RESEARCH PAPER

Exploring the optimum nitrogen partitioning to predict the acclimation of C₃ leaf photosynthesis to varying growth conditions

Xinyou Yin¹*, Ad H.C.M. Schapendonk² and Paul C. Struik¹

¹ Centre for Crop Systems Analysis, Department of Plant Sciences, Wageningen University & Research, PO Box 430, 6700 AK Wageningen, The Netherlands
² Photosyntax B.V., Englaan 8, 6703 EW Wageningen, The Netherlands

* Correspondence: xinyou.yin@wur.nl

Received 9 April 2018; Editorial decision 18 July 2018; Accepted 18 July 2018

Editor: Pierre Martre, INRA, France

Abstract

The distribution of leaf nitrogen among photosynthetic proteins (i.e. chlorophyll, the electron transport system, Rubisco, and other soluble proteins) responds to environmental changes. We hypothesize that this response may underlie the biochemical aspect of leaf acclimation to the growth environment, and describe an analytical method to solve optimum nitrogen partitioning for maximized photosynthesis in C₃ leaves. The method predicts a high investment of nitrogen in Rubisco under conditions leading to excessive energy supply relative to metabolic demand (e.g. low temperature, high light, low nitrogen, or low CO₂). Conversely, more nitrogen is invested in chlorophyll when the energy supply is limiting. Overall, our optimization results are qualitatively consistent with literature reports. Commonly reported changes in photosynthetic parameters with growth temperature were emergent properties of the optimum nitrogen partitioning. The method was used to simulate dynamic acclimation under varying environmental conditions, using first-order kinetics. Simulated diurnal patterns of leaf photosynthetic rates as a result of acclimation differed greatly from those without acclimation (A_without). However, differences in predicted photosynthesis integrated over a day or over the growing season from A_without depended on the value of the kinetic time constant (τ), suggesting that τ is a critical parameter determining the overall impact of nitrogen distribution on acclimated photosynthesis.

Keywords: Acclimation, chlorophyll, electron transport, modelling, optimization, photosynthesis, Rubisco.

Introduction

One of the major underlying components in predicting ecosystem productivity and crop yield is to model photosynthesis of individual leaves in a canopy under fluctuating environmental conditions. A prevailing approach is to use the steady-state photosynthesis model of Farquhar, von Caemmerer, and Berry (Farquhar et al., 1980; ‘the FvCB model’ hereafter). This model predicts photosynthesis as the minimum of the ribulose-1,5-bisphosphate (RuBP)-saturated rate of CO₂ assimilation, which is a function of the maximum carboxylation capacity of Rubisco (V_c,max), and the RuBP regeneration-limited rate, which is a function of the maximum electron transport (J_max) (see Supplementary Appendix A at JXB online).

A substantial body of experimental work has shown a strong empirical correlation between V_c,max or J_max and leaf nitrogen
content (e.g. Harley et al., 1992; Walcroft et al., 1997; Kattge et al., 2009). This is expected because nitrogen is a constituent of many functional protein groups of photosynthesis, such as components of the electron transport chain and enzymes of the Calvin cycle (Evans, 1989). Because nitrogen availability is often limiting to plant growth, it is beneficial, in terms of photosynthetic carbon gain, for plants to use nitrogen efficiently. An investment of nitrogen in a protein compound within a leaf ‘appropriate’ to its environment must be of adaptive significance (Walters, 2005).

There are many reports on photosynthetic acclimation to growth environments, such as irradiance and nitrogen availability (Hikosaka and Terashima, 1996; Warren and Adams, 2001), temperature (Yamasaki et al., 2002; Yamori et al., 2005), and CO₂ levels (Medlyn, 1996; Sharwood et al., 2017). Smith and Dukes (2013) defined acclimation as ‘a physiological, structural, or biochemical adjustment by an individual plant in response to environmental stimulus that is manifested as alteration in the short-term response function of a physiological process’. Evans and Poorter (2001) indicated that to acclimate to their growth environments, plants adjust both biochemical and morphological traits in order to maximize carbon gain. However, in most existing uses of the FvCB model in predicting ecosystem productivity (e.g. Leuning et al., 1995; Lloyd and Farquhar, 1996) and crop growth (e.g. Yin and Struik, 2017; Wu et al., 2018), photosynthetic parameters (e.g. V′c,max and Jmax) are related to overall leaf nitrogen content.

From a meta-analysis of data, Hikosaka et al. (2006) and Kattge and Knorr (2007) showed that most parameters of the FvCB model varied with growth temperature. For example, Hikosaka et al. (2006) showed that activation energy of V′c,max (a parameter showing the sensitivity to measurement temperature, see Equation A6 in Supplementary Appendix A) increased 1010 J mol⁻¹ per °C increase in growth temperature, explaining a large part of the observed increase of the optimum temperature of photosynthesis with the temperature during growth. Therefore, many researchers stressed the need to incorporate this acclimation response to growth environmental variables into ecosystem models (Smith and Dukes, 2013) and crop models (Yin and Struik, 2010). However, any attempt to incorporate this response has been empirically based on experimental (Bernacchi et al., 2003) or meta-analysis results (Kattge et al., 2009; Friend, 2010; Stinziano et al., 2018). This empirical approach is understandable because acclimation is a complex phenomenon involving all physiological, structural, or biochemical adjustments that probably have different time scales. We hypothesize that the partitioning of nitrogen among photosynthetic components underlies the biochemical aspects of acclimation; to analyse the acclimation response of photosynthetic components to environmental changes, it is necessary first to model leaf photosynthesis on the basis of the nitrogen contents in individual compounds. The analysis of nitrogen costs of photosynthetic compounds and their relationships (e.g. Evans, 1989) made it possible to develop such models.

Several studies (Friend, 1991; Hikosaka and Terashima, 1995; Medlyn, 1996; Hikosaka, 1997) have modelled the optimum nitrogen allocation, on the basis of the nitrogen cost of individual photosynthetic compounds. Friend (1991) separated the photosynthetic nitrogen between two compartments (Rubisco and chlorophyll) only. Medlyn (1996) divided the photosynthetic nitrogen into four pools. While Hikosaka and Terashima (1995, 1996) divided the photosynthetic nitrogen into 5–6 protein complexes (core and light-harvesting complexes of PSI, light-harvesting complex II, core complex of PSII, Rubisco, and electron transport and other Calvin cycle enzymes), their analysis used an empirical hyperbolic equation for the light response curve of leaf photosynthesis. Hikosaka (1997) extended the approach by using the FvCB model. Both Medlyn (1996) and Hikosaka (1997) used a numerical routine to determine the optimum nitrogen partitioning that maximizes daily photosynthesis. These studies highlighted the importance of predicting the nitrogen partitioning to understand photosynthetic acclimation with respect to nitrogen use, but did not model acclimation itself. As acclimation is a process in which photosynthetic compounds adjust from their actual level towards their optimum at a given condition (Kirschbaum et al., 1998), the first step in modelling acclimation is to determine the optimum nitrogen partitioning for a specific environmental condition.

In this study, we first develop a simple method that analytically resolves the optimum nitrogen partitioning among photosynthetic compounds in C₃ plants. We then examine to what extent the optimum nitrogen partitioning can explain the experimentally observed acclimation of leaf photosynthesis to environmental variables during growth. Finally, we analyse the potential difference between a model considering dynamic acclimation and the prevailing modelling approach that ignores the acclimation of photosynthesis under field environments.

### Materials and methods

#### Modifying the FvCB model

Our method for determining the optimum nitrogen partitioning is based on the FvCB model for C₃ species (Supplementary Appendix A, with all variables listed in Table 1). In order to find the optimum solution to the nitrogen partitioning, we need to simplify the original non-rectangular formula, Equation (A4) in Supplementary Appendix A, into a Blackman-type equation:

\[
J = \min(\alpha I_{\text{photo}}, J_{\text{max}})
\]

where \(\alpha\) is defined according to Yin et al. (2004). There is experimental support for the linearity between \(J\) and \(I_{\text{photo}}\) within the moderate range of irradiance (Cheeseman and Lexa, 1996; see the Discussion).

#### Nitrogen dependence of component processes

Following Medlyn (1996), we divide total photosynthetic leaf nitrogen (\(N_{\text{leaf}}\)) into four pools. Two of them are in the thylakoid: nitrogen required for the chlorophyll–protein complex (\(N_{C}\)) and for components of the electron transport system (\(N_{E}\)). The other two are soluble proteins related to the activities of the Calvin cycle enzymes, which are divided between Rubisco (\(N_{R}\)) and other soluble protein (\(N_{S}\)).

The amount of \(N_{C}\) determines absorption of photosynthetically active light in the leaf. Based on the equation of Evans (1993) for relating absorption to whole-leaf chlorophyll, the amount of \(I_{\text{photo}}\), in Equation 1 can be calculated by (Medlyn 1996):

\[
I_{\text{photo}} = \frac{a_{C} N_{C}}{a_{C} N_{C} + b_{C}} I_{\text{inc}}
\]

where \(a_{C}\) and \(b_{C}\) are constants.
where $I_{\text{inc}}$ is the incident light fluxes, and $a_c$ and $b_c$ are empirical coefficients (Table 2). Similar to most studies that use the FvCB model, Equation 2 implicitly assumes the absence of a gradient in chloroplast density or in the absorbed light intensity among different layers through the leaf. This assumption is valid only for relatively thin leaves (Farquhar et al., 1980).

$N_T$ collectively refers to the amount of $N_{\text{photo}}$ invested in all the components related to electron transport, including PSI and PSII reaction centres, cytochrome $b_6f$ (Cyt$f$), and other complexes. Note that although PSI and PSII have dual functions, dealing with both light harvesting and electron transport, nitrogen for the chlorophyll in the antennae systems

### Table 1. List of variables used in the model

| Variable | Definition | Unit |
|----------|------------|------|
| $a_c$    | Coefficient in Equation 2 | mmol chlorophyll mol$^{-1}$N |
| $a_J$    | Coefficient in Equation 3 | μmol electron mol$^{-1}$N s$^{-1}$ |
| $A$      | Net leaf photosynthesis rate | μmol CO$_2$ m$^{-2}$ s$^{-1}$ |
| $b_c$    | Coefficient in Equation 2 | mmol chlorophyll mol$^{-1}$ |
| $C_a$    | Atmospheric CO$_2$ concentration | μmol mol$^{-1}$ |
| $C_c$    | Chloroplast CO$_2$ concentration | μmol mol$^{-1}$ |
| $D_J$    | Energy of deactivation for $J_{\text{max}}$ in Equation A7 | J mol$^{-1}$ |
| $E$      | Activation energy for $V_{c,max}$ or $K_{\text{nic}}$ or $K_{\text{iso}}$ | J mol$^{-1}$ |
| $E_{\text{max}}$ | Activation energy for $J_{\text{max}}$ | J mol$^{-1}$ |
| $f_{\text{cyc}}$ | Fraction of cyclic electron transport around PSI | – |
| $I_{\text{abs}}$ | Absorbed photosynthetically active irradiance | μmol photon m$^{-2}$ s$^{-1}$ |
| $I_{\text{inc}}$ | Incident photosynthetically active irradiance | μmol photon m$^{-2}$ s$^{-1}$ |
| $J$ | Rate of linear whole-chain electron transport | μmol electron m$^{-2}$ s$^{-1}$ |
| $J_{\text{max}}$ | Maximum value of $J$ under a saturating irradiance | μmol electron m$^{-2}$ s$^{-1}$ |
| $J_{\text{max25}}$ | | – |
| $J(I)$ | Function for the temperature dependence of $J_{\text{max}}$ | g CO$_2$ g$^{-1}$ Rubisco s$^{-1}$ |
| $K_{\text{C25}}$ | Specific activity of Rubisco at 25 °C | μmol mol$^{-1}$ |
| $K_{\text{Cant}}$ | Michaelis–Menten constant of Rubisco for CO$_2$ | μmol mol$^{-1}$ |
| $K_{\text{C25}}$ | Michaelis–Menten constant of Rubisco for CO$_2$ at 25 °C | μmol mol$^{-1}$ |
| $K_{\text{O2}}$ | Michaelis–Menten constant of Rubisco for O$_2$ | μmol mol$^{-1}$ |
| $K_{\text{O25}}$ | Michaelis–Menten constant of Rubisco for O$_2$ at 25 °C | μmol mol$^{-1}$ |
| $k_B$ | Coefficient in Equation 5 | mol N s μmol$^{-1}$ electron |
| $N_c$ | Leaf nitrogen allocated to chlorophyll | mol N m$^{-2}$ |
| $N_{\text{leaf}}$ | Total nitrogen content in leaves | mol N m$^{-2}$ |
| $N_{\text{leafE}}$ | Physiologically effective total nitrogen content in leaves | mol N m$^{-2}$ |
| $N_{\text{photo}}$ | Photosynthetic nitrogen content in leaves | mol N m$^{-2}$ |
| $N_h$ | Leaf nitrogen allocated to Rubisco | mol N m$^{-2}$ |
| $N_L$ | Leaf nitrogen allocated to other soluble protein | mol N m$^{-2}$ |
| $N_{\text{LRT}}$ | Leaf nitrogen allocated to electron transport system | mol N m$^{-2}$ |
| $O$ | Oxygen concentration (ambient level=210) | mmol mol$^{-1}$ |
| $R$ | Universal gas constant (=8.314) | J K$^{-1}$ mol$^{-1}$ |
| $R_d$ | Day respiration rate | μmol CO$_2$ m$^{-2}$ s$^{-1}$ |
| $S_{\text{R/O2}}$ | Relative CO$_2$/O$_2$ specificity of Rubisco at 25 °C | g CO$_2$ g$^{-1}$Rubisco s$^{-1}$ |
| $S_I$ | Entropy term for $J_{\text{max}}$ in Equation A7 | J K$^{-1}$ mol$^{-1}$ °C |
| $T$ | Leaf temperature | °C |
| $T_{\text{opt}}$ | Optimum leaf temperature for $\Phi_{\text{2LL}}$ | °C |
| $V_c$ | Rubisco activity-limited carboxylation rate | μmol CO$_2$ m$^{-2}$ s$^{-1}$ |
| $V_{\text{C0I}}$ | Function for the $C_c$ dependence of $V_c$ | – |
| $V_{\text{Cmax}}$ | Maximum velocity of Rubisco-limited carboxylation | μmol CO$_2$ m$^{-2}$ s$^{-1}$ |
| $V_{\text{Cmax25}}$ | Maximum velocity of Rubisco-limited carboxylation at 25 °C | μmol CO$_2$ m$^{-2}$ s$^{-1}$ |
| $V_J$ | Function for the temperature dependence of $V_{\text{Cmax}}$ | μmol CO$_2$ m$^{-2}$ s$^{-1}$ |
| $V_{\text{J0}}$ | Electron transport-limited carboxylation rate | μmol CO$_2$ mol$^{-1}$electron |
| $\alpha$ | Efficiency of PSII electron transport on basis of $I_{\text{abs}}$ | mol e$^{-}$ mol$^{-1}$ photon |
| $\epsilon$ | Quantum efficiency for CO$_2$ fixation | mol CO$_2$ mol$^{-1}$electron |
| $\Phi_{\text{2LL}}$ | Quantum efficiency for PSII electron transport | mol e$^{-}$ mol$^{-1}$ photon |
| $\Phi_{\text{2LL/1LL}}$ | Maximum value of that is achieved at $T_{\text{opt}}$ | g Rubisco mol$^{-1}$N |
| $\Phi_{\text{2LL/1LL}}$ | PSII to PSI electron transport efficiency ratio | g Rubisco mol$^{-1}$N min |
| $\nu$ | Coefficient to convert $N_{\text{leaf}}$ to Rubisco | μmol mol$^{-1}$ |
| $\Gamma$ | CO$_2$ compensation point in the absence of day respiration | °C |
| $\Omega$ | Gap between $T_{\text{opt}}$ and temperature at which $\Phi_{\text{2LL}}=\Phi_{\text{2LL/1LL}}/\epsilon$ | – |
of PSI and PSII is attributed to $N_C$, and is not part of $N_V$. This simplifies the calculation of $J_{max25}$, being proportional to $N_V$ only:

$$J_{max25} = a_j N_V$$  \hspace{1cm} (3)$$

where coefficient $a_j$ is the proportion factor.

Assuming that Rubisco is fully activated in vivo, the maximum carboxylation velocity at the reference temperature (25 °C) is given by:

$$V_{c,max25} = 10^6 \nu K_{C25} N_R / 44$$  \hspace{1cm} (4)$$

where $\nu$ is the conversion coefficient from mol $N_R$ to g Rubisco, $K_{C25}$ is the specific activity of Rubisco at 25 °C, and 44 is the molecular weight of CO$_2$.

Activities of the Calvin cycle enzymes, other than Rubisco, are not primary limiting factors of photosynthesis (Farquhar et al., 1980), except for a possible limitation due to triose phosphate utilization (Sharkey, 1985). However, Medlyn (1996) discussed that the likely impact of triose phosphate metabolism on the determination of the nitrogen partitioning is small. It is therefore assumed that the amount of $N_S$ is just sufficient to support the maximum rate of electron transport, $J_{max}$ (Medlyn 1996; Evans and Poorter, 2001):

$$N_S = k_s J_{max}$$  \hspace{1cm} (5)$$

where $k_s$ is the proportion constant.

**Optimization procedure**

Our objective is to determine the optimum distribution of photosynthetic nitrogen ($N_{photo}$) among $N_C$, $N_V$, $N_R$, and $N_S$ such that leaf photosynthesis (A), calculated by Equation A1 in Supplementary Appendix A, is maximal for a given combination of incoming light, chloroplast [CO$_2$], leaf temperature, and leaf nitrogen content. Based on Equation A1 and Equation 1, achieving this optimization is straightforward: the maximal leaf photosynthesis requires a nitrogen distribution over light-harvesting compounds, the electron transport complex, and Rubisco in such a way that:

$$V_i = V_j$$  \hspace{1cm} (6)$$

$$J_{max} = aI_{ps}$$  \hspace{1cm} (7)$$

The rationale for these requirements is that as long as they are not equal, nitrogen has not yet reached the optimum distribution since improvement of $A$ would still be possible by redistribution. From Equations 5, 6, and 7, and a further equation

$$N_C + N_V + N_R + N_S = N_{photo}$$  \hspace{1cm} (8)$$

the optimum value for $N_C$, $N_V$, $N_R$, and $N_S$ can be solved analytically, as given in Supplementary Appendix B. This is the unique solution with biologically realistic values; the other mathematical solution can lead to a negative value for $N_C$.

The above procedure gives the nitrogen distribution that maximizes photosynthesis at a given value of $N_{photo}$. To express model results in terms of $N_{leaf}$, the relationship between $N_{leaf}$ and $N_{leaf}$ has to be specified. The percentage of $N_{leaf}$ allocated as $N_{photo}$ can vary between 50% and 80% (Hikosaka and Terashima, 1995). For simplicity, we used roughly average relationships of $N_{photo}=0.65N_{leaf}$ and $N_{leaf}=N_{leaf}-0.02$ where $N_{leaf}$ is leaf nitrogen effective for physiological activities (Sinclair and Horie, 1989) assuming a base leaf nitrogen of 0.02 mol N m$^{-2}$. The difference between $N_{leaf}$ and $N_{photo}$ refers to some inorganic nitrogen as well as the organic nitrogen used for processes other than photosynthesis.

**Modelling analysis**

The above optimum nitrogen partitioning solution was applied to analyse several experiments reported by Yamori et al. (2005, 2010, 2011), where $N_{leaf}$, net leaf photosynthesis ($A$), and photosynthetic protein components

**Table 2. Indicative values of coefficients used in the model**

| Coefficient | Equation | Unit | Value | Reference |
|-------------|----------|------|-------|-----------|
| $a_1$       | 2        | mmol Chl mol^{-1} N | 25    | Evans (1993); Medlyn (1996) |
| $a_2$       | 3        | μmol e^{-1} mol^{-1} N s^{-1} | 15870 | Medlyn (1996) |
| $a_3$       | 2        | mmol Chl m^{-2} | 0.076 | Evans (1993); Medlyn (1996) |
| $D_j$       | A7       | J mol^{-1} | 200000 | Harley et al. (1992) |
| $E$         | A6       | J mol^{-1} | 65330 (for $V_{c,max}$) | Bernacchi et al. (2002) |
| $E_{max}$   | A7       | J mol^{-1} | 80990 (for $K_{c25}$) | Bernacchi et al. (2002) |
| $f_{cyc}$   | A5       | - | 0.05 | Harley et al. (1992) |
| $k_s$       | 5        | mol N s (μmol e^{-1})^{-1} | 1.25 x 10^{-3} | Medlyn (1996) |
| $K_{C25}$   | 4        | g CO$_2$ g^{-1} Rubisco s^{-1} | 1.6 x 10^{-3} | Farquhar et al. (1980) |
| $K_{c25}$   | A6       | μmol mol^{-1} | 291 | Cousins et al. (2010) |
| $K_{o25}$   | A6       | mmol mol^{-1} | 194 | Cousins et al. (2010) |
| $S_{max}$   | for $\gamma_{25}$ | mmol μmol^{-1} | 3.022 | Cousins et al. (2010) |
| $S_j$       | A7       | J K^{-1} mol^{-1} | 650 | Harley et al. (1992) |
| $T_{opt}$   | A8       | °C | 22.5 | Estimated from Yin et al. (2014) |
| $V$         | 4        | g Rubisco mol^{-1} N | 87.72 | Hikosaka and Terashima (1996) |
| $\tau$      | 9        | min | 40 or variable | Kirschbaum et al. (1998) |
| $\Phi_{ps}/\phi_{ps}$ | A8 | mol e^{-1} photon | 0.78 | Estimated from Yin et al. (2014) |
| $\Phi_{ps}/\phi_{ll}$ | A5 | - | 0.85 | Kingston-Smith et al. (1999) |
| $\Omega$    | A8       | °C | 36.5 | Estimated from Yin et al. (2014) |
were measured for plants that were assumed to have acclimated fully to growth environments.

We assessed to what extent the optimum nitrogen partitioning could explain observed changes of the FvCB parameters with varying growth environment. As acclimation to temperature has been most studied in the literature (e.g. Yamasaki et al., 2002; Yamori et al., 2005; Hikosaka et al., 2006; Katge and Knorr, 2007; Stinziano et al., 2018), we took temperature as the growth environment factor for this analysis. We used the FvCB model coupled with our optimum nitrogen partitioning algorithms to generate a set of photosynthetic CO₂-response curves for plants grown at 15, 20, 25, 30, and 35 °C, respectively. For each growth temperature, CO₂-response curves were generated for seven measurement leaf temperatures (10, 15, 20, 25, 30, 35, and 40 °C), all at \( I_{\text{inc}} \) of 1500 µmol m\(^{-2}\) s\(^{-1}\), and for green leaves (\( N_{\text{leaf}} = 0.15 \text{ mol m}^{-2} \)), mimicking a common measurement protocol for estimating parameter values of the FvCB model. The generated data were then fit to estimate \( V_{c,\text{max}}/J_{\text{max}}^\text{25} \) (represented as \( J_{\text{max}}^\text{25} \) at the \( I_{\text{inc}} \) of 1500 µmol m\(^{-2}\) s\(^{-1}\)), \( E_{c,\text{max}}/J_{\text{max}}^\text{25} \) and \( E_{\text{max}} \) of each growth temperature, while leaving other parameters at their default values as shown in Table 2.

To simulate leaf photosynthesis of plants grown under dynamically fluctuating environments, all \( N_{C}, N_{R}, N_{i}, \text{ and } N_{c} \) are expressed as state variables and a change of their state with time \( (t) \) is modelled using the first-order kinetics:

\[
\frac{dN_{i}}{dt} = \frac{(N_{\text{opt}} - N_{i})}{\tau} \tag{9}
\]

where \( N_{i} \) represents \( N_{C}, \text{ or } N_{R}, \text{ or } N_{i}, \text{ or } N_{c}, \) \( N_{\text{opt}} \) represents the optimum value of these variables as calculated for a given growth condition, and \( \tau \) is a time constant \( (\tau > 0) \). The value of \( \tau \) may differ for different components, but we assume that it is the same for these components so that the sum of simulated \( N_{C}, N_{R}, N_{i}, \text{ and } N_{c} \) is always equal to \( N_{\text{photo}} \). This approach does not consider any metabolic cost of acclimation, which appears to be minor and hard to quantify (Athanasiou et al., 2010). We set \( \tau \) to the value for the Rubisco activity, ~2400 s or 40 min (Kirschbaum et al., 1998). Then actual leaf photosynthetic rates at any given set of environmental conditions can be calculated from the modified FvCB model where photosynthetic absorbance was conditional on the modelled values for \( N_{C} \) (Equation 2), \( V_{c,\text{max}}/J_{\text{max}}^\text{25} \) on the value for \( N_{R} \) (Equation 4), and \( J_{\text{max}}^{\text{25}} \) on the value of \( N_{i} \) and \( N_{c} \) (Equations 3 and 5).

The model was run for wheat for a period of ~50 d, using actual weather data of every 10 min in an experiment under field conditions (Cai et al., 2016), where the average daylength was ~12.2 h. According to the principles discussed by Penning de Vries and van Laar (1982), a time step of 10 min for dynamic simulation was short enough for the process where the characteristic time constant is 40 min (note that the time step has to be ≤\( \tau \); otherwise, simulation with Equation 9 may yield meaningless values such as the oscillating pattern; Penning de Vries and van Laar, 1982). So, the total time steps of our simulation period were ~3650. The modelled ‘acclimating’ leaf photosynthetic rates from using Equation 9 were compared with the predictions by the version of the model without acclimation (‘non-acclimating’), where nitrogen partitioning was static, being fixed to the optimum values for the average environmental conditions of the time steps covered by simulation. The initial values of \( N_{C}, N_{R}, N_{i}, \text{ and } N_{c} \) for dynamic simulation were set to be the same as their values for the ‘non-acclimating’ simulation. Given the uncertainty of \( \tau \) (Sassenrath-Cole and Pearcy, 1994; Kirschbaum et al., 1998), several values of \( \tau \) were used to implement the ‘acclimating’ version of the model. A direct comparison of this ‘acclimating’ model with the original steady-state FvCB model is not useful as many of the input parameters and their values differ between the two models. For all above analyses, as leaf day respiration rate (\( R_{d} \)) is not part of the optimum solutions (see the Discussion), we set \( R_{d} \) at 25 °C to be 0.01 \( V_{c,\text{max}}/J_{\text{max}}^\text{25} \), which is commonly observed (Harley et al., 1992; Yamori et al., 2005; Silva-Pérez et al., 2017; Cai et al., 2018) and used for general prediction (Medlyn et al., 2002; Yin and Struik, 2017).

Results

Illustration of optimization results

Using the above algorithms with values of input parameters (Table 2), we calculated the optimum \( N_{\text{photo}} \) partitioning among the four pools in response to four variables: \( I_{\text{inc}}, N_{\text{leaf}}, C_{c}, \text{ and leaf temperature. Values of some parameters in Table 2, although widely used, are only indicative given their reported variations among species and growth environments (e.g. Silva-Pérez et al., 2017). They are used here merely to illustrate how the nitrogen partitioning varies qualitatively in response to growth environment.

The optimum partitioning pattern versus \( N_{\text{leaf}} \) was calculated at various levels of \( I_{\text{inc}} \) and the results for \( I_{\text{inc}} \) at 1000 µmol m\(^{-2}\) s\(^{-1}\) and 250 µmol m\(^{-2}\) s\(^{-1}\) are given in Fig. 1. The resultant leaf photosynthesis when the optimum partitioning was reached in response to \( I_{\text{inc}} \) and \( N_{\text{leaf}} \) is illustrated in Supplementary Fig. S1. While a non-linearity was not explicitly assumed, our model did predict a non-linear response of leaf photosynthesis to both \( I_{\text{inc}} \) and \( N_{\text{leaf}} \) when partitioning was at the optimum. Specifically, the response to either \( I_{\text{inc}} \) or \( N_{\text{leaf}} \) was a non-rectangular hyperbola (Supplementary Appendix C). However, the curvature of predicted responses to \( N_{\text{leaf}} \) was weak under high light conditions (Supplementary Fig. S1b). The decrease in curvature with increasing light has also been observed experimentally (e.g. Makino et al., 1997).

At high light levels (1000 µmol m\(^{-2}\) s\(^{-1}\)), the optimum partitioning was hardly affected by the variation of \( N_{\text{leaf}} \) (Fig. 1a). At a low \( I_{\text{inc}} \) (250 µmol m\(^{-2}\) s\(^{-1}\)), the nitrogen partitioning became more dependent on \( N_{\text{leaf}} \) itself (Fig. 1b). With low \( I_{\text{inc}} \) and increasing \( N_{\text{leaf}} \) levels, the optimum partitioning required...
N_{\text{photo}}$, increasingly invested preferentially in $N_C$, accompanied by a reducing investment in $N_R$, $N_T$, and $N_S$.

Relationships between relative fractions of $N_C$, $N_R$, $N_T$, and $N_S$ predicted by the model were linear, and these relationships were not altered by the level of $I_{\text{inc}}$ or $N_{\text{leaf}}$ (Supplementary Fig. S2a). At the same leaf temperature and the same $C_c$, the linearity between $N_T$ and $N_S$ can be expected from Equations 3 and 4, and that between $N_R$ and $N_T$ can be expected from Equations B3 and B4 in Supplementary Appendix B. Since the total of $N_C$, $N_R$, $N_T$, and $N_S$ was fixed at a given $N_{\text{leaf}}$, a linearity between $N_C$ and the other three pools was also expected. Overall, the fraction partitioned to $N_C$ was most variable (Supplementary Fig. S2).

For an elevated CO$_2$ condition ($C_c=500$ μmol mol$^{-1}$), a pattern similar to that given in Fig. 1 was obtained (results not shown) and, again, linear relationships between relative fractions of $N_C$, $N_R$, $N_T$, and $N_S$ were predicted (Supplementary Fig. S2b). However, the relative fraction to $N_R$ was reduced, and those to $N_C$, $N_T$, and $N_S$ were increased, relative to the fractions obtained for the default CO$_2$ condition. This predicted effect of CO$_2$ on the $N_{\text{photo}}$ partitioning can be seen from changes in coefficients in linear relationships between relative fractions of $N_C$, $N_R$, $N_T$, and $N_S$, through comparison with those at $C_c=250$ μmol mol$^{-1}$ (Supplementary Fig. S2).

We also examined the effect of leaf temperature on the optimum partitioning by varying the temperature from 5 °C to 40 °C. Figure 2 shows the result of optimization under the condition that $I_{\text{inc}}=500$ μmol m$^{-2}$ s$^{-1}$, $C_c=250$ μmol mol$^{-1}$, and $N_{\text{leaf}}=0.15$ mol m$^{-2}$. For other conditions, the predicted trend was similar. With increasing temperature, the fraction to $N_R$ decreased continuously, but more rapidly so at higher temperature ranges; the fraction to $N_C$ or $N_T$ increased generally, but $N_S$ reached an optimum at ~28 °C. The fraction to $N_T$ first declined, followed by an increase beyond a certain high temperature. This pattern for $N_T$ was due to the assumed optimum response of $I_{\text{max}}$ to temperature (the peaked Arrhenius equation, Equation A7 in Supplementary Appendix A). As expected, the linear relationships between relative fractions of $N_C$, $N_R$, $N_T$, and $N_S$ as shown in Supplementary Fig. S2 did not exist when leaf temperature varied (results not shown). The result of optimization by varying temperature showed interactive effects of temperature with other variables ($I_{\text{inc}}$, $C_c$, and $N_{\text{leaf}}$) on leaf photosynthesis (Supplementary Fig. S3). First, responses of photosynthesis to a change in temperature were stronger when other variables were closer to the favourable level. Secondly, the optimum temperature for photosynthesis varied with other variables. The optimum temperature increased with increasing $I_{\text{inc}}$ or $C_c$ (Supplementary Fig. S3a, c), and declined with increasing leaf nitrogen (Supplementary Fig. S3b).

Comparison of optimized nitrogen partitioning with acclimation experiments

We compared our modelled optimum nitrogen partitioning with actual experimental measurements (Fig. 3; Supplementary Figs S4, S5). For the data set of Yamori et al. (2010), the modelled temperature response curves for tobacco plants grown in three different light levels agreed roughly with the measured curves (Fig. 3a, b). In line with earlier predictions shown in Fig. 1, the modelled $N_C$:$N_{\text{leaf}}$ ratio decreased, while the $N_R$:$N_{\text{leaf}}$ and $N_T$:$N_{\text{leaf}}$ ratios increased, with increasing light levels during growth. Such trends qualitatively agreed with the measured CHL:$N_{\text{leaf}}$, Rubisco:$N_{\text{leaf}}$, and Cyt$f$:$N_{\text{leaf}}$ ratios, respectively, in response to the light levels during growth (Fig. 3c–h), although the measured Rubisco:$N_{\text{leaf}}$ and Cyt$f$:$N_{\text{leaf}}$ ratios did not differ significantly between medium and high light levels (Fig. 3e, g).

For the data set of Yamori et al. (2005) for spinach plants grown in high (HT) and low (LT) temperatures, the modelled temperature response curves under the optimum nitrogen partitioning differed from the measured curves (Supplementary Fig. S4a, b). The LT plants had a much higher $N_{\text{leaf}}$ (0.1269 mol m$^{-2}$) than the HT plants (0.0811 mol m$^{-2}$), resulting in a constantly higher CHL for LT than for HT plants across measurement temperatures (Supplementary Fig. S4b). However, the model predicted a lower optimum temperature for plants grown in the LT than in the HT. The modelled $N_C$:$N_{\text{leaf}}$ ratio hardly differed between HT and LT conditions, but the measured CHL:$N_{\text{leaf}}$ ratio was slightly lower in the LT than in the HT condition (Supplementary Fig. S4c, d). The modelled $N_R$:$N_{\text{leaf}}$ and $N_T$:$N_{\text{leaf}}$ ratios were lower with HT than with LT, in line with the measured Rubisco:$N_{\text{leaf}}$ and Cyt$f$:$N_{\text{leaf}}$ ratios in response to the growth temperature (Supplementary Fig. S4e–h).

For the data set of Yamori et al. (2011) for plants of four species (wheat, rice, spinach, and tobacco) grown in low, medium, and high nitrogen conditions (LN, MN, and HN), we show the average results of four species in order to assess any impact of nitrogen (Supplementary Fig. S5). The modelled temperature response curves under the optimum nitrogen partitioning using the default parameters of Table 2 were somewhat lower than the measured curves (Supplementary Fig. S5a, b). The measured CHL:$N_{\text{leaf}}$ ratio hardly changed, while the measured Rubisco:$N_{\text{leaf}}$ ratio slightly increased and the measured Cyt$f$:$N_{\text{leaf}}$ ratio slightly decreased, with increasing nitrogen supply. 

![Fig. 2. Predicted optimum partitioning of N_{\text{photo}} among N_C, N_R, N_T, and N_S, as affected by leaf temperature, under the condition that I_{\text{inc}}=500$ μmol m$^{-2}$ s$^{-1}$, $C_c=250$ μmol mol$^{-1}$, and $N_{\text{leaf}}=0.15$ mol m$^{-2}$.](image-url)
Modelling photosynthetic acclimation

Using the generated \( A-C_e \) curves at different measurement temperatures as earlier described, values of \( V_{c,\text{max}25} \), \( J_{\text{max}25} \), \( E_{V_{c,\text{max}}} \), and \( E_{J_{\text{max}}} \) of the standard FvCB model were simultaneously fitted for each growth temperature. The model fit improved with increasing growth temperature, with \( R^2 \) of 0.66, 0.77, 0.93, 0.99, and 0.99 for growth temperatures of 15, 20, 25, 30, and 35 °C, respectively. Overall, estimated \( V_{c,\text{max}25} \) and \( J_{\text{max}25} \) decreased only slightly with increasing growth temperature (Fig. 4a). In contrast, estimated \( E_{V_{c,\text{max}}} \) and \( E_{J_{\text{max}}} \) increased with increasing growth temperature, with \( E_{J_{\text{max}}} \) increasing faster than \( E_{V_{c,\text{max}}} \) (Fig. 4b).

Comparison between simulated photosynthesis rates using ‘acclimating’ scenarios and the scenario without acclimation

We ran the model, using Equation 9 to mimic dynamic acclimation.
changed abruptly at a given time step between 1000 μmol m⁻² s⁻¹ and 300 μmol m⁻² s⁻¹ is shown in Supplementary Fig. S6 for three contrasting values of time constant τ. The difference in simulated A among three values of τ was greater when A was increasing than when it was decreasing (Supplementary Fig. S6).

For a more realistic field condition, we implemented simulation for a time period of ~50 d (see the section ‘Modelling analysis’), for upper, middle, and bottom layers of leaves in a canopy, which were assumed to be exposed to 100, 50, and 15% of incoming irradiance levels. As such, we did not consider here any specific change in instantaneous irradiance for leaves as a result of diurnal sun angle, passing clouds, and sunlight, which are often taken into account in other routines of general simulation models. The level of N_photo for these layers was assumed to scale with their exposed irradiance levels, being 0.10, 0.05, and 0.015 mol m⁻², respectively. Irradiance and temperature for a period of four consecutive days, and the equivalent simulated A are shown in Fig. 5 for the upper leaves only, since the pattern was similar for the other two layers except for their lower absolute values of simulated A compared with the upper layer.

As expected, the simulated A assuming acclimation with the default time constant τ (40 min) was lower than the values of A under the optimum nitrogen partitioning (A_opt) (Fig. 5). Increasing τ led the simulated A to deviate more from A_opt, and the difference between A and A_opt was more significant when A was increasing than when A was decreasing within a day (Fig. 5).

The diurnal pattern of simulated A by the version of the model without acclimation (‘non-acclimating’), A_without, differed from that using the versions of the model assuming acclimation (Fig. 5). The simulated A_without could reach a maximum value over certain hours around noon for the days when the incoming irradiance (e.g. the third and fourth days shown in Fig. 5) was high, whereas such a plateau was never simulated using the model versions assuming acclimation.

Daily integrals of A, A_opt, and A_without can be calculated from their simulated instantaneous values. We plotted the A/A_without ratio against the daily A_opt for two values of τ in the upper leaves (Supplementary Fig. S7), where the variation in daily A_opt over the season indicated the day-to-day variation largely

Fig. 4. Estimated values of the FvCB model parameters plotted against growth temperature $T_{growth}$: (a) $V_{c,max25}$ (filled symbols and the solid line) and $J_{max25}$ (open symbols and the dashed line), (b) $E_{Vc,max}$ (filled symbols and the solid line), and $E_{J,max}$ (open symbols and the dashed line). See the text for details of the data that were generated for the analysis shown in this figure.

Fig. 5. Daytime course for randomly selected four consecutive days, of (a) incoming irradiance (filled circles) and temperature (open circles), and (b) simulated net CO₂ assimilation rate (A) under four simulation scenarios: (i) the optimum N partitioning (black circles); (ii) dynamic acclimation using Equation 9 where τ was set to its default value 40 min (open squares); (iii) dynamic acclimation using Equation 9 where τ was extended to 160 min (open triangles); and (iv) the case without acclimation where nitrogen partitioning was static, set to the optimum values for the season-long average environmental conditions (orange-coloured lozenges). The simulation time step was to 10 min, and the total number of time steps was ~3650, equivalent to the daytime period of ~50 d (see the text).
in solar radiation. The $A/A_{\text{without}}$ ratio was close to 1.0 when $A_{\text{opt}}$ was between 0.5 mol m$^{-2}$ d$^{-1}$ and 1.0 mol m$^{-2}$ d$^{-1}$, whereas the ratio tended to increase when $A_{\text{opt}}$ was $<$0.5 mol m$^{-2}$ d$^{-1}$ (cloudy or rainy days) and/or $>$1.0 mol m$^{-2}$ d$^{-1}$ (sunny days). Also, a low $\tau$ increased the $A/A_{\text{without}}$ ratio (Supplementary Fig. S7).

Likewise, the ratios between $A$ and $A_{\text{opt}}$, and between $A$ and $A_{\text{without}}$ integrated over the season decreased with an increase in the value of $\tau$ (Fig. 6). The pattern did not differ much among the three layers of leaves in a canopy. However, the $A/A_{\text{without}}$ ratio was somewhat higher in lower than in upper leaves (Fig. 6b), in line with the most significant variation in the nitrogen partitioning under low irradiance conditions (Fig. 1b). Overall, only when $\tau$ increased towards 120 min did the simulated ‘acclimating $A$’ approximately equal $A_{\text{without}}$ (Fig. 6b).

**Discussion**

**Optimization procedure**

We present an analytical procedure to determine the optimum distribution of $N_{\text{photo}}$ among photosynthetic protein complexes under a specific environmental condition, based on the FvCB model for C$_3$ species. With this procedure, a computationally expensive numerical optimization procedure is avoided. This was achieved by modifying the original FvCB model that uses a non-rectangular hyperbolic equation, Equation A4 in Supplementary Appendix A, to describe the relationship between $J$ and $J_{\text{psii max}}$ (Farquhar and Wong, 1984). This non-rectangular hyperbola requires an empirical parameter (0) to define the curvature of the response, and its value appears to be determined arbitrarily in the literature; for example, 0.67 (Farquhar and Wong, 1984), 0.70 (Evans, 1993), 0.88 (Alonso et al., 2009), 0.90 (Medlyn et al., 2002), and 0.95 (Leuning, 1995). Medlyn (1996) has shown that the optimization result of the nitrogen partitioning is very sensitive to the value of this curvature parameter. Instead of using a non-linear model, we introduced Equation 1, which yielded an additional equation, Equation 7, needed to solve the optimum nitrogen partitioning.

One criticism of using Equation 1 could be that it predicts a constant electron transport efficiency of PSII over the range of irradiance within which $J_{\text{psii max}}$, whereas experimentally measured efficiencies of PSII decline almost linearly with irradiance (e.g. Harbinson et al., 1990; Yin et al., 2014). However, the observed decline of PSII efficiencies could be due to the feedback effect of the limitation set by Rubisco, which increasingly becomes rate limiting with an increase in irradiance. This reasoning is supported by an observation of a sharper decline of PSII efficiencies in nitrogen-starved than nitrogen-enriched leaves (Schependonk et al., 1999) or at a lower temperature (Kingston-Smith et al., 1999), because photosynthesis in nitrogen-starved leaves or at low temperature is more Rubisco limited. Like the original FvCB model, our model does predict a decline in actual PSII efficiencies when $A$ is limited by $V_c$ rather than by $V_c$. The use of Equation 1, rather than a non-rectangular hyperbolic relationship like Equation A4 in Supplementary Appendix A, is supported by an observation that the electron transport rate through PSII increases proportionally with irradiance to at least 1000 μmol m$^{-2}$ s$^{-1}$ if electron transport and CO$_2$ fixation are uncoupled (Cheeseman and Lexa, 1996). Although Equation 1 is linear, our model did generate a hyperbolic non-linear photosynthetic response to irradiance (Supplementary Fig. S1a), suggesting that the nitrogen partitioning may also contribute a part to decreases of the measured PSII efficiency with increasing irradiance.

**Is photosynthetic nitrogen partitioned optimally?**

It is necessary first to point out that the optimum nitrogen-partitioning solution depends on many model coefficients (Table 2) as well as on the coefficients for the relationship between $N_{\text{photo}}$ and $N_{\text{leaf}}$. Values of these coefficients are open to change, given the large variation between species in the nitrogen partitioning (Seemann et al., 1987; Evans, 1989; Hikosaka, 1997). Thus, using only one set of default values of the coefficients contributed to discrepancies between modelled and measured values, either in photosynthetic rates or in nitrogen investments in photosynthetic proteins, for various species (Fig. 3; Supplementary Figs S4, S5). However, the choice of coefficient values does not change the overall trend of model prediction. Our results showed that the optimum nitrogen

**Fig. 6.** Ratios of simulated net CO$_2$ assimilation rate $A$ assuming dynamic acclimation using Equation 9 with different values of time constant $\tau$, either to the simulated net CO$_2$ assimilation rate assuming the optimum N partitioning, $A_{\text{opt}}$ (a), or to the simulated net CO$_2$ assimilation rate without acclimation assumed, $A_{\text{without}}$ (b). Circles, squares, and triangles represent the results for upper, middle, and bottom leaves of a canopy, respectively. Symbols in this figure are drawn from the seasonal integral of simulated values.
partitioning responds to environmental changes according to an induced imbalance between light energy absorbed through photochemistry and the energy utilized through stromal metabolism (Huner et al., 1998). The model predicted a high investment of nitrogen in Rubisco under an environmental change that leads to an excessive energy supply relative to the energy demand by stromal metabolism (e.g. low temperature, high light, low nitrogen, low CO₂). Conversely, more investment in the chlorophyll complex is needed when energy supply is limiting. This response was shown by our findings that fractions partitioned to \( N_C \) and \( N_R \) were most responsive to physiological or environmental changes (Fig. 1; Supplementary Fig. S2), in line with reports that nitrogen partitioning between light-harvesting and carboxylation complexes is nearly optimal (Evans, 1993; Hikosaka and Terashima 1996).

For example, partitioning to \( N_R \) was predicted to decrease in response to an elevated [CO₂] (Supplementary Fig. S2). Substantial experimental evidence (e.g. Schapendonk et al., 2000) has indicated a decline of leaf Rubisco content when plants are grown under elevated CO₂ conditions, which may confirm our optimization result. However, this decline of Rubisco may be a result of a decrease in \( N_{leaf} \) (e.g. Geiger et al., 1999), rather than a reallocation of nitrogen within a leaf. Medlyn (1996) predicted that under a doubling CO₂ concentration from its ambient level, electron transport capacity relative to Rubisco carboxylation velocity (the \( J_{\text{max},25}:V_{\text{c,max},25} \) ratio) should increase by 40%, which is in good agreement with our prediction (Supplementary Fig. S2). Since at elevated CO₂, the efficiency of CO₂ fixation by Rubisco is increased and so less of this enzyme is needed, Sage (1994) indicated that to use nitrogen optimally, the Rubisco content should be decreased and the nitrogen thus freed should be re-allocated to other limiting processes. However, experimental data do not always support this prediction (e.g. Akita et al., 2012). Furthermore, our model also showed that the increase in the \( J_{\text{max},25}:V_{\text{c,max},25} \) ratio in response to the elevated CO₂ became smaller at a lower temperature (results not shown), suggesting a strong interaction between CO₂ and temperature.

Interactions between these environmental variables can be shown, to some extent, even from the steady-state FvCB model. For example, the model predicts increases in the optimum temperature with rising CO₂ or irradiance (Farquhar et al., 1980), and the importance of these shifts in the temperature optimum in predicting impacts of climate changes on ecosystems has often been emphasized (e.g. Long 1991). Here we show changes in the optimum temperature in response not only to CO₂ and irradiance but also to plant nitrogen status (Supplementary Fig. S3). The predicted decline of the optimum temperature with increasing \( N_{leaf} \) is supported by data of Walcroft et al. (1997), which illustrated a higher photosynthetic rate at 25 °C than at 30 °C for plants at high nitrogen, but a lower rate at 25 °C than at 30 °C for those at low nitrogen. However, Sage and Pearcy (1987) showed little apparent change in the optimum temperature with \( N_{leaf} \). Our optimization results generally agree well with previous predictions (Friend, 1991; Hikosaka and Terashima, 1995; Medlyn, 1996) and experimental observations (Seemann et al., 1987; Evans, 1989; Makino et al., 1994, 1997; Warren and Adams, 2001). The exception is the change in the predicted fraction to \( N_R \) under increasing \( N_{leaf} \) conditions; our model predicted a decrease in the fraction to \( N_R \) with an increase in \( N_{leaf} \) (Fig. 1b), the direction opposite to the reports of Friend (1991) and Hikosaka and Terashima (1995). However, our model also indicated that the decreasing partitioning to \( N_R \) under increasing \( N_{leaf} \) only became apparent at a low irradiance level (Fig. 1) and, when irradiance was moderately high (550 µmol m⁻² s⁻¹), the \( N_R/N_{leaf} \) ratio hardly varied with the nitrogen environment (Supplementary Fig. S5f). Experimental evidence of Makino et al. (1994, 1997) showed that the ratio of Rubisco to \( N_{leaf} \) beyond a certain base value is constant (independent of light, temperature, and \( N_{leaf} \)). Yamori et al. (2011) showed that the Rubisco/\( N_{leaf} \) ratio increased with increasing nitrogen supply (Supplementary Fig. S5e). Warren and Adams (2001) also found a consistent overinvestment in Rubisco. Medlyn (1996) hypothesized that leaves tend to maintain high Rubisco levels in order to take advantage of any high light periods because the response of Rubisco–limited photosynthesis to increasing Rubisco is stronger than the response of light-limited photosynthesis to increasing chlorophyll. An alternative explanation would be that Rubisco is not fully activated and its specific activity is lower (Evans, 1989). Thus, our prediction, using the in vitro measured \( K_{c,25} \) value (Farquhar et al., 1980), only indicates the minimum quantity of nitrogen that may be present in Rubisco.

**Can the optimum nitrogen partitioning be used to predict photosynthetic acclimation?**

Our model based on the optimum nitrogen partitioning can explain, at least to a considerable extent, increases in the optimum temperature with increasing growth temperature as reported by, for example, Yamasaki et al. (2002) and Yamori et al. (2005). Such changes can be obtained from the modelled increase in \( V_{c,max} \) and \( J_{\text{max}} \) with increasing growth temperature (Fig. 4b). Following the previous meta-analysis procedure (e.g. Hikosaka et al., 2006), we calculated a linear relationship between \( E_{V_{c,max}} \) and \( E_{J_{\text{max}}} \) and growth temperature (Fig. 4), although the real relationship may be more complex. Our intercept and slope values for \( E_{V_{c,max}} \) were 31.9 933 J mol⁻¹ and 1108 J mol⁻¹ °C⁻¹ (Fig. 4b), which are remarkably similar to 34 100 J mol⁻¹ and 1010 J mol⁻¹ °C⁻¹, respectively, the values of Hikosaka et al. (2006) from their meta-analysis. We did not find equivalent quantitative information in the literature in support of our modelled intercept and slope values for \( E_{J_{\text{max}}} \) shown in Fig. 4b, but the optimum temperature for \( J_{\text{max}} \) increased with growth temperature (Kattge and Knorr, 2007), suggesting that \( E_{J_{\text{max}}} \) may increase with increasing growth temperature as well (Hikosaka et al., 2006). Thus, relationships used in ecosystem models for accommodating photosynthetic thermal acclimation (e.g. Stinziano et al., 2018), which were based on empirical equations like those of Hikosaka et al. (2006) and Kattge and Knorr (2007), can be the emergent properties of our optimum nitrogen partitioning model.

Such empirical relationships only reflect the consequence of acclimation, but do not model the dynamics of acclimation as a process per se, especially not for acclimation to rapidly
varying field environmental conditions. We therefore incorporated algorithms for dynamic adjustment of the nitrogen content of a compound towards its optimum level, to predict leaf photosynthesis in rapidly varying environments. For that, we assumed the first-order kinetics, Equation 9, using a characteristic time constant ($\tau$).

The diurnal course of simulated leaf photosynthesis, $A$, differed from the course generated by the steady-state version of the model without acclimation assumed, $A_{\text{without}}$ (Fig. 5). A major feature of the simulated $A_{\text{without}}$ is that a threshold value was predicted at time steps around noon, which varied little with further increases in irradiance on sunny days, whereas this threshold value was never simulated using the model version of the first-order kinetics. These results imply that the conventional steady-state FvCB model may not suffice for the temporally explicit situations where instantaneous rates of leaf photosynthesis are needed to be predicted accurately. However, daily integrated values of the simulated photosynthetic rate did not always significantly differ between the models with and without acclimation (Supplementary Fig. S7), depending on daily weather conditions and the time constant for simulation. This implies the importance of choosing appropriate environmental ranges in parameterizing the model if the steady-state version is used to simulate photosynthesis under varying conditions. For a further higher temporal scale, seasonally integrated values of the simulated photosynthetic rate, the difference between the models with and without acclimation could totally depend on the time constant $\tau$ (Fig. 6b).

The value for $\tau$ is uncertain and it may vary with species. As our model only considers nitrogen partitioning, $\tau$ may also lump the value for other processes that might contribute to the biochemical aspect of acclimation. In our analysis, we used the same value for $\tau$ for different nitrogen components, and its default value was set to 40 min according to the value for Rubisco-related activity (Kirschbaum et al., 1998). However, $\tau$ for acclimation may depend on growth temperature (Maeva Baumont, INRA, France, personal communication), and the time for the protein turnover may differ among individual compounds (Huner et al., 1998). A possible higher value of $\tau$ for Rubisco, relative to that for other components (Kirschbaum et al., 1998) may contribute to the aforementioned overinvestment in Rubisco. The actual nitrogen partitioning in nature may never be at the optimum, resulting in an imbalance between energy supply and demand in chloroplasts (Huner et al., 1998). As such, plants may always have to engage various photoprotective strategies to minimize photoinhibition (Ort and Baker, 2002).

In addition, our model ignores (i) stomatal conductance; (ii) mesophyll conductance; (iii) triose phosphate utilization limitation; and (iv) the partitioning of $N_{\text{leaf}}$ between $N_{\text{photo}}$ and other physiological nitrogen complexes, which all may play a part in photosynthetic acclimation. Also, in our analysis, $R_{425}$ is assumed to scale with $V_{\text{c,max}}$, which qualitatively agrees with the observation that $R_{425}$ per unit $N_{\text{leaf}}$ is lower for plants grown at high temperature than for those grown at low temperature (data of Yamori et al., 2005), and for plants grown under low light than for those grown under high light (data of Yamori et al., 2010). However, temperature response of respiration may vary among growth conditions (Harley et al., 1992; Walcroft et al., 1997; Tjoelker et al., 2001; Yamori et al., 2005; King et al., 2006; Alonso et al., 2009). There is also a possible response in the partitioning of $N_{\text{leaf}}$ between $N_{\text{photo}}$ and respiratory proteins, to both a daytime and night-time growth environment, which our model does not account for. More importantly, in the longer term, morphological acclimation, such as the variation of specific leaf area, can play a dominant role in determining photosynthetic acclimation (Evans and Poorter, 2001). It was probably due to this morphological acclimation that plants grown in different treatment environments have different values of $N_{\text{leaf}}$ (Fig. 3; Supplementary Figs S4, S5). However, adding these parameters or processes would make it impossible to solve the nitrogen partitioning analytically, and morphological acclimation in particular would need to introduce other mechanisms to model. Nevertheless, since $N_{\text{leaf}}$ is a physiological variable that is often simulated in general plant or crop models (e.g. Yin and Struik, 2017; Wu et al., 2018), our methodology based on the optimum nitrogen partitioning can be incorporated into these models for simulating plant acclimation to varying environmental conditions.

### Supplementary data

Supplementary data are available at *JXB* online.

**Appendix A. Summary of the FvCB model for leaf photosynthesis.**

**Appendix B. Solution to the optimum partitioning of $N_{\text{photo}}$.**

**Appendix C. Model-generated responses of leaf photosynthesis to both $I_{\text{inc}}$ and $N_{\text{leaf}}$.**

Fig. S1. Model-generated leaf photosynthesis rate under the optimum nitrogen partitioning ($A_{\text{opt}}$) in response to irradiance and leaf nitrogen content.

Fig. S2. Relationships between relative fractions of partitioning to $N_C$, $N_R$, $N_I$, and $N_S$ under two levels of CO$_2$.

Fig. S3. Model-generated leaf photosynthesis rate under the optimum nitrogen partitioning, $A_{\text{opt}}$, in response to leaf temperature.

Fig. S4. Observed and simulated temperature response of net CO$_2$ assimilation rate, and observed amounts of photosynthetic proteins per unit $N_{\text{leaf}}$ versus predicted nitrogen in equivalent protein compounds per unit $N_{\text{leaf}}$ in leaves of spinach plants grown in low and high temperature (data from Yamori et al., 2005).

Fig. S5. Observed and simulated temperature response of net CO$_2$ assimilation rate, and observed amounts of photosynthetic proteins per unit $N_{\text{leaf}}$ versus predicted nitrogen in equivalent protein compounds per unit $N_{\text{leaf}}$ in leaves of four species grown in low, medium, and high nitrogen (data from Yamori et al., 2011).

Fig. S6. Kinetics of net leaf photosynthesis $A$ when incoming irradiance is abruptly changed between 1000 nmol m$^{-2}$ s$^{-1}$ and 300 nmol m$^{-2}$ s$^{-1}$ at the 20th time step of simulation.

Fig. S7. The ratio of daily photosynthetic rate simulated assuming acclimation to daily rate simulated without acclimation assumed, plotted against daily photosynthetic rate with the instantaneous optimum N partitioning over a period of $\sim$50 d.
Acknowledgements

This research was conducted within the consortium of the ModCarboStress project, co-financed by NWO - the Netherlands Organisation for Scientific Research (no. 861.15.003), via the ERA-NET Plus action ‘Climate Smart Agriculture: Adaptation of agricultural systems in Europe’.

References

Akita R, Kamiyama C, Hikosaka K. 2012. *Polygonum sachalinense* alters the balance between capacities of regeneration and carboxylation of ribulose-1,5-bisphosphate in response to growth CO₂ increment but not the nitrogen allocation within the photosynthetic apparatus. Physiologia Plantarum 146, 404–412.

Alonzo A, Pérez P, Martínez-Carrasco R. 2009. Growth in elevated CO₂ enhances temperature response of photosynthesis in wheat. Physiologia Plantarum 135, 109–120.

Athanasiou K, Dyson BC, Webster RE, Johnson GN. 2010. Dynamic acclimation of photosynthesis increases plant fitness in changing environments. Plant Physiology 152, 366–373.

Bernacchi CJ, Pimentel C, Long SP. 2003. In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. Plant, Cell & Environment 26, 1419–1430.

Bernacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP. 2002. Temperature response of the Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiology 130, 1992–1998.

Cai C, Li G, Yang H, et al. 2018. Do all leaf photosynthesis parameters of rice acclimate to elevated CO₂, elevated temperature, and their combination, in FACE environments? Global Change Biology 24, 1685–1707.

Cai C, Yin X, He S, et al. 2016. Responses of wheat and rice to factorial combinations of ambient and elevated CO₂ and temperature in FACE experiments. Global Change Biology 22, 856–874.

Cheeseman JM, Lexa M. 1996. Gas exchange: models and measurements. In: Baker NR, ed. Photosynthesis and the environment. Dordrecht: Kluwer Academic Publishers, 223–240.

Cousins AB, Ghannoum O, Van Caemmerer S, Badger MR. 2010. Simultaneous determination of Rubisco carboxylase and oxygenase kinetic parameters in *Triticum aestivum* and *Zea mays* using membrane inlet mass spectrometry. Plant, Cell & Environment 33, 444–452.

Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of *C₃* plants. Oecologia 78, 9–19.

Evans JR. 1993. Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. II. Stability through time and comparison with a theoretical optimum. Australian Journal of Plant Physiology 20, 69–82.

Evans JR, Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant, Cell & Environment 24, 755–767.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of *C₃* species. Planta 149, 78–90.

Farquhar GD, Wong SC. 1984. An empirical model of stomatal conductance. Australian Journal of Plant Physiology 11, 191–120.

Friend AD. 1991. Use of a model of photosynthesis and leaf microenvironment to predict optimal stomatal conductance and leaf nitrogen partitioning. Plant, Cell & Environment 14, 895–905.

Friend AD. 2010. Terrestrial plant production and climate change. Journal of Experimental Botany 61, 1293–1309.

Geiger M, Haake V, Ludewig F, Sonnewald U, Stitt M. 1999. The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. Plant, Cell & Environment 22, 1177–1199.

Harbinson J, Genty B, Baker NR. 1990. The relationship between CO₂ assimilation and electron transport in leaves. Photosynthesis Research 25, 213–224.

Harley PC, Thomas RB, Reynolds JF, Strain BR. 1992. Modelling photosynthesis of cotton grown in elevated CO₂. Plant, Cell & Environment 15, 271–282.

Hikosaka K. 1997. Modelling optimal temperature acclimation of the photosynthetic apparatus in *C₃* plants with respect to nitrogen use. Annals of Botany 80, 721–730.

Hikosaka K, Ishikawa K, Borjigidai A, Muller O, Onoda Y. 2006. Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. Journal of Experimental Botany 57, 291–302.

Hikosaka K, Terashima I. 1995. A model of the acclimation of photosynthesis in the leaves of *C₃* plants to sun and shade with respect to nitrogen use. Plant, Cell & Environment 18, 605–618.

Hikosaka K, Terashima I. 1996. Nitrogen partitioning among photosynthetic components and its consequence in sun and shade plants. Functional Ecology 10, 335–343.

Huner NPA, Oquist G, Sarhan F. 1998. Energy balance and acclimation to light and cold. Trends in Plant Science 3, 224–230.

Kattge J, Knorr W. 2007. Temperature acclimation in a biochemical model of photosynthesis: a reanalysis of data from 36 species. Plant, Cell & Environment 30, 1176–1190.

Kattge J, Knorr W, Raddatz T, Wirth C. 2009. Quantifying photosynthetic capacity and its relationship to nitrogen content for global-scale terrestrial biosphere models. Global Change Biology 15, 976–991.

King AW, Gunderson CA, Post WM, Weston DJ, Wulfschleger SD. 2006. Plant respiration in a warmer world. Science 312, 536–537.

Kingston-Smith AH, Harbinson J, Foyer CH. 1999. Acclimation of photosynthesis, H₂O₂ content and antioxidants in maize (*Zea mays*) grown at sub-optimal temperatures. Plant, Cell & Environment 22, 1071–1083.

Kirschbaum MUF, Küppers M, Schneider H, Giersch C, Néo S. 1998. Modelling photosynthesis in fluctuating light with inclusion of stomatal conductance, biochemical activation and pools of key photosynthetic intermediates. Planta 204, 16–26.

Leuning R, Kelliher FM, De Pury DGG, Schulze E-D. 1995. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. Plant, Cell & Environment 18, 1183–1200.

Lloyd J, Farquhar GD. 1996. The CO₂ dependence of photosynthesis, plant growth responses to elevated atmospheric CO₂ concentrations and their interactions with soil nutrient status. I. General principles and forest ecosystems. Functional Ecology 10, 4–32.

Long SP. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentrations: has its importance been underestimated? Plant, Cell & Environment 14, 729–739.

Makino A, Nakano H, Mae T. 1994. Effects of growth temperature on the responses of ribulose-1,5-bisphosphate carboxylase, electron transport components, and sucrose synthase enzymes to leaf nitrogen in rice, and their relationships to photosynthesis. Plant Physiology 105, 1231–1238.

Makino A, Sato T, Nakano H, Mae T. 1997. Leaf photosynthesis, plant growth and nitrogen allocation in rice under different irradiances. Planta 203, 390–398.

Medlyn BE. 1996. The optimal allocation of nitrogen within the *C₃* photosynthetic system at elevated CO₂. Australian Journal of Plant Physiology 23, 593–603.

Medlyn BE, Dreyer E, Ellsworth D, et al. 2002. Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. Plant, Cell and Environment 25, 1167–1179.

Ort DR, Baker NR. 2002. A photoprotective role for O₂ as an alternative electron sink in photosynthesis? Current Opinion in Plant Biology 5, 193–198.

Penning de Vries FWT, van Laar HH. 1982. Simulation of plant growth and crop production. Wageningen: Pudoc.

Sage RF. 1994. Acclimation of photosynthesis to increasing atmospheric CO₂: the gas exchange perspective. Photosynthesis Research 39, 351–368.

Sage RF, Pearcy RW. 1987. The nitrogen use efficiency of *C₃* and *C₄* plants: II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiology 84, 959–963.

Sassenrath-Cole GF, Pearcy RW. 1994. Regulation of photosynthetic induction state by the magnitude and duration of low light exposure. Plant Physiology 105, 1115–1123.

Schapendonk AHCM, Van Oijen M, Dijkstra P, Pot SC, Jordi WJRM, Stoopen GM. 2000. Effects of elevated CO₂ concentration on photosynthetic acclimation and productivity of two potato cultivars
grown in open-top chambers. Australian Journal of Plant Physiology 27, 1119–1130.

Schapendonk AHCM, Van Oijen M, Pot SC, Van den Boogaard R, Harbinson J. 1999. Nitrogen shortage in a tomato crop: scaling up from effects of electron-transport rate to plant productivity. Zeitschrift für Naturforschung 54c, 840–848.

Seemann JR, Sharkey TD, Wang J, Osmond CB. 1987. Environmental effects on photosynthesis, nitrogen-use efficiency, and metabolite pools in leaves of sun and shade plants. Plant Physiology 84, 796–802.

Sharkey TD. 1985. Photosynthesis in intact leaves of C₃ plants: physics, physiology and rate limitations. Botanical Review 51, 53–105.

Sharwood RE, Crous KY, Whitney SM, Ellsworth DS, Ghannoum O. 2017. Linking photosynthesis and leaf N allocation under future elevated CO₂ and climate warming in Eucalyptus globulus. Journal of Experimental Botany 68, 1157–1167.

Silva-Pérez V, Furbank RT, Condon AG, Evans JR. 2017. Biochemical model of C₃ photosynthesis applied to wheat at different temperatures. Plant, Cell & Environment 40, 1552–1564.

Sinclair TR, Horie T. 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. Crop Science 29, 90–98.

Smith NG, Dukes JS. 2013. Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO₂. Global Change Biology 19, 45–63.

Stinziano JR, Way DA, Bauerle WL. 2018. Improving models of photosynthetic thermal acclimation: which parameters are most important and how many should be modified? Global Change Biology 24, 1580–1598.

Tjoelker MG, Oleksyn J, Reich PB. 2001. Modelling respiration of vegetation: evidence for a general temperature-dependent Q₁₀. Global Change Biology 7, 223–230.

Walcroft AS, Whitehead D, Silvester WB, Kelliher FM. 1997. The response of photosynthetic model parameters to temperature and nitrogen concentration in Pinus radiata D. Don. Plant & Cell Environment 20, 1338–1348.

Walters RG. 2005. Towards an understanding of photosynthetic acclimation. Journal of Experimental Botany 56, 435–447.

Warren CR, Adams M. 2001. Distribution of N, Rubisco and photosynthesis in Pinus pinaster and acclimation to light. Plant, Cell & Environment 24, 597–609.

Wu A, Doherty A, Farquhar GD, Hammer GL. 2018. Simulating daily field crop canopy photosynthesis: an integrated software package. Functional Plant Biology 45, 362–377.

Yamasaki T, Yamakawa T, Yamane Y, Koike H, Satoh K, Katoh S. 2002. Temperature acclimation of photosynthesis and related changes in photosystem II electron transport in winter wheat. Plant Physiology 128, 1087–1097.

Yamori W, Evans JR, Von Caemmerer S. 2010. Effects of growth and measurement light intensities on temperature dependence of CO₂ assimilation rate in tobacco leaves. Plant, Cell & Environment 33, 332–343.

Yamori W, Nagai T, Makino A. 2011. The rate-limiting step for CO₂ assimilation at different temperatures is influenced by the leaf nitrogen content in several C₃ crop species. Plant, Cell & Environment 34, 764–777.

Yamori W, Noguchi K, Terashima I. 2005. Temperature acclimation of photosynthesis in spinach leaves; analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant, Cell & Environment 28, 536–547.

Yin X, Belay DW, van der Putten PE, Struik PC. 2014. Accounting for the decrease of photosystem photochemical efficiency with increasing irradiance to estimate quantum yield of leaf photosynthesis. Photosynthesis Research 122, 323–335.

Yin X, Harbinson J, Struik PC. 2006. Mathematical review of literature to assess alternative electron transports and interphotosystem excitation partitioning of steady-state C₃ photosynthesis under limiting light. Plant, Cell & Environment 29, 1771–1782.

Yin X, Struik PC. 2010. Modelling the crop: from system dynamics to systems biology. Journal of Experimental Botany 61, 2171–2183.

Yin X, Struik PC. 2017. Can increased leaf photosynthesis be converted into higher crop mass production? A simulation study for rice using the crop model GECROS. Journal of Experimental Botany 68, 2345–2360.

Yin X, van Oijen M, Schapendonk AHCM. 2004. Extension of a biochemical model for the generalized stoichiometry of electron transport limited C₃ photosynthesis. Plant, Cell and Environment 27, 1211–1222.