Title:

A System Dynamics approach to model photosynthesis at leaf level under fluctuating light

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ABSTRACT

It has been recognized the need to consider some photosynthetic processes in their transient states since those are more representative of the natural environment. The combination of mathematical models with the available data provides a tool to understand the dynamic responses of plants to fluctuating environments and can be used to make predictions on how photosynthesis would respond to unsteady state conditions. Here we present a leaf level system dynamic photosynthetic model based and validated on an experiment performed on two soybean varieties, the wildtype Eiko and the chlorophyll deficient mutant Minngold, grown in constant and fluctuating light conditions. This mutant is known to have similar steady-state photosynthesis compared to the green wildtype, but it is found to have less biomass at harvest. It has been hypothesized that this might be due to an unoptimized response to non-steady state conditions, therefore this mutant seems relevant to investigate dynamic photosynthesis. The model explained well the photosynthetic responses of these two varieties to fluctuating and constant light conditions and allowed to make relevant conclusions on the different dynamic responses of the two varieties. Furthermore, due to its simplicity, the model could provide the basis of an upscaled dynamic model at plant level.

INTRODUCTION

The continuous rising in population is requiring an increase in agricultural production of at least a 60% (Alexandratos & Bruinsma, 2012). By being the source of food and the responsible of the survival for the majority of life on Earth (Stirbet, Lazár, Guo, & Govindjee, 2019), photosynthesis has recently become a target to improve global food production, since the increase in genetic yield potential seems to be hindered (Foyer, Ruban, & Nixon, 2017; Taylor & Long, 2017). Photosynthesis has been intensively studied in the laboratories but, due to its complex nature, it still provides some challenges (Flexas, Loreto, & Medrano, 2013). Mathematical models can furnish a different tool to better understand the dynamics of this process and can be used to make predictions on how photosynthesis would respond to limiting situations (Stirbet et al., 2019).

Several modelling efforts have been done in order to describe the photosynthesis in its whole. The models can be differentiated by considering the processes at steady state (T N Buckley, Mott, & Farquhar, 2003; Farquhar, von Caemmerer, & Berry, 1980; Ye, Suggett, Robakowski, & Kang, 2013) or non-steady state (Bellasio, 2019; Kirschbaum, Küppers, Schneider, Giersch, & Noe, 1997; Morales et al., 2018); for their spatial scale, leaf scale (Farquhar et al., 1980; Vialet-Chabrand, Silvere R.M.McAusland, Blatt, Lawson, Griffiths, & Matthews, 2017; Zhu, Wang, Ort, & Long, 2013) or canopy scale (Song & Zhu, 2012); and for the different modelling approaches, empirical models (Farquhar et al., 1980; Vialet-Chabrand, Silvere R.M.McAusland et al., 2017), system biology models (Kannan et al., 2019; Petterssons & Ryde−Petterson, 1988; Zhu et al., 2013) and process-based models (Bellasio, 2019; Kaiser, Morales, & Harbinson, 2018; Kirschbaum et al., 1997).

The processes of photosynthesis have been initially tackled in steady-state conditions (Farquhar et al., 1980; Von Caemmerer, 2013). These models are fundamental in understanding physiological characteristics and to answer very specific questions, but usually overestimate total photosynthesis in fluctuating environmental conditions (Timm, Küppers, & Stegemann, 2004). In fact, rarely external conditions are stable in natural environments, so that plants need to continuously adjust to optimize the carbon uptake in these dynamic conditions (Kaiser et al., 2018). Different adjustments can be operated by plants depending on the time scale considered (Kono & Terashima, 2014): in the fast temporal scale plants respond by regulating the processes involved in photochemical (Kaiser et al., 2018; Kono & Terashima, 2014) and non-photochemical processes (Acebron et al., 2020), by activating the Calvin Cycle enzymes (Porcar-Castell, Bäck, Juurola, & Hari, 2006) and by moving their chloroplasts within the leaves (Kaiser et al., 2014); slower adjustments can then be due to the regulation of the stomata (Thomas N Buckley, 2017; Matthews, Vialet-Chabrand, & Lawson, 2018; Silvere Vialet-Chabrand, Matthews, Simkin, Raines, & Lawson, 2017), to the movements of the leaves within the canopy and to the adaptative adjustments in nitrogen and chlorophyll content (Posada, Lechowicz, & Kitajima, 2009; Zhang, Zhong, Wang, Sui, & Xu, 2016) .

One of the main variable conditions is light. Light intensity is continuously changing due to the movements of the clouds and to the wind moving the leaves (Pearcy, 1990; Retkute et al., 2015). Plants need to adapt to these changing in light conditions and some species may be more efficient than others in doing it (Kromdijk et al., 2016; Matsubara, 2018; Urban, Ingwers, McGuire, & Teskey, 2017). One rising question is if a reduction in chlorophyll content might be detrimental or beneficial when dealing with fluctuating light conditions. At canopy level, the role of chlorophyll content has been investigated (Gu et al., 2017; Ort et al., 2015; Slattery, Grennan, Sivaguru, Sozzani, & Ort, 2016; Walker et

al., 2018) and it has been proposed that a reduced chlorophyll content would entail a better distribution of the light in the lower layers of the canopy, therefore increasing the overall photosynthesis. Nevertheless, few have studied the effect of chlorophyll reduction in fluctuating environments (Ferroni et al., 2020).

In this study we focus on the effect of fluctuating light on two soybean varieties: the green wildtype soybean (Eiko) and a chlorophyll deficient mutant (Minngold) which has been firstly described by Campbell et al. (2015). It has been shown (Sakowska et al., 2018) that MinnGold has comparable light curves and A/Ci curves (steady state measurements) at leaf level compared to Eiko but lower biomass was found at harvest. It was hypothesised (Genesio et al., 2020) that a slower adjustment to fluctuating light might cause a lower carbon accumulation at canopy level, and that steady state measurements at leaf level would not be able to capture this difference.

Therefore, in this paper we investigate the role of the chlorophyll content in adjusting to fluctuations in light, combing experimental observations with a modelling framework. To begin with, we implemented a model at leaf level to be a basis in understanding the response of these two varieties to highly fluctuating light environments. We decided to use a process-based approach, based on the principles of system dynamics, according to which a complex system can be represented by flows, compartments (stocks) and feedback loops (Forrester, J. W., 1997).

MATERIAL AND METHODS

Experimental setup

Two soybean varieties have been used in this study with different chlorophyll content: Eiko, the green cultivar used as the wildtype and MinnGold, the chlorophyll deficient mutant (Campbell et al., 2015; Slattery, Vanloocke, Bernacchi, Zhu, & Ort, 2017). The

119 plants were sown in 3 litres pots and grown inside a controlled growth chamber system
120 (Salvatori et al., submitted) for five weeks with either non-fluctuating light or fluctuating
121 light conditions. The light was turned on from 5:00 to 19:00 and the intensity was set to
122 simulate the daily profile of the sun reaching a maximum of 650 µmol m^{-2} s^{-1} for the non-
123 fluctuating light protocol or fluctuating every minute (with a duty cycle of 0.5) with an
124 amplitude of ±20% around the non-fluctuating light intensity value. By doing this, all
125 plants received the same amount of light throughout the day.

126 Then, three plants from each variety and each light protocol were randomly chosen as
127 replicates, from which we selected a young and fully expanded leaf to perform
128 fluorescence analysis combined with gas-exchange using the LI-6800 (Licor Biosciences,
129 Nebraska, USA) equipped with infra-red gas analysers (IRGA) coupled with pulse-
130 amplitude modulation (PAM) fluorometer. In particular, we were interested in recording
131 the carbon assimilation (*A*), the electron transport rate (*ETR*) and the non-photochemical
132 quenching (*NPQ*).

133 We used the following protocol: all plants were dark-adapted overnight, then the light
134 was turned on following either a constant light protocol for 60 minutes at 650 µmol m^{-2} s^{-1}
135 or a fluctuating light protocol with light intensity changing from 780 µmol m^{-2} s^{-1} to 520
136 µmol m^{-2} s^{-1} every minute by simulating the growth conditions. The CO_2 levels were
137 maintained at 400 ppm, vapour pressure deficit (VPD) was kept at 1.8 kPa and leaf
138 temperature at 25°C.

139 The carbon assimilation (A in µmol CO_2 s^{-1}) was calculated as follows:

140 $$A = \frac{\mu_0 \left[c_0 - c_a \left(\frac{1-w_0}{1-w_a} \right) \right]}{s}$$

Where μ_0 is the flow rate (µmol air s^{-1}) entering the leaf chamber, s is the leaf area (m) and c_0 and w_0 are the CO_2 and H_2O concentrations (in µmol CO_2 and mmol H_2O respectively) entering the leaf chamber and c_a and w_a the concentration existing the chamber.

Throughout the protocol, a saturating light pulse of > 5,000 µmol m^{-2} s^{-1} was given to the leaf sample for 800 ms every 30 s, to quantify maximal fluorescence in the light ($F_m^{'}$) and dark (F_m). The operating efficiency of the PSII (Φ_{PSII}) was calculated as follows (Genty, Briantais, & Baker, 1989):

$$\Phi_{PSII} = \frac{\left(F_m^{'} - F_s\right)}{F_m^{'}}$$

where F_s is the steady-state fluorescence.

NPQ was calculated using the equation from Bilger & Björkman (1990) based on Stern-Volmer method, as follows:

$$NPQ = \frac{\left(F_m - F_m^{'}\right)}{F_m^{'}}$$

Finally, the electron transport rate was calculated based on Krall & Edwards (1992), as follows:

$$ETR = I * \alpha * fraction_{PSII} * \Phi_{PSII}$$

Where I is the incident light, $fraction_{PSII}$ is the fraction of absorbed light that is received by the PSII and is normally set to 0.5 (Baker, 2008), α is the absorbance coefficient which was set to 0.55 for MinnGold and 0.78 for Eiko as calculated in the growth chambers (Salvatori et al., submitted).

Model description

Here we present a model of the main processes involved in the regulation of the photosynthesis of leaves of C3 plants exposed to fast changes of light intensity. Figure 1 shows a schematic diagram of the implemented processes, providing a simplified representation of the complex phenomena occurring in photosynthesis. For the sake of simplicity, the presented model essentially considers the main dynamics of a chloroplast as representative of a whole leaf, in a sort of "big chloroplast" approach. Since the modelled leaf is exposed to optimal conditions of CO_2 and average light intensity, we assumed no limitation due to stomatal conductance since this dynamic generally becomes relevant during the induction phase (dark-light transition), nevertheless it is known that in soybean this limitation can be mainly attributed to Rubisco activation (Soleh et al., 2016; Taylor & Long, 2017).

Due to the nature of the experiment conducted, we focused on the limitations imposed by the light reactions. When the light excites the Photosystem 2 (PSII), many pigments (chlorophyll a and b antenna proteins) collect this energy and transfer it to the reaction centre. This number of pigments can be variable from plant to plant and determine the ability of the photosystem to transfer this energy. PSII oxidizes water to O_2 releasing protons into the lumen and thus determining a change in the pH (ΔpH). The electrons are then passed on to the Cytochrome b6f (cytb6f) which delivers them to photosystem 1 (PSI) transporting additional protons into the lumen. For simplicity these last processes involving cytb6f and PSI are not included in the model and therefore not represented in Figure 1.

The energy transported is used to reduce the final acceptor $NADP^+$ to NADPH. The ΔpH generated is then used by the ATP synthase to produce ATP as protons diffuse back from

the lumen to the stroma. This process is generally called linear electron flow (LEF, in Figure 1 defined as ETR, electron transport rate) and the energy produced (ATP and NADPH) is used in the Calvin Cycle to fix CO_2.

The Calvin Cycle is regulated by the enzyme Rubisco, that is itself activated by the ΔpH generated by the electron transport. When there is excess of energy, this can be dissipated as non-photochemical quenching (NPQ). We focused on the energy-dependent quenching (i.e. ΔpH-dependent quenching qE) since it is the most important component of NPQ when regarding fluctuating irradiance, as responds most quickly to its changes (Kaiser et al., 2014), operating in the scale of minutes (Ebenhöh, Fucile, Finazzi, Rochaix, & Goldschmidt-Clermont, 2014). qE is regulated by luminal pH and the xanthophyll cycle pigments. The saturation of the dark reactions causes a decrease in the luminal pH causing the protonation of some PSII proteins (PsbS proteins) (Matuszyńska, Heidari, Jahns, & Ebenhöh, 2016), the release of violaxanthin molecules and their de-epoxidation to antheraxanthin and zeaxanthin. Zeaxanthin then binds to PSII proteins, forming a quenching complex favouring the dissipation of the excitation energy as heat (Porcar-Castell et al., 2006).

Furthermore, the generation of a ΔpH is necessary under environmental stressful conditions, when the dark reactions are saturated, allowing the production of ATP without the reduction of $NADP^+$ (Roach & Krieger-Liszkay, 2014). In such cases the cyclic electron flow (CEF) around the Photosystem 1 (PSI) is activated, increasing electron transfer from PSI to the plastoquinone pool, and again to PSI via the Cytochrome b6/f complex (Yamori, 2016). In C3 plants, CEF is considered negligible at steady state conditions, thus becoming relevant under specific stressful conditions such as low CO_2, high light, drought, or during dark to light transitions (Rochaix, 2011). CEF then becomes

a regulator of NPQ and ETR at non-steady state conditions (Roach & Krieger-Liszkay, 2014; Yamori, 2016).

Mathematical formulation of the model

Using a process-based approach, we represent the described processes by the following equations:

$$\frac{d\,En_{PSII}}{dt} = \overbrace{\alpha \cdot c_{¿} \cdot PAR \cdot \left(1 - \frac{E_{PSII}}{E_{PSII}^{¿}}\right)}^{Energy\ input} - v_{ETR} \cdot E_{PSII} \cdot \overbrace{NADP^{+¿}}^{ETR} - \overbrace{v_d \cdot E_{PSII} \cdot Z \cdot \left(1 - \frac{E_z}{E_Z^{¿}}\right)}^{Energy\ dissipation} ¿$$

Equation 1

which represents the excitation energy in PSII and the transfer of this energy either as linear electron transport (*ETR*), regulated by the amount of final acceptor $NADP^+$, or as dissipation of energy, regulated by zeaxanthin. In fact, the excess energy in PSII can be dissipated only if zeaxanthin has formed the quenching complex with the PSII. This complex is then able to release energy as heat (*NPQ*). The dynamic of the PSII-zeaxanthin complex is described as follows:

$$\frac{dE_z}{dt} = \overbrace{v_d \cdot E_{PSII} \cdot Z \cdot \left(1 - \frac{E_z}{E_Z^{¿}}\right)}^{Energy\ dissipation} - \overbrace{v_{NPQ} \cdot E_z}^{NPQ}$$

Equation 2

Whereas the dynamic of the enzyme is modelled with a saturating curve whose formation depends on the cyclic electron transport (*CEF*):

$$\frac{dZ}{dt} = \begin{cases} \overbrace{v_{za} \cdot (1 - Z)}^{Zeaxanthin\ activation} & if\ CEF > c_y \\ 0\ if CEF \le c_y \end{cases}$$

Equation 3

with

$$CEF = \overbrace{\alpha \cdot c_{¿} \cdot PAR \cdot \left(1 - \frac{E_{PSII}}{E_{PSII}^{¿}}\right)}^{Energy\ input} - v_{ETR} \cdot E_{PSII} \cdot \overbrace{NADP^{+¿}}^{ETR} ¿$$ Equation 4

As previously described, zeaxanthin formation is triggered by a change in ΔpH which occurs when a decoupling of the light reactions with the dark reactions generates an excess in energy which is exploited by the cyclic electron transport (*CEF*) to produce an increase in the ΔpH as well as a production of ATP.

Finally, the energy flowing from PSII to PSI is used to reduce $NADP^+$ (ETR) to become NADPH whose dynamic is described as follows:

$$\frac{dNADPH}{dt} = v_{ETR} \cdot E_{PSII} \cdot \overbrace{NADP^{+¿}}^{ETR} \cdot \eta_{NADP^{+¿} - \overbrace{v_C \cdot R \cdot NADPH}^{A} \cdot \eta_{NADPH}} ¿$$ Equation 5

Whereas the counterpart dynamic of $NADP^+$ is simply described as follows:

$$d\frac{NADP^{+¿}}{dt} = \frac{-dNADPH}{dt} ¿$$ Equation 6

Carbon assimilation (*A*) is therefore regulated by the rate of carboxylation mediated by Rubisco whose activation indirectly depends on the ΔpH generated, and is generally accounted as $\frac{NADPH}{NADP^{+¿}¿}$ (Morales et al., 2018). Therefore equation 5 reads as follows:

$$\frac{dR}{dt} = \overbrace{v_R \cdot (1 - R)}^{Rubisco\ activation} \cdot min\ ¿ \text{ with } \Delta pH = \frac{NADPH}{NADP^{+¿}¿}$$ Equation 7

The description of the six state variables and the parameters with the relative units can be found in Table 1. The model allows the characterization of three quantities measured in gas exchange and fluorescence analysis: *ETR*, *A* and *NPQ*. These three quantities are

fluxes (µmol m^{-2} s^{-1}) and can be derived from the described equations: *ETR* from equation 1, *NPQ* from equation 2 and *A* from equation 5.

Sensitivity analysis

A local sensitivity analysis (Norton, 2015) was performed to analyse the model behaviour under parameter perturbation. The normalized sensitivity index is calculated by changing each parameter of ±5% while keeping all the other constant. The equation for the sensitivity index is the following:

$$SSE_{i,\Delta}=\sqrt{\frac{1}{3*n}\sum_{j=1}^{3}\sum_{i=1}^{n}\left(\frac{X^{j}(p_1,p_2,\ldots,p_i+\Delta,\ldots,p_k)-X^{j}(p)}{max(X^{j}(p))-min(X^{j}(p))}\right)^2} \quad \text{Equation 8}$$

Where $SSE_{i,\Delta}$ is the standardized elementary effect of the parameter p_i with Δ (±5%) perturbation on model outputs and *k* number of parameters (equal to 13); $X^{j}(p)$ are the simulated values of the *j*-th quantity considered (i.e. *NPQ*, *ETR* and *A*) without any parameter perturbation (as in Table 1); *n*is the number of samples per observed quantity (equal for three quantities considered).

RESULTS

Experimental data

The model has been tested on fluorescence data coupled with gas exchange data in the fluctuating light regime for the two varieties Eiko and MinnGold. As described in the methods, the leaf was kept in the dark and then illuminated with fluctuating light at 520 µmol m^{-2} s^{-1} and 780 µmol m^{-2} s^{-1} for 60 minutes. In particular, the changes in electron transport (ETR), carbon assimilation (A) and non-photochemical quenching (NPQ) were recorded (Figure 2). After illumination, ETR and A show an initial slow photosynthetic induction (slower for MinnGold) mainly caused by the activation of the enzyme Rubisco

(Soleh et al., 2016; Taylor & Long, 2017) in which the fluctuations in light are not causing, initially, corresponding fluctuations in these quantities. When Rubisco is fully activated, steady state is reached, and the fluctuations become more evident and constant throughout the experimental period (the last 30 minutes). Regarding NPQ, a faster rise of this quantity is evident with an increase in the amplitude of fluctuations in time connected to the increased level of zeaxanthin. Figure 2B makes a focus on a smaller experimental period, when steady state is already being reached (from minute 45 to 55th). MinnGold results more responsive to fluctuations of light, in the sense that the changing in light intensity is causing higher amplitudes of oscillations in ETR and, to a lesser extent, to A. In NPQ instead it can be observed the opposite behaviour, with fluctuations of light causing smaller amplitudes of oscillations.

Model fitting

For the wildtype Eiko (Figure 3) the model accurately represented the measured dynamics with an R^2 of 0.98 for ETR and A and of 0.94 for NPQ. In this case the model captured both the slow induction dynamic and the fast fluctuating dynamic.

In the case of MinnGold (Figure 4), the model performed well for both ETR and NPQ (R^2=0.93 and 0.91 respectively) whereas it did not capture the slow induction found in A, still having a good R^2=0.84.

Validation

The model has been then validated on gas exchange data in the constant light regime. To validate it, the model has been tested over the data using the parameters found for the fluctuating light protocol in Eiko (Figure 5) and MinnGold (Figure 6) (Table 1). The model performed well also in these conditions, in particular for ETR (R^2=0.96 and 0.98

for Eiko and MinnGold respectively) and A (R^2=0.94 and 0.78), with a slight underperformance for NPQ (R^2=0.65 and 0.76).

Sensitivity

The local sensitivity analysis (Equation 8) allowed to identify the parameters whose change mainly affected the three quantities considered (A, ETR and NPQ). By changing the parameters by a 5% the outcome of the model never deviates more than 4% from the baseline simulation (Figure 7). This means that the model is robust and not much dependent on the changing in the parameters; furthermore, no matters if the percentage change is positive or negative, the outcome is the same.

The sensitivity also showed differences in MinnGold and Eiko. In both cases, the parameter more sensible to changes is $c_{¿}$, the parameter identifying the energy input in PSII and therefore (with α, the absorbance coefficient), the energy entering the photosystem. Differences among MinnGold and Eiko can be found for $E^{¿}_{PSII}$, the carrying capacity of the photosystem 2. A small difference for the two species can also be found for the parameters v_{NPQ}, $E^{¿}_{Z}$ and v_d.

Theoretical evaluation of the model

The model was further validated by performing some theoretical simulations by considering Eiko parameters (Table1). We evaluated how the three quantities (ETR, A and NPQ) would behave when changing the period of the fluctuating light. Figure 8A shows an example of the effect of three different fluctuating periods (30 seconds, 1 minute and 4 minutes fluctuating period with duty cycle equal to 0.5) when compared to the constant light regime. When calculating the cumulative values at steady state (after 40

minutes), A and ETR resulted higher than those for constant light (fluctuating period equal to zero) when light fluctuates with a period higher than 30 seconds and lower than 20 minutes (Figure 8B). Nevertheless, we have an opposite behaviour for NPQ. We also calculated modelled cumulative steady state values with MinnGold parameters (Figure S1). Steady state values of ETR and A decreased as the fluctuation period increased, except for short fluctuating periods in which they increase (of the same order of Eiko, Figure 8B). Nevertheless, in this case we found fluctuations causing a much smaller change in NPQ steady state.

We finally performed simulations with higher fluctuations intensity, with same fluctuating period (1-minute period) (Figure S2). In this case the constant regime results always higher than the fluctuating regime, therefore higher the fluctuation amplitude, lower would be the steady state value.

DISCUSSION

Model assumptions

The model reflects the assumptions of the experimental conditions. Leaves were exposed to optimal CO_2 conditions and average light intensity, therefore we assumed no limitation due to stomatal conductance. Two main conditions are investigated, 1) the photosynthetic induction during the dark-light transition, and 2) the fluctuations of light maintaining the system in a continuous non-steady state condition. One of the main contributions of this paper is found in the modelling of the cyclic electron transport which is thought to be fundamental in the triggering of NPQ when ETR is still limited by the downstream reactions of the Calvin Cycle (Cornic & Baker, 2012; Yamori & Shikanai, 2016). In fact, the dissipation of energy through non-photochemical quenching is possible when zeaxanthin forms a quenching complex with PSII. Zeaxanthin formation is in turn

triggered by a change in ΔpH which, when ETR is limiting, is caused by the cyclic electron transport. The fact that NPQ activation is possible also when ETR is not fully active, is evident from the data, both in the long term and in the short term. Figure 2A in fact shows that NPQ reaches steady state much faster than ETR and A during the dark-light transition. This is also evident in the short term: in fact, during the fluctuations of light (Figure 2B) at steady state, NPQ is still found to be faster than the other quantities in reaching the steady state associated with the specific light intensity.

Model performance

The model performed well in simulating the experimental data both in constant and fluctuating light conditions and in both soybean varieties with R^2s ranging from 0.65 to 0.98 (Figures 3-6). Only two observations were not well fitted by the model (with the lowest R^2s). First, in MinnGold it is found a decoupling of A and ETR in the velocity of induction in both fluctuating (Figure 4) and constant (Figure 6) light conditions. At steady state, the two processes are known to be coupled, since the electron chain starts when electrons are reducing $NADP^+$ which are in turn mainly produced by the Calvin Cycle. Nevertheless, it is known that electrons can also be transferred to other enzymes involved in the regulation of carbon metabolism as well as in the nitrogen and sulfur metabolism (Cornic & Baker, 2012). Since this would need a further discussion and a focus on the nature of this result, we did not aim to capture this dynamic.

The second observation differing from the model is found in NPQ steady state when calculated in constant light. The model in fact overestimated the steady state values in both Eiko and MinnGold (Figure 5 and 6). This might be an adaptation strategy. Since the constant regime is less stressful for the plants, it might be that less energy needs to be dissipated as heat. Since the model was calibrated to the fluctuating light data, a stressful

condition, it might be that the parameters regulating NPQ are set higher than necessary for the constant regime. To capture this difference, it would be probably necessary to introduce a framework regarding the adaptation of the plants based on their growing conditions.

Finally, the theoretical analysis of the model allowed to make some relevant conclusions. When calculating the cumulative values at steady state using Eiko's parameters in respect to different fluctuating light periods (Figure 8B), we found A and ETR steady state values to increase by reaching a maximum at 5 minutes fluctuating period, and then to decrease for fluctuating periods longer than 20 minutes. Therefore, it seems that a certain range of fluctuations of light is favourable for the cumulative steady state carbon assimilation, coherent to Graham, Nguyen, Burdyny, & Sinton (2017). This behaviour is confirmed with MinnGold parameters (Figure S1), but in this case we found much smaller changes in NPQ steady state, meaning probably that NPQ relaxation dynamics in MinnGold are faster than those in Eiko, this being opposed to what proposed by Sakowska et al. (2018). More in general, the understanding of the NPQ influence in regulating dynamic photosynthesis is still controversial. Two recent articles have in fact found an opposite trend in biomass accumulation when accelerating NPQ relaxation time (Garcia-Molina & Leister, 2020; Kromdijk et al., 2016).

Differences in the parameters

Since the model is a theoretical mathematical model, when referring to the values of the parameters it is relevant to look at the relative differences among varieties, whereas the absolute values might be not always coherent with the biology. This is though due to the calibration procedure in finding local minima, therefore other combinations of parameters are possible. Nevertheless, when looking at Table 1, almost all parameters are found to be

comparable among the two varieties, confirming the robustness of the model. Only three parameters differ, E^{i}_{PSII}, v_{ETR} and v_d. E^{i}_{PSII} identifies how much energy can be hold by the photosystem 2 and it represents the number of chlorophyll molecules in the chloroplast, which is known to be different for Eiko and MinnGold (Sakowska et al., 2018; Slattery, Vanloocke, Bernacchi, Zhu, & Ort, 2017). This parameter value therefore is reasonably much higher in Eiko than in MinnGold. Nevertheless, v_{ETR} and v_d are the velocity of activation of ETR and NPQ and are higher in MinnGold. This can be explained by the fact that even if MinnGold has a much lower number of chlorophyll molecules, this number is sufficient to have a responsive ETR and NPQ which can sustain a comparable carbon assimilation. In particular, both the model and the experimental data show MinnGold to be even more responsive to fluctuations of light, in fact the fluctuating light causes higher oscillations in ETR and A (Figure 2).

Comparison with other models

The model presented focuses on the limitations imposed by light reactions, due to the nature of the experiment conducted, therefore the downstream regulation is much simplified. The model therefore is not as comprehensive as preceding models (Bellasio, 2019; Morales et al., 2018) but it demonstrated that a macro representation of the processes is still able to capture well the dynamics found in photosynthesis and helps in unravelling gas exchange and fluorescence data. Furthermore, since the limited number of equations and related parameters, this model could become one of the building blocks of a photosynthesis model at higher scales, both leaf and canopy. Since there are already other system dynamics models, following the same procedure, focused on the dark reactions (Kirschbaum et al., 1997) and on stomatal conductance (S. Vialet-Chabrand et al., 2016), it would be interesting to combine our model with these existing models to

simulate the most dynamic environmental conditions thus allowing an upscaling. In fact, even if relevant canopy level photosynthesis models exist (Song & Zhu, 2012; Van Der Tol, Verhoef, Timmermans, Verhoef, & Su, 2009) none to our knowledge aims to capture the responses of photosynthesis to dynamic environmental conditions, since it would be too complicated with the available models.

Conclusions

We presented here and validated a new system dynamic model based on the light reactions of photosynthesis. Since plants are normally dealing with dynamic environmental conditions, it should be considered to introduce into models such processes in photosynthesis that are usually discarded in steady state models, such as the cyclic electron transport (that we represented in this model) and many other processes - as the water-water cycle, the malate shuttle and the other components of NPQ (Yamori, 2016) - which become limiting when conditions are unsteady.

Furthermore, even if proposed a model at leaf level, due to its simplicity, we aim the model to be one of the building blocks of a photosynthetic model at plant or even canopy scale. Upscaling both the models and the experiments is fundamental since translating these short term leaf scale results into the field is not straightforward (Kaiser et al., 2018; Matsubara, 2018). In particular, in this case we found fluctuations of light to not interfere in the performance of MinnGold in such a short-term analysis even if it is hypothesised that they might have an effect in the long term. Therefore, canopy level data and models become fundamental in unravelling the dynamic photosynthetic processes.

DECLARATIONS

Data availability

435 The datasets and the Matlab codes used during the current study are available from the
436 corresponding author on reasonable request.

437 **Conflict of interest**

438 The authors have no conflict of interest.

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List of Tables

Table 1. *State variables, fixed parameters and calibrated parameters of the model*

	Symbol	Description	Units	Value	
				Eiko	MinnGold
State	En_{PSII}	Energy in photosystem II (t=0)	µmol m^{-2}	0	
	E_z	Energy in PSII-zeax complex (t=0)	µmol m^{-2}	0	
	Z	Zeaxanthin activation level (t=0)	-	0	

	$NADP^{+¿¿}$	$NADP^+$ in chloroplast stroma (t=0)	-	5	
	$NADPH$	NADPH in chloroplast stroma (t=0)	-	5	
	R	Rubisco activation level (t=0)	-	0.001	
Fixed parameters	PAR	Photosynthetically active radiation	µmol $m^{-2}s^{-1}$	520 or 780	
	α	Absorption coefficient	-	0.78	0.54
Calibrated parameters	$c_¿$	Energy input coefficient	-	0.23	0.25
	$E^¿_{PSII}$	PSII energy carrying capacity	µmol m^{-2}	157.56	9.98
	v_{ETR}	Velocity of ETR	s^{-1}	0.78	11.56
	v_d	Velocity of energy dissipation	s^{-1}	0.08	7.00
	$E^¿_Z$	PSII-zeax complex energy carrying capacity	µmol m^{-2}	0.07	0.03
	v_{NPQ}	Velocity of NPQ	s^{-1}	70.58	53.87
	v_{za}	Maximum velocity of zeaxanthin activation	s^{-1}	0.07	0.01
	v_C	Maximum velocity of Calvin Cycle reactions	s^{-1}	11.75	13.04
	η_{NADPH}	Efficiency of NADPH	-	5.07	4.10
	$\eta_{NADP^{+¿¿}}$	Efficiency of $NADP^+$	-	0.89	0.75
	v_R	Maximum velocity of Rubisco activation	s^{-1}	$8.9 \cdot 10^{-4}$	$14 \cdot 10^{-4}$
	d	Maximum H^+ balance value	-	8.40	3.69
	c_y	Minimum necessary cyclic electron flow	-	-4	0

Figure Legends

Figure 1. *Conceptual diagram of the model. The model describes the phenomena occurring in a chloroplast. The six state variables are depicted by the white boxes. The three quantities considered in the simulations are non-photochemical quenching (NPQ), electron transport rate (ETR) and carbon assimilation (A). The dashed arrows describe influences. CEF is the cyclic electron flow transporting back electrons from photosystem I (PSI, not explicitly included in the model, represented in the small grey box) into the linear electron transport (ETR), whose energy*

is exploited to generate ATP (not represented) and influencing thylakoid pH and therefore the activation of Zeaxanthin. The cytoplasmic ΔpH is influenced by the ratio NADPH/NADP$^+$, which activates Rubisco.

Figure 2. *Fluorescence data coupled with gas exchange data in the fluctuating light regime for the two varieties Eiko (in dark green) and MinnGold (in light green)* ***A.*** *Data taken from all the experimental period (60 minutes)* ***B.*** *Focus on the fluctuations from the minute 45 to the 55th.*

Figure 3. Top. *Eiko data (in red) compared to model results (in black). The data are shown as means of three replicates (red continuous line) and their standard error (red shaded area around the mean value).* ***Bottom.*** *Parity plots for ETR, A and NPQ with related R^2.*

Figure 4. Top. *MinnGold data (in red) compared to model results (in black). The data are shown as means of three replicates (red continuous line) and their standard error (red shaded area around the mean value).* ***Bottom.*** *Parity plots for ETR, A and NPQ with related R^2.*

Figure 5. Top. *Eiko data (in red) compared to model results (in black) in constant light. The data are shown as means of three replicates (red continuous line) and their standard error (red shaded area around the mean value).* ***Bottom.*** *Parity plots for ETR, A and NPQ with related R^2.*

Figure 6. Top. *MinnGold data (in red) compared to model results (in black) in constant light. The data are shown as means of three replicates (red continuous line) and their standard error (red shaded area around the mean value).* ***Bottom.*** *Parity plots for ETR, A and NPQ with related R^2.*

Figure *7. Sensibility analysis of the model parameters for both MinnGold and Eiko. The parameters have been perturbed of ±5% around the value in Table 1 and the relative deviation from baseline simulation of the model output was calculated.*

Figure 8. A. *Varying fluctuating period of light in Eiko. Light is fluctuating every 30 seconds, 1 minutes (as in the experiment), and 4 minutes.* ***B.*** *Effect of varying fluctuating light on the steady state variable (cumulative value after 40 minutes). 0 fluctuating period means constant light.*

Supporting Information

Figure S1*. Effect of varying fluctuating light on the steady state variable (cumulative value after 40 minutes) in MinnGold.*

Figure S2. *Varying fluctuating intensity of light in Eiko. Light was either kept constant at 650 PPFD or fluctuating every minute at two different intensities: 650 ± 30% and 650 ± 50%*