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A revised energy partitioning approach to assess the yields of non-photochemical quenching components

C.R. Guadagno, A. Virzo De Santo, N. D'Ambrosio *

Dipartimento di Biologia Strutturale e Funzionale, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cinthia 4, Edificio 7, 80126 Napoli, Italy

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ABSTRACT

Non-photochemical quenching (NPQ) is a complex and still unclear mechanism essential for higher plants. The intensive research on this subject has highlighted three main components of NPQ: energy-dependent process (qE); state transitions to balance the excitation of PSII and PSI (qT); and photoinhibitory processes (qI). Recently, these components have been resolved as quantum yields according to the energy partitioning approach that takes into account the rate constants of every process involved in the quenching mechanisms of excited chlorophylls. In this study a fully extended quantum yield approach and the introduction of novel equations to assess the yields of each NPQ component are presented. Furthermore, a complete analysis of the yield of NPQ in *Beta vulgaris* exposed to different irradiances has been carried out. In agreement with experimental results here it is shown that the previous approach may amplify the yield of qE component and flatten the quantitative results of fluorescence analysis. Moreover, the significance of taking into account the physiological variability of NPQ for a correct assessment of energy partitioning is demonstrated.

1. Introduction

The light energy absorbed by plants can be utilized via photochemistry or dissipated as heat or fluorescence. The above mechanisms are competitive and any variation in the quantum efficiency of one will elicit complementary changes in the yields of the others. Quantifying the fate of excitation energy (energy partitioning) is important for a full understanding of the response of photosynthetic apparatus to environmental factors as well as acclimation mechanisms. Thermal dissipation is ascribed to a composite of processes collectively termed non-photochemical quenching of chlorophyll fluorescence (NPQ) [1]. The major and quickly reversible component of NPQ is the energy-dependent quenching (qE), caused by ΔpH across the thylakoid membrane in the presence of PsbS and zeaxanthin [2,3]. A reversible phosphorylation of light harvesting complex of PSII (LHCII) induces the state transition quenching (qT) which also contributes to NPQ [4]. Another component of NPQ is the quenching mechanism related to photoinhibition (qI) [5–7]. It has been suggested that the long-lasting zeaxanthin-dependent quenching, which occurs under certain environmental conditions, may be combined with qI component because both processes represent a slowly reversible loss of PSII efficiency [8,9]. Initially, the resolution of these components was conducted by the analysis of dark relaxation kinetics of chlorophyll fluorescence quenching [4,5]. In the meantime, a unified approach known as “energy partitioning in PSII complexes” was performed to assess directly the fraction of absorbed energy that is utilized via photochemistry or dissipated through different mechanisms [10–12]. The main benefit of the quantum yield approach is that it takes into account the rate constants of every process involved in the quenching mechanisms of excited chlorophylls [13,14]. For this reason, several methods of energy partitioning has been developed since Genty et al. proposed in 1989 their successful model [12,15,16]. However, one of the most significant contribution in this field was proposed by Hendrickson et al. who introduced the quantum yield of NPQ (Φ_{NPQ}), providing a more quantitative analysis of energy partitioning [9]. Recently, Ahn et al. have extended this approach, expressing the NPQ components as quantum yields (Φ_{qE}, Φ_{qT+qI}) and demonstrating the utility of this resolution [17]. In this work we further extend the quantum yield approach taking into account each component of NPQ (Φ_{qE}, Φ_{qT} and Φ_{qI}). Actually, we demonstrate that the method of Ahn et al. [17] may amplify the yield of qE and flatten the quantitative results of fluorescence analysis because it does not separate Φ_{qT} and Φ_{qI} and considers a standard relaxation time for qE component. Subsequently, the previous method does not effectively take into account the physiological variability of NPQ. Thus, here we determine new equations that more accurately describe the quantum yields of three different NPQ components.

2. Materials and methods

2.1. Plant material

Garden beet (*Beta vulgaris* L.) plants were grown in pots filled with 1:1 peat:soil and watered when required. The plants were grown in a

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* Corresponding author. Tel.: +39 081679139; fax: +39 081679233.
E-mail address: dambrosi@unina.it (N. D'Ambrosio).

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controlled growth chamber with 8/16 h day/night (D/N) photoperiod, 25/20 °C D/N temperature and 65/85% D/N relative humidity (RH), with a growth irradiance of 150 μmol photons m^{-2} s^{-1}.

2.2. Leaf chlorophyll fluorescence measurements

Chlorophyll a fluorescence emissions were measured using a pulse amplitude modulated fluorimeter (PAM-2000, H. Walz, Effeltrich, Germany). After a dark period (30 min), the maximum fluorescence (F_m) was determined applying a saturating pulse (0.8 s) with intensity higher than 5000 μmol photons m^{-2} s^{-1}. Then, the leaves of beet were exposed for 10 min to different photon flux densities (PFDs) (85, 180, 450, 750 and 1200 μmol photons m^{-2} s^{-1}) to obtain F'_m value. After the induction period the recovery of fluorescence signal in darkness for 40 min was monitored, applying seven saturating pulses at different times (2, 5, 10, 15, 20, 30, 40 min) from the beginning of the dark period. The complete experimental protocol for the fluorescence measurements is reported in Fig. 1. NPQ data were expressed as $NPQ = (F_m - F'_m)/F'_m$ [20] and the resolution of the three NPQ components (qE, qT and qI) was performed according to the method of Walters and Horton [18,19] partially modified. NPQ data were reported in a semi-logarithmic plot versus recovery time and the components of NPQ were calculated by linear regression of three exponentially decaying components (Fig. 2).

2.3. Fluorescence parameters and rate constants

In photobiology one of the main conceptions is that the quantum yield of any process, contributing to utilization or dissipation of absorbed light energy, is equal to the ratio of the rate constant for this process to the sum of all rate constants including the other utilization/dissipation processes [10,16,21,22]. So it is very important that useful parameters derived from fluorescence measurements can be defined in terms of ratios of rate constants. F_m and F'_m are maximum levels of fluorescence for a leaf sample in darkness or light conditions, respectively. F_s is the steady-state fluorescence level at light-adapted conditions. These quantities are defined as $F_m = G\frac{k_F}{k_C}$, $F_m = G\frac{k_F}{k_C + k_{NPQ}}$, and $F_s = G\frac{k_F}{k_C + k_{NPQ} + k_P}$, where k_P, k_{NPQ}, k_C are the rate constants for photochemistry, non-photochemical processes and photophysical decay, respectively, and G is the instrumental gain coefficient [14,23]. Recently Ahn et al. [17] further dissected the k_{NPQ} parameter as the sum of $k_{qE} + k_{qT} + k_{qI}$ introducing F''_m. This parameter is the maximum level of fluorescence measured at 10 min of darkness after the illumination period, defined as:

$$F''_m = G\frac{k_F}{k_C + k_{qT} + k_{qI}}. \tag{1}$$

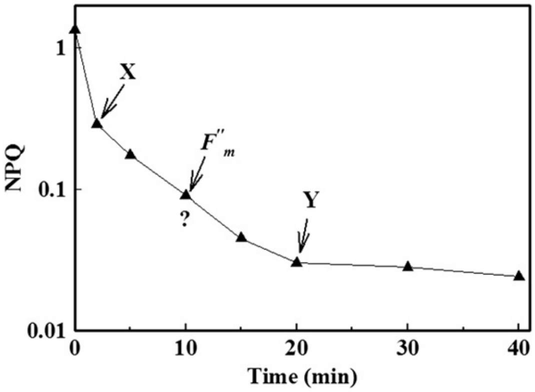

Fig. 2. Semi-logarithmic plot of NPQ values to relaxation time in darkness. The significant changes in the slope of the curve are termed *x* and *y*. No variation in the slope is detected for F''_m at 10 min of relaxation.

From above, the magnitude of NPQ, according to the Stern–Volmer coefficient, corresponds to the ratio of rate constants for the NPQ process relative to the intrinsic photophysical decay (k_C):

$$NPQ = \frac{F_m - F'_m}{F'_m} = \frac{k_{NPQ}}{k_C}. \tag{2}$$

Similarly the ratio of the rate constants for photochemical pathways is equal to [17]:

$$PC = \frac{F'_m - F_s}{F'_m} \times \frac{F_m}{F_s} = \frac{k_P}{k_C}. \tag{3}$$

2.4. Analysis of energy partitioning

The main benefit of the energy partitioning approach is given from the following equation:

$$\Phi_{PSII} + \Phi_{NPQ} + \Phi_C = \frac{k_C + k_{NPQ} + k_P}{k_C + k_{NPQ} + k_P} = 1 \tag{4}$$

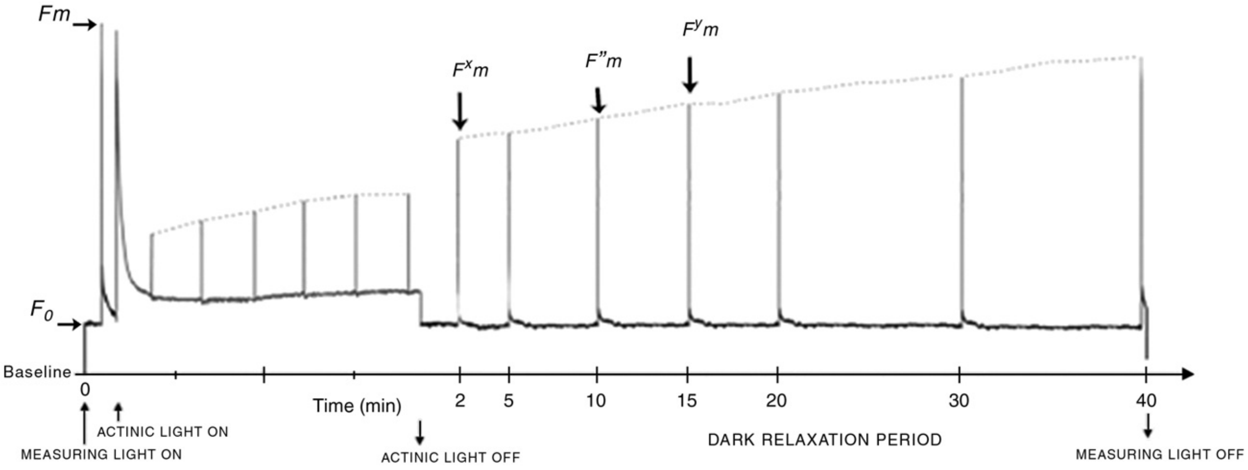

Fig. 1. Example of fluorescence kinetic curve obtained from our experimental protocol in *Beta vulgaris* leaves. F^x_m, F^y_m and F''_m are fluorescence values utilized in our equations and in Ahn et al. [17] approach.

that lets each yield to be solved so that the energy utilized through each pathway can be estimated [14,16,17]. Since the quantum efficiency of PSII photochemistry has been previously defined in terms of the ratio of rate constants as [10,14,16]:

$$\Phi_{\mathrm{PSII}} = \frac{k_{\mathrm{P}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F'_{\mathrm{m}} - F_{\mathrm{s}}}{F'_{\mathrm{m}}} \tag{5}$$

the following ratios can be used to describe the quantum yields of NPQ and of chlorophyll photophysical decay of a light-adapted leaf sample [13,14]:

$$\Phi_{\mathrm{NPQ}} = \frac{k_{\mathrm{NPQ}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F_{\mathrm{m}} - F'_{\mathrm{m}}}{F_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F'_{\mathrm{m}}} \tag{6}$$

$$\Phi_{\mathrm{C}} = \frac{k_{\mathrm{C}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F_{\mathrm{s}}}{F_{\mathrm{m}}}. \tag{7}$$

According to Ahn et al. [17] it is also possible to separate the quantum yields for the different processes of NPQ. For example, Φ_{qE} is defined as:

$$\Phi_{\mathrm{qE}} = \frac{k_{\mathrm{qE}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F''_{\mathrm{m}} - F'_{\mathrm{m}}}{F''_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F'_{\mathrm{m}}} \tag{8}$$

while the sum of Φ_{qT} and Φ_{qI} is obtained from

$$\Phi_{\mathrm{qT+qI}} = \frac{k_{\mathrm{qT}} + k_{\mathrm{qI}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F_{\mathrm{m}} - F''_{\mathrm{m}}}{F_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F''_{\mathrm{m}}} \tag{9}$$

In this paper we demonstrate how the Eq. (9) proposed by Ahn et al. [17] does not take into account the physiological variability of NPQ relaxation kinetic and here we introduce two novel useful fluorescence parameters $F^{x}{}_{\mathrm{m}}$ and $F^{y}{}_{\mathrm{m}}$. These can be defined as the levels of fluorescence corresponding at the time of the first and second variation in the slope of darkness relaxation kinetic of NPQ, respectively [5,18,19].

$F^{x}{}_{\mathrm{m}}$ can be termed as:

$$F^{x}_{\mathrm{m}} = G\frac{k_{\mathrm{F}}}{k_{\mathrm{C}} + k_{\mathrm{qT}} + k_{\mathrm{qI}}} \tag{10}$$

and represents the maximum fluorescence obtained during the dark relaxation, after the qE component of NPQ has completely relaxed, and qT and qI remain as the only active components of NPQ [9,16,24,25].

The maximum fluorescence during the dark period after the total relaxation of qT component is referred to as $F^{y}{}_{\mathrm{m}}$ and can be got out from:

$$F^{y}_{\mathrm{m}} = G\frac{k_{\mathrm{F}}}{k_{\mathrm{C}} + k_{\mathrm{qI}}} \tag{11}$$

where qI is the only NPQ component still active. Using the parameters proposed above, further equations expressing the quantum yield for each of the three NPQ components can be written. The efficiency of the fast component of NPQ (qE) can be defined as:

$$\Phi_{\mathrm{qE}} = \frac{k_{\mathrm{qE}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F^{x}_{\mathrm{m}} - F'_{\mathrm{m}}}{F'_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F^{x}_{\mathrm{m}}} \tag{12}$$

while the quantum yield for state transitions component can be calculated as:

$$\Phi_{\mathrm{qT}} = \frac{k_{\mathrm{qT}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F^{y}_{\mathrm{m}} - F^{x}_{\mathrm{m}}}{F^{x}_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F^{y}_{\mathrm{m}}}. \tag{13}$$

Finally, the quantum yield for the slowest NPQ component results as:

$$\Phi_{\mathrm{qI}} = \frac{k_{\mathrm{qI}}}{k_{\mathrm{C}} + k_{\mathrm{NPQ}} + k_{\mathrm{P}}} = \frac{F_{\mathrm{m}} - F^{y}_{\mathrm{m}}}{F^{y}_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F_{\mathrm{m}}}. \tag{14}$$

The accurate version of energy partitioning approach here proposed is useful to highlight information that could be hidden in the total Φ_{NPQ} parameter.

2.5. Statistical analysis

All data of chlorophyll a fluorescence reported in this work are the average of measurements performed on at least seventeen different plants. Differences between methods were analyzed by the Student's t test based on a significance level of $P<0.05$.

3. Results

In order to assess the light-dependent dynamics of the rate constants (Eqs. (2) and (3)) we plotted the ratios of rate constants (k_{NPQ}, and k_{P} and k_{C}) at the steady-state conditions relative to k_{C}, as estimated in the leaves of *B. vulgaris* exposed to PFDs ranging from 85 to 1200 µmol photons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$ (Fig. 3). As expected, in accordance to previous works [17,26], both $k_{\mathrm{P}}/k_{\mathrm{C}}$ and $k_{\mathrm{NPQ}}/k_{\mathrm{C}}$ demonstrate a dynamic trend in response to the variable PFDs. The $k_{\mathrm{P}}/k_{\mathrm{C}}$ ratio decreases sharply from 85 to 750 µmol photons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$ and gets to an almost constant value at the highest PFD. Differently, the $k_{\mathrm{NPQ}}/k_{\mathrm{C}}$ first increases gradually from 85 to 180 µmol photons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$, then rises up to 750 µmol photons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$ and reaches a value fairly constant at 1200 µmol photons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$. Although the $k_{\mathrm{C}}/k_{\mathrm{C}}$ component is obviously equal to 1.0 over the entire range of PFDs, the sum of all the rate constants ($\sum_i ki$, $i=$ C, NPQ and P) is variable because $k_{\mathrm{P}}/k_{\mathrm{C}}$ and $k_{\mathrm{NPQ}}/k_{\mathrm{C}}$ have different light-dependent dynamics, especially at the lowest PFDs. Then, with the aim of reaching a comprehensive description of energy partitioning we analyzed our results using the quantum yield convention. Interestingly, this approach allows to estimate the fraction of absorbed irradiance consumed through various utilization and dissipation pathways by the application of Eqs. (5)–(7), as underlined from previous authors [9,13,14,17]. The fate of absorbed light energy according to Eq. (4) in beet leaves after 10 min of exposure to irradiances between 85 and 1200 µmol photons $\mathrm{m}^{-2}\ \mathrm{s}^{-1}$ is showed in Fig. 4. The area in dark grey corresponds to Φ_{C} and its value is quite constant at about 0.2 with little variations in the entire range of PFDs considered. As expected, these data demonstrate an increase of thermal dissipation, expressed

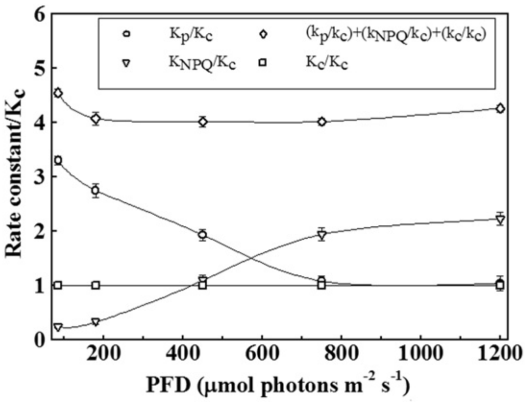

Fig. 3. Plot of rate constants/k_{C} versus PFD. ○ = $k_{\mathrm{P}}/k_{\mathrm{C}}$, □ = $k_{\mathrm{C}}/k_{\mathrm{C}}$; ▽ = $k_{\mathrm{NPQ}}/k_{\mathrm{C}}$, ◊ = the sum of $k_{\mathrm{P}}/k_{\mathrm{C}}$, $k_{\mathrm{NPQ}}/k_{\mathrm{C}}$, $k_{\mathrm{C}}/k_{\mathrm{C}}$ to k_{C}.

as Φ_{NPQ}, strictly correlated to the light intensities. Furthermore, the residual upper section of this yield plot corresponds to Φ_{PSII} whose value decreases in a curvilinear manner with PFD. True to form, these results indicate that the major fraction of absorbed light energy is dissipated by non-photochemical processes over the examined range of PFDs. Values of Φ_{PSII}, Φ_C, Φ_{qE}, and Φ_{qT+qI} for leaves exposed to different PFDs ranging from 85 to 1200 μmol photons $m^{-2}\,s^{-1}$, calculated according to the Eqs. (8) and (9) proposed by Ahn et al. [17], are shown in Fig. 5A. The same data were also processed using our Eqs. (12)–(14) and the results obtained are illustrated in Fig. 5B. Moreover, in both cases the total area occupied by the yields of NPQ components corresponds to the whole Φ_{NPQ} of Fig. 4. Indeed, the plot of Φ_{NPQ}, calculated according to the Eq. (6), versus the sum qE, qT and qI is linear with a slope of one whether they are calculated according to the Eqs. (8) and (9) or whether in reference to the Eqs. (12)–(14). The additivity underlined by Ahn et al. [17] is still maintained as a key benefit of our revisioned approach (Fig. 6). A comparison of the quantitative analysis of the Φ_{NPQ} components according to both procedures is presented in Fig. 7A and B. In order to obtain a good quality corresponding with Φ_{qT+qI}, the value of Φ_{qT} and Φ_{qI} are represented as a sum (Fig. 7B). Although the utilized equations are different, these data demonstrate that the major portion of Φ_{NPQ} over the PFD range is nearly always attributable to Φ_{qE}. Inevitably, the extent of Φ_{qE} is strongly light-dependent and reaches its maximum at the highest PFDs. The quantitative contribution of each component in yield terms according to our revised equations is represented in Fig. 8. In particular, it has to be underlined that Φ_{qE} at the lowest irradiance of 85 μmol photons $m^{-2}\,s^{-1}$ is not the major component of Φ_{NPQ}, then (at 180 μmol photons $m^{-2}\,s^{-1}$) it becomes higher than Φ_{qT} but without any significant difference. Only with the increase of PFD (450 to 1200 μmol photons $m^{-2}\,s^{-1}$) Φ_{qE} value increases significantly and it becomes the main component of thermal dissipation as expected. So, according to a previous work [27] the contribution of Φ_{qT}, depending on state transitions mechanisms, is the most important at the lowest PFDs, and although it becomes saturated interestingly it is still present in a moderate percentage at the higher PFDs. As usual in a short term quenching induction [28,29], Φ_{qI} is almost null up to 750 μmol photons $m^{-2}\,s^{-1}$ and, although it reaches the highest value at 1200 μmol photons $m^{-2}\,s^{-1}$ it is still significantly lower than Φ_{qE}. All these quantitative considerations about the contribution of each component to the total NPQ yield have been clearly pointed out by using our revised approach of energy partitioning, otherwise by the previous method they were partly hidden.

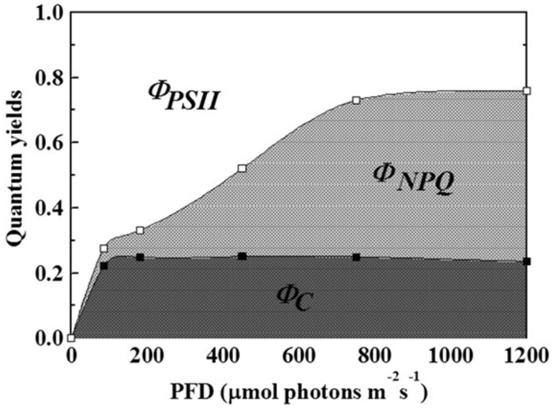

Fig. 4. Estimated fraction of absorbed light energy consumed via various utilization and dissipation pathways in *Beta vulgaris* leaves after 10 min of illumination at different PFDs (85, 180, 450, 750 and 1200 μmol photons $m^{-2}\,s^{-1}$). The measured parameters include photochemistry (Φ_{PSII}), non-photochemical processes (Φ_{NPQ}) and photophysical decay (Φ_C).

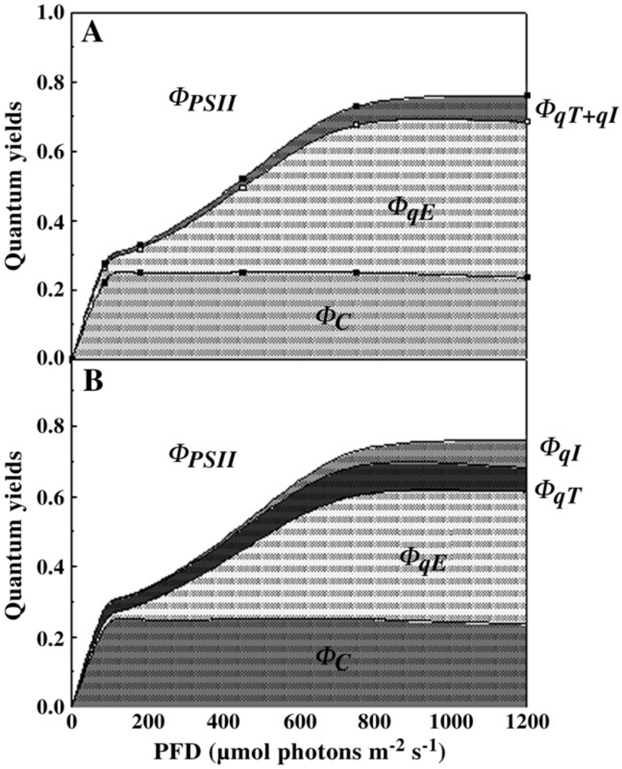

Fig. 5. Quantum yields of photochemistry, qE, qT, qI and the photophysical decay (Φ_{PSII}, Φ_{qE}, Φ_{qT}, Φ_{qI} and Φ_C, respectively) versus PFD in *Beta vulgaris*. Values are reported according to Ahn et al. [17] (A) and according to new equations proposed in this work (B).

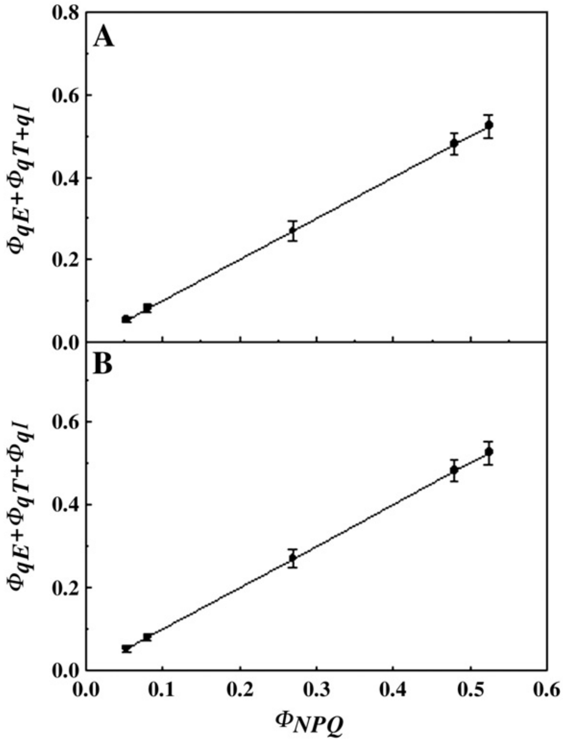

Fig. 6. Plots of the sum of $\Phi_{qE}+\Phi_{qT+qI}$ versus Φ_{NPQ} according to Ahn et al. [17] (A) and $\Phi_{qE}+\Phi_{qT}+\Phi_{qI}$ versus Φ_{NPQ} in our approach (B). Reported data demonstrate that the additivity is detected in both methods.

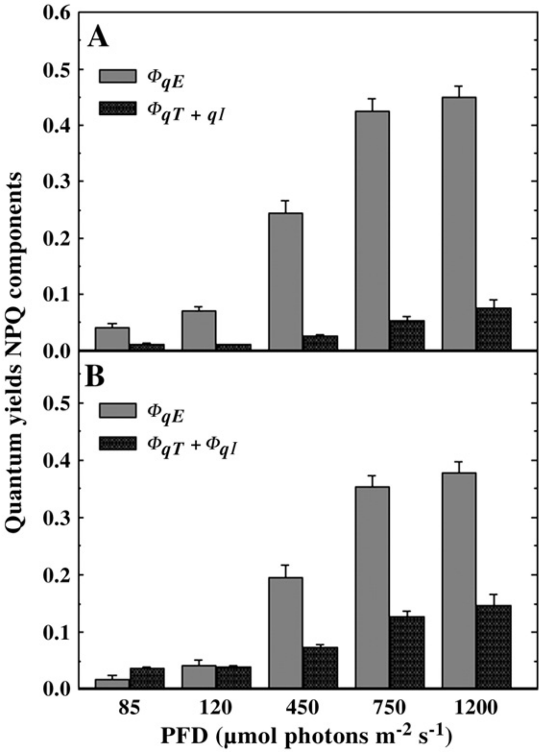

Fig. 7. The contributions of Φ_{qE} and $\Phi_{\mathrm{qT+qI}}$ according to Ahn et al. [17] (A) and the contributions of Φ_{qE} and $\Phi_{\mathrm{qT}}+\Phi_{\mathrm{qI}}$ in our revised approach (B) versus PFD.

4. Discussion

Our revised approach is based on the consideration that NPQ is a physiological mechanism characterized by a great variability. Sometimes changes which naturally occur in NPQ capacity, possibly on genetic basis [30], have been observed in different plant species [31–33]. Sun-acclimated plants have got up to four times as much NPQ capacity as low-light-acclimated plants of the same species [32–38]. The maximum extent of NPQ at saturation irradiance is also dependent on the plant growth conditions, primarily the quality of light, the air temperature and the plant species [31,35]. This variability can influence the total NPQ extent but also the contribution of each single component to total thermal dissipation. Actually, qT and qI components may be larger than qE under certain physiological conditions and in different species as well as various mutants of the same species [17]. Therefore, we extended the energy partitioning approach taking into account the real relaxation time of each component of NPQ. Since the analysis of the kinetic curve of NPQ relaxation in darkness did not reveal any physiological variation in the NPQ components (Fig. 1), we reported NPQ values in a semi-logarithmic plot versus time appreciating the variations in the slope of the curve (Fig. 2). As a result, the detection of two time points, termed as x and y, is achievable; these points correspond to clear changes in the slope and can be associated to the relaxation times of qE and qT component of NPQ [4,5]. Consequently, the $F^{x}{}_{\mathrm{m}}$ and $F^{y}{}_{\mathrm{m}}$, utilized in Eqs. (12)–(14), are the fluorescence values at the time points x and y and are dependent on the environmental conditions and plant species. On the contrary, the point which corresponds to 10 min of dark relaxation, associated to $F''{}_{\mathrm{m}}$ is not characterized by any relevant variation in the NPQ value (Fig. 2). So, we demonstrate that the application of a semi-logarithmic plot of NPQ values during the time of dark relaxation is the basis for a careful choice of the relaxation time of NPQ components. In fact, the use of $F''_{\mathrm{m}} = G\frac{k_{\mathrm{F}}}{k_{\mathrm{C}} + k_{\mathrm{qT}} + k_{\mathrm{qI}}}$ [17] is not suitable for assessing a correct analysis of the yield of NPQ components. The $F''{}_{\mathrm{m}}$ is a too inflexible parameter because it is always taken after 10 min of relaxation in darkness of NPQ. At that time it is possible that qT component too is just relaxed and a consequent overestimation of Φ_{qE} value may occur. Moreover, by this method it is evidently not possible to specify if the increase in $\Phi_{\mathrm{qT+qI}}$ originates with qT, qI or both components [17]. Using the new features, $F^{x}_{\mathrm{m}} = G\frac{k_{\mathrm{F}}}{k_{\mathrm{C}} + k_{\mathrm{qT}} + k_{\mathrm{qI}}}$ and $F^{y}_{\mathrm{m}} = G\frac{k_{\mathrm{F}}}{k_{\mathrm{C}} + k_{\mathrm{qI}}}$, we were able to choose the more appropriate value. Our results clearly show that the value of Φ_{qE} calculated through the previous method is overestimated by 2 to 7% with respect to the data calculated by our approach. The significant overestimation goes up with the increase of PFDs considered. To the same extent the value of $\Phi_{\mathrm{qT+qI}}$ (Fig. 7A) is decreased significantly respect to $\Phi_{\mathrm{qT}}+\Phi_{\mathrm{qI}}$ (Fig. 7B) with a consequent flattening of the contribution of state transitions and photoinhibitory quenching to total NPQ. Therefore, our method unveils a significant overestimation for Φ_{qE} and a parallel as much as significant flattening in the sum of the other two components ($\Phi_{\mathrm{qT}}+\Phi_{\mathrm{qI}}$) in the results analyzed according to the Ahn et al. procedure [17]. It has to be underlined that the differences observed in our results compared to those reported by Ahn et al. [17] are not due to the different optical features of the leaves. Therefore our proposed method of energy partitioning can be applied to any plant species. Furthermore, our analysis comes up to the requirement of a complete separation for the contributions to total thermal dissipation of the three NPQ components as quantum yields (Fig. 8). In fact, we fully succeeded in the separation of Φ_{qT} and Φ_{qI} introducing the revised equations $\Phi_{\mathrm{qT}} = \frac{F^{y}_{\mathrm{m}} - F^{x}_{\mathrm{m}}}{F^{x}_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F^{y}_{\mathrm{m}}}$ and $\Phi_{\mathrm{qI}} = \frac{F_{\mathrm{m}} - F^{y}_{\mathrm{m}}}{F^{y}_{\mathrm{m}}} \times \frac{F_{\mathrm{s}}}{F_{\mathrm{m}}}$. Our revised energy partitioning approach allows easily the separation of Φ_{qE}, Φ_{qT} and Φ_{qI} but does not modify the extent of Φ_{PSII} and Φ_{C} (Fig. 5A and B) considering that these latter parameters are not derived from the relaxation kinetic. However, the results of our reconsideration of the previously proposed equations for the calculation of the quantum yields for NPQ components do not change the additivity benefit (Fig. 6) that still remains as a key gain of our approach. In summary, this original repartition leads to a complete analysis of energy partitioning giving the right importance to each NPQ component and demonstrates that is possible to fully analyze Φ_{NPQ} by using the new equations [Eqs. (12)–(14)] here introduced. We propose that this revised approach of energy partitioning will facilitate a better understanding of the natural photoprotection and of such a variable mechanism as NPQ.

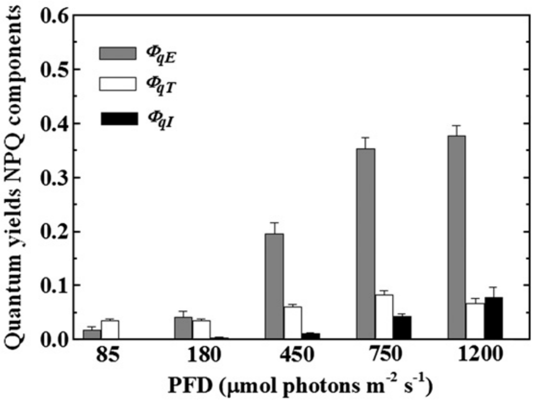

Fig. 8. The repartition into Φ_{qE}, Φ_{qT} and Φ_{qI} in leaves of *Beta vulgaris* exposed to different PFDs ranging from 85 to 1200 μmol photons m^{-2} s^{-1}.

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