Can increased leaf photosynthesis be converted into higher crop mass production? A simulation study for rice using the crop model GECROS

Xinyou Yin* and Paul C. Struik
Centre for Crop Systems Analysis, Department of Plant Sciences, Wageningen University & Research, PO Box 430, 6700 AK Wageningen, The Netherlands

* Correspondence: Xinyou.Yin@wur.nl
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Abstract
Various genetic engineering routes to enhance C₃ leaf photosynthesis have been proposed to improve crop productivity. However, their potential contribution to crop productivity needs to be assessed under realistic field conditions. Using 31 year weather data, we ran the crop model GECROS for rice in tropical, subtropical, and temperate environments, to evaluate the following routes: (1) improving mesophyll conductance (gₘ); (2) improving Rubisco specificity (S₉/₅₅); (3) improving both gₘ and S₉/₅₅; (4) introducing C₄ biochemistry; (5) introducing C₄ Kranz anatomy that effectively minimizes CO₂ leakage; (6) engineering the complete C₄ mechanism; (7) engineering cyanobacterial bicarbonate transporters; (8) engineering a more elaborate cyanobacterial CO₂-concentrating mechanism (CCM) with the carboxysome in the chloroplast; and (9) a mechanism that combines the low ATP cost of the cyanobacterial CCM and the high photosynthetic capacity per unit leaf nitrogen. All routes improved crop mass production, but benefits from Routes 1, 2, and 7 were ≤10%. Benefits were higher in the presence than in the absence of drought, and under the present climate than for the climate predicted for 2050. Simulated crop mass differences resulted not only from the increased canopy photosynthesis competence but also from changes in traits such as light interception and crop senescence. The route combinations gave larger effects than the sum of the effects of the single routes, but only Route 9 could bring an advantage of ≥50% under any environmental conditions. To supercharge crop productivity, exploring a combination of routes in improving the CCM, photosynthetic capacity, and quantum efficiency is required.

Key words: Crop modelling, crop productivity, GECROS, genetic transformation, photosynthesis, radiation use efficiency, simulation, water use efficiency, yield potential.

Introduction
Yields of major crops have increased steadily during the last decades. Fischer et al., (2014) claimed that, in order to ensure food and energy security for a growing and increasingly demanding population, staple crop production will need to grow by 60% from 2010 to 2050, with the greatest increases in the next 20 years. They further stressed that higher rates of increase than the current rate should be aimed for to drive faster reductions in world hunger and to guard against unanticipated negative contingencies, for example as a result of increasing frequencies of extreme weather under global climate change. Crop yield per area of land is the production of mass per unit area multiplied by harvest index. Yield gains associated
with the first Green Revolution in cereal crops such as wheat and rice were mainly due to increased harvest index by introducing (semi-)dwarfing genes (e.g. Miflin, 2000; Sadras and Lawson, 2011). For further progress to be made, improvement in crop mass production via increasing leaf and canopy photosynthetic capacity and efficiency should be explored (Long et al., 2006; Murchie et al., 2009; Parry et al., 2011; Ort et al., 2015). Evidence suggests that genetic variation in leaf photosynthesis has not been exploited to be incorporated into crop cultivars (e.g. Driever et al., 2014), except for recent releases in which yield gains were accompanied, to some extent, by traits related to increased leaf photosynthesis (Fischer et al., 2014).

Arguably, exploiting natural genetic variation is still the most feasible approach to improve yield traits including photosynthesis (Flood et al., 2011). For example, exploring natural variation in mesophyll conductance for CO₂ diffusion (gₘ) may improve photosynthesis (Gu et al., 2012; Flexas et al., 2013; Chen et al., 2014). However, very often there is little correlation between leaf photosynthesis and crop productivity across germplasm (Driever et al., 2014; Koester et al., 2016) or across individual lines of a segregating population (e.g. Gu et al., 2014a, b), partly because natural variation in leaf photosynthesis and underlying traits is generally small (Driever et al., 2014). To enhance crop productivity at a greater pace, genetic engineering and synthetic biology approaches to improving leaf photosynthesis should be explored (Long et al., 2006, 2015; Singh et al., 2014; Ort et al., 2015; Kromdijk and Long 2016).

Major crops such as rice follow the pathway of C₃ photosynthesis. Compared with C₄ crops such as maize, C₃ crops have lower photosynthetic productivity primarily because ~20–35% of the carbohydrate is lost through photorespiration (Long et al., 2006; Walker et al., 2016), resulting from the oxygenation of ribulose-1,5-biphosphate by Rubisco, the primary enzyme for CO₂ fixation. Various genetic engineering routes to enhance C₃ leaf photosynthesis have been proposed to suppress photorespiration, thereby improving crop productivity. These include: replacing Rubisco with foreign forms with higher specificity for CO₂ relative to O₂ (Sₑ/Sᵣ) (Whitney et al., 2001; Zhu et al., 2004; Parry et al., 2011), designing a photorespiratory bypass (Kebeish et al., 2007), and transforming the C₄ CO₂-concentrating mechanism (CCM) (e.g. von Caemmerer et al., 2012) or the cyanobacterial bicarbonate-based CCM (Price et al., 2011, 2013; Lin et al., 2014) into main C₃ crops.

Some of the engineering approaches have already made progress and were evaluated experimentally for mass production using model plants, such as Arabidopsis thaliana engineered with a photorespiratory bypass (Kebeish et al., 2007) and tobacco with a cyanobacterial bicarbonate transporter (Pengelly et al., 2014) and with accelerated recovery from photoprotection (Kromdijk et al., 2016). Any progress for major crops may not be expected in the near future, and modelling should be considered as an important tool to assess the potential of yield improvement by these photosynthesis-enhancing routes. Many researchers (e.g. Zhu et al., 2004; Song et al., 2013; Kromdijk and Long, 2016) have published modelling studies to assess the potential benefit of using various routes in improving photosynthesis; but most of their analyses were based on the simulation of canopy photosynthesis at a fixed leaf area index (LAI) for a given environmental condition.

During the growth cycle of annual field crops, LAI expands initially, reaches its maximum size, and then senesces, and complex interactions and feedback mechanisms can occur between photosynthesis and other physiological components (Yin and Struik, 2008). These complexities should be considered when scaling up from instantaneous leaf assimilation to daily canopy photosynthesis and to total mass production over the growing season (Boote et al., 2013). To that end, Gu et al. (2014a) ran numerical simulations using a full crop growth model, GECROS (Yin and van Laar, 2005), and examined the potential of exploiting the natural genetic variation in leaf photosynthesis within a single segregating population for contributing to crop productivity in rice (Oryza sativa L.) under field conditions.

Here we ran the crop model GECROS to quantify the extent to which improved leaf photosynthesis, predominantly from genetic engineering to suppress photorespiration, can result in an expected increase in crop mass production under well-watered, as well as water-limited, field conditions, using rice as an example.

### Materials and methods

Model algorithms and approach in applying GECROS (v4.0) for this study are outlined below. Model parameters, if not defined in the text, are given in Table 1.

#### C₃ photosynthesis model

The model of Farquhar et al., (1980) the FvCB model hereafter calculates net CO₂ assimilation rate (A) as the minimum of the Rubisco-limited (Aᵣ) and e⁻ transport-limited (Aᵣ) rates. The two limiting rates can be expressed collectively as:

\[
A = \frac{(C_ᵣ - Γ_i) x_i}{C_i + x_i} - R_d \tag{1}
\]

where \(Aᵣ\), \(x_i = V_{max} + x_i = K_{mC}_i(1 + O/K_{mC})\); \(Aᵣ\), \(x_i = [1 – f_{pe}/(1 – f_{oc})]J/4 + x_i = 2O_{VPD/}, \) where \(x_i\) is written according to the FvCB model extended by Yin et al. (2004) to be compatible with a C₄ model for which accounting for \(f_{oc}\) is required (see later). In the model, \(C_i\) and \(O\) are the CO₂ and O₂ level, respectively, at the carboxylation sites of Rubisco, \(J\) is the total PSII e⁻ transport rate, and \(Γ_i\), defined as \(O_{VPD/}\) (where \(Γ_i\) is half of the inverse of \(S_{oc}\)) is the CO₂ compensation point in the absence of day respiration \(R_d\).

The submodel for stomatal conductance for CO₂ transfer \(g_c\) is:

\[
g_c = g_0 + \frac{A + R_d}{C_i - C'_r} f_{vpd} \tag{2}
\]

where \(g_0\) is the residual value of \(g_c\), when irradiance approaches zero, \(C'_r\) is the intercellular CO₂ level \(C_i\) at which \(A + R_d = 0\), and \(f_{vpd}\) is the relative effect of leaf-to-air vapour difference (VPD) on \(g_c\) (see later). CO₂ transfer from \(C_i\) (the ambient CO₂ level) to \(C'_r\) can be written as (Flexas et al., 2013):

\[
C_i = C'_r – A/(1/g_m + 1/g_c) \tag{3}
\]

\[
C'_r = C_i – A/g_m \tag{4}
\]
Table 1. Input parameter values for various parts of biochemical leaf photosynthesis models

| Category | Symbol | Definition (unit) | \( C_3 \) | Reference | \( C_4 \) | Reference |
|----------|--------|-------------------|----------|-----------|----------|-----------|
| e⁻ transport | \( \Phi_{\text{ILL}} \) | Quantum efficiency of PSII e⁻ transport under limiting light (mol mol⁻¹) at \( T_{\text{opt}} \) | 0.78 | Yin et al., (2014) | 0.78 | Assumed to be the same as for \( C_3 \) |
| | \( r_{2/1} \) | Ratio of \( \Phi_{\text{ILL}} \) to quantum efficiency of PSII e⁻ transport under limiting light (⁻) | 0.85 | Genty and Harbinson (1996) | 0.85 | Assumed to be the same as for \( C_3 \) |
| | \( \theta \) | Convexity of irradiance response of PSII e⁻ transport rate (⁻) | 0.8 | Yin et al., (2009) | 0.8 | Assumed to be the same as for \( C_3 \) |
| | \( f_{\text{cyc}} \) | Fraction of total PSI e⁻ flux that follows cyclic e⁻ transport (⁻) | 0.05 | Yin et al., (2006) | 0.45² | Yin and Struik (2012) |
| | \( f_{\text{psuedo}} \) | Fraction of total PSI e⁻ flux that follows pseudocyclic e⁻ transport (⁻) | 0.10 | Yin et al., (2006) | 0.05 | Yin and Struik (2012) |
| | \( f_o \) | Fraction of total plastocynnone e⁻ flux that follows the Q-cycle (⁻) | NU | NU | 1 | Furbank et al., (1990) |
| | \( h \) | H⁺ required per ATP production (mol mol⁻¹) | NU | NU | 4 | Yin and Struik (2012) |
| | \( \alpha \) | Fraction of O₂ evolution in bundle-sheath cells (⁻) | NA | NA | 0.1 | Standard value for \( C_4 \) species such as maize |
| | \( x \) | Fraction of ATP used for CCM (⁻) | NA | NA | 0.4² | von Caemmerer and Furbank (1999) |
| | \( \psi \) | Extra ATP required for the CCM per CO₂ fixed (mol mol⁻¹) | NA | NA | 2² | von Caemmerer and Furbank (1999) |
| | \( T_{\text{opt}} \) | Optimum temperature for \( \Phi_{\text{ILLL}} \) (°C) | 23 | Data of Yin et al., (2014) | 34 | Data of Yin et al., (2016) |
| | \( \Omega \) | Difference between \( T_{\text{opt}} \) and the temperature at which \( \Phi_{\text{ILL}} \) falls to e⁻⁻ of its maximum (°C) | 36.8 | Data of Yin et al., (2014) | 38.4 | Data of Yin et al., (2016) |
| Enzyme kinetics and activity | \( S_{\text{c/o25}} \) | Relative CO₂/O₂ specificity of Rubisco at 25 °C (mol mol⁻¹) | 3022 | Cousins et al., (2010) | 2862 | Cousins et al., (2010) |
| | \( \gamma_{25} \) | Half the reciprocal of \( S_{\text{c/o25}} \) (mol mol⁻¹) | 0.5/3022 | By definition | 0.5/2862 | By definition |
| | \( K_{\text{m25}} \) | Michaelis–Menten constant of Rubisco for CO₂ at 25 °C (μmol mol⁻¹) | 291 | Cousins et al., (2010) | 485 | Cousins et al., (2010) |
| | \( K_{\text{m25}} \) | Michaelis–Menten constant of Rubisco for O₂ at 25 °C (μmol mol⁻¹) | 194 | Cousins et al., (2010) | 146 | Cousins et al., (2010) |
| | \( \chi_{\text{vmax25}} \) | Linear slope of maximum Rubisco activity at 25°C (\( V_{\text{vmax25}} \)) versus (\( n_n \))(μmol s⁻¹ g⁻¹) | 75 | Derived from data of Yin et al., (2009) | 93 | 1.24 times that for \( C_3 \) (Cousins et al., 2010; Perdomo et al., 2015) |
| | \( \chi_{\text{vmax25}} \) | Linear slope of maximum PSII e⁻ transport rate at 25 °C (\( J_{\text{vmax25}} \)) versus (\( n_n \))(μmol s⁻¹ g⁻¹) | 100 | Harley et al., (1992); Yin et al., (2009) | 200 | Derived from data of Yin et al., (2011) |
| | \( \chi_{\text{v25}} \) | Linear slope of PEP carboxylation efficiency at 25 °C (\( V_{\text{v25}} \)) versus (\( n_n \))(μmol s⁻¹ g⁻¹) | NA | NA | 0.791 | Derived from data of Yin et al., (2011) |
| Leaf respiration | \( R_{\text{D25}} \) | Day respiration at 25 °C (μmol m⁻² s⁻¹) | 0.01\( V_{\text{vmax25}} \) | Common assumption | 0.01\( V_{\text{vmax25}} \) | Assumed to be the same as for \( C_3 \) (von Caemmerer and Furbank, 1999) |
| | \( R_{\text{m}} \) | Respiration rate occurring in mesophyll cells (μmol m⁻² s⁻¹) | NA | NA | 0.5\( R_{\text{s25}} \) | von Caemmerer and Furbank, 1999 |
| Category         | Symbol | Definition (unit)                                                                 | $C_2$ Value | Reference           | $C_3$ Value | Reference           |
|------------------|--------|-----------------------------------------------------------------------------------|--------------|---------------------|--------------|---------------------|
| CO₂ diffusion    | $g_0$  | Empirical residual stomatal conductance if light approaches zero (mol m⁻² s⁻¹)    | 0.01         | Leuning (1995)      | 0.01         | Assumed to be the same as for $C_3$ |
|                  | $a_1$  | Empirical constant for $g_1$ response to VPD (--)                                 | 0.9          | Derived from Morison and Gifford (1983) | 0.9          | Set the same as for $C_3$ crops² |
|                  | $b_1$  | Empirical constant for $g_1$ response to VPD (kPa⁻¹)                              | 0.15         | Derived from Morison and Gifford (1983) | 0.15         | Set the same as for $C_3$ crops² |
|                  | $\chi_{gbs25}$ | Linear slope of mesophyll conductance at 25 °C ($g_{b25}$) versus (n₀–n) (mol s⁻¹ g⁻¹) | 0.125       | Derived from data of Yin et al., (2009); Gu et al., (2012) | NU           | NU                  |
|                  | $\chi_{gbs25}$ | Linear slope of bundle-sheath conductance at 25 °C ($g_{b25}$) versus (n₀–n) (mol s⁻¹ g⁻¹) | NA          | NA                  | 0.007⁶       | Yin et al., (2011) |
|                  | $u_{g25}$ | Coefficient lumping diffusivities and solubilities of CO₂ and O₂ in H₂O at 25 °C | NA          | NA                  | 0.047        | von Caemmerer and Furbank (1999) |
| Temperature response | $E_T$ | Activation energy for $\gamma$ (J mol⁻¹)                                          | 24 460       | Bernacchi et al., (2002) | 27 417       | Yin et al., (2016) |
|                  | $E_{VCmax}$ | Activation energy for $V_{cmax}$ (J mol⁻¹)                                        | 65 330       | Bernacchi et al., (2001) | 53 400       | Yin et al., (2016) |
|                  | $E_{Kmc}$ | Activation energy for $K_{mc}$ (J mol⁻¹)                                          | 80 990       | Bernacchi et al., (2002) | 35 600       | Perdomo et al., (2015) |
|                  | $E_{Kmd}$ | Activation energy for $K_{md}$ (J mol⁻¹)                                          | 23 720       | Bernacchi et al., (2002) | 15 100       | Yin et al., (2016) |
|                  | $E_{R2}$ | Activation energy for $R_2$ (J mol⁻¹)                                             | 46 390       | Bernacchi et al., (2001) | 41 853       | Yin et al., (2016) |
|                  | $E_{i2}$ | Activation energy for $I_{i2}$ (J mol⁻¹)                                           | 88 380⁶      | Yin and van Laar (2005) | 116 439      | Yin et al., (2016) |
|                  | $D_{i2}$ | Deactivation energy for $I_{i2}$ (J mol⁻¹)                                         | 200 000      | Harley et al., (1992) | 135 982      | Yin et al., (2016) |
|                  | $S_{i2}$ | Entropy term for $I_{i2}$ (J K⁻¹ mol⁻¹)                                            | 650          | Harley et al., (1992) | 458.7        | Yin et al., (2016) |
|                  | $E_{i3}$ | Activation energy for $I_{i3}$ (J mol⁻¹)                                           | NA          | NA                  | 51 029       | Data of Yin et al., (2016) |
|                  | $D_{i3}$ | Deactivation energy for $I_{i3}$ (J mol⁻¹)                                         | NA          | NA                  | 130 363      | Data of Yin et al., (2016) |
|                  | $S_{i3}$ | Entropy term for $I_{i3}$ (J K⁻¹ mol⁻¹)                                            | NA          | NA                  | 425.6        | Data of Yin et al., (2016) |
|                  | $E_{iyn}$ | Activation energy for $I_{iyn}$ (J mol⁻¹)                                         | 49 600       | Bernacchi et al., (2001) | NU           | NU                  |
|                  | $D_{iyn}$ | Deactivation energy for $I_{iyn}$ (J mol⁻¹)                                       | 437 400      | Bernacchi et al., (2002) | NU           | NU                  |
|                  | $S_{iyn}$ | Entropy term for $I_{iyn}$ (J K⁻¹ mol⁻¹)                                         | 1400         | Bernacchi et al., (2002) | NU           | NU                  |
|                  | $E_{gbs}$ | Activation energy for $g_{bs}$ (J mol⁻¹)                                         | NA          | NA                  | 116 767      | Yin et al., (2016) |
|                  | $D_{gbs}$ | Deactivation energy for $g_{bs}$ (J mol⁻¹)                                       | NA          | NA                  | 264 604      | Yin et al., (2016) |
|                  | $S_{gbs}$ | Entropy term for $g_{bs}$ (J K⁻¹ mol⁻¹)                                                 | NA          | NA                  | 860          | Yin et al., (2016) |
|                  | $E_{unc}$ | Activation energy for $u_{inc}$ (J mol⁻¹)                                      | NA          | NA                  | –1630        | Yin et al., (2016) |
| Base leaf N      | $n_b$  | Base leaf nitrogen, at and below which leaf photosynthesis is zero (g m⁻²)      | 0.3          | Sinclair and Horie (1989) | 0.3          | Assumed to be the same as for $C_3$

NA, not applicable; NU, not used by the model presented herein.

* These parameter values need to be adjusted if the $C_3$ model is used for simulating the cyanobacterial CCM (see the text and Table 2).

² Data of Morison and Gifford (1983) showed that stomatal sensitivity to VPD could differ between $C_3$ and $C_4$; such a difference can be mimicked by our stomatal conductance model, Equation 2 for $C_2$ and Equation 11 for $C_3$ leaves, when using the same values of $a_1$ and $b_1$.

³ Parameter set in GECROS to be dependent on crop species; the value 88 380 was set as default for rice (Yin and van Laar, 2005).
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Combining Equations 1–4 gives a standard cubic equation, as shown in Supplementary Text 1 at JXB online.

**C₄ photosynthesis model**

The C₄ model of von Caemmerer and Furbank (1999), as modified by Yin and Struik (2009, 2012), is used here. In C₄ plants, CO₂ is fixed initially in the mesophyll by phosphoenolpyruvate (PEP) carboxylase into C₄ acids that are then decarboxylated to supply CO₂ to Rubisco, which is localized in the bundle-sheath chloroplasts. The co-ordinated functioning of the ‘Kranz’ anatomy and C₄ biochemistry enables an effective CCM. The extra ATP consumption for sustaining the CCM requires a higher fₑ in C₄ than in C₃ photosynthesis (Yin and Struik, 2012; Nakamura et al., 2013). The rate of PEP carboxylation (\(V_p\)) could be limited either by the PEP carboxylase or by the rate of e⁻ transport (Yin and Struik, 2009):

\[ V_p = \min (\varepsilon_p C_i, x J_2 z / \phi) \]  

where \(\varepsilon_p\) is the initial carboxylation efficiency of the PEP carboxylase, \(\phi\) is the extra ATP required for the CCM per CO₂ fixed, and \(z = 2 + f_{\text{dec}}/f_{\text{trans}}\) if (1–\(f_{\text{dec}}\))/[(1–\(f_{\text{trans}}\)] (here \(h\) is the H⁺:ATP ratio: Yin et al., 2004; Yin and Struik, 2012), and \(x\) represents the fraction of ATP partitioned to the reactions associated with the operation of \(V_p\). In the standard C₃ model for malic-enzyme subtypes such as crop plants maize and sorghum, \(x\) was set to 0.4, arising from \(q_\text{NADP}/(3 + q_\text{NADP})\), where \(q_\text{NADP} = 2\), and 3 is mol ATP required for the Calvin cycle to fix 1 mol CO₂.

An effective CCM requires a small bundle-sheath conductance (\(g_{sb}\)) as \(g_m^b\) determines the CO₂ leakage from the bundle sheath to the mesophyll (\(L\)) that affects CO₂ assimilation (von Caemmerer and Furbank, 1999):

\[ L = g_m^b (C_i - C_c) \]

\[ A = V_p - L - R_m \]

Equations 5–7 can be combined to result in:

\[ A = a C_i + (b - A - R_m) / g_m \]  

where \(a = 1 + \varepsilon_p/g_m\) and \(b = 0\) if \(V_p\) is PEP carboxylase limited, and \(a = 1 \) and \(b = x J_2 z / \phi\) if \(V_p\) is e⁻ transport limited (Yin and Struik, 2009).

The rate of CO₂ fixation by Rubisco is modelled in the same way as for C₃ photosynthesis:

\[ C_i = a C_i + (b - A - R_m) / g_m \]

\[ A = \frac{(C_i - \gamma O) x_i}{C_i + x_i O + x_i} - R_i \]

where \(\gamma = \frac{f_{\text{dec}}}{f_{\text{trans}}}\) (Yin et al., 2011), which would predict a monotonic increase in ATP production rate, thus in an e⁻ transport-limited carboxylation rate, with increasing \(f_{\text{dec}}\). This does not agree with the more efficient CCM in terms of ATP use (e.g. cyanobacterial CCM; Price et al., 2011). Using the NADPH-limited form allows a revised C₄ model to simulate photosynthesis of other CCM systems (see below) and to be consistent with the C₃ photosynthesis modelling where the NADPH-limited form is predominantly used.

A relationship for \(O\), partial pressure between the intercellular air space (\(O\)) and the sites around Rubisco in bundle-sheath cells (\(O\)) is described as (von Caemmerer and Furbank, 1999):

\[ O = \alpha A (\mu g_m + \mu g_m^b) + O \]  

A model for \(g_m\) of C₄ leaves was formulated in a way that slightly differed from Equation 2 of the C₃ counterpart, to solve analytically for \(A\) in C₄ photosynthesis (Yin and Struik, 2009):
where \( C_i \) is the CO\(_2\) level at leaf surface, and \( C_r \) is the \( C_r \)-based CO\(_2\) compensation point in the absence of \( R_{0} \) and can be calculated as \( [g_n \gamma - \Omega R(1 + \gamma - \alpha)u_R]R + R_{0}[g_n + e_p] \) (Yin and Struik, 2009).

Equation 11 for \( C_i \) and Equation 2 for \( C_r \), although both empirical, can reproduce experimentally observed linear relationships between \( A \) and \( g \) across various levels of irradiance and nutrients (e.g. Wong et al., 1985) (see Supplementary Fig. S1).

Equation 3 also applies to \( C_4 \) photosynthesis. Combining Equations 3 and 8–11 can yield the standard cubic equation that gives the prediction of \( A \) (Supplementary Text 1).

### Algorithms common to \( C_3 \) and \( C_4 \) photosynthesis

Some common algorithms were used for \( C_3 \) and \( C_4 \) models. First, \( J_r \) is described as a function of absorbed irradiance \( I_{abs} \) as (Yin et al., 2006; Yin and Struik, 2012):

\[
J_r = (\alpha_{rs} I_{abs} + J_{max} - \sqrt{(\alpha_{rs} I_{abs} + J_{max})^2 - 4\theta J_{max} \alpha_{rs} I_{abs}}) / (2\theta)
\]  

(12)

where \( \alpha_{rs} \) and \( b_l \) represent the \( C_r \)–\( C_i \) ratio in water vapour-saturated air and the slope of the decrease of this ratio with increasing VPD, respectively, if \( g_0 \) in Equation 2 or 11 approaches nil.

Thirdly, a number of parameters are related to leaf temperature (\( T_1 \)), and some of these can be described by the Arrhenius equation normalized with respect to 25 °C:

\[
\text{Parameter} = 
\frac{1}{1}
\frac{1}{1 - \text{max}(a_1 - b_1 \cdot \text{VPD}, 0.01) + 1}
\]  

(13)

where \( a_1 \) and \( b_1 \) represent the \( C_r \)–\( C_i \) ratio in water vapour-saturated air and the slope of the decrease of this ratio with increasing VPD, respectively, if \( g_0 \) in Equation 2 or 11 approaches nil.

### Revising the \( C_4 \) model for simulating the cyanobacterial CCM

The single-cell \( C_4 \) photosynthesis model of von Caemmerer and Fur Burbank (2003; see also Supplementary Text 2) can be used for simulating cyanobacterial photosynthesis (Price et al., 2011). However, this model is hard to solve once it is coupled to a \( g_0 \) model (Equation 2 or 11). We therefore revise the above \( C_4 \) model to simulate the cyanobacterial CCM, based on the model concept of Price et al. (2011). These revisions are: (i) set \( g_m \) to a high value to mimic \( g_0 \) (conductance of the chloroplast envelope to CO\(_2\)); (ii) set \( V_{\text{cyc}} \) as it stands for the combined rate of cyanobacterial bicarbonate transporters; (iii) set \( R_m = R_c \); and (iv) re-estimate \( f_{\text{cyc}} \) and \( x \), in view of the fact that extra ATP required for the cyanobacterial CCM also comes from the cyclic e- pathway (Shikmants, 2007). The ATP cost of bicarbonate transport may be lower than that of the \( C_4 \) CCM (Price et al., 2013; Fur Burbank et al., 2015). Two single-gene transporters (BicA and SbtA) that have been well characterized in cyanobacteria are considered here, and Price et al. (2011) estimated that the two transporters require 0.25 and 0.50 ATP per transport event, respectively (so, \( q \) in Equation 5 is 0.75). We re-estimated \( x \) as 0.2 and \( f_{\text{cyc}} \) as 0.18 (Table 1), where 0.2 arises from 0.75/(3 + 0.75), and 0.18 arises from the \( C_4 \) model of Yin and Struik (2009) for the balanced NADPH:ATP ratio assuming \( h = 4 \). This revised \( C_4 \) model gives simulated rates of \( A \) virtually identical to the model of Price et al., (2011) using the same set of parameter values (Supplementary Text 2) under normal and elevated [CO\(_2\)] conditions.

### Setting scenarios of improved leaf photosynthesis for simulation

Major routes in enhancing photosynthesis will be examined, except for the photorespiratory bypass. Modelling this bypass would require more complicated algorithms and parameters (von Caemmerer, 2013), which cannot be straightforwardly implemented to simulate field environments where modelling of \( g_0 \) is also needed. We examined the impact of improving \( g_m \) (Tholen et al., 2012; Flexas et al., 2013), improving \( S_{\text{in}} \) (Zhu et al., 2004; Parry et al., 2011), introducing the \( C_4 \) mechanisms into \( C_3 \) crops (von Caemmerer et al., 2012), and using cyanobacterial bicarbonate transporters and the CCM (Price et al., 2011, 2013). Given that efforts to engineer these routes, especially the latter two, into new crops will most probably make progress step-wise, we propose the following nine routes (Table 2):

1. Improving \( g_m \) versus leaf N (\( g_{m25} \), see Equation 16) is set from its default value 0.125 (Table 1) to be three times higher (i.e. 0.375).
Improving $S_{\text{clo}}$, where $S_{\text{clo25}}$ is set from its $C_3$ default value 3022 (Table 1) to 4427, the observed $S_{\text{clo25}}$ for the non-green alga *Griffithsia monilis* (Whitney et al., 2001).

Improving $s_m$ as well as $S_{\text{clo}}$, where $X_{\text{km25}}$ of 0.375 and $S_{\text{clo25}}$ of 4427 are combined.

Introducing $C_4$ biochemistry, where the $C_4$ photosynthesis model is used with $C_3$ kinetic constants (Table 1) while setting $g_m$ as high as the $C_3$ default $g_m$ (i.e., setting the slope of $g_m$ versus leaf N; $X_{\text{km25}}$ see Equation 16) to 0.125.

Making $C_4$ Kranz anatomy function effectively to minimize CO$_2$ leakage, where the low $X_{\text{km25}}$ (0.007) is combined with $C_3$ enzyme kinetic constants (Table 1).

Engineering the complete $C_4$ mechanism, where $C_4$ kinetic constants (Table 1) combined with a low $X_{\text{km25}}$ (0.007) is used in the $C_4$ photosynthesis model.

Engineering cyanobacterial single-subunit bicarbonate transporters (BiCA and SbtA), where the above revised $C_4$ model is combined with the default $C_3$ parameters with $X_{\text{km25}} = 0.125$ and the revised values for $g_f$, $x$, $R_g$, and $f_{\text{g}}$ (Table 2).

Adding a more elaborate cyanobacterial CCM, whereby the carboxysome shell proteins are expressed in chloroplasts to enrich the CO$_2$ level around Rubisco similar to the level in the $C_4$ bundle-sheath compartment. This route assumes that the chloroplast $C_3$ Rubisco can be reorganized into effective carboxysome structures and other requirements for carboxysome to function are optimized (Price et al., 2011). So, the same model and parameter values as for Route 7 are used, except for $X_{\text{km25}}$ which is now set to a lower value of 0.007 as for $C_4$ bundle-sheath conductance.

A complete cyanobacterial CCM installed. The complete cyanobacterial CCM will require replacement of the $C_3$ Rubisco with a cyanobacterial Rubisco in order to make use of the potential kinetic properties in a high-CO$_2$ carboxysome (Long et al., 2011). Based on the expectation of engineering the cyanobacterial CCM that approaches photosynthetic capacities typical of $C_4$ plants (Price et al., 2013), we used $X_{\text{km25}}$ and $X_{\text{kmmax25}}$ of $C_4$ photosynthesis (Table 1) for this route. So, this route has the low ATP cost of the cyanobacterial CCM as well as a high enzymatic capacity per unit N to mimic the complete cyanobacterial CCM.

Simulation results of all nine routes will be compared with those of the default in which the $C_4$ photosynthesis model with the $C_3$ parameter values in Table 1 is used.

**Modelling daily canopy photosynthesis and transpiration**

In GECROS, instantaneous canopy photosynthesis and transpiration were calculated using the sun/shade model of de Pury and Farquhar (1997), in which the sunlit and shaded portions of the canopy each are considered as a big leaf, and the above leaf-level model is applied. Assuming an exponential profile of leaf N, total photosynthetically active N for each portion was calculated, and N-dependent photosynthetic parameters $V_{\text{max}}$, $J_{\text{max}}$, $\delta$, $R_m$, and $E_p$ (see Equation 16) were then scaled up accordingly to each portion of the canopy. Instantaneous rates were scaled up to daily total, using the Gaussian integration (Goudriaan, 1986) to account for any asymmetric diurnal courses of radiation and temperature. These approaches for spatial and temporal extensions apply to the case in the absence of water limitation.

In the presence of water limitation, the available water is partitioned between sunlit and shaded leaves according to the relative share of their potential transpiration $(E_T)$ to obtain their actual transpiration $(E_r)$. The diurnal course of available water is assumed to follow that of radiation. Based on the Penman–Monteith equation, the actual transpiration is transformed into the actual level of stomatal resistance to water vapour $(r_{w,a})$ (Yin and van Laar, 2005):

$$r_{w,a} = \frac{(E_p - E_r)(\delta + \gamma_{sw})}{(\gamma'E_r) + r_{w,a}E_p / E_r}$$

where $r_{w,a}$ is the stomatal resistance to water vapour in the absence of water limitation [ = 1/(1.6$g_s$)], where $g_s$ is solved from the algorithm in Supplementary Text 1; $r_{w,a}$ and $r_{w,a}$ are the boundary-layer resistance to heat and to water vapour, respectively; $\gamma$ is the psychrometric constant; and $s$ is the slope of the saturated vapour pressure as a function of temperature (kPa °C$^{-1}$). The actual $r_{w,a}$ was converted into the actual $g_s$, which can be used as input to the analytical quadratic model (see Supplementary Text 3) to estimate the instantaneous actual photosynthesis of the sunlit and shaded leaves. The Gaussian integration was again used to obtain the daily total of the actual photosynthesis. Equation 18 suggests that the impact of water deficit is mainly via stomatal conductance: any non-stomatal effect of water deficit is not modelled in GECROS, except when accounting for changes in $T_d$ under drought.

**Crop simulation approaches**

Simulations were conducted for three sites, Los Baños (14°6'N, 121°9'E; the Philippines), Nanjing (32°56'N, 118°59'E; China), and Shizukuishi (39°41'N, 140°57'E; Japan), representing tropical, sub-tropical, and temperate rice-growing conditions, respectively, using 31 year (1980–2010) baseline weather data and the present atmospheric CO$_2$ concentration. We also ran the model under the climate scenario for 2050, at which the expected CO$_2$ is ~550 μmol mol$^{-1}$ and air temperature is 2 °C higher than the baseline (Li et al., 2015). As we only examined the impact of changed leaf photosynthesis on crop productivity, we decoupled the GECROS soil module and used only the crop module for simulation to avoid any confounding effects from uncertainties in simulating soil processes. Potential production was simulated by setting the daily water supply to the crop as non-limiting. Water-limited production was simulated by setting the daily available water for evapotranspiration to no more than 50% of seasonal average daily transpiration simulated for the potential production, which was 1.97, 1.63, and 1.34 mm H$_2$O d$^{-1}$ for Los Baños, Nanjing, and Shizukuishi, respectively. Daily N supply was set in such a way that the accumulated N uptake by the crop followed the sigmoid curve of Yin et al. (2003) and that the total uptake at maturity reached 20 g N m$^{-2}$, equivalent to the N uptake in high-yielding rice experiments (Setter et al., 1994).

Model parameters for phenology were calibrated (Table 3) so that simulated baseline crop duration was in line with that of the standard cultivar at each site (Li et al., 2015). Crop models are less accurate in predicting spikelet number and therefore harvest index than in predicting crop mass (Boote et al., 2013; Li et al., 2015). To minimize the impact of this uncertainty, we used the simulated total shoot mass (excluding dead leaves) at maturity as the proxy for crop productivity. Input parameters were set as the default values of GECROS for rice (Yin and van Laar, 2005) and those relevant to our study are given in Table 3. As $C_4$ enzyme kinetic parameters, especially their temperature responses, are less certain than the $C_3$ counterparts (Boyd et al., 2015), additional analysis was conducted for $C_4$ simulation (Supplementary Text 4). Similarly, because the exact ATP cost of bicarbonate transport is uncertain (Fridlyand et al., 1996; McGrath and Long, 2014), sensitivity analysis was conducted for Route 9 with regard to this cost (Supplementary Text 5). Further details about GECROS are given in Supplementary Text 6, and source codes of the full GECROS model can be obtained upon request.

**Results and Discussion**

**Simulated leaf photosynthesis**

All routes could increase $A$ in the light-saturated region, especially Routes 6 and 9 (Fig. 1). In the light-limited region, the impact of the routes was smaller, and Routes 4, 5, and 6 in fact had a negative effect (see the inset of Fig. 1). This negative impact
is associated with the two extra ATPs required for PEP regeneration in the C₄ cycle (von Caemmerer and Furbank, 1999), for which a high $f_{\text{cyc}}$ is required (Yin and Struik, 2012; Table 1). In Route 4 where this high ATP cost was not compensated by an effective CCM to suppress photorespiration, the negative effect was particularly high. Because these routes act differently for the light-saturated and limited regions, the curvature in the light response curve was diverse (Fig. 1) despite the same curvature factor $\theta$ (0.8; Table 1) used for Equation 12 describing the light response of PSII $e^-$ transport rate for all these curves.

### Table 3. Values of some input parameters of the GECROS crop model relevant to this study

| Parameter | Definition (unit) | Value |
|-----------|------------------|-------|
| $S_n$ | Specific leaf area constant for newly emerging leaves ($m^2\ g^{-1}$) | 0.03 |
| $n_{\text{Rmin}}$ | Base value of root nitrogen concentration ($g\ g^{-1}$) | 0.005 |
| $n_{\text{Rmax}}$ | Base value of stem nitrogen concentration ($g\ g^{-1}$) | 0.005 |
| $n_{\text{Riv}}$ | Nitrogen concentration in plant reserves ($g\ g^{-1}$) | 0.0015 |
| $S_p$ | Potential weight of a single grain (g) | 0.025 |
| $n_{\text{G}}$ | Potential nitrogen concentration in grains ($g\ g^{-1}$) | 0.0145 |
| $H_{\text{max}}$ | Maximum final plant height (m) | 1.0 |
| $T_{\beta}$ | Time constant for senescence ($d$) | 2 |
| $T_o$ | Base temperature for phenology (°C) | 8 |
| $T_{\alpha}$ | Optimum temperature for phenology (°C) | 30 |
| $T_{\tau}$ | Ceiling temperature for phenology (°C) | 42 |
| $m_{\text{f}}$ | Minimum number of days for post-flowering period (thermal day$^a$) | 70, 85, 48$^b$ |
| $m_{\text{p}}$ | Minimum number of days for pre-flowering period (thermal day$^a$) | 28, 32, 22$^b$ |
| STTIME | Starting time of simulation, equivalent to day number (from 1 January) for seedling emergence | 10, 145, 125$^b$ |

$^a$ One thermal day is equivalent to one calendar day if the temperature at each moment of the day is always at the optimum.

$^b$ Values used for Los Baños (the Philippines), Nanjing (China), and Shizukuishi (Japan), respectively. The STTIME value for Los Baños is for the dry season there (which is the season with the high yield potential), and that for Nanjing is for single-cropping rice (that is predominant in the region, compared with the double-cropping rice where rice is planted twice per year).

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**Simulated canopy photosynthesis**

Not surprisingly, the calculated daily canopy photosynthesis ($A_{\text{canopy,d}}$) increased with increasing LAI (Fig. 2), due to a higher interception of photosynthetically active radiation (PAR) at higher LAI. Also, the light response curve of $A_{\text{canopy,d}}$ became increasingly linear with increasing LAI, because at high LAI, leaves in the canopy are predominantly light limited, and within the light-limited range leaf photosynthesis increases almost linearly with light level (Fig. 1). Because the difference in leaf photosynthesis among the routes was mainly recognized in the light-saturated region (Fig. 1), the ratio of $A_{\text{canopy,d}}$ of photosynthesis-enhancing routes to that of the default $C_3$ route increased with increasing radiation level, and decreased with increasing LAI (Fig. 2). $A_{\text{canopy,d}}$ of Route 4, compared with the default $C_3$ route, was notably lower, regardless of the radiation level, when LAI was ≥3 (Fig. 2).

**Default simulation for crop durations and mass production**

Using GECROS, we simulated crop duration and mass production. A 2 °C warming for 2050, relative to the present climate, was simulated to shorten crop duration by ~5, 10, and 20 d, for tropical, subtropical, and temperate environments, respectively (Table 4). This different effect across the environments is due to the fact that temperature during the growing season in the tropics is around the optimum value, at which warming is expected to have a smaller effect than at the other sites where the growing season temperature is mostly in the range where development rate increases greatly with warming.

Despite the shorter duration, simulated aboveground mass at crop maturity increased for 2050 compared with the present climate (Table 4), largely due to CO₂ elevation from 400 μmol mol$^{-1}$ to 550 μmol mol$^{-1}$. This is because we implicitly
assumed that future breeding can develop rice cultivars capable of coping with any effect of warming on spikelet sterility, so the effect of CO$_2$ elevation was dominant. However, a recent FACE (free-air CO$_2$ enrichment) study (Cai et al., 2016) using a present cultivar showed that yields of rice were decreased by 17–35% under the combination of elevated CO$_2$ and temperature, compared with the ambient condition, due to fewer filled grains at the elevated temperature. As expected, water limitation decreased mass production, but increased water use efficiency (WUE) (Table 4). The WUE differed little among the three sites, but was higher for 2050 than for the present climate, partly due to increased $A_{canopy}$, and partly due to generally decreased canopy transpiration under the 2050 climate (Table 4). The reduced canopy transpiration for the 2050 climate was largely a result of partial stomatal closure induced by higher [CO$_2$] (e.g. Wong et al., 1985).

Impact of photosynthesis-enhancing routes on crop mass production

Compared with the default C$_3$ photosynthesis, all routes increased aboveground mass production, except for three cases for the potential production–2050 climate combination where the benefit from Route 5 was virtually nil or slightly negative (Table 5). In general, the benefit from Routes 1, 2, and 7 was ≤10%. All routes resulted in higher benefits in the presence of drought than in the absence of drought, and under the present climate than for 2050. This could be explained by the shape of a diminishing return for $A$–$C_i$ curves, because drought and the present climate both result in a lower $C_i$ compared with the potential production level and the 2050 climate, respectively.

Route combinations had an equal effect to, or a larger effect than, the sum of the routes acting alone. For example, the benefit from Route 3 (improving both $g_m$ and $S_{cl/o}$) was about the sum of the benefits from Route 1 (improving $g_m$) and Route 2 (improving $S_{cl/o}$) for the potential production and was higher than the sum of the two for the water-limited condition (Table 5).

The benefit from Route 6 (the complete C$_4$ mechanism) was considerably higher than the sum of the benefits from Route 4 (C$_4$ biochemistry components) and Route 5 (Kranz anatomy components for low $g_{bs}$) for any condition (Table 5). This result suggests that the ongoing programme of installing C$_4$ photosynthesis into C$_3$ crops (von Caemmerer et al., 2012), if successful, needs to engineer the complete C$_4$ mechanism. The benefit of a partial engineering is only marginal or even counter-productive under future high-CO$_2$ environments (see Route 5 in Table 5), because of the high ATP cost for operating the C$_4$ cycle. In an earlier preliminary simulation analysis

![Figure 2](https://academic.oup.com/jxb/article-abstract/68/9/2345/3079355)
(Yin and Struik, 2008), we showed that a low $g_{\text{so}}$ alone would increase rice yield in the tropics by ~25%. However, that analysis used an arbitrarily low $g_{\text{so}}$ and a version of the C$_4$ model that assumes an H$^+$:ATP ratio of 3, whereas a recent analysis suggested that this ratio is most probably 4 (Yin and Struik, 2012), suggesting that the model version Yin and Struik (2008) used may have underestimated quantum requirement for the C$_4$ CCM. From our present analysis, even with the complete C$_4$ mechanism, its advantage over the C$_3$ default was simulated to be >50% only under the combination of water limitation and the present climate; for other conditions, its advantage ranged between 22% and 40% (Table 5).

As engineering for the complete Krantz anatomy is challenging, the CCM in cyanobacteria, which is probably less expensive energetically, has been suggested as an obvious alternative to engineer (Price et al., 2011, 2013; Furbank et al., 2015). Our simulation showed that the simplest form for the cyanobacterial CCM with bicarbonate transporters, Route 7, had a marginal advantage (Table 5). Pengelly et al. (2014) showed that tobacco plants transformed with the BicA transporter had no discernible effect on CO$_2$ assimilation rates, suggesting that BicA was either not located or not activated correctly. Our simulation (Table 5) showed that a more elaborate cyanobacterial CCM, where the carboxysome shell proteins were expressed to enrich the CO$_2$ level around Rubisco in chloroplasts (Route 8), had a higher advantage than the equivalent C$_4$ CCM (Route 5), largely because of a lower ATP cost assumed for the cyanobacterial CCM. However, its benefit was lower than from the complete C$_4$ mechanism, namely Route 6, which includes the additional mechanism that C$_4$ plants have a considerably high carboxylation rate per unit leaf N (Evans and von Caemmerer, 2000; Makino et al., 2003). The complete cyanobacterial mechanism (Route 9), which has the low energy cost for the CCM as well as the high carboxylation and e$^-$ transport capacity per unit N (presumably as high as for C$_4$ plants), was the only route that could bring an advantage of ≥50% under any environmental conditions (Table 5). However, as the exact ATP cost for the cyanobacterial CCM is uncertain, the simulated benefits of Routes 7–9 should be considered as tentative and their real benefits might be lower (Supplementary Text 5).

**Effects of enhanced leaf photosynthesis on some other crop traits**

The benefit from all routes for simulated season-long canopy photosynthesis ($A_{\text{anony}}$), shown in the upper rows of Fig. 3, differed from that shown in Table 5 for aboveground mass. This difference suggests that other traits were also affected by altered photosynthesis. Aboveground mass can be calculated as:

$$\text{Aboveground mass} = \left( \frac{\text{PAR}_{\text{int}} \times \text{PLUE} - \text{RESP}}{1 - F_{\text{root}}} \right) - \text{SENES}$$

where PAR$_{\text{int}}$ is the season-long intercepted PAR by the crop (MJ m$^{-2}$), PLUE is the overall photosynthetic light use efficiency ($= A_{\text{anony}} / \text{PAR}_{\text{int}} / \text{g CO}_2 \text{MJ}^{-1} \text{PAR}$), RESP is season-long crop respiration (g CO$_2$ m$^{-2}$), $F_{\text{root}}$ is fraction of mass for roots.
Next, we evaluated the extent to which these secondary effects also contributed to differences in the simulated aboveground mass. This was done from the difference in the significance level of individual terms of multiple linear regression of aboveground mass versus PAR\text{int}, PLUE, RESP, F\text{root}, and SENES (Table 6). Although the decreasing effect of SENES on mass could not be identified (because of the collinearity between mass and SENES), the significant effect of the other four terms (PLUE, PAR\text{int}, RESP, and F\text{root}) was well estimated. While the primary PLUE had the strongest effect under both potential and water-limited conditions, the secondary PAR\text{int} always had the second strongest effect. These results on the importance of the secondary effects on crop-level traits suggest that most existing simulation studies on assessing the impact of engineering photosynthetic targets are incomplete, because computation was only done for leaf photosynthesis (e.g. McGrath and Long, 2014) or for canopy photosynthesis (Zhu et al., 2004; Song et al., 2013).

Assessing the importance of individual biochemical targets

While secondary traits were affected, after all one would assess how the primary PLUE is affected by individual biochemical targets or parameters of photosynthesis. We regressed PLUE against individual photosynthetic parameters, with site included as covariate in the regression to remove any effect of possible site differences.

The regression analysis based on simulation results using the C\text{3} model (Routes 1–3 plus the default) indicated that manipulating S_{clo} affected PLUE more than manipulating g_m (results not shown), consistent with the result that the percentage change in PLUE by Route 2 was higher than that by Route 1 (Fig. 3). However, the relative impact of manipulating S_{clo} and g_m depends on the extent to which they could actually be changed. Furthermore, an improvement in S_{clo} may be at the cost of decreasing V_{\text{cmax}}, because of the often observed negative correlation between Rubisco

Table 5. The percentage increase of the 31 year average aboveground mass by nine photosynthesis-enhancing routes, relative to that shown in Table 4 for the default route, in rice crop simulated for the present climate and the 2050 climate, under either potential or water stress environments, in three representative sites

| Site          | Los Baños (tropics) | Nanjing (subtropics) | Shizukuishi (temperate) |
|---------------|---------------------|----------------------|-------------------------|
|               | Potential          | Water limited        | Potential               | Water limited       | Potential               | Water limited       |
| Production level | Present 2050       | Present 2050         | Present 2050            | Present 2050        | Present 2050            | Present 2050        |
| Route #       | 1                   | 2                    | 3                       | 4                       | 5                       | 6                       |
| Route #       | 7                   | 8                    | 9                       | 10                      | 11                      | 12                      |
| Route #       | 4                   | 5                    | 6                       | 7                       | 8                       | 9                       |
| Route #       | 1                   | 2                    | 3                       | 4                       | 5                       | 6                       |
| Route #       | 7                   | 8                    | 9                       | 10                      | 11                      | 12                      |
| Route #       | 1                   | 2                    | 3                       | 4                       | 5                       | 6                       |
| Route #       | 7                   | 8                    | 9                       | 10                      | 11                      | 12                      |

a Route numbers correspond to those defined in Table 2.

and SENES is aboveground mass lost after leaf senescence (g DM m^{-2}). We obtained the data for these five traits from the GECROS output, and calculated the percentage change of each of these traits for each route relative to the C\text{3} default (Fig. 3).

The enhanced leaf photosynthesis from most routes also resulted in increased PAR\text{int}, although to a small extent only, up to a maximum of 8.3\% (Fig. 3). As a result, the percentage increase in PLUE by the routes was generally slightly lower than that in A_{\text{canopy}} (Fig. 3). The increased PAR\text{int} stemmed from an increased LAI in the early growth phase (results not shown), in line with the recent result of Kromdijk et al. (2016) showing that increased leaf photosynthesis also resulted in increased leaf area. The increased canopy photosynthesis, on the one hand, increased the component of growth respiration; on the other hand, it decreased the component of maintenance respiration which is modelled in GECROS dependent on crop N status. The net result is that most routes decreased RESP (Fig. 3). The simulated F\text{root} generally became higher with photosynthesis-enhancing routes (Fig. 3), as expected from the classical functional equilibrium theory (Brouwer, 1983). However, in the presence of water limitation, F\text{root} could be lower compared with the default values, probably because the crop maintained a comparatively high N status under drought. Because a higher photosynthesis resulted in a lower N:C ratio in the crop and GECROS modelled leaf senescence depending on the relative magnitude of N- and C-determined LAI, the most effective enhancing routes, such as Routes 6 and 9, resulted in increased leaf senescence (Fig. 3). This simulation result is in analogy to the faster senescence and reduced LAI in later stages of development for C\text{3} crops grown under elevated [CO\text{2}] in FACE experiments (e.g. Kim et al., 2003). Sinclair et al., (2004) simulated that a 33\% increase in leaf photosynthesis may translate into only a 5\% increase in soybean grain yield, or a \textasciitilde6\% change in grain yield in the absence of additional N, presumably associated with more leaf senescence. Long et al., (2006) reported experimentally a lower than expected crop yield stimulation with rising [CO\text{2}].
Fig. 3. Heat map for the percentage change (%) of the 31 year average trait value for each of the nine photosynthesis-enhancing routes (route numbers defined in Table 2), relative to that for the default route, in a rice crop simulated for the present climate and the 2050 climate, under either potential or water-limited environments, at three representative sites. Traits shown are: $A_{\text{canopy,s}}$, season-long canopy photosynthesis; PAR$_{\text{int}}$, season-long intercepted PAR; PLUE, overall photosynthetic light use efficiency defined as $A_{\text{canopy,s}}$ divided by PAR$_{\text{int}}$; RESP, season-long crop respiration; $F_{\text{root}}$, fraction of mass for roots; SENES, shoot mass lost due to leaf senescence; and WUE, water use efficiency. (Colours: white for no change, green for decrease, red for increase, and colour intensity for the magnitude of decrease or increase.)
S\textsubscript{c\textsubscript{lo}} and carboxylase turnover rate (e.g. Kubien \textit{et al.}, 2008; Perdono \textit{et al.}, 2015). This negative correlation was not considered here, in view of the fact that the non-green alga \textit{G. monilis} has a high S\textsubscript{c\textsubscript{lo25}} while maintaining a Rubisco turnover rate similar to \textit{C}_{3} plants (Whitney \textit{et al.}, 2001). The impact of the trade-off between Rubisco S\textsubscript{c\textsubscript{lo}} and carboxylase turnover on canopy photosynthesis was analysed by Zhu \textit{et al.}, (2004).

Our simulations using the \textit{C}_{4} model (Routes 4–9) involved changes in values of a set of parameters. The most important ones are \(\chi_{\text{gb25}}\) (which determines the effectiveness of the CCM), \(\phi\) (extra ATP requirement for the CCM, which determines the required \(f_{\text{cyc}}\) and, therefore, light-limited photosynthetic efficiency), and \(\chi_{J_{\text{max25}}}\) or \(\chi_{J_{\text{max25}}}\) (which determine light-saturated photosynthetic capacity). Other parameters (e.g. some \textit{C}_{3} parameters used for Route 5) had little impact on the shape and values of light response curves (results not shown).

We therefore conducted the analysis of regressing PLUE versus \(\chi_{\text{gb25}}\), \(3 + \phi\) (total ATP requirement per mol CO\textsubscript{2} assimilated, ATP\textsubscript{req}), and \(\chi_{J_{\text{max25}}}\) (Table 7). There was little effect of sites on PLUE. All three parameters were important for any production level–climate combination. Comparatively, the CCM parameter \(\chi_{\text{gb25}}\) became most important for water-limited production, because a more effective CCM to elevate the CO\textsubscript{2} level around Rubisco can more effectively overcome the negative effect of low \(C_{i}\) under drought. Under the potential production, especially combined with high [CO\textsubscript{2}] of the 2050 climate, the photosynthetic capacity parameter \(\chi_{J_{\text{max25}}}\) and quantum efficiency parameter ATP\textsubscript{req} were comparatively more important (Table 7). These results suggest that photosynthetic capacity, quantum efficiency, and CCM strength all need improving in order to turbocharge canopy photosynthesis. Based on natural variation of leaf photosynthesis, Gu \textit{et al.}, (2014a) showed that quantum efficiency parameters had even higher effects than capacity parameters on rice productivity.

### Table 6. The coefficients (with probability of significance in parentheses) of linear regression of 31 year average simulated aboveground mass against the simulated values of five component traits, for either potential or water-limited environments, or using the pooled data for the two environments

| Coefficient (unit) | Potential | Water-limited | Pooled data |
|-------------------|-----------|---------------|-------------|
| \(b_0\) (g DM m\textsuperscript{-2}) | -3435.24 (5.92 \times 10^{-6}) | -1907.22 (5.34 \times 10^{-6}) | -1751.43 (1.57 \times 10^{-5}) |
| \(b_1\) (g DM MJ\textsuperscript{-1} PAR) | 4.495 (2.00 \times 10^{-5}) | 3.064 (7.19 \times 10^{-6}) | 3.342 (9.92 \times 10^{-6}) |
| \(b_2\) (g DM m\textsuperscript{-2} (g CO\textsubscript{2} MJ\textsuperscript{-1} PAR\textsuperscript{-1})) | 415.65 (9.36 \times 10^{-6}) | 339.49 (3.47 \times 10^{-6}) | 328.83 (1.32 \times 10^{-5}) |
| \(b_3\) (g DM (g CO\textsubscript{2})) | -0.2635 (0.045) | -0.5735 (6.53 \times 10^{-5}) | -0.7445 (1.22 \times 10^{-3}) |
| \(b_4\) (g DM m\textsuperscript{-2}) | -1996.04 (5.05 \times 10^{-3}) | -539.44 (0.001) | -1245.07 (2.92 \times 10^{-5}) |
| \(b_5\) (g DM g\textsuperscript{-1} DM) | -1.1938 (0.153) | 1.6387 (6.84 \times 10^{-5}) | 1.9219 (0.001) |
| \(R^2\) | 0.992 | 0.999 | 0.993 |
| Data points | 60 | 60 | 120 |

### Table 7. The coefficients with their probability of significance of linear regression of 31 year average simulated PLUE (overall photosynthetic light-use efficiency as defined in Table 6) against three biochemical parameters (\(\chi_{\text{gb25}},\) ATP\textsubscript{req}, and \(\chi_{J_{\text{max25}}}\) representing the strength of the CCM, quantum requirement, and photosynthetic capacity, respectively) used in the \textit{C}_{4} photosynthesis model, for four cases where potential or water-limited environments were combined with present or 2050 climate conditions

| Coefficient | Present climate | 2050 climate | Water-limited | Present climate | 2050 climate |
|-------------|----------------|--------------|---------------|----------------|--------------|
| Intercept   | 11.674         | 12.108       | 9.019         | 2.31 \times 10^{-12} | 9.385         |
| Nanjing\textsuperscript{a} | 0.097         | 0.102       | -0.078       | 0.48          | -0.112       |
| Shizukushu\textsuperscript{a} | 0.167         | 0.415       | -0.200       | 0.09          | -0.051       |
| \(\chi_{\text{gb25}}\) | -12.751        | -10.574      | -10.262       | 1.84 \times 10^{-8} | -8.406       |
| ATP\textsubscript{req} \textsuperscript{b} | -1.237        | -1.244       | -0.659       | 1.24 \times 10^{-6} | -0.676       |
| \(\chi_{J_{\text{max25}}}\) | 0.0162        | 0.0150       | 0.0083       | 1.22 \times 10^{-6} | 0.0072       |
| \(R^2\) | 0.963         | 0.965       | 0.961         | 0.976         | 0.976 |
| Data points | 18            | 18          | 18            | 18            | 18            |

\textsuperscript{a} Site was included as the covariate in regression, with Los Baños as the reference having a coefficient of zero.

\textsuperscript{b} Total ATP requirement per CO\textsubscript{2} assimilated (= 3 + \(\phi\)), i.e. 5 for \textit{C}_{4} photosynthesis and 3.75 for cyanobacterial photosynthesis (see the text).
Concluding remarks

We simulated the likely impact of major routes in ongoing programmes using transgenic technology to improve photosynthesis (Table 2). Our analysis showed that improving leaf photosynthesis can result in an increased rice mass production to a different extent (Table 5), thereby also resulting in different improvements in resource use efficiency such as WUE (Fig. 3). However, to supercharge photosynthesis significantly, engineering for a single improvement route can hardly be effective. Some single routes may be counter-productive at the canopy level. For example, installing C4 biochemistry, if not combined with an effective CCM, is only beneficial for upper leaves of the canopy, while it has no or even a negative impact for lower shaded leaves because such a mechanism requires extra ATP for the C4 cycle. Note that the standard C4 model of von Caemmerer and Furbank (1999) for e- transport limitation does not explicitly consider the increased cyclic e- transport due to the extra ATP costs relative to C3 photosynthesis, and, therefore, cannot recognize the little advantage of C4 photosynthesis under shade. Similarly, the simulation by McGrath and Long (2014) in assessing the potential of cyanobacterial CCM took no account of the extra ATP required by bicarbonate transporters. Our simulation also showed that manipulating photosynthesis may result in unwanted secondary effects on some traits at crop level (e.g. inducing faster senescence if nutrient uptake is not increased). Therefore, the beneficial effect of the single route for high photosynthesis on increasing crop productivity may have previously been overestimated. To supercharge crop productivity, combined routes for improved CCM, photosynthetic capacity, and quantum efficiency are required.

Supplementary Data

Supplementary data are available at JXB online.

Text 1. Analytical solution to the cubic equation as a result of combined stomatal conductance, CO2 diffusion, and biochemical leaf photosynthesis models.

Text 2. Revising the C4 photosynthesis model for simulating the cyanobacterial CCM.

Text 3. Analytical solution to the quadratic equation as a result of combined CO2.

Text 4. Analysis with respect to temperature response parameters of C4 enzyme kinetics.

Text 5. Sensitivity analysis with respect to ATP cost for the cyanobacterial CCM.

Text 6. Description of the crop model GECROS (version 4.0).

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