Electronic Supplementary Material (ESM):
The limiting factors and regulatory processes that control the environmental responses of C₃, C₃-C₄ intermediate, and C₄ photosynthesis

Jennifer E. Johnson¹ · Christopher B. Field¹, ² · Joseph A. Berry¹

Abstract The expressions in Appendices I-V are given for idealized C₃, Type I C₃-C₄, and NADP-ME C₄ cases. These cases allow us to focus on developing an explicit representation of chloroplast electron transport, while maintaining an implicit representation of mitochondrial electron transport. Appendix I gives expressions that describe the whole-leaf net assimilation rate (A), in relation to net CO₂ assimilation in the mesophyll (Aₘ) and the bundle sheath (Aₛ). These expressions are relevant under all environmental conditions. Appendices II, III, and IV give additional expressions that describe the dependence of Aₘ and Aₛ on light, pCO₂, and pO₂. Appendix II gives expressions for a pure light-limited state, and Appendix III gives expressions for a pure light-saturated state. Appendix IV gives expressions for mixed light-limited/light-saturated states, and Appendix V gives the solution to the model. For the analyses in this paper, the equations in Appendices I-V were implemented in MATLAB (2020b, The MathWorks, Natick, MA, USA). The Symbolic Math Toolbox was used to generate analytical solutions to the systems of equations specified in Appendix V, and the Global Optimization and Parallel Computing Toolboxes were used to fit the model to data.

¹Department of Global Ecology, Carnegie Institution for Science, 260 Panama Street, Stanford CA 94305 USA
²Woods Institute for the Environment, Stanford University, 473 Via Ortega, Stanford CA 94305 USA

Correspondence: Jennifer E. Johnson, Email: jjohnson@carnegiescience.edu, ORCID ID: 0000-0003-2181-8402
**Appendix I: Governing rate equations**

**Steady-state electron transport**

We begin by describing a metabolic state in which the supplies of Fd, NADPH, and ATP from chloroplast electron transport are dynamically coordinated with the energetic demands from carbon metabolism. For the C$_3$ and the Type I C$_3$-C$_4$ mesophyll, the rates of electron transport through PS II and PS I depend on the activity of the photosynthetic carbon reduction (PCR) and photosynthetic carbon oxidation (PCO) cycles:

\[
J_{P680m} = 4 \cdot V_{cm} + 4 \cdot V_{om} \quad (1a)
\]

\[
J_{P700m} = (3 \cdot V_{cm} + 3.5 \cdot V_{om} - J_{P680m} \cdot n_L) / n_C + J_{P680m} \quad (1b)
\]

where $J_{P680m}$ is the total rate of linear electron flow (LEF) through mesophyll PS II (mol e$^{-}$ m$^{-2}$ s$^{-1}$), $J_{P700m}$ is the total rate of LEF and cyclic electron flow (CEF1) through mesophyll PS I (mol e$^{-}$ m$^{-2}$ s$^{-1}$), $V_{cm}$ and $V_{om}$ are the carboxylation and oxygenation rates of mesophyll Rubisco (mol CO$_2$ or O$_2$ m$^{-2}$ s$^{-1}$), and $n_L$ and $n_C$ are the ATP coupling efficiencies of LEF and CEF1, respectively (mol ATP mol$^{-1}$ e$^{-}$).

For the Type I C$_3$-C$_4$ bundle sheath, the analogous expressions are given by:

\[
J_{P680s} = 4 \cdot V_{cs} + 4 \cdot V_{os} \quad (1c)
\]

\[
J_{P700s} = (3 \cdot V_{cs} + 3.5 \cdot V_{os} - J_{P680s} \cdot n_L) / n_C + J_{P680s} \quad (1d)
\]

where $J_{P680s}$ is the total rate of LEF through bundle sheath PS II (mol e$^{-}$ m$^{-2}$ s$^{-1}$), $J_{P700s}$ is the total rate of LEF and CEF1 through bundle sheath PS I (mol e$^{-}$ m$^{-2}$ s$^{-1}$), and $V_{cs}$ and $V_{os}$ are the carboxylation and oxygenation rates of bundle sheath Rubisco (mol CO$_2$ or O$_2$ m$^{-2}$ s$^{-1}$). For the C$_4$ mesophyll, the corresponding expressions are:

\[
J_{P680m} = 2 \cdot V_{pm} \quad (2a)
\]

\[
J_{P700m} = 2 \cdot V_{pm} \cdot (1 - n_L / n_C + 1 / n_C) \quad (2b)
\]

where $V_{pm}$ is the mesophyll activity of PEPC (mol CO$_2$ m$^{-2}$ s$^{-1}$). In Eqns. 2a-2b, the partitioning between LEF and CEF1 in the mesophyll reflects the requirement for 2 ATP and 1 NADPH to drive the NADP-ME type C$_4$ cycle. For the C$_4$ bundle sheath, the corresponding expressions are:

\[
J_{P680s} = 4 \cdot V_{cs} + 4 \cdot V_{os} - 2 \cdot V_{ds} \quad (2c)
\]

\[
J_{P700s} = (3 \cdot V_{cs} + 3.5 \cdot V_{os} - J_{P680s} \cdot n_L) / n_C + J_{P680s} \quad (2d)
\]

where $V_{ds}$ is the bundle sheath activity of NADP-ME (mol CO$_2$ m$^{-2}$ s$^{-1}$). In Eqns. 2c-2d, the partitioning between LEF and CEF1 in the bundle sheath reflects the energetic requirements to drive the PCR and PCO cycles as well as the partial suppression of LEF due to NADPH production by NADP-ME.

**Steady-state gas-exchange**

We continue by defining expressions that link the activity of carbon metabolism to gas-exchange. For a C$_3$, Type I C$_3$-C$_4$, or C$_4$ leaf, a general expression for the net assimilation rate ($A$) is given by the sum of net CO$_2$ assimilation in the mesophyll ($A_m$) and the bundle sheath ($A_s$):

\[
A = A_m + A_s \quad (3a)
\]

\[
A_m = V_{em} - 0.5 \cdot V_{om} - R_m \quad (3b)
\]

\[
A_s = V_g + V_p - L \quad (3c)
\]

where $A$, $A_m$, and $A_s$ can have units of mol assimilated C m$^{-2}$ s$^{-1}$ or mol CO$_2$ m$^{-2}$ s$^{-1}$. In Eqn. 3c, $V_g$ is the net rate at which the glycolate shuttle transfers assimilated C from the mesophyll into the bundle sheath (mol C m$^{-2}$ s$^{-1}$), $V_p$ is the net rate at which the malate shuttle transfers assimilated C from the mesophyll into the bundle sheath (mol C m$^{-2}$ s$^{-1}$), and $L$ is the rate at which CO$_2$ leaks out of the bundle sheath via diffusion (mol CO$_2$ m$^{-2}$ s$^{-1}$). The rate of the glycolate shuttle is determined by mesophyll Rubisco oxygenase activity that is coupled to bundle sheath GDC activity, and the rate of the malate shuttle is based on mesophyll PEPC activity that is coupled to bundle sheath NADP-ME activity:

\[
V_g = 0.5 \cdot V_{om} - V_{gm} \quad (4a)
\]

\[
V_p = V_{pm} - V_{dm} \quad (4b)
\]

where $V_{gm}$ is the rate of GDC activity in the mesophyll (mol CO$_2$ m$^{-2}$ s$^{-1}$), $V_{pm}$ is the rate of carboxylation via mesophyll PEPC (mol CO$_2$ m$^{-2}$ s$^{-1}$) and $V_{dm}$ is mesophyll C$_4$-decarboxylase activity that results in futile cycling (mol CO$_2$ m$^{-2}$ s$^{-1}$). Combining Eqns. 3-4 then yields a second expres-
tion for the overall net CO₂ assimilation rate \( A \):

\[
A = V_{cm} - V_{gm} + V_{pm} - V_{dm} - L - R_m
\]  
(5)

which can represent photosynthesis in C₃, any of the various C₃-C₄, or C₄ plants. If all of the Rubisco is localized to the mesophyll, this expression reduces to the regular C₃ expression described by Farquhar et al. (1980). If some of the Rubisco and all of the GDC are localized to the bundle sheath, the reduced expression is equivalent to the Type I C₃-C₄ expression described by von Caemmerer (1989). If all of the Rubisco is localized to the bundle sheath and there is no futile C₄ cycling, the reduced expression is equivalent to the C₄ expression described by Berry and Farquhar (1978). In the dark, the leak rate \( L \) is equivalent to the rate of bundle sheath respiration, \( R_s \).

**Linking electron transport with gas-exchange**

The electron transport rates can now be linked directly to the net CO₂ exchange rates using the relationship:

\[
\frac{V_o}{V_c} = \frac{1}{S} \frac{O}{C} = \frac{K_c \cdot k_o \cdot O}{K_o \cdot C}
\]  
(6)

where \( O \) and \( C \) are the \( pO₂ \) and \( pCO₂ \) in the chloroplast (bar), \( S \) is the specificity of Rubisco for carboxylation relative to oxygenation (mol CO₂ mol⁻¹ O₂), \( k_c \) and \( k_o \) are the catalytic constants of Rubisco for CO₂ and O₂ (mol CO₂ or O₂ mol⁻¹ sites s⁻¹), and \( K_c \) and \( K_o \) are the Michaelis-Menten constants for CO₂ and O₂ (bar). For the C₃ and Type I C₃-C₄ mesophyll, combining Eqs. 1a, 1b, 5, and 6 gives:

\[
J_{P_{680m}} = \frac{J_{P_{700m}}}{1 - \frac{n_L}{n_C} + \left[ \frac{3 + 7 \cdot O_s / (2 \cdot S \cdot C_s)}{(4 + 4 \cdot O_s / (S \cdot C_s)) \cdot n_C} \right]}
\]  
(7a)

\[
A_m = \frac{J_{P_{680m}} \cdot [1 - O_m / (2 \cdot S \cdot C_m)]}{4 \cdot [1 + O_m / (S \cdot C_m)]} - R_m
\]  
(7b)

where \( O_m \) and \( C_m \) are the mesophyll \( pO₂ \) and \( pCO₂ \) (bar). For the Type I C₃-C₄ bundle sheath, the analogous expressions are derived by combining Eqs. 1c, 1d, 5, and 6:

\[
J_{P_{680s}} = \frac{J_{P_{700s}}}{1 - \frac{n_L}{n_C} + \left[ \frac{3 + 7 \cdot O_s / (2 \cdot S \cdot C_s)}{(4 + 4 \cdot O_s / (S \cdot C_s)) \cdot n_C} \right]}
\]  
(8a)

\[
A_s = \frac{J_{P_{680s}} \cdot [1 - O_s / (2 \cdot S \cdot C_s)]}{4 \cdot [1 + O_s / (S \cdot C_s)]} - R_s
\]  
(8b)

where \( O_s \) and \( C_s \) are the bundle sheath \( pO₂ \) and \( pCO₂ \) (bar). For the C₄ mesophyll, the expressions for linear electron flow through PS II and the net CO₂ assimilation reduce to:

\[
J_{P_{680m}} = \frac{J_{P_{700m}}}{1 - \frac{n_L}{n_C} + \left[ \frac{3 + 7 \cdot O_s / (2 \cdot S \cdot C_s)}{(4 + 4 \cdot O_s / (S \cdot C_s)) \cdot n_C} \right]}
\]  
(9a)

\[
A_m = -R_m
\]  
(9b)

which are derived from Eqns. 2a, 2b, and 5. For the C₄ bundle sheath, the corresponding expressions for the linear electron flow through PS II and the net CO₂ assimilation expand to:

\[
J_{P_{680s}} = \frac{J_{P_{700s}} - J_{P_{680m}} \cdot \left[ \frac{3 + 7 \cdot O_s / (2 \cdot S \cdot C_s)}{(4 + 4 \cdot O_s / (S \cdot C_s)) \cdot n_C} \right]}{4 \cdot [1 + O_s / (S \cdot C_s)]} - R_s
\]  
(10a)

\[
A_s = \frac{J_{P_{680s}} + J_{P_{700s}} \cdot [1 - O_s / (2 \cdot S \cdot C_s)]}{4 \cdot [1 + O_s / (S \cdot C_s)]}
\]  
(10b)

which are derived from Eqns. 2c, 2d, 5, and 6. Under different environmental conditions, Eqns. 7-10 can be solved using the expressions in Appendices II, III, IV, and V.

**Appendix II: Pure light-limited state**

**Expressions for net CO₂ assimilation \( A_{mj} \) and \( A_{sj} \)**

In a pure light-limited state, the net CO₂ assimilation rates of the mesophyll and bundle sheath \( A_{mj} \) and \( A_{sj} \) are kinetically controlled by the maximum activity of Cyt b₅f and the excitation balance of PS I and PS II. For the C₃, Type I C₃-C₄, and C₄ cases, the light-limited rates of PS I electron transport in the mesophyll and bundle sheath \( \frac{J_{P_{700m}}}{A_{mj} \text{ and } A_{sj}} \) are given by:

\[
J_{P_{700m}} = \frac{V_{\text{max}} (\text{CB6F}) \cdot Q}{\alpha_{1m} \cdot K_{P_1} / (K_{P_1} + K_{D_1} + K_{F_1}) + Q}
\]  
(11a)

\[
J_{P_{700s}} = \frac{V_{\text{max}} (\text{CB6F}) \cdot Q}{\alpha_{1s} \cdot K_{P_1} / (K_{P_1} + K_{D_1} + K_{F_1}) + Q}
\]  
(11b)

where \( Q \) is the photosynthetically active radiation incident on the leaf (mol incident PPFD m⁻² s⁻¹), \( \alpha_{1m} \) and \( \alpha_{1s} \) are the absorbance cross-sections associated with the PS I bed in the mesophyll and bundle sheath (mol PPFD absorbed by PS I mol⁻¹ incident PPFD), \( K_{P_1} / (K_{P_1} + K_{D_1} + K_{F_1}) \) is the maximum photochemical yield of PS I (mol energy dissipated
mol$^{-1}$ energy absorbed), and $V_{m,\text{max}}$ (CB6F) and $V_{m,\text{max}}$ (CB6F) are the maximum activities of Cyt b$_6$f in the mesophyll and bundle sheath (mol e$^-$ m$^{-2}$ s$^{-1}$).

Since the total electron flow through PS I may be comprised of any combination of linear and cyclic components, it represents the upper limit for linear electron flow through PS II ($J_{P680,mj}$, $J_{P680,bj}$). For the C$_3$, Type I C$_3$-C$_4$, and C$_4$ mesophyll, Eqn. 11a can be substituted into Eqn. 7a-7b and Eqn. 9a-9b to calculate the rate of electron flow through PS II and the corresponding rate of net CO$_2$ assimilation in the mesophyll ($A_{mj}$) from Q, O$_m$, and C$_m$. For the Type I C$_3$-C$_4$ and C$_4$ bundle sheath, Eqn. 11b can be substituted into Eqn. 8a-8b and Eqn. 10a-10b to calculate the rate of electron flow through PS II, and the corresponding rate of net CO$_2$ assimilation in the bundle sheath ($A_{sj}$) from Q, O$_s$, and C$_s$. The expressions for $C_s$ and O$_s$ are given below.

### Expressions for bundle sheath CO$_2$ ($C_s$)

For the Type I C$_3$-C$_4$ and C$_4$ cases, the steady-state CO$_2$ concentration of the bundle sheath is a balance between CO$_2$ supply (a function of glycine or malate shuttling) and demand (a function of net assimilation and diffusive leakage). However, the supply of CO$_2$ to the bundle sheath depends on the state of the mesophyll. For the Type I C$_3$-C$_4$ case, the light-limited rate of glycine shuttling is given by:

$$V_{gj} = \frac{J_{P680,mj} \cdot [O_m / (2 \cdot S \cdot C_m)]}{4 \cdot [1 + O_m / (S \cdot C_m)]} \tag{12a}$$

which is dependent on the energy supply in the mesophyll. For the C$_4$ case, the light-limited rate of malate shuttling is given by:

$$V_{pj} = \frac{J_{P700,mj}}{2 \cdot \left[1 - \frac{n_L}{n_C} + \frac{1}{n_C}\right]} \tag{12b}$$

which is also dependent on the energy supply in the mesophyll. On the demand side, the rate of diffusive leakage ($L$) is given by:

$$L = \frac{g_{bs} \cdot (C_s - C_m)}{P_{tot}} \tag{13}$$

where $g_{bs}$ is the conductance of the bundle sheath cell wall to CO$_2$ (mol CO$_2$ m$^{-2}$ s$^{-1}$), $C_s - C_m$ is the CO$_2$ partial pressure gradient between the bundle sheath and the mesophyll (bar), and $P_{tot}$ is the total atmospheric pressure (bar). Writing a conservation equation and rearranging yields:

$$C_s = C_m + [(V_{gj} + V_{pj} - A_{sj}) \cdot P_{tot} / g_{bs}] \tag{14}$$

where $C_s$ is represented as a function of $A_{sj}$.

### Expressions for bundle sheath O$_2$ ($O_s$)

Just as for CO$_2$, the steady-state O$_2$ concentration of the bundle sheath is a balance between O$_2$ supply (a function of splitting of water at PS II) and demand (a function of Rubisco oxygenase activity, respiration, and diffusive leakage). For the Type I C$_3$-C$_4$ case, we specify that net O$_2$ evolution is proportional to net CO$_2$ assimilation ($A_s$). For the C$_4$ case, we specify that net O$_2$ evolution is proportional to net CO$_2$ assimilation less the O$_2$ evolution that is suppressed by NADP-ME activity ($A_s - V_p/2$). For the diffusive leak, $L_o$ is given by:

$$L_o = \frac{g_o \cdot (O_s - O_m)}{P_{tot}} \tag{15a}$$

where $g_o$ is the conductance of the bundle sheath cell wall to O$_2$ (mol O$_2$ m$^{-2}$ s$^{-1}$), and $O_s - O_m$ is the O$_2$ partial pressure gradient between the bundle sheath and the mesophyll (bar). The conductance of the bundle sheath cell wall to O$_2$ is linked to the conductance of the bundle sheath cell wall to CO$_2$ by:

$$g_o = g_{bs} \cdot \frac{D_o \cdot H_o}{D_c \cdot H_c} \tag{15b}$$

where $D_o$ and $D_c$ are the diffusivities of O$_2$ and CO$_2$ in water (m$^2$ s$^{-1}$), and $H_o$ and $H_c$ are the solubilities of O$_2$ and CO$_2$ in water (bar$^{-1}$). Writing a conservation equation and rearranging yields the steady-state O$_2$ concentration of the bundle sheath as:

$$O_s = O_m + \frac{(A_{sj} - V_{pj}/2) \cdot P_{tot}}{g_o} \tag{16}$$

where $O_s$ is represented as a function of $A_{sj}$.

### Appendix III: Pure light-saturated state

### Expressions for net CO$_2$ assimilation ($A_{m,c}$ and $A_{sc}$)

In a pure light-saturated state, the CO$_2$ assimilation rates of the mesophyll and bundle sheath ($A_{m,c}$, $A_{sc}$) are kinetically controlled by the maximum activity of Rubisco and/or...
Expressions for bundle sheath CO\textsubscript{2} and O\textsubscript{2} (C\textsubscript{S} and O\textsubscript{S})

As before, the CO\textsubscript{2} supply depends on the state of the mesophyll. For the Type I C\textsubscript{3}-C\textsubscript{4} case, the light-saturated rate of glycine shuttling (V\textsubscript{gc}) is given by:

\[ V_{gc} = \frac{V_{\text{max}} \cdot [O_m/(2 \cdot S)]}{K_G \cdot (1 + O_m/K_G) + C_m} \]  

(18a)

where all of the terms have been defined previously. For the C\textsubscript{4} case, the light-saturated rate of malate shuttling (V\textsubscript{pc}) is given by:

\[ V_{pc} = \frac{V_{\text{max}} \cdot C_m}{K_p + C_m} \]  

(18b)

where V\textsubscript{max} is the maximum mesophyll activity of PEPC (mol CO\textsubscript{2} m\textsuperscript{-2} s\textsuperscript{-1}), and K\textsubscript{p} is the Michaelis-Menten constant of PEPC for CO\textsubscript{2} (bar).

The expression for the partial pressure of CO\textsubscript{2} in the bundle sheath is then given by:

\[ C_s = C_m + \frac{(V_{gc} + V_{pc} - A_{sc}) \cdot P_{tot}}{g_{bs}} \]  

(19)

where C\textsubscript{s} is represented as a function of A\textsubscript{sc}. The corresponding expression for the partial pressure of O\textsubscript{2} in the bundle sheath is given by:

\[ O_s = O_m + \frac{(A_{sc} - V_{pc}/2) \cdot P_{tot}}{g_o} \]  

(20)

where O\textsubscript{s} is also represented as a function of A\textsubscript{sc}.

Appendix IV: Mixed light-limited/light-saturated states

For the Type I C\textsubscript{3}-C\textsubscript{4} and C\textsubscript{4} cases, we also define two ‘mixed states’: one in which the mesophyll is light-limited while the bundle sheath is light-saturated, and the other in which the mesophyll is light-saturated while the bundle sheath is light-limited. In the first mixed state, the CO\textsubscript{2} supply to the bundle sheath is controlled by the light-limited rates of glycine shuttling (V\textsubscript{gj}) or malate shuttling (V\textsubscript{pj}) while the rate of net CO\textsubscript{2} assimilation in the bundle sheath is controlled by the light-limited rate of Rubisco activity (A\textsubscript{gj}). In this state, the partial pressures of CO\textsubscript{2} and O\textsubscript{2} in the bundle sheath are given by:

\[ C_s = C_m + \frac{(V_{gj} + V_{pj} - A_{sc}) \cdot P_{tot}}{g_{bs}} \]  

(21a)

\[ O_s = O_m + \frac{(A_{sc} - V_{pj}/2) \cdot P_{tot}}{g_o} \]  

(21b)

which are solved with substitution from Eqns. 12a, 12b, and 17b. In the second mixed state, the CO\textsubscript{2} supply to the bundle sheath is controlled by the light-limited rates of glycine shuttling (V\textsubscript{gc}) or malate shuttling (V\textsubscript{pc}) while the rate of net CO\textsubscript{2} assimilation in the bundle sheath is controlled by the light-limited rate of Rubisco activity (A\textsubscript{gj}). In this state, the partial pressures of CO\textsubscript{2} and O\textsubscript{2} in the bundle sheath are given by:

\[ C_s = C_m + \frac{(V_{gc} + V_{pc} - A_{gj}) \cdot P_{tot}}{g_{bs}} \]  

(22a)

\[ O_s = O_m + \frac{(A_{gj} - V_{pc}/2) \cdot P_{tot}}{g_o} \]  

(22b)

which are solved with substitution from Eqns. 8a, 8b, 11b, and 18a for the Type I C\textsubscript{3}-C\textsubscript{4} case and with substitution from Eqns. 10a, 10b, 11b, and 18b for the C\textsubscript{4} case.

Appendix V: Solution for pure and mixed states

Once the potential limiting states are defined, the model is solved in three steps. In the first step, the realized net CO\textsubscript{2}
assimilation rate of the mesophyll is calculated as:

\[ A_m = \min\{A_{mj}, A_{mc}\} \quad (23) \]

where \( \min\{} \) denotes the minimum of the potential light-limited \((A_{mj})\) and light-saturated \((A_{mc})\) rates. In the second step, the realized net CO\(_2\) assimilation rate of the bundle sheath is calculated. We first calculate the potential light-limited net CO\(_2\) assimilation rate using:

\[ A_{sj} = \begin{cases} \frac{f(V_{gj}, V_{pj})}{f(V_{gc}, V_{pc})}, & \text{if } A_m = A_{mj} \\ \frac{f(V_{gc}, V_{pc})}{f(V_{gj}, V_{pj})}, & \text{if } A_m = A_{mc} \end{cases} \quad (24a) \]

to account for the pure state where the mesophyll and bundle sheath are both light-limited as well as the mixed state where the mesophyll is light-saturated and the bundle sheath is light-limited. We then calculate the potential light-saturated net CO\(_2\) assimilation rate using:

\[ A_{sc} = \begin{cases} \frac{f(V_{gj}, V_{pj})}{f(V_{gc}, V_{pc})}, & \text{if } A_m = A_{mj} \\ \frac{f(V_{gc}, V_{pc})}{f(V_{gj}, V_{pj})}, & \text{if } A_m = A_{mc} \end{cases} \quad (24b) \]

to account for the mixed state where the mesophyll is light-limited and the bundle sheath is light-saturated as well as the pure state where the mesophyll and bundle sheath are both light-saturated. The realized net CO\(_2\) assimilation rate of the bundle sheath is then given by:

\[ A_s = \min\{A_{sj}, A_{sc}\} \quad (24c) \]

where \( \min\{} \) denotes the minimum of the potential light-limited \((A_{sj})\) and light-saturated \((A_{sc})\) rates. In the third step, the mesophyll and bundle sheath rates from Eqns. 23 and 24c are combined:

\[ A = A_m + A_s \quad (25) \]

which gives the overall rate of net CO\(_2\) assimilation.

Once the limiting states have been identified, the expressions in Johnson and Berry (2021) can be used to derive many other aspects of the internal state of the electron transport system (e.g., the photochemical yields of PS II and PS I, the redox poise of the mobile electron carrier pools in the intersystem chain, the level of non-photochemical quenching of PS II, and the state of photosynthetic control of Cyt \( b_6 f \)). These internal states then provide a basis for simulating observable fluorescence- and absorbance-based signals. Example calculations are provided in the model code. Note that for simplicity and clarity, all of these calculations assume that: (i) LEF is always accompanied by the amount of CEF1 required to balance the energy supply with the demands of carbon metabolism; and (ii) the absorption cross-sections of PS II and PS I are static rather than dynamic. See Johnson and Berry (2021) for discussion of alternatives.

References

Berry JA, Farquhar GD (1978) The CO\(_2\) concentrating function of C\(_4\) photosynthesis: A biochemical model. In: Hall D, Coombs J, Goodwin T (eds) Proceedings of the Fourth International Congress on Photosynthesis, Reading, England. The Biochemical Society, London, pp 119–131

Farquhar GD, von Caemmerer S, Berry JA (1980a) A biochemical model of photosynthetic CO\(_2\) assimilation in leaves of C\(_3\) species. Planta 149:78–90. 
https://doi.org/10.1007/bf00386231

Johnson JE, Berry JA (2021) The role of Cytochrome b\(_6\)f in the control of steady-state photosynthesis: a conceptual and quantitative model. Photosynth Res 148:101–136. 
https://doi.org/10.1007/s11120-021-00840-4

von Caemmerer S (1989) A model of photosynthetic CO\(_2\) assimilation and carbon-isotope discrimination in leaves of certain C\(_3\)-C\(_4\) intermediates. Planta 178:463–474. 
https://doi.org/10.1007/bf00963816