Supplementary Data

Supplementary appendix A. Basic equations in the C₄ photosynthesis model of von Caemmerer & Furbank (1999) and analytical solutions of the model given by Yin et al. (2011)

The basic equations of the C₄ model (von Caemmerer & Furbank 1999) for net photosynthesis rate (A) are:

\[ A = V_p - L - R_m \]  \hspace{1cm} (A1)

\[ L = g_{bs}(C_c - C_m) \]  \hspace{1cm} (A2)

\[ C_m = C_i - A / g_m \]  \hspace{1cm} (A3)

A can be written in terms of the Rubisco carboxylation rate (Vₖ) and oxygenation rate (Vₒ):

\[ A = V_k - 0.5V_o - R_d = \frac{(C_c - \gamma O_c)x_1}{C_c + x_2 O_c + x_3} - R_d \]  \hspace{1cm} (A4)

where \( x_1, x_2, \) and \( x_3 \) are intermediate constants or variables: for the enzyme (Rubisco)-limited rate, \( x_1 = V_{Cmax}, \) \( x_2 = K_mC / K_mO, \) \( x_3 = K_mC; \) for the e⁻ transport-limited rate, \( x_1 = (1-x)J_{atp}/3, \) \( x_2 = 7\gamma/3 \) and \( x_3 = 0. \)

The O₂ partial pressure in eqn (A4), \( O_c, \) is described as

\[ O_c = \alpha A/(u_{oc} g_{bs}) + O_m \]  \hspace{1cm} (A5)

where \( O_m \) is the mesophyll O₂ partial pressure (which we set here the same as \( O_i, \) the intercellular air-space O₂ partial pressure). The variable \( u_{oc} \) in eqn (A5) represents the coefficient that lumps diffusivities and solubilities of CO₂ and O₂ in water (von Caemmerer & Furbank 1999), and its possible dependence on temperature is quantified in Appendix B.

In the context of the modified model, eqn (1) in the main text, \( V_p \) is described by:

\[ V_p = \begin{cases} \frac{C_m V_{pmax}}{C_m + K_p} & \text{if } A < \min(A_{TE}, A_{TT}) \\ xJ_{atp}/2 & \text{if } A = \min(A_{TE}, A_{TT}) \end{cases} \]  \hspace{1cm} (A6)

The analytical solutions for individual terms of eqn (1) in the main text for C₄-photosynthesis are different, depending on whether \( V_p \) is limited by enzyme activity or by e⁻ transport. If the rate is limited by e⁻ transport, the solution is quadratic. If the rate is limited by PEPc activity, the solution is cubic.

The quadratic solution to \( A_{TE} \) or \( A_{TT} \)

The standard quadratic expression for \( A \) (referring to either \( A_{TE} \) or \( A_{TT}, \) for which \( V_p \) is limited by e⁻ transport) is (Yin et al. 2011):
The cubic expression and its solution to $A_{EE}$ or $A_{ET}$

In this solution, $x_1, x_2$ and $x_3$ are defined according to the text below eqn (A4), depending on whether the rate of the $C_3$ cycle is limited by the Rubisco activity or by $e^-$ transport.

Three roots for the above cubic equation are:

$$A_i = -2\sqrt{Q}\cos(\psi/3) - p/3$$
\[ A_2 = -2\sqrt{Q} \cos[(\psi + 2\pi)/3] - p/3 \]
\[ A_3 = -2\sqrt{Q} \cos[(\psi + 4\pi)/3] - p/3 \]

where \( Q = (p^3 - 3q)/9 \)

\[ \psi = \arccos\left(\frac{U}{\sqrt{Q^3}}\right) \]
\[ U = (2p^3 - 9pq + 27r)/54 \]

We found the root \( A_1 \) suitable for calculating either \( A_{EE} \) or \( A_{ET} \) under any combinations of \( C_i \), \( I_{\text{inc}} \) and \( O_i \).
Supplementary appendix B. Quantifying temperature dependence of diffusivities and solubilities of CO₂ and O₂ in water

The coefficient $u_{oc}$ in eqn (A5) in Appendix A actually lumps:

$$u_{oc} = \frac{D_{O2} S_{O2}}{(D_{CO2} S_{CO2})}$$

(B1)

where $D_{O2}$ and $D_{CO2}$ are the diffusivities for O₂ and CO₂ in water, respectively, and $S_{O2}$ and $S_{CO2}$ are their respective solubilities in water. $u_{oc}$ at 25°C is 0.047 (von Caemmerer & Furbank 1999).

von Caemmerer & Evans (2015) provided an equation, their eqn (3), describing the decrease in $S_{CO2}$ with increasing temperature, based on data at the site (http://en.wikipedia.org/wiki/Henry’s_law). Here we re-formulate it using the standard Arrhenius equation normalized at 25°C as:

$$S_{CO2} = S_{CO2,25} \cdot e^{\frac{19.95}{R} \left(\frac{1}{298} - \frac{1}{273.15}\right)}$$

(B2)

where the universal gas constant $R = 0.008314$ kJ K⁻¹ mol⁻¹. The data provided at the same website also allows quantifying the decrease of $S_{O2}$ with increasing temperature:

$$S_{O2} = S_{O2,25} \cdot e^{\frac{14.13}{R} \left(\frac{1}{298} - \frac{1}{273.15}\right)}$$

(B3)

Based on the report of Frank et al. (1996), von Caemmerer & Evans (2015) provided an equation describing the increase of $D_{CO2}$ with increasing temperature. We rewrite the equation here normalized at 25°C as:

$$D_{CO2} = D_{CO2,25} \cdot e^{\frac{16.90}{R} \left(\frac{1}{298} - \frac{1}{273.15}\right)}$$

(B4)

Data of Han & Bartels (1996) show that $D_{O2}$ also increases with increasing temperature, from which we obtained:

$$D_{O2} = D_{O2,25} \cdot e^{\frac{9.45}{R} \left(\frac{1}{298} - \frac{1}{273.15}\right)}$$

(B5)

Combining eqns (B2-B5) into eqn (B1) gives:

$$u_{oc} = 0.047 \cdot e^{\frac{1.63}{R} \left(\frac{1}{298} - \frac{1}{273.15}\right)}$$

(B6)

With such small value of activation energy for $u_{oc}$ (i.e., -1.63 kJ mol⁻¹), the model predicts that $u_{oc}$ decreases from 0.048 at 13.5°C to 0.046 at 39°C of our experimental temperature range.
The photosynthetic rate in the initial part of an $A$-$C_i$ curve can be approximated by (von Caemmerer & Furbank 1999):

$$A = C_m V_{p\text{max}}/(C_m+K_p) - R_m - g_{bs}C_m$$

where $C_m$ is the level of CO$_2$ in the mesophyll cells. Because $g_{bs}$ is low (0.0009 to 0.0065 mol m$^{-2}$ s$^{-1}$ in our cases, Fig. 6) and the CO$_2$ levels for the initial part of an $A$-$C_i$ curve are also low (Fig. S1), the last term of the above equation is negligible.

The difference in $C_m$ from $C_i$ is described by:

$$C_m = C_i - A/g_m$$

Combining this equation with Eqn (C1) yields a model:

$$A = \frac{g_m(C_i + K_p) + V_{p\text{max}} - R_m - g_{bs}C_{m}}{4g_m[C V_{p\text{max}} - R_m (C_i + K_p)]/2}$$

The advantage of this model, eqn (C2), compared with the one described in the main text $dA/dC_i = K_p V_{p\text{max}}/(C_i+K_p)^2$, is that it does not assume that $g_m$ is infinite and the initial part of the $A$-$C_i$ curves is strictly linear. Its disadvantage is that $g_m$ has to be assumed beforehand.

When we assumed a most likely value $g_{m_{25}} = 1.0$ mol m$^{-2}$ s$^{-1}$ (Kromdijk et al. 2010), we estimated $E_{Kp}$ when the model was combined with Eqn (3) for $K_p$ and Eqn (4) for $V_{p\text{max}}$. Using three sets of $V_{p\text{max}}$ parameters from Chinthapalli et al. (2003), Massad et al. (2007) and Boyd et al. (2015), the estimated $E_{Kp}$ based on data of the initial part of the $A$-$C_i$ curves, and subsequently estimated $V_{c\text{max}25}$ and $g_{bs}$ at six temperatures based on the full data set, are shown in Table S1.

### Table S1

|                   | $E_{Kp}$ (kJ mol$^{-1}$) | $V_{c\text{max}}$ (μmol m$^{-2}$ s$^{-1}$) | $g_{bs}$ (mmol m$^{-2}$ s$^{-1}$) |
|-------------------|-------------------------|----------------------------------------|----------------------------------|
|                   |                         |                                        | 13.5°C  | 18°C   | 25°C   | 30°C   | 34°C   | 39°C   |
| Chinthapalli et al. (2003) | 66.3(4.1)              | 49.0(0.9)                              | 0.98(0.39) | 0.92(0.26) | 2.56(0.36) | 5.54(0.49) | 6.40(0.48) | 4.79(0.71) |
| Massad et al. (2007)         | 79.5(4.4)              | 49.3(0.9)                              | 0.94(0.39) | 0.98(0.27) | 2.66(0.37) | 5.93(0.51) | 6.53(0.49) | 4.39(0.69) |
| Boyd et al. (2015)           | 73.3(4.1)              | 49.0(0.9)                              | 0.92(0.38) | 0.94(0.26) | 2.58(0.37) | 5.61(0.49) | 6.41(0.48) | 4.52(0.69) |
**Figure S1** The initial linear section of $A-C_i$ curves of 2% $O_2$ at six measurement temperatures. Bars represent standard errors of the mean of four replicated leaves (if larger than symbols).
Figure S2 Comparison between modelled (curve) and measured (points) $A$-$I_{inc}$ and $A$-$C_i$ curves at six leaf temperatures under the condition of 21% $O_2$. The curves are drawn from connecting two nearby values calculated by the model.
Figure S3 Temperature response of bundle-sheath conductance $g_{bs}$ estimated using four contrasting temperature responses of mesophyll conductance $g_m$ as reported for C$_3$ photosynthesis by Bernacchi et al. (2002) for tobacco, Walker et al. (2013) for tobacco, Walker et al. (2013) for Arabidopsis, and Evans & von Caemmerer (2013) for tobacco.
Figure S4 Comparison of temperature response of bundle-sheath conductance $g_{bs}$ normalised to 1.0 at 25°C between Kiirats et al. (2002) for *Amaranthus edulis* (open symbols) and our study for maize (solid symbols based on data shown in Figure 6 in the main text).