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Dynamics of the energy flow through photosystem II under changing light conditions: a model approach

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Abstract. Several biochemical models of photosynthesis exist that consider the effects of the dynamic adjustment of enzymatic and stomatal processes on carbon assimilation under fluctuating light. However, the rate of electron transport through the light reactions is commonly modelled by means of an empirical equation, parameterised with data obtained at the steady state. A steady-state approach cannot capture the dynamic nature of the adjustment of the light reactions under fluctuating light. Here we present a dynamic model approach for photosystem II that considers the adjustments in the regulative non-photochemical processes. The model is initially derived to account for changes occurring at the seconds-to-minutes time-scale under field conditions, and is parameterised and tested with chlorophyll fluorescence data. Results derived from this model show good agreement with experimentally obtained photochemical and non-photochemical quantum yields, providing evidence for the effect that the dark reactions exert in the adjustment of the energy flows at the light reactions. Finally, we compare the traditional steady-state approach with our dynamic approach and find that the steady-state approach produces an underestimation of the modelled electron transport rate (ETR) under rapidly fluctuating light (1 s or less), whereas it produces overestimations under slower fluctuations of light (5 s or more).

Keywords: chlorophyll fluorescence, dynamic model, electron transport rate, fluctuating light, non-photochemical quenching, xanthophyll-cycle.

Introduction

Photosynthetic carbon assimilation is estimated with the help of biochemical models (Farquhar *et al*. 1980; Gross 1982; Hari *et al*. 1986; Pearcy *et al*. 1997; Allen and Pearcy 2000; Hari and Mäkelä 2003). These models include enzymatic and stomatal adjustments in the dark reactions of photosynthesis to follow changes in the carbon assimilation rate, where light intensity is the independent input variable. However, these approaches do not integrate the dynamic nature of the light reactions of photosynthesis, and the electron transport rate (ETR) is generally modelled by an empirical equation, where the parameters are derived from the steady-state light-response curve of photosynthesis. To our understanding, this steady-state approach for the light reactions may encounter problems under conditions of natural fluctuating light. In this study we address this question by developing a dynamic model for photosystem II (PSII), and using the model to compare a dynamic approach with the traditional steady-state approach.

Due to the passing of clouds and movement of sunflecks inside and below the canopy, plant leaves in the field are exposed to frequent, short-term fluctuations in the

Abbreviations used: α, Parameter for the efficiency of light absorption; a_c, effective absorption area of a chlorophyll molecule (m^2); c, rate of light capture (photons s^{-1}); $Chla^{ON}$, $Chla^{OFF}$, $Chlb^{ON}$, $Chlb^{OFF}$, number of chlorophyll a/b molecules carrying an exciton (ON), or in the ground state (OFF); Chl_T, total number of chlorophyll molecules in our model system; d, rate of excitation loss by constitutive heat dissipation processes (excitons s^{-1}); E, efficiency of NPQ; ETR, electron transport rate; f, rate of excitation loss by chlorophyll fluorescence emission (excitons s^{-1}); γ, parameter representing the rate constant of re-oxidation of the quinone-equivalent pool (s^{-1}); I, light intensity (μmol photons m^{-2} s^{-1}); I_{MB}, constant I of the modulating beam; φ_a, overall efficiency of energy capture by chlorophyll molecules; k_f, k_d, k_n, k_p, parameters representing the rate constants associated with fluorescence emission, constitutive heat dissipation, heat dissipation by NPQ, and photochemistry, respectively (s^{-1}); λ_b, λ_r, parameters related to the building and relaxation of NPQ; NPQ, non-photochemical quenching; n, rate of excitation loss by NPQ (excitons s^{-1}); p, rate of excitation loss by photochemistry (excitons s^{-1}); Q, oxidised fraction of the quinone-equivalent pool; Q^{ON}, Q^{OFF}, number of quinone-equivalents reduced (ON) or oxidised (OFF); S^{ON}, S^{OFF}, number of active and inactive quenching sites.

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light environment. These fluctuations necessitate a series of acclimation processes in the leaf to retain a high photochemical efficiency while minimising the risks of photo-oxidation associated with excess light (Demmig-Adams and Adams 1996; Gilmore 1997; Eskling *et al*. 2001). Short-term acclimation processes operate in leaves over the time scale of seconds to minutes and include both structural and biochemical adjustments related to the movement of light-harvesting complexes or to concentration of specific xanthophyll pigments (Huner *et al*. 1998; Kruse 2001; Nixon and Mullineaux 2001).

When a photon is captured by a chlorophyll *a* (Chla) or chlorophyll *b* (Chlb) molecule of PSII, the energy of the exciton may be directed to different pathways. In a dark-acclimated and non-photoinhibited leaf, the exciton can be transferred to another pigment molecule or to the primary acceptor pheophytin and subsequently used in photochemistry. Alternatively, the exciton may also be emitted as fluorescence, dissipated as heat by internal conversion (constitutive heat dissipation), or may experience intersystem crossing and form a chlorophyll triplet state (Parson and Nagarajan 2003). All these energy-consuming processes compete with each other and the overall rate constant of excitation decay is the sum of each of the process rate constants (Govindjee 2004).

In a light-acclimated leaf under illumination, the rate of ATP and NADPH formation by the light reactions may exceed their consumption in the dark reactions, increasing the ratio of chloroplast stromal [ATP] / [ADP], leading to accumulation of protons in the thylakoid lumen and increasing the redox state of the plastoquinone pool (Nixon and Mullineaux 2001). As a result, the rate of charge separation in the reaction centre of PSII decreases, increasing the yields of the alternative energy-consuming processes, including chlorophyll triplet states formation. The latter can react with molecular oxygen resulting in highly reactive singlet-oxygen, which may cause oxidative damage to the thylakoid membranes (Demmig-Adams and Adams 1996; Havaux and Niyogi 1999; Lawlor 2001). Therefore, to avoid damage when the photochemical pathway saturates, protective mechanisms are needed to deactivate the excess excitons and minimise the rate of formation of hazardous chlorophyll triplet states.

The main energy-consuming processes in PSII (see Fig. 1) are often classified into photochemical (when the energy is used to reduce the plastoquinone pool) and non-photochemical (when the energy is dissipated via another pathway) processes. Non-photochemical processes include fluorescence emission, constitutive heat dissipation, formation of chlorophyll triplet states and light-induced heat dissipation. The latter is often referred to in the literature as non-photochemical quenching (NPQ) (Krause and Weis 1991; Demmig-Adams *et al*. 1996; Müller *et al*. 2001). NPQ processes can be thought of as protective mechanisms of the plant to cope with excess excitation and they are classified into three types (see Fig. 1).

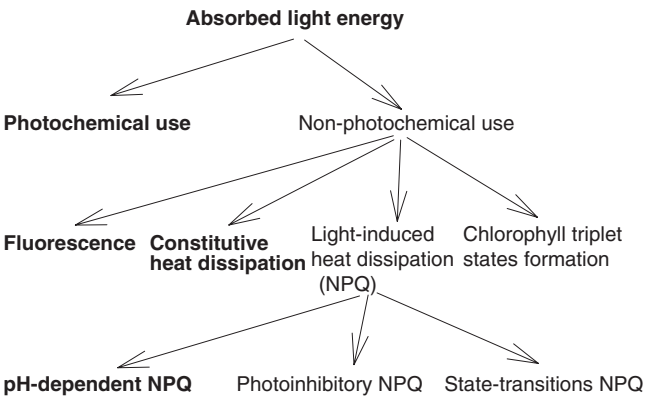

Fig. 1. Schematic classification of the alternative processes where absorbed light energy can be utilised. In the present work we study the dynamic variations in the photochemical usage, fluorescence, constitutive heat dissipation and pH-dependent NPQ.

The first type of NPQ is the pH-dependent NPQ, regulated by lumen pH and the xanthophyll cycle pigments (Demmig-Adams *et al.* 1996; Eskling *et al*. 2001). When lumen pH decreases due to saturation of the dark-reactions, some PSII proteins are protonated and violaxanthin molecules released and de-epoxidised to antheraxanthin and zeaxanthin. Zeaxanthin then binds to PSII proteins, where it forms a quenching complex that favours the dissipation of excitation energy as heat. This process has been suggested to be bimodal, where an initial rapid component of NPQ is caused by the protonation of PSII proteins and a slower component appears after zeaxanthin is bound to the protonated proteins (Gilmore 1997; Müller *et al*. 2001; Morosinotto *et al*. 2003). However, the exact mechanism of heat dissipation has not yet been identified (Bruce and Vasil'ev 2004). Overall, changes in pH-dependent NPQ occur within minutes after a change in light intensity (Krause and Weis 1991; Müller *et al*. 2001).

The second type of NPQ is regulated by state transitions and appears when the absorption of light in PSII is higher than in PSI. Under these conditions, some of the weakly bound light-harvesting complexes from PSII (LHCII) migrate to regions richer in PSI, balancing the absorption rates of both photosystems (Haldrup *et al*. 2001; Kruse 2001). This process operates on a time scale of minutes (Haldrup *et al*. 2001; Kruse 2001) and due to the ability of the reaction centre of PSI to dissipate excitons as heat, even when it is oxidised (Krause and Weis 1991; Dau 1994), the process is classified as NPQ because the LHCIIs that are newly bound to PSI quench the fluorescence emission.

Finally, the third type of NPQ is induced by photoinhibition and found after prolonged exposure to excess light. Photoinhibitory NPQ is a slow, reversible process (i.e. hours to days) related both to the damage of the D1 protein in

the reaction centre (Aro *et al*. 1993) and to a sustained form of pH-dependent NPQ (Demmig-Adams *et al*. 1998; Krause and Jahns 2003).

Chlorophyll fluorescence techniques are widely used to study the dynamics of the energy-consuming processes in PSII, described above. From the standard fluorescence yield equation, where the rate constant associated with fluorescence (k_f) is divided by the sum of the rate constants of each of the energy-consuming processes in PSII (Kitajima and Butler 1975; Govindjee 2004), one can derive equations to calculate the quantum yield of photochemistry (Genty *et al*. 1989), the overall yield of thermal dissipation (Demmig-Adams *et al*. 1996), or the energy fluxes into the main energy-consuming processes in PSII (Laisk *et al*. 1997; Hendrickson *et al*. 2004; Kramer *et al*. 2004). Following the same fundamental assumptions as for the derivation of these equations, we derive here a dynamic equation for the continuous estimation of the yields and energy fluxes of each of the PSII energy-consuming processes.

The aim of this study was to develop a dynamic model that describes the continuous adjustments of the energy flow in photosystem II to changing light conditions occurring at a time scale of seconds to minutes, able to continuously estimate the energy partitioning into photochemistry and non-photochemical processes. The model is intended to cover the existing gap between the integration of the dynamics of the light reactions into the modelling of photosynthesis in field conditions.

Studying the energy flow in PSII

Structure of the model

In order to analyse the adjustments of PSII as a response to variations in light intensity occurring in field conditions during the course of the day, we concentrate initially on the time scale of seconds to minutes. Consequently, we consider a population of *N* PSII units where each PSII has a given amount of Chl*a*, Chl*b* and xanthophyll molecules, as well as associated quinone and plastoquinone pools. We assume that apart from the xanthophylls, all other stoichiometries remain constant at this time scale. The quinone and plastoquinone pools are treated together since the analysis of the redox state of the different quinone forms requires a much faster temporal scale and falls out of the scope of the present study. Consequently, we represent the different forms as a pool of quinone-equivalent molecules capable of accepting one electron each. Furthermore, for the derivation of our model dynamic equations, we consider the case where the system of *N* PSII units follow a lake-type organisation model (Kitajima and Butler 1975; Dau 1994; Bernhardt and Trissl 1999) and excitons can move freely between PSII units.

Processes

In the proposed structure (Fig. 2), pigment molecules and quinone-equivalents shift from an OFF to an ON state after accepting a photon, an exciton, or an electron, returning to the OFF state after losing it. Excitation transfer between

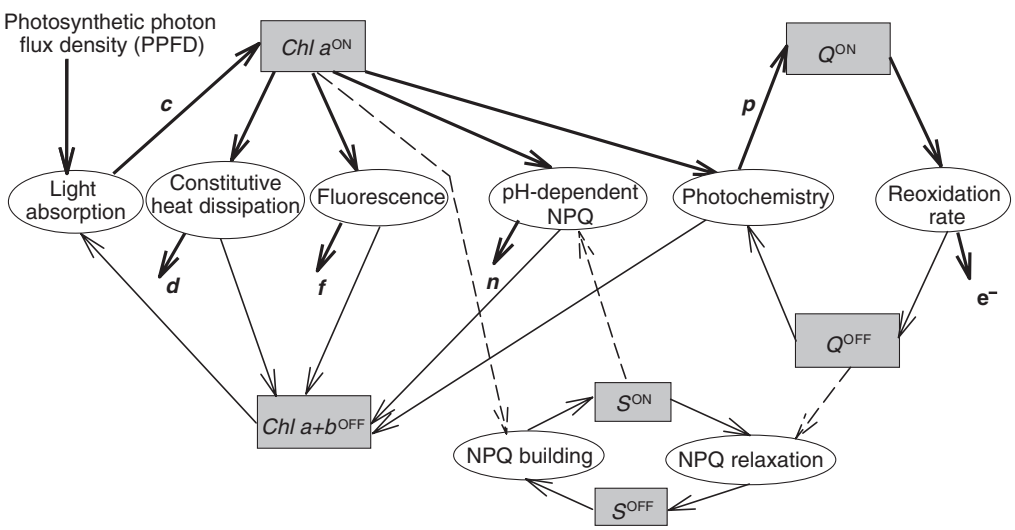

Fig. 2. Proposed PSII model. Rectangular boxes represent amounts of a substance in a determined state, and ellipsoids represent processes that have associated rate constants. Thick arrows represent the flow of energy, and broken arrows indicate an effect. $Chla^{ON,OFF}$ represents the number of chlorophyll *a* molecules in the model system carrying an exciton (ON) or in the ground state (OFF); $Chla{+}b^{OFF}$ is the sum of chlorophyll *a* and *b* molecules in the system in the ground state; Q^{ON}, Q^{OFF} are the number of quinone-equivalents in reduced or oxidised state, respectively; S^{ON}, S^{OFF} are the number of active or inactive quenching sites, respectively; and *d*, *f*, *n*, and *p* are the rates of constitutive heat dissipation, fluorescence, non-photochemical quenching processes and reduction of the quinone-equivalents, respectively.

Chl*b* and Chl*a* is unidirectional and occurs in the order of picoseconds (Dau 1994; Amerongen and Dekker 2003). Thereby, at our time scale, Chl*b* operates only in light absorption and excitons are always assumed to be located in Chl*a*. From Chl*a* the exciton can follow different paths; it can be re-emitted as fluorescence, lost as constitutive heat, dissipated by non-photochemical quenching processes, or captured by an oxidised quinone-equivalent. Finally, the reduced (ON) quinone-equivalents are re-oxidised by the downstream electron transport, returning to the OFF state.

Assumptions

Initially we consider the formation of chlorophyll triplet states as a part of the constitutive heat dissipation component. Furthermore, photoinhibitory NPQ is not yet included in the model, since it takes place at a slower time scale. And finally, the state-transitions form of NPQ, which is much smaller than the pH-dependent form of NPQ (Krause and Weis 1991), is not considered here for simplicity reasons.

Dynamic equations

The rate of light capture (c) is determined by the light intensity (I), and the number of chlorophyll molecules in the ground state:

$$c = \alpha I(Chla^{\mathrm{OFF}} + Chlb^{\mathrm{OFF}}), \tag{1}$$

where I is the photosynthetic photon flux density (PPFD) at the leaf surface ($\mu mol\, m^{-2}\, s^{-1}$), $Chla^{\mathrm{OFF}}$ and $Chlb^{\mathrm{OFF}}$ are numbers of Chl*a* and Chl*b* in the ground state calculated from the selected stoichiometry and number of PSII units (see the Materials and methods section), and α is an efficiency parameter (m^2 chlorophyll $molecule^{-1}$).

The rate of constitutive heat dissipation (d) (excitons s^{-1}) is linearly proportional to the number of excitons ($Chla^{\mathrm{ON}}$):

$$d = k_d Chla^{\mathrm{ON}}, \tag{2}$$

where k_d is a parameter known as the rate constant of heat dissipation. The rate of fluorescence (f) is:

$$f = k_f Chla^{\mathrm{ON}}, \tag{3}$$

where k_f is a parameter known as the rate constant of fluorescence.

The efficiency of NPQ varies along the light conditions, increasing or decreasing with the light intensity. Changes in the efficiency of NPQ are introduced into the model with the variable NPQ-efficiency, denoted with E, where E changes from zero (NPQ is absent) to one (NPQ operates at maximum efficiency). Thus, the rate of NPQ processes (n) is calculated as:

$$n = k_n E\, Chla^{\mathrm{ON}}, \tag{4}$$

where k_n is a parameter that corresponds to the rate constant associated with NPQ processes.

The efficiency of photochemistry varies according to the redox state of the plastoquinone pool: when the pool of plastoquinone is totally reduced the rate of photochemistry is zero and this rate is maximum when the plastoquinone pool is totally oxidised. These variations are included in our model as the oxidised fraction of the quinone-equivalent pool, denoted by Q, analogous to the commonly used fraction of open reaction centres (Barber *et al*. 1989; Kramer *et al*. 2004). The fraction Q ranges between zero (all the quinone-equivalents reduced) and one (all the quinone-equivalents oxidised). Thus, the rate of photochemistry (p) is:

$$p = k_p Q\, Chla^{\mathrm{ON}}, \tag{5}$$

where k_p is a parameter that corresponds to the rate constant associated with the photochemical process.

Combining eqns 1–5, we obtain the differential equation for the number of excitons ($Chla^{\mathrm{ON}}$):

$$\frac{dChla^{\mathrm{ON}}}{dt} = \alpha I(Chla^{\mathrm{OFF}} + Chlb^{\mathrm{OFF}}) - k_f Chla^{\mathrm{ON}} - k_d Chla^{\mathrm{ON}} - k_n E\, Chla^{\mathrm{ON}} - k_p Q\, Chla^{\mathrm{ON}}. \tag{6}$$

Estimation of rate constants

Variations in the efficiency of NPQ (E), and in the fraction of oxidised quinone-equivalents (Q), take place around nine orders of magnitude more slowly than changes in the number of excitons ($Chla^{\mathrm{ON}}$). Thus, over a time scale of milliseconds to seconds the fast processes involved in changes in $Chla^{\mathrm{ON}}$ can be considered to be in the steady state and $dChla^{\mathrm{ON}}/dt = 0$. Under natural light there is no exciton accumulation in PSII and a single exciton can be found per PSII at any point in time (Dau 1994), thereby the sum of $Chla^{\mathrm{OFF}}$ and $Chlb^{\mathrm{OFF}}$ remains approximately constant and equal to the total number of chlorophyll molecules in our system (Chl_T). The number of excitons ($Chla^{\mathrm{ON}}$) can then be solved from eqn 6 as:

$$Chla^{\mathrm{ON}} = \frac{\alpha I\, Chl_T}{k_f + k_d + k_n E + k_p Q}. \tag{7}$$

Conventional modulated fluorometers include an actinic light source and a modulated beam of constant light intensity (I_{MB}), the latter serves to record the variations in the fluorescence yield induced by the actinic light. Subsequently, by combining eqns 3 and 7, the rate of chlorophyll fluorescence emission (f), proportional to the fluorescence signal (F) recorded by a fluorometer, is obtained as:

$$f = \frac{k_f\, \alpha\, I_{MB}\, Chl_T}{k_f + k_d + k_n E + k_p Q}, \tag{8}$$

analogous to the fluorescence yield equation (in that case, divided by the light absorption term), which can be found

in diverse formulations depending on the application (Barber *et al*. 1989; Laisk *et al*. 1997; Parson and Nagarajan 2003; Govindjee 2004; Hendrickson *et al*. 2004; Kramer *et al*. 2004). Next, after keeping a leaf in the dark for long enough, we assume that all the pH-dependent NPQ has relaxed and $E = 0$. In addition, the quinone-equivalents are fully oxidised and $Q = 1$. Consequently the minimum fluorescence rate (f_o) under beam light, can be expressed as:

$$f_o = \frac{k_f \alpha I_{MB} Chl_T}{k_f + k_d + k_p}, \quad (9)$$

and after a saturating light pulse, which completely reduces the quinone-equivalent pool ($Q = 0$), the maximum fluorescence rate (f_m) is:

$$f_m = \frac{k_f \alpha I_{MB} Chl_T}{k_f + k_d}. \quad (10)$$

Conversely, after exposing a leaf to excessive light (i.e. light that saturates the energy consumption in the dark reactions) for several minutes at ambient temperatures we assume that the maximum efficiency of pH-dependent NPQ processes is reached (E_{max}), and by then applying a saturating light pulse we obtain the maximum fluorescence rate in the light (f'_m), as:

$$f'_m = \frac{k_f \alpha I_{MB}\, Chl_T}{k_f + k_d + E_{max} k_n}. \quad (11)$$

Next, substituting the previous fluorescence rates (f) with their proportional measured modulated fluorescence signal (F), and combining eqns 9 and 10, we obtain the rate constant for photochemistry (k_p) as:

$$k_p = \frac{F_m}{F_o}(k_d + k_f) - (k_d + k_f), \quad (12)$$

and combining eqns 10 and 11, we obtain the rate constant for non-photochemical quenching processes (k_n) as:

$$k_n = \frac{\frac{F_m}{F'_m}(k_d + k_f) - (k_d + k_f)}{E_{max}}. \quad (13)$$

At wavelengths greater than 700 nm (which most portable modulated fluorometers record) the recorded fluorescence signal includes a significant component from PSI, which accounts for ~6% of the fluorescence at the F_m state for C_3 plants (Pfündel 1998). It is not clear how, if at all, the pH-dependent and xanthophyll cycle-mediated NPQ affects PSI, or if it operates in a way similar to PSII (Eskling *et al*. 2001; Morosinotto *et al*. 2003). In this study, as a first approximation, we considered that the pH-dependent NPQ operates similarly in both photosystems, thus, the component of PSI fluorescence will correspond to the 6% of the prevailing F_m, or F'_m in the case of an illuminated leaf. Subsequently, the effect of PSI fluorescence cancels out in eqn 13. However, for obtaining the k_p associated with PSII, eqn 12 needs to be reformulated as:

$$k_p = \frac{F_m - 0.06F_m}{F_o - 0.06F_m}(k_d + k_f) - (k_d + k_f). \quad (14)$$

Next, if f_m is the maximum fluorescence rate and c the rate of light absorption, combining eqns 1 and 10 (with $I = I_{MB}$) the maximum fluorescence yield ΦF_m can be expressed as:

$$\Phi F_m = \frac{f_m}{c} = \frac{k_f}{k_f + k_d}. \quad (15)$$

Since the maximum fluorescence yield (ΦF_m) for chlorophyll molecules associated with PSII *in vivo* is 10% (Barber *et al*. 1989) and the rate constant for k_f is $6.7 \times 10^7\ s^{-1}$ (Rabinowich and Govindjee 1969), the rate constant k_d can be estimated from eqn 15.

Dynamic equations for NPQ and reoxidation of the quinone-equivalents

Variations in pH-dependent NPQ can be explained as a function of the combined effect of both the protonation of binding sites in PSII proteins and the amount of zeaxanthin and antheraxanthin bound to these sites (Gilmore 1997; Morosinotto *et al*. 2003). As a first approximation we define a quenching site as a site where NPQ heat dissipation may take place. These sites can be in an active state, when they are actively dissipating excitation energy as heat, or in an inactive state. We denote the number of quenching sites in the active state by S^{ON} and the number of inactive sites by S^{OFF}. In our model, we use the fraction of active quenching sites [$S^{ON}/(S^{ON} + S^{OFF})$] (See Fig. 2), as equivalent to the efficiency of NPQ (E). The rate of violaxanthin / zeaxanthin interconversion and the protonation of PSII proteins, are driven by the thylakoid lumen pH (Demmig-Adams *et al*. 1996; Eskling 2001; Müller *et al*. 2001). In this study, thylakoid lumen pH is not yet represented as a variable in the calculations, however, we consider that under physiological conditions variations in the lumen pH are proportional to variations in $Chla^{ON}$ (related to the excitation lifetime), and to the oxidised fraction of the quinone-equivalent pool (Q). This approximation is justified because large excitation lifetime results come directly from over-reduced states of the plastoquinone pool, which at the same time is correlated with the accumulation of protons in the thylakoid lumen (Nixon and Mullineaux 2001).

We assume that the building process of NPQ (activation of inactive sites) is proportional to the product of $Chla^{ON}$ and S^{OFF}, and the relaxation process (inactivation of active sites) is proportional to the product of Q and S^{ON}. With these assumptions the variations in the fraction of active

quenching sites and correspondingly variations in E are calculated as:

$$\frac{dE}{dt} = \frac{\lambda_b \, Chla^{ON} \, S^{OFF}}{S^{OFF} + S^{ON}} - \frac{\lambda_r \, Q \, S^{ON}}{S^{OFF} + S^{ON}}, \qquad (16)$$

where the first term of the equation refers to the building of NPQ and the second term to the relaxation; λ_b and λ_r are experimentally obtained parameters associated with the building and relaxation processes, respectively, and the denominator in both terms is the total number of quenching sites.

The rate of electron transport to PSI determines the rate at which the plastoquinone pool is re-oxidised. And the rate of photochemistry (p) determines the rate at which the plastoquinone pool is reduced. To calculate the variations in the oxidised fraction of the quinone-equivalent pool (Q), we define Q^{ON} and Q^{OFF} as the number of quinone-equivalents in a reduced and oxidised state, respectively. We assume that the re-oxidation process is proportional to Q^{ON}, and the reduction process corresponds to p (eqn 5), as:

$$\frac{dQ}{dt} = \frac{\gamma \, Q^{ON}}{Q^{ON} + Q^{OFF}} - \frac{k_p \, Q \, Chla^{ON}}{Q^{ON} + Q^{OFF}}, \qquad (17)$$

where the first term of the equation refers to the re-oxidation of the plastoquinone pool (increase in Q) and the second to the reduction of the plastoquinone pool (decrease in Q), and γ is a parameter representing the rate constant for the re-oxidation of the quinone-equivalent pool.

Materials and methods

Plant material

We used three European alder trees [*Alnus glutinosa* (L.) Gaertn.], 7 m tall and 7 years old, located in the Viikki Science Campus of the University of Helsinki for performing the experiments during 7–9 July 2004. Leaves from the lower canopy facing south were used for all experiments.

Estimation of the rate constants for photochemical and non-photochemical quenching processes

Four leaves per tree were dark-acclimated *in situ* for 2 h with special leaf-clips (Hansatech Ltd, Kings Lynn, UK). Leaves with the clip attached were then excised and F_o and F_m were measured with a portable chlorophyll fluorometer (FMS-2, Hansatech Ltd). F_m was obtained with a saturating light pulse of 3500 μmol m^{-2} s^{-1} of PPFD at the leaf surface. Subsequently, the leaf was provided with a PPFD of 2250 μmol m^{-2} s^{-1} for 6 min to attain E_{max}, and F'_m was measured (longer periods did not result in lower F'_m, data not shown).

For the estimation of k_n and k_p, E_{max} was set to 0.8 as a first approximation, as an equivalent of the maximum de-epoxidation rate for most plants (Eskling *et al*. 2001). We estimated k_n and k_p for alder leaves from eqns 13 and 14, as the average value of the four measurements in each of the three trees ($n = 12$).

Estimation of the parameters associated with NPQ building and relaxation

In a similar way, after 2 h of dark-acclimation, leaves with the clips attached were excised and F_o and F_m were measured. Following this measurement the leaves were supplied with different light intensities for 400 s (0, 200, 1200 and 1800 μmol m^{-2} s^{-1}). Subsequently, the actinic light was disconnected and the leaf left to recover in the dark for 450 s. The dynamics of NPQ were followed by applying a series of saturating pulses during both the induction and recovery periods. After excision all fluorescence measurements were carried out indoors at 20°C.

The parameters λ_b and λ_r were derived from the measurements at a PPFD of 1200 μmol m^{-2} s^{-1}. The parameter λ_b corresponded to the value at which modelled non-photochemical yield or n/c (See eqns 1 and 4), and experimental average NPQ yield, calculated according to Hendrickson *et al*. (2004), were equal after 400 s of illumination. Likewise, the value of λ_r fulfilled the condition that the regression line between modelled and experimentally obtained NPQ values during the relaxation period was of the form $x = y$ (modelled = measured).

Estimation of the parameter representing the re-oxidation of the quinone-equivalent pool

The parameter γ was determined from the measurements at 1200 μmol m^{-2} s^{-1}, as the value at which modelled photochemical yield or p/c (See eqns 1 and 5), and experimentally obtained average photochemical yield after 400 s of illumination (Genty *et al*. 1989; Hendrickson *et al*. 2004) were equal.

Estimation of the light absorption efficiency parameter

From the effective absorption area of a chlorophyll molecule, denoted here as a_c, one can estimate that under a PPFD of 2000 μmol m^{-2} s^{-1}, a chlorophyll molecule will theoretically be hit by photons at a rate of 46 s^{-1}. However, only two or three photons are captured by a chlorophyll molecule in 1 s under high light conditions (Lawlor 2001). Therefore as a first approximation we estimate the overall efficiency of energy capture by chlorophyll molecules, denoted here by φ_a, as 0.05 (5%).

The efficiency parameter α (eqn 1) is expressed as:

$$\alpha = \varphi_a \, a_c \, 6.023 \times 10^{17}, \qquad (18)$$

where φ_a is 0.05, a_c equals to 3.8×10^{-20} m^2 (Lawlor 2001) and 6.023×10^{17} is a conversion factor to transform micromoles of photons into absolute units.

Model stoichiometry

For the model simulations each PSII was assumed to have 148 Chl*a*, 82 Chl*b*, and 1 xanthophyll molecule (Amerongen and Dekker 2003, and references therein), as well as 1 quinone A (Q_A), 1 quinone B (Q_B), and 10 plastoquinone molecules (Govindjee 2004). The charge in Q_A was assumed to be in equilibrium with the reaction centre, and since Q_B and plastoquinone can both accept two electrons, the total number of electrons needed to fully reduce all the plastoquinone pool is 22, thus, we proposed that each PSII had 22 quinone-equivalent molecules associated with it. The number of quenching sites (S) per PSII was preliminary set equal to the number of xanthophyll molecules. And the number (N) of PSII units used in the simulations was 5000.

Results and discussion

Rate constants and parameters

Alder leaves used in the experiments could be considered to be non-photoinhibited and after 2 h of dark acclimation, when pH-dependent NPQ can be considered non-existent, maximum quantum yield of PSII (F_v/F_m) was 0.825 ± 0.006 (mean ± standard error). The rate constants and parameters associated with each of the energy-consuming processes in PSII are shown in Table 1.

Table 1. Rate constants associated with fluorescence (k_f), constitutive heat dissipation (k_d), non-photochemical quenching (k_n), photochemistry (k_p), parameter values for the building (λ_b) and relaxation of NPQ (λ_r) and for the rate of reoxidation of the plastoquinone pool (γ)

Values of k_n and k_p are measured means ± s.e. ($n = 12$). k_f from Rabinowich and Govindjee (1969), k_d calculated from eqn 15

Rate constant or parameter	Value
k_f (s^{-1})	6.7×10^7
k_d (s^{-1})	6.03×10^8
k_n (s^{-1})	$2.92 \pm 0.12 \times 10^9$
k_p (s^{-1})	$4.94 \pm 0.26 \times 10^9$
λ_b	0.0087
λ_r	835
γ	2.74

The rate constants of constitutive heat dissipation (k_d) and fluorescence (k_f) are commonly assumed to remain constant. Accordingly, we defined (eqns 13 and 14) the rate constants associated with NPQ (k_n) and to photochemistry (k_p), and assumed that they also remain constant at a time scale of seconds to minutes. The rate constant k_n represents the maximum rate of heat dissipation by NPQ processes, for a given size of the xanthophyll-cycle pool and structural properties of PSII. Similarly, the rate constant associated with photochemistry, k_p, represents the maximum rate of photochemistry in the absence of photoinhibition and it is directly proportional to the well known $(F_m - F_o)/F_m$ fluorescence parameter, since they are calculated from the same data (see eqn 14). Our assumptions and the following results can be utilised for the study of short-term variations in the energy flow at PSII in non-photoinhibited leaves. To apply this model to the study of long-term acclimations (e.g. Krause *et al*. 2004) one should include, among others, variations in the chlorophyll contents, which affect the absorption of light (eqn 1), changes in the xanthophyll pigment pool and structural rearrangements at the thylakoid membrane, which affect the maximum rate of heat dissipation (k_n) (eqn 4), and the effect of an inactive or photoinhibited fraction of reaction centres, which decrease the maximum rate of photochemistry (k_p) (eqn 5).

Energy flow through PSII after varying the light intensity

The model reproduced well the slow dynamic build-up and relaxation of NPQ at the light levels of 1200 and 1800 $\mu mol\, m^{-2}\, s^{-1}$ (see Fig. 3*A*, *B*). Measured values of photochemical yield were calculated from fluorescence data according to Genty *et al*. (1989) and NPQ yields were estimated from fluorescence data according to Hendrickson *et al*. (2004). The comparison to the control treatments (PPFD = 0) indicated that variations observed in the fluorescence yields under illumination and subsequent dark recovery could be fully attributed to the effect of light. When leaves were illuminated after dark acclimation, measured

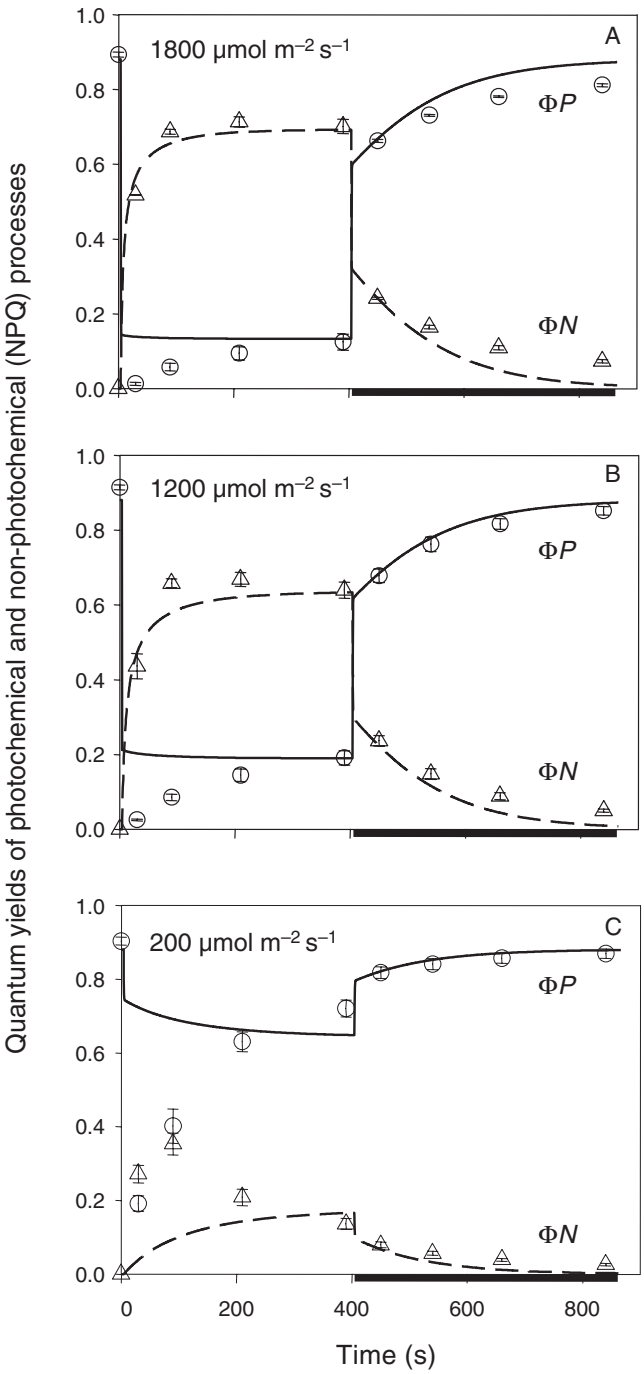

Fig. 3. Measured and modelled quantum yields of photochemistry and NPQ in dark-acclimated alder leaves after illumination at different light levels. Solid lines represent modelled photochemical yield (ΦP), dashed line represent modelled NPQ (ΦN); circles and triangles represent measured ΦP and ΦN, respectively. Error bars are s.e. ($n = 3$). ΦP was calculated from Genty *et al*. (1989), modified to exclude the PSI fluorescence component, $\Phi P = [(F_m - 0.06F_m) - (F - 0.06F_m)]/(F_m - 0.06F_m)$, and the yield of NPQ processes was calculated according to Hendrickson *et al*. (2004) as $\Phi N = [(F_s/F'_m) - (F_s/F_m)]$. Thick horizontal bar indicates dark periods (PPFD = 0.001 $\mu mol\, m^{-2}\, s^{-1}$), otherwise the illumination was as indicated in the figure.

values of the photochemical yield reached a minimum and recovered subsequently until a higher steady-state value was attained (Fig. 3). This behaviour may be attributed to the enzymatic activation of the dark reactions and stomatal opening after the lights are switched on (Pearcy *et al*. 1997; Allen and Pearcy 2000; Maxwell and Johnson 2000). Furthermore, this pattern in the photochemical quantum yield seems to produce a response in the measured yield of NPQ, which initially increases to a higher level and decreases later when photochemistry is activated (Fig. 3*A–C*). These observations show the feedback of the dark reactions on the energy partitioning at the light reactions. Such an interaction could be further studied with our model by linking it to the dark reactions through the rate of re-oxidation of the plastoquinone pool (γ).

Interestingly, at low light intensities (Fig. 3*C*) the model largely underestimated the yield of NPQ. First, this could be evidence of the role of state-transitions NPQ under low light conditions, which were not included in our model. State-transitions have been found to play a significant role in quenching the chlorophyll fluorescence under low light conditions (Krause and Weis 1991; Haldrup *et al*. 2001; Kruse 2001), whereas under high light intensities phosphorylation of LHCII (state-transitions) apparently does not take place (Rintamäki *et al*. 1997). Second, the momentary underestimation of NPQ (Fig. 3*C*) could also imply that under low light, the effect of the fast-protonation of PSII proteins probably accounts for a greater share of the total NPQ than under higher light intensities. This would be in agreement with the suggestion that at least two light-dependent processes are involved in the pH-dependent NPQ (Gilmore 1997; Müller *et al*. 2001).

In Fig. 4 we present the result of a simulation with our model, which produces continuous estimates for all PSII-associated energy-consuming processes: photochemistry, NPQ, constitutive heat dissipation and fluorescence. However, the modelled yield of fluorescence differs from measured values during changes in light intensity (data not shown). The main cause for this discrepancy is that in the derivation of the model equations we assumed a perfect connectivity between PSII units, allowing exciton transfer from closed to open reaction centres, when in fact a limited degree of connectivity exists (Krause and Weis 1991; Dau 1994; Bernhardt and Trissl 1999). In spite of this, since the parameterisation of our model was done with fluorescence data obtained in either the F_o or F_m state, when all reaction centres are open or closed, respectively, the exciton transfer from closed to open reaction is by definition absent. Consequently, under these conditions the degree of connectivity or type of organisational model has no implication on the level of fluorescence (Kitajima and Butler 1975; Dau 1994) and we can say that the photochemical and non-photochemical yield estimates of our model are not affected by the lake model assumption.

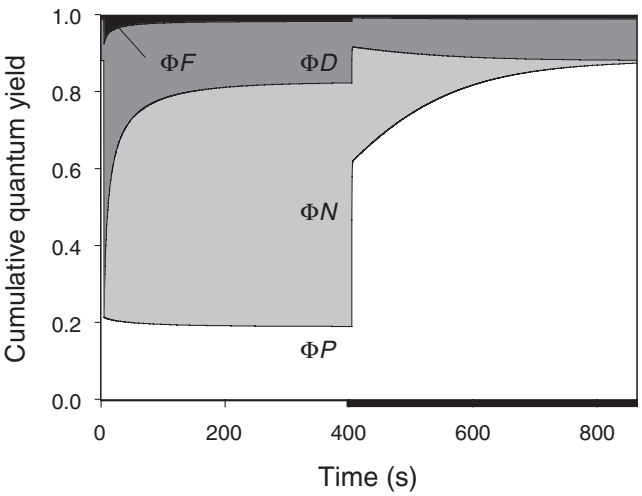

Fig. 4. Modelled development of the yields of fluorescence (Φ*F*), constitutive heat dissipation (Φ*D*), non-photochemical quenching processes (Φ*N*), and photochemistry (Φ*P*) in previously dark-acclimated alder leaves. Thick horizontal bar indicates dark period (PPFD = 0.001 μmol m^{-2} s^{-1}), otherwise the illumination was 1200 μmol m^{-2} s^{-1}.

The results show that when the light intensity varies, changes in the yields of the processes are not instantaneous and it takes several minutes before a new steady-state level is attained (Fig. 4), evidence of the slow dynamic characteristics of NPQ (Demmig-Adams and Adams 1996; Maxwell and Johnson 2000), as well as changes in the capacity of the dark reactions (Pearcy *et al*. 1997; Allen and Pearcy 2000; Maxwell and Johnson 2000).

Dynamic and steady-state approaches

The pattern of the steady-state electron transport rate (ETR) under different flashing light regimes has fundamental differences with our dynamic approach (Fig. 5). The steady-state approach shifts immediately to the higher or lower ETR according to the PPFD. In contrast, we observe a different pattern of variation in our dynamic estimates. At the time scale of hundreds of milliseconds the ETR varies after a change in light intensity due to the gradual reduction or oxidation of the plastoquinone pool, causing the rapid increase or decrease in the ETR (Fig. 5), whereas at a longer time scale (tenths of seconds, minutes) one can observe gradual changes in the ETR caused by the building or relaxation of NPQ (see the variation in the maximum ETR levels estimated in e.g. Fig. 5*C*, *F*). Also, our dynamic approach produces different estimates depending on the recent light history of the leaf, thus, in leaves acclimated to higher light (Fig. 5*A–C*), the dynamic approach produces lower ETR compared with the steady-state approach during the periods of low light, since the NPQ is only slowly acclimating to the new light conditions. Conversely, in leaves acclimated to the low light levels (Fig. 5*D–F*), the dynamic

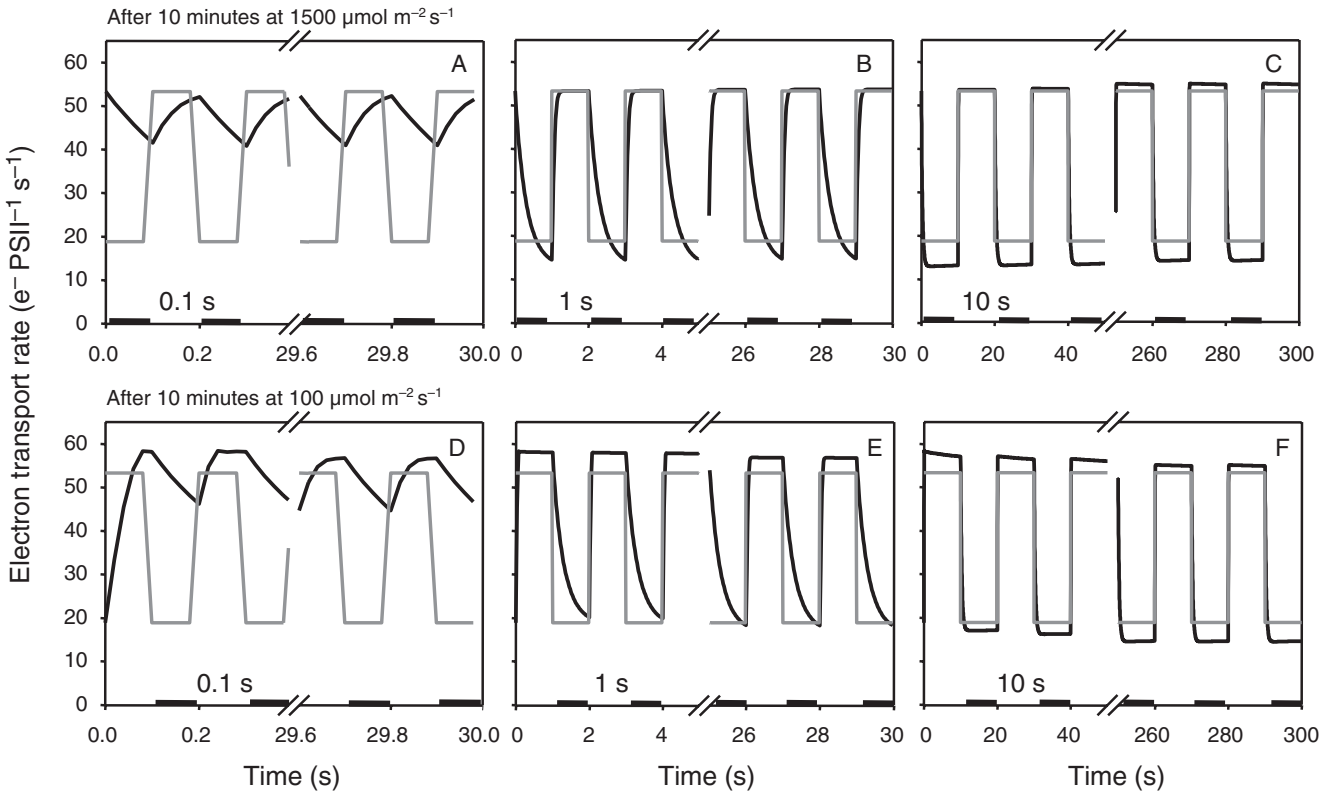

Fig. 5. Modelled electron transport rate (ETR) under fluctuating light following our dynamic approach (black line), or under a steady-state approach (grey line). Values for the steady-state levels correspond to our model estimates after 15 min of illumination. ETR (e^- $PSII^{-1}$ s^{-1}) is calculated from eqn 5 divided by the number N of PSII units and the duration of the timestep under consideration (here 0.02 s). Light fluctuates between 100 (thick horizontal line in the abscissa) and 1500 µmol m^{-2} s^{-1}. The duration of the light interval is 0.1 s (*A*, *D*), 1 s (*B*, *E*) or 10 s (*C*, *F*). The simulation starts on leaves previously acclimated for 15 min to a PPFD of 1500 µmol m^{-2} s^{-1} (*A–C*) or 100 µmol m^{-2} s^{-1} (*D–F*). The *x*-axes are cut to show the trends in the beginning of the simulation and after several fluctuations.

approach gives initially higher ETR estimates during the high-light intervals compared with the steady-state approach, due again to the slow acclimation of NPQ.

In Fig. 6 we present a more quantitative comparison between the steady-state and dynamic approach. The analysis shows that in those conditions where the light intensity changes at intervals faster than 1 s the steady-state approach significantly underestimates the modelled electron transport as obtained by our dynamic approach. The effect reverses for light fluctuations occurring every few seconds, where the steady-state approach overestimates the number of electrons transported compared with the dynamic approach. These results are comparable to those obtained in carbon assimilation studies, where steady-state models have been compared with dynamic models considering enzymatic and / or stomatal limitations of the dark reactions of photosynthesis (Gross 1982; Roden and Pearcy 1993; Pearcy *et al*. 1997; Naumburg and Ellsworth 2002). Under an interval of rapid sunflecks, the use of a steady-state approach underestimates the overall carbon assimilation (e.g. Roden and Pearcy, 1993), due to the utilisation of the stored energy pools during the periods of low light. Our results reveal a similar behaviour in the light reactions under rapidly fluctuating light (Fig. 6), mostly induced by the utilisation of the electrons stored in the plastoquinone pool during the low light period (see Fig. 5*A*, *B*, *D*, *E*). For light fluctuations taking place at larger intervals, the dynamic nature of enzymatic and stomatal processes of photosynthesis also shows that the steady-state approaches tend to overestimate the carbon assimilation (Gross 1982; Pearcy *et al*. 1997; Naumburg and Ellsworth 2002). Thus, both under rapid or slow fluctuations in the light intensity the dynamics of the electron transport through the light reactions seem to follow the same pattern as the dynamics of the overall carbon assimilation. Thereby the question arises whether part of the observed effect of fluctuating light on the carbon assimilation dynamics could be explained by the dynamics of light reactions.

Finally, we simulated the effect of fluctuating light of different frequencies on leaves acclimated to low light (leaves inside or below a canopy), and leaves acclimated to high light (upper canopy leaves). Interestingly, leaves acclimated to low light had larger underestimation errors associated with the

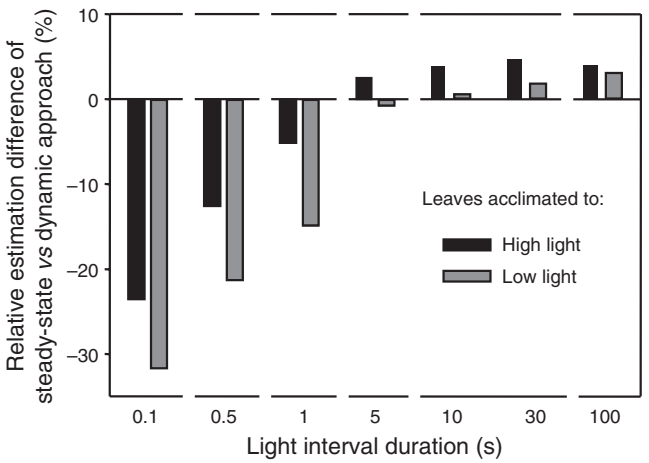

Fig. 6. Relative estimation difference (*RED*) in the electron transport of a steady-state approach *v.* our dynamic approach, depending on the frequency of the light change and for light fluctuating between a PPFD of $1500\,\mu mol\,m^{-2}\,s^{-1}$ and $100\,\mu mol\,m^{-2}\,s^{-1}$. Two different scenarios are simulated with leaves acclimated for 15 min to high light ($1500\,\mu mol\,m^{-2}\,s^{-1}$) and to low light ($100\,\mu mol\,m^{-2}\,s^{-1}$). *RED* was calculated over an integration period (i) comprising several light fluctuation intervals, as: $\mathrm{RED} = \left\{100\left[\int_{t=i}^{t=0} ETR_s(t)\,dt - \int_{t=i}^{t=0} ETR_d(t)\,dt\right]\right\} \Big/ \int_{t=i}^{t=0} ETR_d(t)\,dt$, where the subindexes s and d refer to the steady-state and dynamic approaches, respectively.

rapid sunflecks, whereas leaves acclimated to high light had larger overestimation errors associated with slower changes in light. Considering this behaviour together with the fact that most light fluctuations in the lower part of the canopy are fast, whereas light fluctuations at the top of the canopy are slower, it can be concluded that applying a steady-state approach might lead to overestimation of the ETR in leaves at the top of the canopy and underestimation at the lower part of the canopy.

Concluding remarks

The model presented here successfully reproduced the dynamics of the photochemical and NPQ yields after a change in light environment at the time scale of seconds to minutes. The results show that both the changes in light intensity and the feedback from the dark-reactions influence the partitioning of energy in the light-reactions. This model is intended to serve as a basis for the dynamic modelling of the light reactions of photosynthesis and subsequently for the dynamic estimation of the ETR under natural field conditions. Therefore, the basic model equations presented here can be further developed to account for, *inter alia*, changes in pigment contents, photoinhibition of PSII, structural changes affecting the efficiency of NPQ or the connection with the dark reactions. The model provides a tool to study the seasonal adjustments of the light reactions and its effects on the seasonality of carbon assimilation under field conditions. Furthermore, the model can be used to interpret easily acquirable chlorophyll fluorescence data, because it establishes a direct link between measured fluorescence parameters (F_o and F_m) and the actual processes and composition of the thylakoid system.

Finally, the analysis of the dynamic approach presented here *v.* a traditional steady-state approach for the light reactions, revealed that the steady-state approach underestimates the dynamically modelled ETR under rapidly fluctuating light (1 s or less), and overestimates the same ETR under slower fluctuations of light (more than 5 s), demonstrating the advantages of a dynamic modelling approach under natural conditions in the field.

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