sharp initial reduction in modelled $A$, followed by a hyperbolic increase to a new steady state value (Figure 5b). The steady state modelled $A$ slightly underestimated the measured rate (Figure 5b). A similar pattern was followed by $f(RuBP)$ (Figure 5d) and [ATP] (Figure 5h), although they reached steady state faster and slower than $A$, respectively. The ATP and NADPH production rates (Figure 5b) and [NADPH] (Figure 5h) reached steady state almost immediately after an initial spike. The model closely resembled the measured slow decrease in $g_s$ (Figure 5d). The response of Rubisco activation state (Figure 5d) was similar, although faster, than the observed trend in $g_s$. [PGA] sharply increased in the initial seconds after light reduction, and then decreased to a new steady state value where [Pi] was higher than the initial value at high PPFD (Figure 5f). The initial sharp increase in [PGA] was possible due to a high Rubisco activation state. This depleted the pool of RuBP, which could not be regenerated because of insufficient light. The trend in [DHAP] was comparable to the simulations of Laisk et al. (1989) [Figure 11 in Laisk et al. (1989)]. In contrast to this model, Laisk et al. (1989) model predicted that [ATP], [Pi] and intermediates of the RPP cycle had smooth transitions to steady state after perturbation without local maxima or minima. My simulations are perhaps more realistic as they resemble measurements of [Pi] and [ATP] by Santarius and Heber (1965), although with slower kinetics.

4. Dynamic responses to an increase and decrease in $C_a$

Model predictions for a steep increase (from 350 to 1500 µmol mol$^{-1}$) or decrease (from 1500 to 350 µmol mol$^{-1}$) in $C_a$ were compared with data by Laisk et al. (1991). CS was timed with a first order exponential delay function analogous to Eqn 41 with a time constant of 35 s. After the sudden increase in $C_a$, $A$ increased above 40 µmol m$^{-2}$ s$^{-1}$ for ~1s, which I attribute to the dissolution of CO$_2$ into the leaf, then stabilised ~36 µmol m$^{-2}$ s$^{-1}$ for ~30s, which I attribute to the carboxylation of the pool of phosphorylated metabolites. Finally $A$ reached a minimum (Figure 6a) coincident with a minimum in [Pi] (Figure 6e), $v_{\text{ATP}}$ (Figure 6a) and [ATP] (Figure 6g). After these three phases, all modelled quantities approached steady state smoothly.

After a steep decrease in $C_a$ (Figure 6b, d, f, g), $A$ decreased for ~1s below the steady state value before the perturbation, which can be explained by the stripping of dissolved CO$_2$ out of the leaf.
Subsequently $A$ smoothly approached a new steady state value. The [PGA] reached a minimum after $\sim 80$ s, which determined a maximum in $[P_i]$ and a consequent maximum in $v_{ATP}$ and $[ATP]$.

Overall, the model captured the dependence of $A$ on $P_i$ dynamics, which underpins the so–called 'photosynthetic oscillations' (Walker 1992). Further, modelled $v_{ATP}$ (which is a function of the reciprocal of leaf fluorescence) replicated the pattern of fluorescence shown by the simulations of Laisk and Eichelmann (1989) [their Figure 5]. However, neither the model of Laisk and Eichelmann (1989) nor mine captured the measured response of $A$ beyond $30$ s of induction, consisting of a very deep trough in $A$ lasting $10–20$ s, followed by $4–5$ dampened oscillations with a period of $\sim 60$ s leading to a new steady state.

5. Dynamic responses to a decrease in atmospheric $O_2$ concentration

A simulation was run to replicate the experiment of Bellasio et al. (2014), which involved assessing the response of $A$ and $Y$(II) to a decrease in $[O_2]$ in a tobacco leaf. The model accurately captured $A$ and $Y$(II) at steady state before and after the reduction in $[O_2]$. The dynamic response of $Y$(II) was also closely reproduced (Figure 7). However, modelled $A$ failed to capture the initial spike measured in $A$ immediately after the reduction in $[O_2]$, which may be a measurement artefact that originated during adjustments of the infrared gas analyser.

Discussion

A newly derived process–based stock–and–flow biochemical model of photosynthesis was coupled to a dynamic hydro–mechanical model of stomatal behaviour. The new photosynthesis model features time–explicit constraints on $J_{ATP}$, $g_s$ and Rubisco activation state. Steady state metabolite concentrations are determined by environmental drivers and the kinetic parameters of enzymes, but not by initial metabolite concentrations. The coupled model achieved a stable and realistic rate of light–saturated $A$. After a perturbation in an environmental driver (e.g. $PPFD$), the model was able to regain a specific steady state. The model successfully replicated gas exchange experiments, including $A$–$C_i$ and $A$–$PPDF$ curves, and transient responses to steep changes in $[O_2]$, $C_a$, and $PPDF$.

Simplifying assumptions

The mathematical description of dark reactions was simplified from Zhu et al. (2007) by reducing the number of metabolites and reactions simulated, and removing some of the feedback loops. Offloading of the RPP cycle to photosynthetic sinks was simplified into a single process called carbohydrate synthesis ($CS$). Additionally, the reactions of the photorespiratory cycle were assumed non–limiting. Additional feedbacks from sedoheptulose–1,7–bisphosphatase (SBPase) and fructose–bisphosphate (FBP) (Wang et al. (2014b) were not included in the model. Feedbacks characterised in vivo involve redox regulation [e.g. Zhang and Portis (1999)]. However, in the
model, the dynamics of PGA depend solely on the equilibrium between its formation by Rubisco and reduction. This approach was able to reproduce the response of PGA to PPFD observed by von Caemmerer and Edmonson (1986) (Figure 4c). The pool of phosphorylated metabolites includes PGA, DHAP RuP and RuBP. Additional pools of sugar phosphates were added in pilot simulations, but for simplicity, were not included in the final model, nor was the activity of the malate shuttle (Foyer et al. 1992).

Dynamic simulation of electron transporters can be computationally demanding (Zaks et al. 2012), therefore the flows associated with light reactions were described with classical equations, in line with Wang et al. (2014a). This simplification implies that responses are instantaneous, which is physiologically plausible because the speed of light reactions is higher than that of dark reactions (Trinkunas et al. 1997). The model also ignored chloroplast movements, which have been shown to dynamically vary in some species (Davis et al. 2011; Morales et al. 2018). Respiration was assumed to be supplied by new assimilates (3-phosphoglyceric acid, PGA) following the original formulation of Bellasio and Griffiths (2014) and subsequent developments (McQualet al. 2016; Bellasio 2017). The ATP and NADH produced during respiration were neglected because they are likely to be consumed by basal metabolism. Although CS was made partially reversible (Eqn 28), a calibration of metabolite replenishment in the dark is required before the model can be used for long simulations around or below light– or CO2–compensation points. For further details on assumptions, see Bellasio et al. (2017) and Bellasio (2017).

Comparison with other models

The model presented here characterises biochemical processes more comprehensively than preceding models (Pearcy et al. 1997; Gross et al. 1991; Gross 1982) that featured phenomenological pseudoreactions (Morales et al. 2018) not mechanistically linked to enzyme activity. Additionally, my model is simpler, freely available, and therefore more readily applicable than earlier models (Wang et al. 2014b; Zhu et al. 2013; Zhu et al. 2007; Laisk et al. 2009; Laisk and Edwards 2000; Laisk and Eichelmann 1989). Like the models of Wang et al. (2017) and Morales et al. (2018), my model also responds to PPFD and external CO2 concentration, even at limiting levels. Importantly, in my model, light reactions can respond to transitions in atmospheric [O2] (Figure 6). In addition to linking Rubisco activation to PPFD (mediated by rubisco activase), a feature that some other models encompass, a distinctive feature of my model is including a description of Rubisco inactivation at low [CO2] (mediated by decarbamylatation). I made both these drivers time–dependent with empirical functions. Lastly, and uniquely, my model includes the process–based description of stomatal responses to a range of environmental drivers such as humidity and soil water availability.

There are two main differences between the dynamic photosynthesis model of Morales et al. (2018) and the model described here. In the model by Morales et al. (2018), there are two dynamic
processes: Rubisco and a pseudo–reaction associated to RuBP regeneration. In my model, there are
nine dynamic reactions in the dark phase, which are mechanistically dependent on the concentration
of 12 metabolites. In Morales et al. (2018), light reactions are simulated dynamically with explicit
description of quenching phenomena. In my model, there is full integration between dark reactions
and the electron transport chain, including: feedbacks at the level of cyclic electron flow
engagement; at the link between O2 concentration and electron flow through the glutathione –
ascorbate peroxidase (APX) cycle; and at the level of $Y(II)$. The latter is dependent on [ATP],
[NADPH] and [Pi] mediated by the kinetics of ATP synthesis.

In most dynamic and steady state models, feedbacks are accounted for with a discontinuous
function selecting between the ‘minimum of’ two or more quantities. For instance, Busch and Sage
(2017) calculated $A$ by selecting between three limiting factors: light, enzyme capacity, or triose
phosphate availability. In Wang et al. (2014a) and preceding models, the calculation of $V_C$ is
underpinned by the selection between RuBP or CO2 limitations. In my model, all biochemical
feedbacks operate continuously. The transition from light– to enzyme–limitation is smooth,
pivoting around the poise between regeneration and use of RuBP. This poise is captured by a
quadratic function, $f(RuBP)$, which depends on the concentration of RuBP relative to the
concentration of Rubisco catalytic sites. This function was originally developed by Farquhar et al.
(1980) ($V_C/W_C$ in their notation, representing the actual, relative to the RuBP–saturated, rate of
carboxylation), but it has rarely been implemented in its full quadratic form. The transition to TPU
limitation is also smooth but in this case, is underpinned by a decreasing amount of Pi liberated by
CS, thereby reducing [Pi] thats feedbacks directly on $\nu_{ATP}$ and indirectly over $Y(II)$. Under TPU
limitation, assimilation is controlled by $V_{MAX,CS}$.

Simulation processing time

On a standard desktop or laptop computer (~4 GB of RAM and ~2 GHz CPU speed), the model
cycles at ~1000 Hz, with the actual time taken for a simulation run depending on the integration
time step. The model is ‘stiff’, therefore the steplength is constrained by stability requirements,
rather than those of accuracy. The model becomes unstable when the fluxes accrued over the time
step become comparable with the corresponding stocks. Of course, the fastest reactions such as
those involving CO2 diffusion and hydration are the most affected. If carbonic anhydrase (CA) is
included in calculations, the model is unstable at time steps greater than 0.5 ms. Using an
integration step of 0.3 ms, it takes ~35 s of computation time to simulate 10 s transition, and 42
hours to simulate a 12–hr photoperiod.

Stability can be improved by ignoring CA activity, which makes the model stable for time steps
shorter than 2 ms. CA may be relevant at timescales shorter than 0.1 s and was included in the
model because it has been deemed important in a number of recent studies [e.g. (Ho et al. 2015)];
however, excluding CA did not change the model outputs presented here. Using a step of 1.5 ms, it takes ~7 s of processing time to simulate 10 s and 8.4 hours to simulate a 12–hr photoperiod.

Stability can also be ameliorated by incrementing the stocks. In this model, the residual leaf volume, occupied by cell walls and apoplastic solution, is all assumed to be intercellular air. A higher volume allows better model stability, because the flux of incoming CO₂ is buffered by a larger pool of air. Thickness and porosity may need to be adjusted for specific applications, for instance, for simulating airspace patterning manipulation (Lehmeier et al. 2017). Improvements in speed can also be achieved by assuming that CO₂ in the liquid phase is in equilibrium with bicarbonate, forming a common stock.

The Excel® workbook incorporates a selection feature allowing the user to include or exclude these simplifications and automatically amends the calculations according to the selection. Ignoring CA and assuming a common pool with bicarbonate, the maximum time step scales with the reciprocal of $g_s$ (determining the entrant flow of CO₂) and the model can be used reliably with a 5 ms resolution under a range of conditions.

**Future developments**

I am currently working on simulating photosynthetic oscillations. This involves adding complexity to the description of $CS$, new feedback loops, and perhaps allowing multiple timings for signalling functions. Subsequently, I plan to model the triose phosphate utilisation to reproduce the patterns experimentally described by Busch et al. (2017). In the long term, this model will form the core of an emerging C₄ model, encompassing all C₃ features presented here plus a dynamic description of C₄ metabolite diffusion.

In my model, stomatal conductance follows first–order kinetics, but it could be adapted to allow for the sigmoidal kinetics used in other studies (Kirschbaum et al. 1997; Vialet-Chabrand et al. 2013). Another development for the model is the mechanistic implementation of a simplified, dynamic and integrated electron transport chain building on the basis of previous work (Zaks et al. 2012; Laisk and Eichelmann 1989; Zhu et al. 2013; Morales et al. 2018), but including some of the continuous feedbacks present in this model and others (Joliot and Johnson 2011; Roach and Krieger-Liszkay 2014; Foyer et al. 2012).

Shorter processing time could be achieved if all calculations were performed directly using the Excel®–embedded VBA® (Visual Basic for Applications, or another suitable software). This would avoid the need for VBA® and Excel® to interact at every cycle. I choose to keep all the equations in the Excel® workbook to maximise transparency, and to allow straightforward model modification and parameterisation without the necessity of modifying the code, which only iterates results. Further gains in processing speed could be achieved by substituting the simple Euler integration,
whereby the model is calculated at each time increment, with more sophisticated calculus involving stiff solvers and parallel integration with a range of suitable time steps.

Many of the parameters used to model photosynthesis are temperature dependent. Given the large number of parameters and the difficulty in experimentally resolving the dependency of individual quantities, I opted for not including temperature at this stage, but it should be addressed in the future.

Conclusion

Models are descriptions of natural systems that trade–off comprehensiveness with the simplicity and ease of use. Traditional steady–state models are simple but inherently unsuitable for assessing rapid responses of photosynthesis to environmental drivers. Dynamic models are more complex, but are needed to study rapid responses to environmental perturbation. I derived a dynamic process–based photosynthetic model for C3 leaves simplifying wherever possible while integrating and expanding the functionalities of recently published dynamic models. In particular, my model combines a hydromechanical model of stomatal behaviour with dynamic descriptions of dark and light reactions. The model is presented in a transparent format and can be run as a freely downloadable, stand–alone workbook in Microsoft® Excel®. The model successfully replicated complete gas exchange experiments featuring both short lag times and full photosynthetic acclimation, as well as dynamic transitions between light, CO₂ and oxygen levels. The model has the potential to supersede steady state models for detailed or time–dependent ecophysiological studies and I encourage its use for basic research in photosynthesis. Steady–state models will remain useful for larger scale simulations.

Availability

The model, coded in Microsoft® Excel®, is freely available from the Supplementary Information associated with the online version of this paper. A video tutorial is available on Youtube at the following link: (to be added when available).
Figures.

**Figure 1. Schematic model.** The leaf is represented by three uniform compartments: the external atmosphere, the mesophyll and the intercellular space. The intercellular space communicates with the surroundings through stomata, which regulate CO₂ diffusion and respond to biochemical and hydro–mechanical forcing. Intercellular CO₂ dissolves, reaching a mesophyll CO₂ pool (all pools are represented by boxes) and may undergo enzymatic hydration. Rubisco carboxylation ($V_c$, with two arrows to symbolise the production of two PGA molecules) and oxygenation ($V_o$) reactions consume RuBP and produce PGA and PGLA. PGLA is recycled through the photorespiration cycle eventually regenerating PGA (concentrations of intermediate metabolites are not calculated). PGA is the substrate for respiration ($R_{LIGHT}$) and is reduced ($PR$) to triose phosphate (DHAP), which is the substrate of carbohydrate synthesis ($CS$). In this model, carbohydrates – the final product of photosynthesis – are generic triose that vanishes once synthesised. The majority of DHAP enters the sugar conversion phase of the reductive pentose phosphate cycle (RPP), which is simulated as a single reversible step. Light reactions (depicted in the top left–hand side) supply the ATP and NADPH pools. The concentration of O₂ is assumed to equal the ambient concentration.
Figure 2. Schematic of the light reactions submodel. Light is shown in solid yellow, electron fluxes in black, protein complexes are drawn as boxes, and proton delivery to the lumen is depicted by block arrows. A fraction of the light incident on the leaf (PPFD), $I$, is absorbed by PSI ($I_1$) or PSII ($I_2$). Electrons flowing through PSII ($J_2$) reach the plastoquinone and plastoquinol pool (PQ and PQH$_2$), simultaneously taking up protons from the stroma. Electrons flow to the Cytochromes b$_{bf}$ where they may undergo so-called ‘Q–cycling’, which results in the translocation of one additional proton (shown in grey), eventually reaching PSI through plastocyanin (not shown). Here, electrons may either be cycled back through cyclic electron flow, CEF ($J_{Cyc}$) to PQ; be used for alternative sinks (these include O$_2$ and NO$_3$ in this model); or be used by photosynthetic dark reactions. CEF can also follow two different paths, either the PGR5–mediated CEF or through the NDH complex, which translocates two additional protons across the thylakoid membrane. ATP synthase regenerates one molecule of ATP for each $h$ proton returned to the stroma.
Figure 3. Simulated $A-C_i$ and $A-PPFD$ response curves. Gas exchange experiments simulated with time increments of 1.5 ms. Modelled responses of assimilation rate, $A$ (a–b), stomatal conductance, $g_s$ (c–d), and NADPH production rate, $v_{NADPH}$ (e–f) to incident irradiance, $PPFD$ (left) and $[\text{CO}_2]$ in the intercellular space, $C_i$ (right) at 21% $O_2$ (open circles) and at 2% $O_2$ (open triangles). The model is plotted against data from Bellasio et al. (2016b) measured under 21% $O_2$ (closed circles) and at 2% $O_2$ (filled triangles). Error bars show ±1 SE. Details of parameterisation are given in supporting Tables S1 and S2.
Figure 4. **Metabolite concentrations at steady state.** The model was run for 1800 s at different levels of PPFD (left–hand panels) or $C_a$ (right–hand panels) until quasi steady state was reached. Panels (a–b): RuBP concentration; Panels (c–d): PGA concentration; Panels (d–e): relative Rubisco activity calculated as $f[RuBP] \times R_{act}$. The model (open symbols) is plotted against data from von Caemmerer and Edmondson (1986) (closed symbols) measured under 21% O$_2$ (circles) and at 2% O$_2$ (triangles). Parameterisation was maintained from previous simulation, see supporting Tables S1 and S2.
Figure 5. Response to a transition from low– to high–light and from high– to low–light. Circles show the average of \( n=3 \) measurements taken on spinach (\textit{Spinacia oleracea}, courtesy of Ross Deans, \textit{unpublished}). The leaf was acclimated under a \textit{PPFD} of 50 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (left) or 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (right) until steady state was reached, then \textit{PPFD} was increased to 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) or decreased to 50 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and the variation in leaf level assimilation, \( A \) (a, b), stomatal conductance, \( g_{s} \) (c, d) and \( \text{CO}_2 \) concentration in the intercellular space, were recorded every 10 s. Lines show model outputs: rate of ATP and NADPH synthesis, and \( A \) (panels a and b); \( g_{s}, f(\text{RuBP}) \) and Rubisco activation state (c and d); concentrations of metabolites (e and f); ATP and NADPH concentrations (g and h). For simulations, \( C_{a} \) was the same as in the measurement cuvette (350 \( \mu \text{mol mol}^{-1} \)), \( V_{\text{CMAX}} = 0.18 \text{ mmol m}^{-2} \text{s}^{-1} \), stomatal characteristics were adjusted at \( \chi_{\beta} = 0.8 \) mol air MPa\(^{-1} \), \( \tau_{0} = -0.12 \), \( K_{i} = 3600 \text{ s} \); \( K_{d} = 1200 \text{ s} \) all other parameters were maintained from previous simulations (supporting Tables S1 and S2).
Figure 6. Modelled response to a transition from ambient– to high–CO$_2$ and from high– to ambient CO$_2$. The model was run at a $C_a$ of 350 µmol mol$^{-1}$ (left) or of 1500 µmol mol$^{-1}$ (right) until steady state was reached. Then $C_a$ was increased to 1500 µmol mol$^{-1}$ or decreased to 350 µmol mol$^{-1}$. Lines show modelled rates of ATP and NADPH synthesis, and $A$ (panels a and b); gs, $f$(RuBP) and Rubisco activation state (c and d); concentrations of metabolites (e and f); ATP and NADPH concentrations (g and h). Parameters were the same as in Figure 5.
Figure 7. Response to a transition from ambient to low atmospheric oxygen concentration.
Open symbols show tobacco measurements from Bellasio et al. (2014) where the leaf was acclimated under a $PPFD = 300 \, \mu\text{mol m}^{-2} \, \text{s}^{-1}$ and $C_a = 200 \, \mu\text{mol mol}^{-1}$ until steady state was reached. Then the background gas was switched to 2% O$_2$ and the variation in leaf level assimilation ($A$, triangles), and yield of photosystem II ($Y(II)$, squares) were recorded every ~17 s. The model replicated this experiment. The solid line shows modelled leaf level assimilation, while dots show modelled $Y(II)$ calculated as $Y(II) \approx \frac{V_{\text{NADPH}}}{0.45 \cdot PPFD \cdot s}$, where $s$ is an energy conversion coefficient. In the model, $C_a$ was set to equal $C_a$ in the measurement cuvette, while all other parameters were maintained from Figure 3 and 4 (supporting Tables S1 and S2).
Appendix – model details

1. Flows

1.1. A submodel for light reactions of photosynthetic CO₂ assimilation in C₃ leaves: potential ATP and NADPH production rate

The submodel calculates \(I_1, I_2, J_1, J_2, J_{\text{ATP}}, \text{ and } J_{\text{NADPH}}\) when \(f_{\text{Cyc}}, f_{\text{Pseudocyc}}, f_Q, f_{\text{NDH}}, Y(\Pi)_{\text{LL}}, s, h, \alpha_V, V_{0V}\) and \(\theta_V\) are known. \(I_1\) and \(I_2\) are the light absorbed by PSI and PSII, respectively. \(J_1\) and \(J_2\) are the electron flow though PSI and PSII, respectively. \(J_{\text{ATP}}\) and \(J_{\text{NADPH}}\) are the steady state rates of ATP and NADPH production, respectively. \(f_{\text{Cyc}}\) is the proportion of electron flow at PSI which follows CEF, \(f_{\text{Pseudocyc}}\) is the fraction of \(J_1\) used by alternative electron sinks (APX cycle and nitrate reduction), \(f_0\) is the level of Q–cycle engagement, and \(f_{\text{NDH}}\) is the fraction of \(f_{\text{Cyc}}\) flowing through NDH. The \(Y(\Pi)_{\text{LL}}\) is the initial yield of PSII extrapolated under zero \(PPFD\), \(s\) is the combined energy partitioning coefficient described in Yin et al. (2009), and \(h\) is the number of protons required to synthesize each ATP. \(\alpha_V, V_{0V}\) and \(\theta_V\), define the slope, the offset and the curvature of the function \(f'(PPFD)\), expressing the \(PPFD\) dependence of \(Y(\Pi)\).

When \(f_{\text{Cyc}}=0\), \(I_1, I_2, J_1\) and \(J_2\) take the values \(I_1, 0, I_2, 0, J_1, 0\) and \(J_2, 0\) respectively and \(J_{1,0}=J_{2,0}\). Then \(I_{2,0}\) and \(I_{1,0}\) can be expressed as (Yin et al. 2004, 2009):

\[
I_{2,0} = PPFD \cdot s, \tag{1}
\]

\[
I_{1,0} = \frac{I_{2,0} \cdot Y(\Pi)_{\text{LL}}}{Y(\Pi)_{\text{LL}}}, \tag{2}
\]

The total light absorbed by both PSI and PSII is \(I = I_{1,0} + I_{2,0}\), and \(I<PPFD\).

When CEF is engaged, \(I_1\) increases by a quantity \(\chi\) (Yin et al. 2004):

\[
I_1 = (1 + \chi) I_{1,0}, \tag{3}
\]

where \(\chi\) is calculated as a function of \(f_{\text{Cyc}}\) as (Yin et al. 2004):

\[
\chi = \frac{f_{\text{Cyc}}}{1 + f_{\text{Cyc}} + Y(\Pi)_{\text{LL}}}. \tag{4}
\]

I simulate the rate of cyclic electron flow through a tentative function as:

\[
f_{\text{Cyc}} = \max(0, -1 + 15 \frac{v_{\text{ATP}}}{J_{\text{ATP}}} - \frac{v_{\text{NADPH}}}{J_{\text{NADPH}}}). \tag{4b}\]

When the ratio of actual ATP production relative to the potential \(\frac{v_{\text{ATP}}}{J_{\text{ATP}}}\) is greater than the ratio of actual NADPH production relative to the potential \(\frac{v_{\text{NADPH}}}{J_{\text{NADPH}}}\), indicating ATP demand greater than NADPH demand, \(f_{\text{Cyc}}\) will be greater than zero. Eqn 4b yields values very close to zero in C₃ plants, and further testing will be necessary before application with other photosynthetic types.

If \(I\) is constant, \(I_2, J_2\) and \(J_1\) are calculated as (Yin et al. 2004):
Where $Y(II)$ is the yield of photosystem II, which depends on PPFD and feedbacks from dark reactions through the novel process–based function:

$$Y(II) = Y(II)_{LL} \frac{v_{ATP}}{J_{ATP}} \frac{v_{NADPH}}{J_{NADPH}} \left(1 - \max[0, f'(PPFD)]\right).$$

The rationale of Eqn 8 is that $Y(II)$ has a maximum operational value, $Y(II)_{LL}$, and is quenched by three distinct factors (Müller et al. 2001): 1) the slowing down of ATP synthesis caused by limiting availability of phosphate or ADP (described by $\frac{v_{ATP}}{J_{ATP}}$); 2) the reduction of the plastoquinon pool (described by $\frac{v_{NADPH}}{J_{NADPH}}$); 3) reaching the maximum capacity for electron transport (described by $f'(PPFD)$, which responds to PPFD as a non–rectangular hyperbola). $f'(PPFD)$ is calculated with Eqn 20, below, but with different parameterisation, see Table S2). Parameter values were adjusted by fitting modelled assimilation against $A/PPFD$ response curves (Figure 3, Table S2).

The proton flow to the lumen includes: one proton per electron from water oxidation, one proton from electron flow through the cytochromes ($J_{Cyt}$), two protons from the electron flow through the Q–cycle ($J_Q$, Yin et al. 2004) and two protons from the electron flow through NDH ($J_{NDH}$, Kramer and Evans 2011). The rate of ATP production is:

$$J_{ATP} = \frac{j_2 + j_{Cyt} + 2j_Q + 2j_{NDH}}{h},$$

where $h$ is the number of protons required to synthesize each ATP molecule, the flow through the $Q$–cycle is $J_Q=f_Q J_1$; the complement, directly flowing to the $b_{6}f$ complex, is $J_{Cyt}=(1-f_Q) J_1$; and the flow through the NDH complex is $J_{NDH}=f_{Cyc}f_{NDH} J_1$.

The total NADPH production can be expressed as (Yin et al. 2004):

$$J_{NADPH} = J_1 \frac{1-f_{Cyc}}{2} \frac{f_{Pseudocyc}}{J_{NADPH}}.$$

The alternative electron sinks include nitrogen metabolism (chiefly reduction) as well as the APX cycle. The APX cycle is known to depend on $O_2$ concentration and the availability of PSI acceptors (Miyake and Yokota 2000; Schreiber and Neubauer 1990). I describe $f_{Pseudocyc}$ as a linear function of $O_2$ concentration and $\frac{v_{NADPH}}{J_{NADPH}}$ as:
\[ f_{\text{Pseudocyc}} = f_{\text{Pseudocyc NR}} + 4 \{O_2\} (1 - \frac{\nu\text{NADPH}}{J_{\text{NADPH}}}), \]

where the coefficient 4 was fitted empirically to yield a value of \( f_{\text{Pseudocyc}} \approx 0.1 \) under ordinary ambient conditions, and the fraction of \( f_{\text{Pseudocyc}} \) partitioned to nitrate reduction (\( f_{\text{Pseudocyc NR}} \)) was set at \( \approx 0 \) for simplicity.

The time dependence of \( J_{\text{ATP}} \) and \( J_{\text{NADPH}} \) was modelled after Bellasio et al. (2017) as:

\[
\begin{cases}
J_{\text{ATP or NADPH}}(t+dt) = J_{\text{ATP or NADPH}}(t) + \frac{J_{\text{ATP or NADPH}}(t) - J_{\text{ATP or NADPH}}(t-1)}{K_{J \text{ATP or NADPH}}} dt & \text{if } J_{\text{ATP or NADPH}}(t) < J_{\text{ATP or NADPH}}(t-1) \\
J_{\text{ATP or NADPH}}(t) = J_{\text{ATP or NADPH}} & \text{else}
\end{cases}
\]

where \( K_{J \text{ATP or NADPH}} \) is the time constant for an increase in \( J_{\text{ATP or NADPH}} \), \( J_{\text{ATP or NADPH}}(t) \) and \( J_{\text{ATP or NADPH}}(t+dt) \) are the values at the time step \( t + dt \) or at the previous step \( t \), respectively; \( J_{\text{ATP or NADPH}} \) are the steady state values (Eqns 9 and 10).

### 1.2. Actual rates of ATP and NADPH production

The actual rates of ATP (\( \nu_{\text{ATP}} \)) and NADPH (\( \nu_{\text{NADPH}} \)) production are calculated after Wang et al. (2014b):

\[
\nu_{\text{ATP}} = \frac{J_{\text{ATP}} \left( [\text{ATP}] [P] - [\text{ADP}] \right)}{K_m \text{ADP} K_m \text{Pi} \left( 1 + \frac{[\text{ADP}]}{K_m \text{ADP}} + \frac{[\text{ATP}]}{K_m \text{ATP}} + \frac{[P_i]}{K_m \text{Pi}} + \frac{[\text{ATP}] [P_i]}{K_m \text{ADP} K_m \text{Pi}} \right)}
\]

\[
\nu_{\text{NADPH}} = \frac{J_{\text{NADPH}} \left( [\text{NADPH}] - [\text{NADP}] \right)}{K_m \text{NADPH} \left( 1 + \frac{[\text{NADP}]}{K_m \text{NADPH}} + \frac{[\text{NADPH}]}{K_m \text{NADPH}} \right)}
\]

where square brackets indicate metabolite concentration, \( K_m \) represents the Michaelis–Menten constant for a given metabolite, and \( K_e \) is the equilibrium constant of the reaction (Table S1).

### 1.3. CO₂ diffusion, dissolution and hydration

The rate of CO₂ diffusion through the stomata is:

\[
\text{CO}_2 \text{ stomatal diffusion} = \frac{g_s (C_a - C_l)}{1000},
\]

where \( g_s \) is stomatal conductance to CO₂ (mol m⁻² s⁻¹); \( C_a \) and \( C_l \) are the CO₂ concentrations (μmol mol⁻¹) external to the leaf and in the intercellular space, respectively; and the 1000 is used to convert the units from micromoles to mmol m⁻² s⁻¹.

The rate of CO₂ dissolution in aqueous media within the leaf is:

\[
\text{CO}_2 \text{ dissolution} = \frac{g_m (C_l - [\text{CO}_2]) K_{h\text{CO}_2}}{1000},
\]

where \( g_m \) is mesophyll conductance to CO₂ diffusion (mol m⁻² s⁻¹), \([\text{CO}_2]\) is the CO₂ concentration in mesophyll cells (mM), which is assumed to be spatially uniform, \( K_{h\text{CO}_2} \) is CO₂ volatility (the
reciprocal of solubility) (µbar mM⁻¹) and 1000 is used to convert the units into mmol m⁻² s⁻¹, the unit of all subsequent rates.

The rate of CO₂ hydration to bicarbonate is (Wang et al. 2014b):

\[
CA = \frac{v_{\text{MAX CA}} \left[ \frac{[\text{HCO}_3^-][\text{H}^+]}{k_m \text{CO}_2 \left(1 + \frac{[\text{CO}_2]}{k_m \text{CO}_2 + k_m \text{HCO}_3^-}\right)} \right]}{1000}
\]

where \(v_{\text{MAX CA}}\) is the maximum hydration rate.

1.4. Reaction rates

The rate of Rubisco carboxylation (\(V_C\)) was modified from Wang et al. (2014b) as:

\[
V_C = \frac{v_{\text{C MAX}} \cdot R_{\text{act}} \cdot f(RuBP) \cdot [\text{RuBP}][\text{CO}_2]}{\left(k_m' \text{CO}_2 + [\text{CO}_2]\right)\left(k_m' \text{RuBP} + [\text{RuBP}]\right)}
\]

where \(v_{\text{C MAX}}\) is the maximum carboxylation rate. In the \(v_{\text{C MAX}}\) used in Farquhar et al. (1980), Rubisco is assumed fully activated and also fully RuBP saturated in the ‘enzyme–limited’ case. Here, \(v_{\text{C MAX}}\) is more closely comparable to the in vitro rate. \(R_{\text{act}}\) is the Rubisco activation state, a time dependent variable calculated as:

\[
R_{\text{act}} t + dt = R_{\text{act}} t + \left(\frac{R_{\text{act eq}} - R_{\text{act}} t}{\tau_i}\right) dt \quad \text{if } R_{\text{act}} t \leq R_{\text{act eq}}
\]

\[
R_{\text{act}} t + dt = R_{\text{act}} t + \left(\frac{R_{\text{act eq}} - R_{\text{act}} t}{\tau_d}\right) dt \quad \text{else}
\]

where \(\tau_i\) and \(\tau_d\) are the time constants for Rubisco induction and deactivation, respectively (Seemann et al. 1988) and the steady state \(R_{\text{act}}\) value is:

\[
R_{\text{act}} = f(PPFD) \cdot f([\text{CO}_2]),
\]

where \(f(PPFD)\) simulates activation state of Rubisco independently of CO₂ concentration, and I included \(f([\text{CO}_2}]\) to capture the inactivation of Rubisco observed in vivo at low CO₂. The \(f(PPFD)\) and \(f([\text{CO}_2}]\) were modelled with non–rectangular hyperbolas (Gross et al. 1991):

\[
f(PPFD) = V_0 + \frac{\alpha_P \cdot PPFD + 1 - V_0 - \sqrt{(\alpha_P PPFD + 1 - V_0)^2 - 4\alpha_P PPFD \theta_P}}{2 \theta_P}
\]

\[
f([\text{CO}_2]) = V_\theta C + \frac{\alpha_C [\text{CO}_2] + 1 - V_\theta C - \sqrt{(\alpha_C [\text{CO}_2] + 1 - V_\theta C)^2 - 4\alpha_C [\text{CO}_2] \theta_C}}{2 \theta_C}
\]

where \(V_0, \alpha_P, \text{ and } \theta_P\) are empirical parameters of the hyperbola for \(f(PPFD)\) defining the initial activity in the dark, the slope of the dependency and the curvature, respectively; \(V_\theta C, \alpha_C, \text{ and } \theta_C\) are the equivalent parameters for \(f([\text{CO}_2}]\).

The \(f(RuBP)\) is a function of RuBP concentration, relative to the concentration of Rubisco active sites, which was modelled using a non–rectangular hyperbola after Farquhar et al. (1980):

\[
f(RuBP) = \frac{E_T + K_m' \text{RuBP} + [RuBP] - \sqrt{(E_T + K_m' \text{RuBP} + [RuBP])^2 - 4[RuBP]E_T}}{2E_T},
\]
where $E_T$ is the total concentration of Rubisco catalytic sites, calculated from $V_{C\text{MAX}}$ and turnover rate after Wang et al. (2014b). The Michaelis–Menten constant for RuBP and CO$_2$ are:

$$K'_m_{\text{RuBP}} = K_m\text{RuBP} \left(1 + \frac{|\text{PGA}|}{K_m\text{PGA}} + \frac{|\text{NADP}|}{K_{I\text{NADP}}} + \frac{|\text{ADP}|}{K_{I\text{ADP}}} + \frac{|P_i|}{K_{I\text{Pi}}}\right),$$

where $K_i$ are the constants for the competitive inhibition.

The rate of Rubisco oxygenation ($V_o$) was calculated after Farquhar et al. (1980) as:

$$V_o = V_C 2\gamma^* \frac{|O_2|}{|CO_2|},$$

where $\gamma^*$ is half the reciprocal Rubisco specificity, calculated in the liquid phase (von Caemmerer 2000) using constants from Sander (2015) and Warneck and Williams (2012). In this model, the glycine decarboxylase (GDC) decarboxylation rate equals $V_o$; for a justification and possible stoichiometric variants, see Bellasio (2017).

The rate of RuP phosphorylation was modified from Wang et al. (2014b) as:

$$RuP_{\text{phosp}} = \frac{v_{MAX} |\text{ATP}| |\text{RuP}| - |\text{ATP}| |\text{RuBP}|}{|\text{ATP}| + K_m\text{ATP}(1 + |\text{ADP}|) \left[|\text{RuP}| + K_m\text{RuP}(1 + |\text{PGA}|) + |\text{RuBP}| + |P_i|\right]},$$

The reducing phase of the reductive pentose phosphate pathway was modelled as a single–step pseudoreaction. The rate of PGA reduction ($PR$) was calculated by fusing the rates of PGA phosphorylation and DPGA reduction from Wang et al. (2014b) as:

$$PR = \frac{v_{MAX} |\text{ATP}| |\text{PGA}| |\text{NADPH}|}{|\text{PGA}| + K_m\text{PGA}(1 + |\text{ADP}|) \left[|\text{ATP}| + K_m\text{ATP}(1 + |\text{ADP}|)\right] |\text{NADPH}| + K_m\text{NADPH}(1 + |\text{ADP}|)}.$$
The interconversion phase of the RPP was modelled as a single step pseudoreaction through a generic Michaelis Menten equation for equilibrium reaction (Zhu et al. 2007) as:

$$RPP = \frac{v_{\text{MAX}} [\text{DHAP}] (1 - [\text{RuBP}])}{([\text{DHAP}] + K_{m, \text{DHAP}})}.$$  \hspace{1cm} 28b

The constants in Eqn 28b were adapted from the original separate reactions in Wang et al. (2014b) to operate and maintain physiologically realistic concentrations of substrates (Table 1).

2. Stocks

2.1. Change in metabolite concentrations

The change of metabolite concentrations in time $\frac{d[\text{met}]}{dt}$ was described by a set of ordinary differential equations based on the stoichiometry of Bellasio (2017) informed with the reaction rates described above and converted from variation in leaf level pool to variation in concentration using the mesophyll volume as described in Wang et al. (2014b).

The rates of change in concentrations of CO₂, bicarbonate (HCO₃⁻), RuBP, PGA, DHAP, ATP, and NADPH were calculated as:

$$\frac{d[\text{CO}_2]}{dt}V_M = \text{CO}_2 \text{ Dissolution} + R_{\text{LIGHT}} - V_C + 0.5 GDC - CA$$  \hspace{1cm} 29

$$\frac{d[\text{HCO}_3^-]}{dt}V_M = CA$$  \hspace{1cm} 30

$$\frac{d[\text{RuBP}]}{dt}V_M = RuP_{\text{phosp}} - V_C - V_0$$  \hspace{1cm} 31

$$\frac{d[\text{PGA}]}{dt}V_M = 2V_C + V_0 + 0.5 GDC - PR - \frac{1}{3}R_{\text{LIGHT}}$$  \hspace{1cm} 32

$$\frac{d[\text{DHAP}]}{dt}V_M = PR - CS - \frac{5}{3}RuP_{\text{phosp}}$$  \hspace{1cm} 33

$$\frac{d[\text{ATP}]}{dt}V_M = v_{\text{ATP}} - RuP_{\text{phosp}} - V_0 - PR - 0.5 CS$$  \hspace{1cm} 34

$$\frac{d[\text{NADPH}]}{dt}V_M = v_{\text{NADPH}} - PR - 0.5 V_0$$  \hspace{1cm} 35

$$\frac{d[\text{RuP}]}{dt}V_M = RPP - RuP_{\text{phosp}},$$  \hspace{1cm} 36

where $V_M$ is mesophyll volume per meter square of leaf (L m⁻²) calculated after considering the leaf half–full of mesophyll (Lawlor 1993), $R_{\text{LIGHT}}$ is light respiration and is input to the model as described in Bellasio (2017), and all the other flux rates have been previously described: CO₂ Dissolution (Eqn 15), $CA$ (Eqn 16), $RuP_{\text{phosp}}$ (Eqn 26), $V_C$ (Eqn 17), $V_0 (=GDC$, Eqn 25), $PR$ (Eqn 27), $CS$ (Eqn 28), $v_{\text{ATP}}$ (Eqn 12), $v_{\text{NADPH}}$ (Eqn 13) and $RPP$ (Eqn 28b). Equations 29–30 were derived in this study and Equations 31 to 36 are modified from (Bellasio 2017).
2.2. Concentrations determined from total metabolite pools

The concentrations of ADP, NADP+, and phosphate ([P_i]) are calculated simply by subtraction from a total pool:

\[
[\text{ADP}] = A_{\text{Tot}} - [\text{ATP}]
\]

\[
[\text{NADP}^+] = N_{\text{Tot}} - [\text{NADPH}]
\]

\[
[P] = P_{\text{ITot}} - [\text{PGA}] - [\text{DHAP}] - [\text{RuP}] - 2[\text{RuBP}] - [\text{ATP}],
\]

where \(A_{\text{Tot}}, N_{\text{Tot}},\) and \(P_{\text{ITot}}\) are the total pools of adenylates, nicotinamides and phosphate, respectively.

3. The hydro–mechanical model of stomatal behaviour

The model calculates \(g_S\) after Bellasio et al. (2017) as:

\[
g_S = \max \left( g_S, \frac{\chi \beta \tau (\Psi_{\text{Soil}} + \pi_e)}{1 + \chi \beta \tau R_b \partial_S} \right),
\]

where \(\chi \beta\) is a combined parameter scaling turgor–to–conductance and the hydromechanical–to–biochemical response; \(\tau\) is the sensor of biochemical forcing; \(\Psi_{\text{Soil}}\) is soil water potential; \(\pi_e\) is epidermal osmotic pressure; \(R_b\) is the effective hydraulic resistance to the epidermis, calculated as \(1/K_h\), the corresponding hydraulic conductance; and \(\Delta S\) is the leaf–to–boundary layer H2O mole fraction gradient, a measure of vapour pressure deficit, \(VPD\). The parameter \(\tau\) encompasses the biochemical components of the model and is calculated from \(f(RuBP)\) as:

\[
\tau = \tau_0 + f(RuBP),
\]

where \(\tau_0\), the basal level of \(\tau\), was manually assigned. Stomatal dynamics were accounted for by describing the time dependence of \(g_S\) with a set of recursive equations (Bellasio et al. 2017):

\[
g_{S_t+dt} = g_{S_t} + \begin{cases} 
\frac{g_s - g_{S_t}}{K_i} dt & \text{if } g_{S_t} < g_s \\
\frac{g_s - g_{S_t}}{K_d} dt & \text{else}
\end{cases}
\]

where \(g_{S_t+dt}\) and \(g_{S_t}\) are the \(g_S\) values at the time step \(t+dt\) or at the previous step \(t\), respectively; \(g_s\) is the steady state value (Eqn 40), \(K_i\) and \(K_d\) are the time constants for an increase and decrease in \(g_S\), respectively.