S.1. Extended model justification and overview

S.1.1. Elements of the model

Our model considers a single spherical Bienertia mesophyll cell, divided into three concentric compartments. In the centre, we have the CCC composed of mitochondria and Rubisco-rich chloroplasts. It is surrounded by one large concentric vacuole interspersed with cytoplasmic channels that connect the central region with the periphery. We do not model these channels explicitly, but merge them with the vacuole interior into an effective medium through which gases can diffuse. The peripheral region, contains chloroplasts rich in enzymes that fix inorganic carbon into aspartate as part of the C₄ photosynthetic pathway. We refer to the three compartments as “the core”, “the vacuole”, and “the periphery”. We note that Bienertia mesophyll cells are not actually spherical. However, since our aim is to look at the general effects of size on the efficiency of a C₄ pathway, a simpler model (which is also more amenable to numerical investigation) will suffice. Within this geometry, we consider the diffusion of carbon dioxide and oxygen in the cell, as well as the processes of fixation of carbon into C₄ acids in the periphery and its subsequent release in the core, and the processes of carboxylation and oxygenation of RuBP by Rubisco. The latter starts a complex chain of photorespiratory reactions, resulting in a loss of carbon from the cell’s sugar store, which is released as CO₂ in the mitochondria in the CCC. We also consider oxygen production by photosystem-II in the CCC chloroplasts, which is associated with the production of NADPH that is needed for RuBP regeneration and photorespiration.

It is important to emphasise that, although we conceptually divide the cell’s interior into distinct spatial regions, the model places no intracellular barriers that would hinder diffusion of oxygen and CO₂ between these regions. This is a deliberate choice, to test the viability of the C₄ pump when there is nothing but spatial separation to provide diffusive resistance to gases in the liquid phase. It is clear that placing additional barriers would further improve the efficiency of the C₄ photosynthesis by decreasing the leakage of CO₂ from the core, provided the transport of substrates and C₄ acids is unhindered.

Since the details of the C₄ pathway vary among plant species (von Caemmerer and Furbank, 2003; Jenkins et al., 1989; Sage, 2004), we abstract the pathway into its two essential steps. First is the fixation of atmospheric CO₂ into a C₄ acid by PEPC in the periphery. The second is the decarboxylation of the C₄ acid by NAD-ME in the
core region, which frees the captured carbon. By ignoring the remaining steps in the \( \text{C}_4 \) cycle we implicitly assume they are not rate limiting. This, for instance, implies that the base \( \text{C}_3 \) substrate (alanine) as well as the \( \text{C}_4 \) product (aspartate) are abundant within the cell - a necessary condition for optimal functioning of the \( \text{C}_4 \) pathway in any case.\(^1\) Similar assumptions are placed on all the steps of the \( \text{C}_3 \) pathway which involve Rubisco, prior to carboxylation or oxygenation of RuBP (i.e. Rubisco activation, RuBP binding, etc.), since carbon-fixation is the limiting step there.

Our model thus explicitly considers three enzymes: Rubisco (active site concentration \( c_R \) in the CCC), PEPC (active site concentration \( c_P \) in the periphery), and NAD-ME (parametrised by a \( \text{CO}_2 \) mitochondrial current \( \Psi_{\text{mito}} \) in the CCC). The model also explicitly considers two mobile inorganic molecular species - carbon dioxide and oxygen - whose concentrations in steady-state, \( c_C \) \((r) \) and \( c_O \) \((r) \), will vary with distance, \( r \), from the cell centre. The enzymatic reactions of \( \text{CO}_2 \) and oxygen follow Michaelis-Menten kinetics. Since detailed kinetics data for \textit{Bienertia}'s Rubisco are not presently available, we use kinetic parameters for maize (\textit{Zea mays}) (Cousins et al., 2010), a well-studied \( \text{C}_4 \) plant (the carboxylation catalytic rates, \( k_{\text{cat}C} \), for maize and \textit{Bienertia} Rubisco are similar, and higher than in \( \text{C}_3 \) plants (Rosnow et al., 2015)). PEPC and NAD-ME kinetic parameters are taken for \textit{Zea mays} and \textit{Arabidopsis thaliana} respectively (Kai et al., 1999; Tronconi et al., 2008). Values of all the physical and chemical parameters used in the simulations are listed in Table 1. In addition to these explicitly treated enzymatic processes, we also take into account the release of carbon dioxide in mitochondria (photorespiration) and oxygen in the core chloroplasts (Hill reaction at PS-II). The photorespiratory \( \text{CO}_2 \) release is set to match half the Rubisco's oxygenation current, while the Hill reaction is set to produce oxygen by water cleavage by the amount needed to replenish the NADPH lost running the Calvin-Benson and the photorespiratory cycle (this amounts to one oxygen molecule produced for every RuBP carboxylation or oxygenation event).

The efficacy of the photosynthetic pathway will be determined by three principal factors, firstly the \( \text{C}_4 \) pump reaction kinetics (PEPC concentration, NAD-ME concentration), secondly the Rubisco concentration, and thirdly the cell geometry, i.e. the radii of the three compartments. To analyse the effects of the geometry, we optimise the \( \text{C}_3 \) pump reaction kinetics, i.e. for a given choice of geometry and the core Rubisco concentration, we tweak the biochemistry of the \( \text{C}_4 \) pump by varying the concentration of PEPC in the periphery to optimize its performance during steady state carbon fixation, see S.2. The concentration of NAD-ME in the core is automatically adjusted, for any given PEPC concentration, so as to balance the PEP-carboxylation in the periphery with the malate-decarboxylation in the core.\(^2\) This implies a regulatory mechanism for NAD-ME expression, based on the concentration of \( \text{C}_4 \) acids (aspartate and/or malate) within the central compartment.

\[ S.1.2. \ CO_2 \text{ and HCO}_3^- \]

We do not explicitly model bicarbonate in the cell. This is because it effectively decouples from \( \text{CO}_2 \) except in the periphery, where the presence of carbonic-anhydrase (CA) ensures rapid equilibration. Elsewhere, the absence of

\(^1\)It also means that the ATP consumption connected to pyruvate-to-PEP conversion via PPDK enzyme is now effectively associated with the carbon capture step which it precedes.

\(^2\)In the limit of high malate concentration (\( \geq 100\text{K}_M = 30\text{mM} \)) that we are interested in, NAD-ME will be saturated by malate, and the malate-decarboxylation current will be directly proportional to NAD-ME concentration.
CA means the interconversion between CO$_2$ and HCO$_3^-$ will be slow - much slower than other (diffusive and kinetic) processes. In the periphery, HCO$_3^-$ will be at equilibrium with local CO$_2$ concentration, as CA-assisted CO$_2$ ↔ HCO$_3^-$ interconversion is much faster than PEPC carboxylation (Heinhorst et al., 2006). Bicarbonate can then be taken 'out of the equation', as it only affects the effective PEPC Michalis-Menten constant for CO$_2$, $K_{P(CO_2)} = \frac{K_P(CO_2)}{1 + \frac{[HCO_3^-]}{K_P(HCO_3^-)}} \approx 1.20K_P(HCO_3^-)$ at pH = 7.5.3

This approximation is valid if CO$_2$ ↔ HCO$_3^-$ interconversion can be neglected in the core and vacuole regions. To see this is indeed the case, let us consider the fate of a CO$_2$ molecule released from the core mitochondria (either as a photorespiration product or from malate-to-pyruvate conversion). Without CA it converts to HCO$_3^-$ slowly (Johnson, 1982) - much slower than the time it takes for it to diffuse out of the core region or to react with Rubisco. The relevant lengthscale will be the average CO$_2$ diffusion distance, $\lambda = \sqrt{\frac{D_{CO_2}}{k_{CO_2}}}$, where $k_{CO_2}$ is the overall CO$_2$ → HCO$_3^-$ conversion rate. The rate depends on pH, but will generally be below $7 \cdot 10^{-2} \text{ s}^{-1}$ for pH≤8.5 (Johnson, 1982), giving $\lambda \geq 160 \mu \text{m}$. This is well above the largest Bienertia cell radius considered in the paper. Once in the periphery, the conversion becomes rapid, thanks to CA. The fate of HCO$_3^-$ molecules in the cytoplasm outside the periphery will be similar. The back-reaction $HCO_3^- \rightarrow CO_2$ is roughly twenty times slower than the forward reaction, while diffusion constants of CO$_2$ and HCO$_3^-$ are comparable (Mazarei and Sandall, 1980; Walker et al., 1980), so any HCO$_3^-$ molecule will most likely end up captured by PEPC.

Note that, while the model assumes no CA in the core region, the conclusions would remain the same if CA were present in the stroma of the core plastids. This is because, even though CA would cause rapid equilibration of CO$_2$ and HCO$_3^-$, the HCO$_3^-$ within the stroma is effectively trapped (it is confined to individual chloroplasts) and it does not react with any other enzymes in the model. At steady-state, the HCO$_3^-$ concentration within the stroma would thus simply be proportional to the CO$_2$ concentration.

To improve the treatment of CO$_2$/HCO$_3^-$ kinetics, one would also need to consider (and have the experimental knowledge of) the variation in pH and the CA distribution amongst the different compartments, as well as explicit modelling of transport through cytoplasmic channels. This would greatly increase the complexity of the model, and the uncertainties in the numerous newly introduced parameters would have to be addressed.

S.1.3. Determination of the photon cost

There are several efficacy measures that can be used to evaluate the net carbon fixation. We use the photon cost of carbon fixation (the inverse of the base quantum yield), which we refer to simply as ‘the photon cost’. This is the minimal number of photons that, on average, need to be collected by the linear and cyclic photosystems to regenerate the ATP and NADPH used in the process of net fixation of one carbon atom into sugar.4 It covers the cost of RuBP regeneration, the photorespiratory cycle, and the C$_4$ pump operation. If the optimal cost is achieved at non-vanishing

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3This results in a rescaling of the effective PEPC-CO$_2$ reaction rate, $k_{onP} = 20 \frac{k_{catP}}{K_P}$

4We do not consider additional costs - for example, due to the inefficiencies in photon collection by the photosystem's antenna complex. These considerations fall outside the scope of this paper.
PEPC and NAD-ME concentrations (i.e. when the C₄ pump is active), we can say that the C₄ photosynthetic pathway is a viable and preferable alternative to C₃-only photosynthesis for the selected cell geometry.

The net photon cost can be expressed as

\[ \varphi = \frac{\varphi_C \Phi_C + \varphi_O \Phi_O + \varphi_{C4} \Phi_{C4}}{\Phi_C - \Phi_O/2} \]  
(S1)

where \( \varphi_C, \varphi_O, \) and \( \varphi_{C4} \) are base costs of RuBP regeneration after one Rubisco-carboxylation event, of the photorespiratory salvage cycle after an oxygenation event, and of one pyruvate-to-PEP conversion (which has to occur for each PEP-carboxylation event in the steady-state). These values are estimated at \( \varphi_C = 8, \varphi_O = 9, \) and \( \varphi_{C4} = 4 \) (Zhu et al., 2010). \( \Phi_C, \Phi_O, \) and \( \Phi_{C4} \) are the total RuBP-carboxylation current, RuBP-oxygenation current and the C₄ current.

These values correspond to optimal utilisation of the linear and cyclic electron transfer chains in producing the required amounts of ATP and NADPH to run the above processes. The amounts are 3 ATP and 2 NADPH for each carbon atom assimilated via Calvin-Benson cycle, 3.5 ATP and 2 NADPH for the photorespiratory cycle after each oxygenation event, and 2 ATP for every pyruvate-to-PEP conversion (Farquhar et al., 1980; Zhu et al., 2010; Kramer and Evans, 2011). The ATP and NADPH requirements translate into photon cost as follows. One turn of the linear electron transfer chain involves absorption of 4 photons (2 by photosystem I and 2 by photosystem II), which are used to transfer 6 protons into the thylakoid lumen and reduce one NADP⁺ molecule (Zhu et al., 2010; Kramer and Evans, 2011). ATP synthase uses the resulting proton concentration gradient to phosphorylate ADP. Production of one ATP molecule requires a down-gradient transfer of 4 protons (Zhu et al., 2010).⁵ The cyclic electron transfer chain only maintains the proton gradient, so it can be used to produce additional ATP (above the ATP/NADPH ratio of 3/2). The commonly accepted scenario involves transfer of 4 protons for every 2 photons absorbed by photosystem I (Zhu et al., 2010).⁶ Combining the ATP/NADPH requirements of photosynthetic processes with the productivities of the transfer chains, we obtain the stated photon cost values.

S.1.4. External environment

We consider the cell placed in one of two possible environments. One is a gas phase (air), the other is water. In the case of a gaseous environment, we place a diffusion barrier, in the form of the plasma membrane and cell wall, that hinders the exchange of CO₂ and oxygen between the cell and the gas phase. By varying the permeability of this barrier we can also effectively account for partial occlusion of our cell by its neighbours. We make the standard assumption that the gas solvation at the cell boundary is a fast process (Tholen and Zhu, 2011), so that the concentrations of CO₂ and O₂ in a thin hydrated layer immediately beyond the cell wall are in equilibrium with their partial pressures

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⁵The exact efficiency of ATP synthase is a matter of active research. There are indications that production of an ATP molecule requires more than 4 protons on average, and that the actual efficiency varies among species (Kramer and Evans, 2011). Four protons per ATP is a commonly used value when estimating the efficiency of photosynthesis (Zhu et al., 2010).

⁶There are indications that the cyclic electron transfer chain is in fact twice as efficient as commonly assumed (Kramer and Evans, 2011). If so, the photon cost of processes which involve only ATP consumption - such as the C₄ pump operation - would be halved. As our general goal is to provide a conservative estimate of the single-cell C₄ photosynthetic pathway efficiency, we assume the lower productivity (of 2 protons per photon) for the cyclic chain.
in air. In the second scenario, the cell is immersed in water. By considering the functioning of the C₄ pump in an aqueous environment, where diffusion is slow to the point of limiting the CO₂ flux, we can assess the efficacy of carbon-concentrating mechanisms in relation to cell size in single-cell C₄ aquatic plants (von Caemmerer et al., 2014; Reinfelder et al., 2000). The external concentrations of dissolved CO₂ and O₂ are, in this case, set to be at equilibrium with their partial pressures in air at a large distance from the cell, and will notably deviate from equilibrium in the cell’s proximity.

S.2. Equations of the model

We model the *Bienertia* mesophyll cell as a spherically symmetric system of three concentric compartments and an exterior. The inner compartment (“the core”), is a sphere of radius \( r_i \). It is surrounded by vacuole mantle, up to radius \( r_v \) from the centre. The vacuole is followed by a thin shell (“the periphery”) up to the external radius of the cell, \( r_e \). Beyond, we have “the outside”, which can be either air or water.

S.2.1. Within the cell

We treat the core as an homogeneous mixture of mitochondria and chloroplasts. Rubisco is spread evenly throughout the core, with concentration \( c_R \). It reacts with CO₂ and oxygen, following a Michaelis-Menten type kinetics, with bimolecular reaction rates \( k_{onC} \) and \( k_{onO} \), and saturating concentrations \( K_C \) and \( K_O \), of CO₂ and O₂ respectively (for parameter values see Table 1). The mitochondria provide a spatially uniform release of CO₂ stemming from photorespiration and malate decarboxylation. The chloroplasts likewise produce a uniform release of oxygen equal to NADPH consumption by carbon fixation and photorespiration. These release rates per unit volume, \( \psi_{mitoC} \) and \( \psi_{chlorO} \), are, in steady-state, determined self-consistently by current-balance conditions (Eq. S23 and S24).

To derive the steady-state equations for carbon dioxide and oxygen distributions, \( c_C(r) \) and \( c_O(r) \), we start from a general set of time-dependent diffusion-reaction equations for the core region \( r < r_i \),

\[
\frac{\partial c_C}{\partial t} = D_C \nabla^2 c_C - k_{outC}c_C (c_R - c_{RC} - c_{RO}) + \psi_{mitoC} \\
\frac{\partial c_O}{\partial t} = D_C \nabla^2 c_O - k_{outO}c_O (c_R - c_{RC} - c_{RO}) + \psi_{chlorO} \\
\frac{\partial c_{RC}}{\partial t} = k_{onC}c_R (c_R - c_{RC} - c_{RO}) - k_{catC}c_{RC} \\
\frac{\partial c_{RO}}{\partial t} = k_{onO}c_O (c_R - c_{RC} - c_{RO}) - k_{catC}c_{RO}
\]

where \( c_{RC} \) and \( c_{RO} \) are concentrations of Rubisco-RuBP-CO₂ and Rubisco-RuBP-O₂ complexes, and \( k_{catC} = K_Ck_{outC} \) and \( k_{catO} = K_Ok_{outO} \) are Rubisco carboxylation and oxygenation catalysis rates. By setting all the time derivatives to zero and simplifying the Laplace operator for the case of a spherically symmetric system, we get the steady-state equations for the radially varying concentrations of CO₂ and oxygen, \( c_C \) and \( c_O \), within the core region, \( r < r_i \),

\[
D_C \frac{1}{r} \frac{d^2}{dr^2} (rc_C) = k_{outC}c_RK_C \frac{c_CK_O}{K_CK_O + c_CK_O + K_Cc_O} - \psi_{mitoC}
\]
Within the vacuole no reactions take place, so the concentrations of CO$_2$ and oxygen are affected by diffusion only. In the steady-state, the Laplace equation holds for $r_l < r < r_e$,

$$D_O \frac{1}{r} \frac{d^2}{dr^2} (rc_O) = k_{o_m} c_R K_O \frac{K_CC_O}{K_CC_O + K_C K_O + K_C c_O} - \psi_{chlorO}$$  \hspace{1cm} (S7)

In the periphery, CO$_2$ indirectly reacts with the PEPC enzyme. We assume abundant PEP supply. We also use a simpler form of the reaction rate, linear in $c_C$.

Starting from the time-dependent equations,

$$\frac{\partial c_C}{\partial t} = D_C \nabla^2 c_C - k_{o_m} P_E c_P c_C$$  \hspace{1cm} (S9)

$$\frac{\partial c_O}{\partial t} = D_C \nabla^2 c_O$$  \hspace{1cm} (S10)

we straightforwardly obtain the steady-state equations valid for $r_e < r < r_c$,

$$D_C \frac{1}{r} \frac{d^2}{dr^2} (rc_C) = k_{o_m} P_E c_P c_C$$  \hspace{1cm} (S11)

$$D_O \frac{1}{r} \frac{d^2}{dr^2} (rc_O) = 0$$  \hspace{1cm} (S12)

Solutions to the differential equations for $c_C$ and $c_O$ have to match smoothly at the boundaries between the intracellular regions, i.e. we have conditions $c_C (r \to r_l) = c_C (r \to r_e)$ and $\frac{dc_C}{dr} \bigg|_{r \to r_l} = \frac{dc_C}{dr} \bigg|_{r \to r_e}$, with analogous conditions at the $r = r_e$ boundary. To fully determine $c_C$ and $c_O$, we also need to set the boundary conditions at the centre, $r = 0$, and the cell boundary, $r = r_e$. The first is simply the smoothness requirement at $r = 0$,$^8$

$$\frac{dc_C}{dr} \bigg|_{r \to 0} = \frac{dc_O}{dr} \bigg|_{r \to 0} = 0.$$  \hspace{1cm} (S13)

The situation at the $r = r_e$ boundary depends on our choice of external conditions.

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$^7$ The use of a linear form permits an analytical solution of the resulting diff. equations, which is handy since the numerical integration of the equations becomes unstable when (as is usually the case) the CO$_2$ concentration in the periphery is low or vanishing. Where this is not the case, the PEPC concentration at which the optimal solution is found will be underestimated, but the optimal solution itself (in particular the optimal photon cost) should not change, since it is determined by flux-balance and cost considerations. A correction to the optimal PEPC concentration can be estimated by equating the total PEP carboxylation currents when linear and M-M reaction rates are used, assuming the same average CO$_2$ concentration in the periphery (i.e. $c_{P_E}(MM)_{Caw}/(K_{PCO2} + c_{Caw}) = c_{P_E}(lin)_{Caw}/K_{PCO2}$). We do not apply this correction in Fig. 2(a) because it is not exact. The correction becomes notable (a rescaling by a factor between 2 and 4) away from the optimal-geometry line, in the region where the optimal PEPC concentration is small (< 0.01 mM).

$^8$ A finite slope at $r = 0$ would imply the presence of an infinite-strength source or drain at the origin. The quickest way to see this is to note that the concentration gradient (and hence the current density) would be discontinuous at the origin. The continuity equation, $\nabla \cdot j = \dot{q}$, then implies a divergent right hand side. Another way would be to consider the current into a vanishingly small sphere ($r \to 0$) around the origin. The current through the sphere is proportional to $\frac{1}{4} \pi c (r \to 0) r^2$, which should be equal to the source term $S (r \to 0) r^2$, where $S (r \to 0)$ is the source strength at the origin. Hence, $\frac{1}{4} \pi c (r) \propto r^3 (r)$ as $r \to 0$.
S.2.2. The choice of exterior

In the case of air outside and a diffusion barrier at \( r_e \), we can safely assume a constant gas concentration outside the cell due to the significantly higher diffusion coefficients in a gaseous phase. We make the standard assumption that the concentrations of \( \text{CO}_2 \) and \( \text{O}_2 \) in a thin hydrated layer immediately beyond the cell wall are in equilibrium with their partial pressures in air: \( c_C (r > r_e) = c_{\text{eq}} \) and \( c_O (r > r_e) = c_{\text{eq}} \). In this case the flow (per unit area) of dissolved \( \text{CO}_2 \) (\( j_C \)) and oxygen (\( j_O \)) at the cell boundary will be determined by the cell wall and cell membrane permeability, \( \sigma_B \). This flow must match the diffusive flow within the cell, at the cell membrane

\[
\begin{align*}
    j_C &= \sigma_B \left( c_{\text{eq}} - c_C(r \to r_e) \right) = D_C \frac{dc_C}{dr} \bigg|_{r \to r_e} \quad \text{(S14)} \\
    j_O &= \sigma_B \left( c_{\text{eq}} - c_O(r \to r_e) \right) = D_O \frac{dc_O}{dr} \bigg|_{r \to r_e} \quad \text{(S15)}
\end{align*}
\]

In the case of a water environment, the concentrations of \( \text{CO}_2 \) and oxygen outside the cell will noticeably vary with distance (following the Laplace equation, Eq. S8), due to the limitations of diffusive transport. The boundary condition at the infinity requires that \( c_C \) and \( c_O \) approach their equilibrium dissolved values, \( c_{\text{eq}} \) and \( c_{\text{eq}} \). Using the Laplace equation outside the cell, we obtain a matching condition at \( r = r_e \):

\[
\begin{align*}
    r_e \frac{dc_C}{dr} \bigg|_{r_e} + c_C(r_e) &= c_{\text{eq}} \\
    r_e \frac{dc_O}{dr} \bigg|_{r_e} + c_O(r_e) &= c_{\text{eq}}
\end{align*}
\]

S.2.3. The currents

The final ingredients are the determination of mitochondrial \( \text{CO}_2 \) release rate \( \Phi_{\text{mito}} \) and the plastid oxygen release rate \( \Phi_{\text{chlorO}} \). At steady-state, the total mitochondrial \( \text{CO}_2 \) release current (\( \Phi_{\text{mito}} \)) must match the \( \text{C}_4 \) acid current (\( \Phi_C \)) and the photorespiratory release current, which is half the Rubisco oxygenation current (\( \Phi_O \)). The oxygen release current (\( \Phi_{\text{chlorO}} \)) must produce enough NADPH to support both the Rubisco carboxylation (\( \Phi_C \)) and Rubisco oxygenation current (i.e. both the Calvin-Benson cycle and the photorespiratory requirements for reducing power). Two NADPH molecules are needed per each RuBP carboxylation or oxygenation event, and one NADPH is produced oxygenation current (i.e. both the Calvin-Benson cycle and the photorespiratory requirements for reducing power). Two NADPH molecules are needed per each RuBP carboxylation or oxygenation event, and one NADPH is produced for each \( \text{H}_2\text{O} \) molecule split in the Hill process; hence two are produced for every \( \text{O}_2 \) evolved.

The currents are given by

\[
\begin{align*}
    \Phi_C &= \int_0^{r_e} 4\pi r^2 \cdot k_{\text{act}} c_C K_C \frac{c_C K_O + c_C K_O + c_{\text{eq}}}{K_C K_O + c_C K_O + c_{\text{eq}}} \, dr \quad \text{(S18)} \\
    \Phi_O &= \int_0^{r_e} 4\pi r^2 \cdot k_{\text{act}} c_O K_O \frac{K_{C\text{CO}}}{K_C K_O + c_C K_O + c_{\text{eq}}} \, dr \quad \text{(S19)}
\end{align*}
\]

\(^9\)We ignore the bicarbonate pool outside the cell because \( \text{HCO}_3^- \) cannot pass the cell membrane in any significant amount.

\(^{10}\)Concentrations of oxygen and \( \text{CO}_2 \) outside follow a simple diffusion law, \( \frac{1}{r^2} \frac{d}{dr} (rc) = 0 \), (i.e. same as Eq. S12). The solution is of the form \( rc(r) = Ar + B \). By noting that \( A = c(r \to \infty) \) and \( \frac{A}{r} = -\frac{B}{r} \), we can get a general expression, valid for any \( r \geq r_e \): \( r^2 c(r) + c(r) = c(r \to \infty) \).
\[
\Phi_{C_4} = \int_{r_i}^{r_e} 4\pi r^2 \cdot k_{catPCPC} \, dr \\
\Phi_{\text{mitoC}} = \frac{4\pi}{3} r_i^3 \psi_{\text{mitoC}} \\
\Phi_{\text{chlorO}} = \frac{4\pi}{3} r_i^3 \psi_{\text{chlorO}} 
\]

(S20)

(S21)

(S22)

The balance conditions can now be stated as

\[
\Phi_{\text{mitoC}} = \Phi_{C_4} + \Phi_O/2 \\
\Phi_{\text{chlorO}} = \Phi_C + \Phi_O 
\]

(S23)

(S24)

The differential equations S6-S8 and S11-S12, along with the boundary (S13 and S16-S17 or S14-S15) and current-balance (S23-S24) conditions uniquely determine the solution \((c_C(r) \text{ and } c_O(r))\) distributions for a given choice of radii, \(r_i, r_v, \text{ and } r_e\), enzyme concentrations, \(c_R\) and \(c_P\), and specified exterior conditions.

The concentration of NAD-ME in the core can be calculated by noting that \(\Phi_{C_4}\) must match the total malate-decarboxylation rate, under conditions of saturation by malate,

\[
c_N = \frac{\Phi_{C_4}}{4\pi r_i^3 k_{catN}} 
\]

(S25)

S.2.4. Case of abundant PEPC

In the abundant PEPC region, minimal cost is achieved in the numerically unreachable \(c_P \to \infty\) limit. To get an exact solution in this case, a modified problem is solved. Since now the entirety of PEP carboxylation occurs in vanishingly thin layers at \(r = r_v\) and \(r = r_e\), Equation S11 is simplified: \(c_C(r_v < r < r_e) = 0\). The malate current is instead determined by the diffusive flow of CO\(_2\) through the \(r_v\) and \(r_e\) boundaries,

\[
\Phi_{C_4} = 4\pi r_e^2 j_C - 4\pi r_v^2 D_C \left. \frac{dc_C}{dr} \right|_{r=r_v} 
\]

(S26)

where \(j_C\) is given by\(^{11}\) \(j_C = D_C \left. \frac{dc_C}{dr} \right|_{r=r_v} = D_C c_{eq}/r_v\), if water is outside, or by \(j_C = \sigma_R c_{eq}\), if air is outside. All other equations remain the same.

S.2.5. Expressions for CO\(_2\) leakage and net assimilation rate

CO\(_2\) leakage represents the part of the carbon delivered via the malate shuttle that subsequently escapes the core as CO\(_2\). By its definition, it is a meaningful quantity only when the C\(_4\) pump is active and the concentration of CO\(_2\) within the core is larger than in the periphery. The escaping CO\(_2\) current is

\[
\Phi_{esc} = \Phi_{\text{mitoC}} - \Phi_C 
\]

(S27)

\(^{11}\)The expression is obtained from \(\frac{d}{dr} c(r) + c(r) = c(r \to \infty)\) (see footnote 10), by setting \(r = r_e\) and \(c(r_e) = 0\).
The part of it that is due to malate decarboxylation (as opposed to photorespiration) is \( \Phi_{esc} / \Phi_{mitoC} \). The relative leakage is then:

\[
\phi_{leak} = \frac{\Phi_{esc}}{\Phi_{mitoC}} = 1 - \frac{\Phi_C}{\Phi_{C4} + \Phi_O/2}
\]  
(S28)

Expressions for the net assimilation rates per Rubisco and per unit volume are:

\[
\text{assimilation per Rubisco} = \Phi_C - \Phi_O/2
\]

\[
= \frac{4\pi}{3} r_i^3 c_{icR} (S29)
\]

\[
\text{assimilation per volume} = \Phi_C - \Phi_O/2
\]

\[
= \frac{4\pi}{3} r_e^3 c_{e} (S30)
\]

S.3. Numerical implementation

The photon cost landscapes shown in the figures were evaluated on a grid of 101×101 points ((\( r_i, r_c - r_i \)) pairs). For each point, differential equations (S.2) were solved using a standard shooting method, i.e. by varying the concentration of \( c_C \) and oxygen at the origin, \( c_C(r = 0) \) and \( c_O(r = 0) \), and their release rate in the core, \( \psi_{mitoC} \) and \( \psi_{chlorO} \), so as to find a solution that (I) satisfies the boundary conditions at \( r_e \) (Eq. S16 and S17 or S14 and S15), and (II) satisfies the current balance conditions, Eq. S23 and S24. The equations were numerically integrated in the core region; beyond \( r_i \) the solution could be expressed analytically. The equations were solved for \( c_P = 0 \) and \( c_P \to \infty \), to get the \( C_3 \) and abundant-PEPC solutions where possible. An adaptive sweep was also done across a range of finite \( c_P \) values for each point to find a finite-PEPC photon cost minimum (if present). This procedure was implemented in Python, using SciPy libraries for ODE integration and root finding (scipy.integrate.odeint and scipy.optimize.fsolve).

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