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1	A model for describing the light response of the non-photochemical quenching of chlorophyll
2	fluorescence
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23 Abstract

25	The operation of photosynthetic energy-dissipating processes is commonly characterised by
26	measuring the light response of the non-photochemical quenching (NPQ) of chlorophyll
27	fluorescence, or NPQ vs. E curves. This study proposes a mathematical model for the
28	quantitative description of the generic NPQ vs. E curve. The model is an adaptation of the Hill
29	equation and is based on the close dependence of NPQ on the xanthophyll cycle (XC). The
30	model was tested on NPQ vs. E curves measured in the plant Arabidopsis thaliana and the
31	diatom Nitzschia palea, representing the two main types of XC, the violaxanthin-
32	antheraxanthin-zeaxanthin (VAZ) type and the diadinoxanthin-diatoxanthin (DD-DT) type,
33	respectively. The model was also fitted to a large number of published light curves, covering the
34	widest possible range of XC types, taxa, growth conditions, and experimental protocol of curve
35	generation. The model provided a very good fit to experimental and published data, coping with
36	the large variability in curve characteristics. The model was further used to quantitatively
37	compare the light responses of NPQ and of PSII electron transport rate, ETR, through the use of
38	indices combining parameters of the models describing the two types of light-response curves.
39	Their application to experimental and published data showed a systematic large delay of the
40	build-up of NPQ relatively to the saturation of photochemistry. It was found that when ETR
41	reaches saturation, NPQ is on average still below one fifth of its maximum attainable level,
42	which is only reached at irradiances about three times higher. It was also found that organisms
43	having the DD-DT type of XC appeared to be able to start operating the XC at lower irradiances
44	than those of the VAZ type.
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48	Keyword index: Chlorophyll fluorescence; modelling; non-photochemical quenching;
49	photoacclimation; photoprotection; xanthophyll cycle
50	

51 Introduction

52

53	Photosynthesis requires a balance between maximizing light absorption and minimizing
54	damages caused by excessively absorbed light energy. Under natural conditions, exposure to
55	sunlight involves unavoidable risks to the photosynthetic apparatus due to the formation of
56	reactive oxygen species of photosynthetic origin produced under excess light (Osmond et al.
57	1997; Ort 2001; Demmig-Adams and Adams 2006). The sustainment of prolonged
58	photosynthetic activity under high solar irradiances is ensured by the regulation of the
59	repartition of absorbed light energy between photochemistry and energy-dissipating pathways.
60	The latter function as photoprotective process against permanent damages to the photosynthetic
61	apparatus, or photoinhibition, and are therefore of considerable interest for understanding the
62	resistance of photoautotrophs to environmental stress (Demmig-Adams and Adams 2006;
63	Lavaud 2007; Li et al. 2009).

64 One of the most important photoprotective processes is the non-photochemical 65 quenching (NPQ). NPQ groups several pathways among which the thermal dissipation of excess 66 energy (or $q_{\rm E}$, the energy-dependent quenching, Müller et al., 2001) is considered as the most important. q_E takes place in the light-harvesting antenna of the photosystem II (PS II). It relies 67 68 on the presence of specific PS II antenna proteins (PsbS in plants, Li et al. 2000; LhcSR in 69 green microalgae, Peers et al. 2009; Lhcx in diatoms, Bailleul et al. 2010), the building of a 70 transthylakoid proton gradient (ΔpH), and the subsequent enzyme-mediated operation (de-71 epoxidation) of the xanthophyll cycle (XC) (Goss and Jakob, 2010). The XC produces de-72 epoxidised forms of xanthophylls which are essential regulatory partners of $q_{\rm E}$, especially in 73 some groups of microalgae (like the diatoms) (Lavaud, 2007; Li et al. 2009; Goss and Jakob 74 2010). In plants, green algae and some Heterokontophyta (brown algae, Chrysophyceae), the 75 XC is based on the reversible conversion of pigment violaxanthin (Vx) to the $q_{\rm F}$ -involved 76 zeaxanthin (Zx), passing through the intermediate form antheraxanthin (Ax) (VAZ type of XC); 77 in other algal groups, like the diatoms, dinoflagellates, Xanthophyceae and Haptophyta, the XC 78 consists in the conversion between only two pigments, diadinoxanthin (DD) and the $q_{\rm E}$ -involved

diatoxanthin (DT) (DD-DT type of cycle) (Olaizola and Yamamoto 1994; Lavaud et al. 2004;
Lavaud 2007). In phycobilisome-containing organisms, there is no XC (cyanobacteria) or its
presence is uncertain (red algae) (Goss and Jakob 2010).

82 The operation of NPQ processes is usually quantified using variable chlorophyll 83 fluorescence (Pulse Amplitude Modulation fluorometry, PAM), by calculating the fluorescence 84 index NPQ, which is based on the relative difference between the maximum fluorescence 85 measured in the dark-adapted state, F_m , and upon exposure to light, F_m' (see Table 1 for 86 notation):

$$NPQ = \frac{F_m - F_m}{F_m}$$
(1)

89

90 This index is a rearrangement of the Stern-Volmer equation and reflects the assumption that the 91 reciprocal of fluorescence yield is proportional to the Zx or DT concentration (Bilger and 92 Björkman 1990; Lavaud et al. 2002; Baker and Oxborough 2004). The NPQ index has been 93 routinely used to quantify the operation of photoprotective processes as well as the extent of 94 photoinhibitory damages. Under experimental conditions allowing to assume that the prevailing 95 processes causing the quenching of fluorescence are of photoprotective nature, NPQ has been 96 used as a measure of the overall photoprotective capacity of the photosynthetic apparatus (e.g. 97 Dimier et al. 2007b; Lavaud et al. 2007).

98 One common way to characterise the operation of photoprotective processes or the 99 susceptibility to photoinhibition is to quantify the light response of NPQ. This is done by 100 constructing NPQ vs. E curves that record the development of NPQ with increasing incident 101 irradiance. These curves are analogous to the more frequently measured light-response curves of 102 the PSII electron transport rate (ETR) in the sense that they represent the variation of steady 103 state photosynthetic activity between different light levels, thus not informing on the kinetics of 104 NPQ generation, but on the NPQ attainable under each irradiance. The shape of NPQ vs. E 105 curve varies widely, both regarding its overall shape as well as the absolute NPQ values

106 reached. Typically, NPQ increases monotonically with irradiance, varying from zero (measured 107 in darkness) to maximum values that vary greatly with taxonomical groups, physiological state, 108 environmental constraints, or light levels applied. The curve presents a variable degree of 109 sigmoidicity, ranging from cases when NPQ starts to increase steeply from the lowest light 110 levels, following a simple saturation-like pattern (no sigmoidicity), to cases when NPQ remains 111 close to zero for a range of low light levels, then showing an abrupt increase only for 112 intermediate irradiances before stabilizing at maximum values (highly sigmoid). Often, 113 although the stabilization of NPQ is evident, a constant value is not reached within the range of 114 irradiances applied and the maximum attainable NPQ cannot be estimated. 115 Light-response curves of NPQ have been analysed and interpreted on the basis of

115Eight-response curves of Ni Q nave been analysed and interpreted on the basis of116arbitrarily chosen features, by qualitatively describing its shape (e.g. low or high sigmoidicity),117or by selecting NPQ values reached at particular *E* levels (e.g. NPQ at maximum applied *E*).118The absence of an adequate descriptive model impedes the characterization of the curve along119the whole range of irradiances applied. Also, the lack of a commonly-used set of descriptive120parameters makes it difficult to compare curves measured in different studies or experimental121conditions or taxonomic groups.

122 The present study proposes a simple mathematical model for the quantitative 123 description of the generic NPQ vs. E curve, by means of the estimation of a small number of 124 physiologically-meaningful parameters. The model is based on the close, but not absolute, 125 dependence of NPQ on the operation of the XC, through the light-induced de-epoxidation of PS 126 II antenna pigments Vx or DD. To illustrate the main features and variability of the NPQ vs. E 127 curve, as well as the interpretation of model parameters, light-response curves were measured 128 on a plant (Arabidopsis thaliana (L.); VAZ type XC) and a diatom (Nitzschia palea (Kütz) W. 129 Smith; DD-DT type XC) grown under experimental conditions expected to induce a large 130 variability in curve shape and NPQ absolute values. The adequacy of the model was further 131 tested by fitting it to published NPQ vs. E curves measured on a large variety of photosynthetic 132 organisms and experimental conditions, and covering the widest possible range of NPQ values 133 and curve shapes. The usefulness of the model was also illustrated by exploring the relationship

- between NPQ vs. *E* curves and the photoacclimation status, characterized by light-response
 curves of the PSII electron transport rate, ETR.
- 130
- 138 Materials and Methods
- 139
- 140 Model rationale
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142 The model proposed to describe the light-response curve of NPQ is an adaptation of the Hill 143 equation, originally derived in the context of ligand binding to macromolecules with multiple 144 binding sites. This equation describes the variation of the number of filled binding sites with the 145 increase in ligand concentration, and is routinely used to characterise the cooperativity of 146 enzymatic reactions (Voet and Voet 1990). The use of the Hill equation for modelling the NPQ 147 vs. E curve is based on the main assumption that NPQ is mostly due to the operation of the XC 148 coupled to the build-up of the transthylakoid ΔpH , or energy-dependent quenching (q_E ; Müller 149 et al. 2001), and that the other potential components of NPO, the quenching due to state 150 transitions $(q_{\rm T})$ or to photoinhibition $(q_{\rm I})$ are not expected to significantly affect the NPQ vs. E 151 curves (see Discussion). The analogy underlying this rationale is to consider the epoxidized XC 152 pigments Vx and DD as representing the macromolecule of the Hill model, and the 153 transthylakoidal Δp H-dependent protonation of specific light-harvesting complex (LHC) 154 antenna sites as corresponding to the ligand concentration that bind to their multiple binding 155 sites. At steady state, each irradiance level corresponds to a stable transthylakoidal ΔpH and 156 resulting Vx or DD de-epoxidation and activation through protonation of LHC sites (the so-157 called 'activation' of Zx and DT; Horton et al. 2000; Goss et al. 2006; Lavaud and Kroth 2006; 158 Horton et al. 2008), and thus to a defined NPQ value. The NPQ vs. E curve is thus proposed to 159 be described by the equation:

NPQ (E) = NPQ
$$_{m} \frac{E^{n}}{E_{50}^{n} + E^{n}}$$
 (2)

163 where NPQ_m is the maximum NPQ value reached during the light curve, E_{50} is the irradiance 164 level for which NPQ attains 50% of NPQ_m, and n is the Hill coefficient, characterizing the 165 sigmoidicity of the curve. Whilst the parameters E_{50} and *n* are directly adopted from the Hill 166 equation (originally having only these two parameters), a third parameter, NPQ_m, had to be 167 considered to account for the variability in the absolute values of NPQ. Under the analogy with 168 the ligand binding context of the Hill equation, NPQ(E) is considered to represent the fraction 169 of Vx or DD molecules de-epoxidized into Zx or DT and which have been 'activated'. As such, 170 E_{50} represents the irradiance level necessary to de-epoxidize and 'activate' 50% of convertible 171 Vx or DD pool (ligand concentration resulting in half occupation of binding sites) necessary to 172 reach the maximal NPQ level, NPQ_m. Of potential importance for the characterization of the 173 physiological processes underlying the NPQ vs. E curve is the sigmoidicity parameter n, which 174 measures the type and extent of the reaction cooperativity. In the case of n < 1, the curve 175 displays a saturation-like increase asymptotically towards NPQ_m, indicating the presence of a 176 negatively cooperative reaction; if n > 1, the curve starts to present a sigmoidal shape, the 177 sigmoidicity increasing with n, indicating a positively cooperative, or allosteric, reaction; if n178 =1, the model is reduced to the Michaelis-Menten equation, indicative of a non-cooperative 179 reaction. 180

181 Measurement of light-response curves

182

183 To test the adequacy of the model, Eq. 2 was first fit to NPQ vs. E curves generated for 184 organisms representative of the two types of XC, a plant (A. thaliana; VAZ type of XC) and a 185 diatom (*N. palea*; DD-DT type of XC), grown under light conditions expected to induce large 186 variations in NPQ absolute values and in the shape of the NPQ light-response curve. Plants of A. thaliana (ecotype Columbia) were grown under controlled growth chamber conditions for 4-187

188	5 weeks under a 16:8 h light/dark cycle and 70% relative humidity. The light/dark temperatures
189	were 22/20 °C. Three irradiance levels were applied: 10 ('low light', LL), 75 ('moderate light',
190	ML) and 150 µmol photons m ⁻² s ⁻¹ ('high light', HL). The diatom Nitzschia palea (Kütz.) W.
191	Smith (collection of the Department of Biology, University of Aveiro) was grown
192	photoautotrophically in unialgal semi-continuous batch 100 mL cultures in sterile natural
193	seawater enriched with f/2 nutrients (Guillard and Ryther 1962). Cultures were grown at 20 $^{\circ}$ C,
194	under 20 (LL), 100 (ML) and 400 μmol photons $m^{-2}~s^{-1}$ (HL) in a 12:12 h light/dark cycle. Cells
195	were harvested by centrifugation (3000 \times g, 10 min) during the exponential phase of growth,
196	and were resuspended in fresh growth medium supplemented with NaHCO ₃ to a final
197	concentration > 10 μ g Chl <i>a</i> ml ⁻¹ .
198	Light-response curves of NPQ and ETR were generated by exposing the samples to 7-
199	12 levels of actinic light, up to 920 μ mol m ⁻² s ⁻¹ . Samples were dark-adapted for 30 min before
200	the start of the light curve to allow determination of fluorescence levels F_0 (minimum
201	fluorescence) and $F_{\rm m}$, required for the calculation of NPQ (Eq. 1). Samples were light-activated
202	before the start of each light-response curve, through exposure to low light of 54 μ mol photons
203	$m^{-2} s^{-1}$ until a steady state in fluorescence was reached (minimum 15 min). Under each light
204	level, a saturation pulse (0.8 s for A. thaliana and 0.6 s for N. palea) was applied and
205	fluorescence levels $F_{\rm s}$ (steady state fluorescence) and $F_{\rm m}$ ' were recorded and used to calculate
206	NPQ (Eq. 1) and ETR, using (Genty et al. 1989):

 $ETR = E \frac{F_m - F_s}{F_m}$ (3)

209

208

210 A different sample (plant leaf or algal culture aliquot) was used for measuring NPQ and ETR 211 under each light level. Three replicated measurements were made for each light level. Light-212 response curves of ETR were described by fitting the model of Eilers and Peeters (1988), and by 213 estimating the parameters α (the initial slope of the curve), ETR_m (maximum ETR) and E_k (the 214 light-saturation parameter). The parameter E_k is commonly interpreted as a measure of the light 215 level to which a sample is acclimated to, and commonly used to characterise its

216 photoacclimation status (Behrenfeld et al. 2004).

Chlorophyll fluorescence yield was measured using a PAM fluorometer comprising a
computer-operated PAM-Control Unit (Walz) and a WATER-EDF-Universal emitter-detector
unit (Gademann Instruments GmbH, Germany), using a modulated blue light (LED-lamp
peaking at 450 nm, half-bandwidth of 20 nm) as source for measuring, actinic, and saturating
light (Cruz and Serôdio 2008). Fluorescence was measured using a 6 mm-diameter Fluid Light
Guide fiberoptics. In the case of *N. palea*, the fiberoptics was connected to a fluorescence
cuvette (KS-101, Walz, Effeltrich, Germany).

225 Published light-response curves

226

227 The model was also fitted to published NPQ vs. E curves, covering a wide range of taxonomic 228 groups (higher plants, mosses, green algae, diatoms, dinoflagellates, others), XC types (VAZ, 229 DD-DT, absence of XC), mutants with variable degrees of impairment of the XC operation, 230 habitats (land and aquatic higher plants; planktonic and benthic microalgae), growth conditions 231 (e.g. low and high light), physiological state (heat stress or XC inhibitors) and fluorescence 232 light-response curve measuring protocols (steady state and rapid light curves, RLCs). Only 233 species with a known functional XC were considered. Studies presenting light curves having 234 less than seven data points were not considered, because of the large errors in the model fitting 235 and parameter estimation. For each study, when three or more light curves were available for 236 each species and experimental treatment, only the two curves presenting the most extreme 237 (higher and lower) NPQ values were used. Detailed information on the data used in the meta-238 analysis is summarised in Table 3. When available, the corresponding ETR vs. E curves were 239 also characterised by fitting the model of Eilers and Peeters (1988) and by estimating its 240 parameters.

Some studies have reported the formation of NPQ in the dark (Perkins et al. 2010). This
phenomenon was observed on diatoms (Jakob et al. 1999) and brown algae (Mouget and

243	Tremblin 2002), and results in NPQ vs. E curves showing a distinctive bi-phasic pattern, with
244	NPQ decreasing from initial high values measured in the dark to a minimum under low to
245	moderate light levels (e.g. Geel et al. 1997; Serôdio et al. 2006). Being relevant only for a
246	limited number of taxonomic groups and physiological conditions, this type of NPQ light-
247	response curve was not considered in the present study.
248	
249	Model fitting and parameter estimation
250	
251	The light-response curve models were fitted using a procedure written in Microsoft Visual Basic
252	and based on Microsoft Excel Solver. Model parameters were estimated iteratively by
253	minimizing a least-squares function, forward differencing, and the default quasi-Newton search
254	method. The model can be easily fitted using commonly available software packages. On
255	preliminary tests, this method was compared with the fitting procedures implemented in
256	Sigmaplot 9.0 (Systat Software, Inc., San Jose, USA) and Statistica 8.0 (Statsoft, Inc., Tulsa,
257	USA) and no significant differences were found between the estimates of model parameters.
258	The standard errors of the parameter estimates were calculated following Ritchie (2008)
259	and are asymptotic standard errors. For nonlinear models such as the one here tested, asymptotic
260	standard errors may not be adequate, as they possibly underestimate the actual parameter
261	uncertainty and cannot evaluate the eventual asymmetry of the confidence regions of estimated
262	parameters (Johnson 2008). A number of alternative approaches exist, the best being based on
263	Monte Carlo methods, such as the Boostrap (Press et al. 1996; Johnson 2008). However, the
264	benefits of pursuing alternative methods, requiring substantially more computation time, depend
265	on the magnitude of the uncertainty associated to parameter estimation. In this study, the use of
266	asymptotic standard errors was justified by the finding that they were on average relatively low,
267	in a majority of cases below 10% of parameter estimates (see below, Results), in which case
268	they can be considered acceptable (Tellinghuisen 2008).
269	
270	Results

2/1

272 Model fitting to NPQ vs. *E* curves

273

274 The light-response curves of NPQ measured in the plant A. thaliana and in the diatom N. palea 275 acclimated to different growth light conditions showed a wide range of curve shapes, varying 276 between two main types: curves with low sigmoidicity, presenting a simple saturation-like 277 pattern (e.g. Fig. 1a, HL), and curves with high sigmoidicity, presenting an initial period of low 278 values, followed by a phase of rapid increase leading to a stage of plateau of maximum values 279 (e.g. Fig. 2a, HL). In all cases, the model provided an excellent fitting to the experimental data 280 throughout the whole range of light levels explaining always more than 99.1% of the data 281 variability (Table 2). Although the residuals (Figs. 1b, 2b) showed in some cases a clearly non-282 random pattern of variation, values were typically low, between \pm 5%. The fitting of the model 283 resulted in a large variation of model parameters with species and growth light conditions, 284 following some consistent trends. Both for A. thaliana and N. palea, the increase in growth light 285 resulted in an increase in NPQ_m (Fig. 1a, 2a; Table 2). This increase in NPQ_m could be detected 286 despite the fact that in most cases the curves did not reach their maximum values within the 287 range of irradiances applied (e.g. Fig. 1a). Another effect of increasing growth light was the 288 increase in the irradiance level for which the curves reached maximum values. For LL-grown 289 samples, maximum NPO was reached for lower irradiances, with the curves showing an overall 290 lower sigmoidicity, while for HL-grown samples, curves reached saturation at much higher light 291 levels. This trend could be well described by model parameter E_{50} , which increased with growth 292 light in both species (Table 2).

The most noticeable difference between the NPQ light-response curves of *A. thaliana* and *N. palea* was the apparent curve sigmoidicity, with less sigmoid curves being found for the former and more sigmoid curves (and a larger variation) for the latter. This variation in curve sigmoidicity was well characterized by the model parameter *n*, which averaged 1.2 for *A. thaliana* (varying from 0.98 to 1.44), whilst reaching an average value of 2.11 for *N. palea*

298	(varying from 1.61 to 2.61). However, a different pattern of variation was found for each
299	species, with <i>n</i> decreasing with growth light in <i>A</i> . <i>thaliana</i> , and increasing in <i>N</i> . <i>palea</i> (Table 2).
300	
301	Model fitting to published NPQ vs. <i>E</i> curves

303 The compilation of published light-response curves of NPQ allowed gathering a large number 304 of datasets covering a wide range of NPQ absolute values and curve shapes. The model 305 provided a very good fit to the published data, coping with the large diversity in curve 306 characteristics resulting from different combinations of maximum NPQ values attained (NPQ_m), 307 irradiance range of NPQ build-up (E_{50}), and curve sigmoidicity (n) (Table 3). Considering all 308 cases, the model explained more than 96% of data variability, independently of type of XC (and 309 XC impaired mutants), species, growth conditions, and experimental protocol used for the 310 generation of light curves. A summary of the meta-analysis carried out on the data set of 311 published NPQ vs. E curves is presented in Table 4. NPQ_m was the most variable parameter 312 (c.v. 112.5%), mostly responding to growth conditions, averaging 2.89 but reaching maximum 313 values above 9 for diatoms and even higher for some mosses (Table 3). E_{50} ranged from 30-40 μ mol photons m⁻² s⁻¹ to above 3500 μ mol m⁻² s⁻¹, in all cases attaining values well above growth 314 315 irradiances (see below). However, with few exception (the diatoms Skeletonema costatum and *Phaeocystis antarctica*, and the high light-grown A. *thaliana*), all values of E_{50} above 1200 316 317 μ mol m⁻² s⁻¹ were obtained for *A. thaliana* mutants with impaired operation of the XC (Table 3). 318 n was the least variable parameter (c.v. 50.8%), meaning that the curve shape remained 319 relatively unaltered, and that the variability in the NPQ vs. E curves was mainly due to changes 320 in NPQ maximum values and in NPQ onset along the irradiance range. n averaged 1.7, 321 corresponding to a noticeable degree of sigmoidicity, although values around 1.0 (no 322 sigmoidicity) or slightly lower (mostly mutants with impaired XC) as well as above 3.0 (very 323 high sigmoidicity) were also found (Table 3).

324 Despite the low number of data points forming most NPQ vs. *E* curves, standard errors
325 of parameter estimates were on average relatively low (15.6, 27.3 and 12.2% of estimates for

326 NPQ_m, E_{50} and *n*, respectively). However, standard errors were typically much higher in the 327 cases when XC was impaired (24.5, 43.9 and 13.5% for NPQ_m, E_{50} and *n*, respectively), 328 indicating that a high level of precision in the estimation of model parameters can be expected 329 for samples under natural conditions.

330 When comparing different XC types, no clear differences were found between model 331 parameters, due to the large overlap of their range of variation. Model parameters appeared to 332 depend mostly on growth conditions, as larger variations could be found within the same 333 species than amongst different taxonomic groups. The model also described very well the light-334 response curves of NPQ for composite samples (phytoplankton, microphytobenthos). Model 335 parameters varied independently from each other, as no significant correlations were found 336 between NPQ_m, E_{50} or *n*, either when considering all data pooled together, or when considering 337 for separate for each XC type.

338 Considering the published data, the effects of growth light levels on model parameters 339 were identical to those described for the experimental data of this study. When compared to 340 lower light conditions, NPQ light-response curves of samples acclimated to high light showed 341 higher values of NPQ_m (Bilger and Bjorkman 1990; Burritt and MacKenzie 2003; Müller et al. 342 2004; Ralph and Gademann 2005; Rodríguez-Calcerrada et al. 2007; Cruz and Serôdio 2008) 343 and, with the exception of Ralph and Gademann (2005), all these studies showed a similar 344 increase in E_{50} with growth light. The model also fitted very well the light-response curves of 345 NPQ with impaired functioning of the XC, illustrating the usefulness of estimating model 346 parameters to characterize quantitatively the effects on NPQ of inhibitors (e.g. Bilger and 347 Bjorkman 1990), mutations (e.g. Niyogi et al. 1998), or changes in temperature (Ralph et al. 348 2001).

Finally, the model was also found to be adequate to describe NPQ vs. *E* curves generated under non-steady state conditions, i.e. derived from RLCs. The comparison of the model parameters estimated for light curves of different light steps showed the same pattern on VAZ and DD-DT samples, with shorter light steps resulting in lower NPQ_m and higher E_{50} , whilst *n* not showing any consistent trend (Ralph and Gademann 2005; Perkins et al. 2006).

355 Relationship between NPQ and ETR light-response curves

356

357 To illustrate the application of the model to evaluate to what extent the NPQ response to light is 358 related to the photoacclimation status, the light levels required for induction of NPQ and for 359 photochemistry or ETR saturation were compared. A simple form of achieving this is by the 360 direct comparison of model parameters E_{50} and E_k estimated for the two types of light-response 361 curves. These parameters have the advantage of being measured in the same scale (PAR irradiance, μ mol photons m⁻² s⁻¹) and of being independent of the units used to measure 362 363 photosynthetic rates, ETR or NPQ, which can vary with the method in use (the case of 364 photosynthesis) or instrument settings (the case of ETR).

365 For the experimental data obtained in this study, the increase in growth irradiance 366 induced a substantial change in the photoacclimation status, noticeable by a clear change in the 367 ETR vs E curves. As a result, the increase observed for E_{50} was followed by a similarly large 368 increase in E_k , (due to the decrease of α and the increase of ETR_m), both in A. thaliana (Fig. 3) 369 and in N. palea (Fig. 4). However, E_{50} was found to be in all cases higher than E_k , by 3.7 and 370 2.5 times, on average, for A. thaliana and N. palea, respectively (2.90 for the whole data). This 371 result indicates that the light-induced build-up of substantial values of NPQ started only after 372 linear electron transport reached near saturation. Furthermore, it shows that a large fraction of 373 E_{50} variability was related to variations in the photoacclimation status due to different growth 374 light conditions, as E_{50} increased linearly with E_k , both for the plant as for the diatom (although 375 the correlation was not statistical significant, but a clear linear trend is obvious; Fig. 5). This 376 analysis also showed that the slope of the regression line of E_{50} on E_k was higher for A. thaliana 377 than for N. palea, indicating that, for the same photoacclimation status (the same E_k) the plant 378 requires a higher light level for NPQ to reach 50% of its maximum capacity. Or, in other words, 379 that NPQ is induced latter in the range of irradiances in the plant than in the diatom.

Interestingly, the analysis of the subset of published data presenting both NPQ and ETR
light-response curves, showed the same generic trend. When pooling together the published and

382experimental data for samples with fully operational XC (i.e. excluding mutants or samples383treated with XC inhibitors) and E_k estimates based only on steady state light curves (i.e.384excluding RLCs as E_k estimation is largely dependent on light step duration; Serôdio et al.3852006), the ratio E_{50}/E_k was found to be higher on organisms having a VAZ type of XC and to386differ significantly from those having a DD-DT type (3.32 and 2.39, respectively; *t*-test P =3870.038; Fig. 6a).

388 Another way to compare the light responses of NPQ and ETR is to calculate the fraction 389 of NPQ that is formed when ETR approaches saturation (i.e. when $E = E_k$), or:

390

 $391 NPQ _{Ek} = \frac{NPQ (E_k)}{NPQ_m} (4)$

392

393 Low values of NPQ_{Ek} indicate that when photochemistry saturates, NPQ is still not significantly 394 developed (corresponding to high E_{50}/E_k), whilst high values indicate that NPQ responds more 395 promptly to light increase and the approach of ETR saturation (corresponding to low E_{50}/E_k). 396 Considering both published and experimental data (the data subset used above for comparing 397 E_{50}/E_k), NPQ_{Ek} averaged 0.16, and remained below 0.35, indicating that when $E = E_k$, NPQ 398 hardly reached over one third of the maximum attainable level. Confirming the expected 399 inverted relationship between NPQ_{Ek} and E_{50}/E_k , significantly lower values were found for 400 organisms with a VAZ type of XC than for those with the DD-DT type (0.14 and 0.21, 401 respectively; *t*-test, P = 0.023; Fig. 6b). 402 403 Model fitting and parameter estimation

404

405Despite the usually reduced number of data points in the analyzed NPQ light-response curves406(average 9.7, maximum 13), the model proposed in this study was found to be easily fitted to407experimental curves, in most cases yielding parameter estimates virtually independently from

408 the start values used in the iterative fitting procedure (although realistic start values allows a

409	more rapid and precise parameter estimation). The main problem found when trying to fit Eq. 2
410	to NPQ vs. E curves occurred when the curves were very close to linear, showing no clear
411	features like sigmoidicity or saturation. This resulted in that a similarly good fit could be
412	reached with different combinations of model parameters values. Still, this occurred only in
413	relatively small number of cases, like the case of the diatom Skeletonema costatum (Lavaud et
414	al. 2007) and some A. thaliana mutants with severe impairment of XC operation, for which
415	some degree of uncertainty remain associated to the presented parameter estimates.
416	This problem seems to result from the common situation of having light-response
417	curves constructed with the main purpose of characterizing the ETR vs. E curves, NPQ having
418	only a secondary interest. As it often happens that the NPQ approaches its maximum for light
419	levels much above the range used for measuring ETR, the NPQ vs. E curve may result truncated
420	and its complete shape may not be available for model fitting. Yet, the relationship established
421	between NPQ and ETR light curves in similar data may be used to impose boundaries to the
422	model parameter estimates, and thus help to reach a single set of meaningful values.
423	
424	
425	Discussion
426	
427	Model assumptions
428	
429	The model proposed for describing the NPQ vs. E curve is based on the main assumption that
430	NPQ is mostly due to $q_{\rm E}$, the 'energy-quenching' associated to the build-up of the transthylakoid
431	ΔpH and the operation of the XC. This assumption is generally supported by the finding of
432	strong linear relationships between NPQ and de-epoxidized xanthophylls in all groups
433	investigated (plants, green algae, diatoms, Chrysophyceae) and under a wide range of
434	experimental conditions.
435	However, besides q_E , NPQ also quantifies the fluorescence quenching caused by state
436	transitions (q_T) or by photoinhibition (q_I) which may potentially significantly affect the NPQ vs.

437 *E* curves. $q_{\rm T}$ is relevant in phycobilisome-containing organisms (cyanobacteria and red algae) 438 (Campbell et al. 1998; Mullineaux and Emlyn-Jones 2004), although not as much in higher 439 plants and green algae (Pfannschmidt 2005; Eberhard et al. 2008; Ruban and Johnson 2009), 440 and it does not occur for the Heterokontophyta (Lavaud 2007). When occurring, $q_{\rm T}$ is not 441 significant in light conditions triggering $q_{\rm E}$ -related NPQ (Mullineaux and Emlyn-Jones 2004; 442 Tikkanen et al. 2006; Ruban and Johnson 2009). With the exception of some land plants 443 (overwintering conifers and tropical evergreen species), q_1 origin is not clearly defined and it 444 requires particular conditions such as prolonged environmental stress (Müller et al. 2001; 445 Demmig-Adams and Adams 2006; Horton et al. 2008).

446 The effects of $q_{\rm T}$ or $q_{\rm I}$ on the light-response curve of NPQ would likely consist in a 447 general change in curve shape, through the increase of NPQ at low light levels (for $q_{\rm T}$) and of 448 NPQ_m (for q_I). However, these components of NPQ may be expected not to significantly affect 449 the NPQ vs. E curves measured under the usually applied experimental protocols. This is 450 because the duration of the light steps commonly applied ($\leq 2-3$ min, and much shorter in the 451 case of RLCs) correspond to light doses clearly different than those required to induce both $q_{\rm T}$ 452 or q_1 (Campbell et al. 1998; Müller et al. 2001; Demmig-Adams and Adams 2006; Horton et al. 453 2008; Ruban and Johnson 2009).

454 The same reasoning applies to another implicit assumption of the model, that during the 455 generation of a light curve $q_{\rm E}$ is due solely to the development of the $\Delta p H$ and the subsequent 456 de-epoxidation of the present (and susceptible of being de-epoxidized) pool of xanthophylls. In 457 fact, it is well established that exposure to high light can induce the *de novo* synthesis of XC 458 pigments (e.g. Olaizola and Yamamoto 1994; D'Haese et al. 2004; Lavaud et al. 2004; 459 Demmig-Adams and Adams 2006). This process causes the accumulation of de-epoxidized 460 forms, which could result in the linear increase of NPQ in the high-light part of the curve. 461 Again, the *de novo* synthesis requires the prolonged exposure to very high irradiances (e.g. 462 Lavaud et al. 2004; Demmig-Adams and Adams 2006; Lavaud 2007), well above the light 463 doses applied during the construction of NPQ light-response curves.

464	Nevertheless, even if significant q_T , q_I or <i>de novo</i> pigment synthesis do occur, the
465	resulting curve may still be of the same general shape and describable by the model. In fact, this
466	study showed that the model fits very well to data obtained in a wide range of experimental
467	conditions, in which the occurrence of any of these processes cannot be completely ruled out
468	(Table 3). In this case, however, care should be taken in interpreting and comparing the model
469	parameters estimated for different species or condition, as they may be affected differently by
470	processes other than the build-up of the ΔpH and the XC. In the case of organisms with
471	impaired operation of the XC (mutants or inhibitor-treated samples), the model was nevertheless
472	shown to be of value, providing a useful quantitative characterization of the light response of
473	NPQ.

474 Another major assumption of the model is that the formation of $q_{\rm E}$, through the de-475 epoxidation of Vx or DD generated by the acidification of the lumen relates to incident 476 irradiance following the generic conditions of application of the Hill equation, i.e. that NPQ can 477 be considered as allosterically regulated by irradiance through the build-up of the 478 transthylakoidal ΔpH . This assumption is partially supported by the finding that, in isolated 479 plant chloroplasts, the regulation of $q_{\rm E}$ is of allosteric nature, resulting in response curves of $q_{\rm E}$ 480 as a function of ΔpH of sigmoidal shape, shown to be adequately described by the Hill equation 481 (Pfündel and Dilley 1993; Ruban et al. 2001; Takizawa et al. 2007; Pérez-Bueno et al. 2008). 482 This sigmoidicity is attributed to the protonation of binding sites of the LHC protein Psbs 483 induced by lumen acidification, and subsequent conformational changes within the LHC, 484 switching the de-epoxidized xanthophylls to an 'activated' state. It is taken as an indication of 485 allosteric regulation of $q_{\rm E}$, resulting from proton binding showing positive cooperativity, and the 486 'activated' de-epoxidized xanthophylls Zx (Horton et al. 2000; Horton et al. 2008; Li et al. 487 2009) or DT (Goss et al. 2006; Lavaud and Kroth 2006) acting as a positive effector. 488 The strong physiological basis of the proposed model is likely to explain its success to

489 cope with a large diversity of light-response curve characteristics. Other mathematical models
 490 have also been used to successfully describe NPQ vs. *E* curves, namely an exponential
 491 saturation function, the Michaelis-Menten function, and a hyperbolic tangent function (Ritchie

492 2008). However, these models have only been tested on a reduced dataset, and on light curves
493 of low sigmoidicity. Interestingly, the model that yielded the better fit to experimental data was
494 the Michaelis-Menten equation (Ritchie 2008), a particular case of the model proposed in the
495 present study.

496

497 Model fitting to NPQ vs. *E* curves

498

499 The usefulness of the model was demonstrated by the possibility to estimate NPQ_m despite the 500 fact that in most cases the curves did not reach a saturation plateau within the range of 501 irradiances applied (e.g. Fig. 1a). When assuming NPQ to represent mostly $q_{\rm E}$, the maximum 502 NPQ reached by a sample quantifies its photoprotective potential via the dissipation of 503 excessive light energy. By applying the model, curves measured under different experimental 504 conditions can be readily compared, and changes in NPQ_m (e.g. following changes in growth 505 light conditions) can be quantified, which would be otherwise impossible. The accurate 506 estimation of NPQ_m is also required for quantifying E_{50} , which, in some cases of strict 507 relationship (e.g. for most Heterokontophyta; Lavaud et al. 2004; Lavaud 2007), is interpretable 508 as the light level corresponding to the de-epoxidation and 'activation' of the xanthophyll 509 convertible pool necessary to induce half of the maximal NPQ. The size of the xanthophyll 510 convertible pool can be very different in VAZ and DD-DT organisms varying with species and 511 growth conditions, ranging between 50 and 70% (Lavaud 2007; Goss and Jakob 2010). Again, 512 the fit of Eq. 2 to NPQ vs. E curves provided an adequate form of characterizing how promptly 513 the XC is ΔpH -activated when responding to an increase in incident irradiance.

However, while NPQ_m and E_{50} can be grossly estimated by visual inspection of the curves (at least when saturation is reached for the light levels applied), the curve sigmoidicity can hardly be characterized quantitatively without the fit of the model and estimation of *n*. The sigmoidicity coefficient was found to vary around 1.5-2.0, values corresponding to positive cooperativity and thus indicating the allosteric regulation of NPQ by light. Minimum *n* values averaged about 1 (reaching significantly lower values only for cases of XC impairment),

520	indicate that negative cooperativity is not involved in the regulation of processes underlying
521	NPQ. These results generally support that the allosteric nature known for the more fundamental
522	regulation of q_E by ΔpH in plants (Ruban et al. 2001; Li et al. 2009) holds for the relationship of
523	NPQ to irradiance in a large diversity of photosynthetic organisms.
524	The Hill coefficient has been interpreted as representing the number of allosteric
525	regulators (D'Haese et al. 2004). Considering the diversity of factors likely to contribute to the
526	NPQ vs. E curve (Demmig-Adams and Adams 2006; Eberhard et al. 2008; Horton et al. 2008;
527	Li et al. 2009), some of them not fully identified in some photosynthetic taxa (Lavaud 2007), n
528	should be considered simply as an empirical coefficient informing on the degree of allostery and
529	serving as a practical descriptor of the curve shape.
530	
531	Relationship of NPQ light response to photoacclimation status
532	
533	The modelling of the NPQ vs. E curves opens the new possibility of quantitatively comparing
534	the light responses of NPQ and of photochemistry or ETR. Photosynthesis and ETR light-
535	response curves are very commonly used as a form of characterizing the photoacclimation status
536	of photosynthetic organisms, through the estimation of the parameters of a number of available
537	models (Henley 1993; Behrenfeld et al. 2004; Ralph and Gademann 2005; Guarini and Moritz
538	2009; Perkins et al. 2010). By describing the NPQ vs. E curve by a small set of meaningful
539	parameters, the light response of NPQ can be characterized relatively to the one of ETR or
540	photosynthetic rate.
541	This possibility is useful for the definition of the light levels corresponding to the onset
542	of NPQ (e.g. activation of the XC) relatively to the saturation of photochemistry. As the light
543	response of NPQ may depend greatly on the photoacclimation status, the characterization and

comparison of NPQ vs. E curves should be preferably normalized to parameters indicative of

photochemistry saturation. Furthermore, the comparison of the NPQ and ETR light responses is

also of interest because it provides insight on the way an organism combines the responses of

photochemistry and of photoprotective processes to changes in ambient light. However, without

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adequate parameterization of the NPQ light-response, this question can be answered onlyapproximately.

550 Despite its simplicity, the indices E_{50}/E_k and NPQ_{Ek} here proposed provide an efficient 551 form of comparing the light levels for which NPQ reaches significant values and photochemistry reaches saturation. The application of these indices allowed quantifying the 552 553 delay of the light response of NPQ relatively to the saturation of photochemistry. Under the 554 assumption that NPQ mostly represents $q_{\rm E}$, it showed that it takes about three times the 555 irradiance at E_k for half of q_E to develop and that, at E_k , the formation of q_E is still at relatively 556 low levels, providing typically less than one fifth of maximum photoprotective capacity. For the 557 cases when $q_{\rm E}$ is strictly related to the XC operation (e.g. in most of Heterokontophyta; Lavaud 558 et al. 2004; Lavaud 2007), the values of the indices E_{50}/E_k and NPQ_{Ek} can be interpreted in 559 terms of the de-epoxidation state of the convertible pool of xanthophylls and their 'activation' 560 upon LHC protonation. Yet, the modelling of the NPQ vs. E curve may allow for the 561 development of other indices that better characterize this relationship.

Interestingly, the meta-analysis carried out in this study showed consistent differences between the relationships of NPQ light response to photoacclimation status in organisms with VAZ and DD-DT types of XC. The fact that these differences were found despite the diversity in taxa and growth conditions may indicate that they are due to fundamental differences in the way the two types of organisms cope with high light, especially regarding the underlying q_E mechanism and regulation of the XC, illustrating the advantages provided by the quantitative description of the NPQ vs *E* curve.

569

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571

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820	Table 1. Notation
821	
822	α – Initial slope of the ETR vs. <i>E</i> curve
823	Ax – Antheraxanthin
824	DD – Diadinoxanthin
825	DT – Diatoxanthin
826	E - PAR irradiance (µmol photons m ⁻² s ⁻¹)
827	E_{50} – Irradiance level corresponding to 50% of NPQ _m in a NPQ vs. <i>E</i> curve
828	$E_{\rm k}$ – Light-saturation parameter of the ETR vs. <i>E</i> curve
829	ETR – PSII relative electron transport rate
830	ETR_m – Maximum ETR in a ETR vs. <i>E</i> curve
831	$F_{\rm o}$, $F_{\rm m}$ – Minimum and maximum fluorescence of a dark-adapted sample
832	$F_{\rm s}, F_{\rm m}'$ – Steady state and maximum fluorescence of a light-adapted sample
833	NPQ – Non-photochemical quenching
834	NPQ_m – Maximum NPQ value reached in a NPQ vs. <i>E</i> curve
835	NPQ_{Ek} – Fraction of NPQ_m reached when $E = E_k$
836	n – Sigmoidicity coefficient of the NPQ vs. E curve
837	PSII – Photosystem II
838	RLC – Rapid light-response curve
839	VAZ – Vx-Ax-Zx XC
840	Vx – Violaxanthin
841	XC – Xanthophyll cycle
842	Zx – Zeaxanthin

844
845 Table 2. Summary of the results of the fitting of the model to NPQ vs. *E* curves measured in *A*.
846 *thaliana* and *N. palea* grown under low (LL), moderate (ML) and high light (HL). Parameter
847 estimates ± one standard error.

		Model parameters						
		NPQ _m	E_{50}	n	r ²			
A. thaliana	LL	3.91 ± 0.82	209.9 ± 8.31	1.44 ± 0.52	0.999			
	ML	4.63 ± 0.24	542.0 ± 49.2	1.21 ± 0.04	0.999			
	HL	5.90 ± 1.04	1141.0 ± 372.1	0.98 ± 0.06	0.998			
N. palea	LL	2.70 ± 0.10	91.7 ± 7.01	1.61 ± 0.19	0.991			
	ML	3.65 ± 0.05	312.6 ± 5.59	2.16 ± 0.05	0.999			
	HL	3.86 ± 0.11	551.4 ± 15.8	2.55 ± 0.08	0.999			

850	Table 3. Results of fitting of Eq. 2 to published NPQ vs. <i>E</i> curves. Composite: samples containing organisms with different XC types. LC: light curve protocol
851	(SS: steady state LL; RLCx: rapid light curve, x seconds light step; N-SLC: non-sequential light curve). Treatment: conditions applied before the measurement
852	of the light curves (PAR, temperature); growth conditions when more than one curve available for the same taxon (HL: high light; LL: low light; DTT: XC
853	inhibitor dithiothreitol; LL/HL: transfer from LL to HL; Dense: plants from dense forest stand; Edge: plants from forest edge; IL: intermittent light; CL:
854	continuous light; wt: wild type; aba1, crr6, CRR6, lut1, lut2, lut2, npq1, npq2, npq4, npq5, vtc2: mutants; LI-1, Sf-2, Col-0, Ws-2: Arabidopsis accessions;
855	Mixing: water column mixing). Parameter estimates ± one standard error.

XC type	Taxon		LC	Treatment	NPQ _m	E ₅₀	n	r^2	Reference
VAZ	Magnoliophyta	Arabidopsis thaliana	SS	wt 10 °C	2.07 ± 0.04	113.6 ± 6.0	1.11 ± 0.08	0.998	Havaux and
	(flowering plants)			wt 25 °C	2.98 ± 0.40	1118.0 ± 293.8	1.09 ± 0.11	0.996	Kloppstech
				<i>npq1</i> 10 °C	1.68 ± 0.25	636.5 ± 587.0	0.40 ± 0.06	0.998	(2001)
				<i>npq1</i> 25 °C	1.16 ± 0.22	867.1 ± 557.5	0.63 ± 0.10	0.995	
			SS	Li-1	3.76 ± 0.18	563.8 ± 40.6	1.83 ± 0.16	0.998	Jung and
				Sf-2	4.12 ± 0.16	661.9 ± 33.7	2.03 ± 0.13	0.999	Niyogi (2009)
				Col-0	3.29 ± 0.19	683.7 ± 55.0	1.84 ± 0.16	0.998	
				Ws-2	3.32 ± 0.19	599.8 ± 49.1	1.96 ± 0.21	0.997	
			SS	wt	2.06 ± 0.08	184.1 ± 13.4	2.21 ± 0.32	0.993	Lokstein et al.
				lut1	1.52 ± 0.06	195.5 ± 13.7	2.28 ± 0.33	0.994	(2002)
				lut2	1.76 ± 0.10	231.0 ± 22.2	1.72 ± 0.22	0.995	
				aba1	2.36 ± 0.19	251.1 ± 43.2	1.14 ± 0.13	0.996	
				lut2aba1	1.17 ± 0.07	214.7 ± 30.6	1.08 ± 0.10	0.997	

Model parameters

	SS	wt	3.22 ± 0.22	822.2 ± 90.9	1.46 ± 0.15	0.993	Müller-Moulé
		vtc2	3.86 ± 0.22	1606.8 ± 137.5	1.32 ± 0.05	0.957	et al. (2002)
	SS	wt LL	2.99 ± 0.22	993.6 ± 96.6	1.95 ± 0.21	0.998	Müller-Moulé
		vtc2 LL	4.52 ± 1.08	2492.1 ± 700.8	1.49 ± 0.15	0.999	et al. (2004)
		vtc2npq4 LL	2.03 ± 0.32	2556.4 ± 402.8	1.73 ± 0.08	0.991	
		vtc2npq1 LL	2.51 ± 1.41	2597.0 ± 1777.5	1.43 ± 0.26	0.998	
		wt HL	4.55 ± 6.55	4082.0 ± 5997.8	1.47 ± 0.37	0.998	
		vtc2 HL	6.82 ± 2.35	4133.0 ± 1851.3	1.24 ± 0.10	0.999	
		vtc2npq4 HL	1.82 ± 1.00	3527.3 ± 2314.0	1.39 ± 0.21	0.996	
		vtc2npq1 HL	1.80 ± 1.84	3502.8 ± 4087.1	1.39 ± 0.32	0.996	
	SS	wt	2.21 ± 0.10	457.0 ± 26.5	1.48 ± 0.16	0.993	Munekage et
		pgr5	1.77 ± 3.20	1478.1 ± 5634.7	0.81 ± 0.45	0.957	al. (2002)
	SS	wt	2.25 ± 0.11	503.2 ± 39.4	1.44 ± 0.07	0.999	Munshi et al.
		crr6	2.00 ± 0.07	397.7 ± 22.5	1.60 ± 0.08	0.999	(2006)
		crr6CRR6	2.01 ± 0.08	365.2 ± 24.2	1.65 ± 0.11	0.999	× ,
	SS	wt	4.47 ± 1.24	1894.4 ± 746.9	1.34 ± 0.17	0.995	Niyogi et al.
		npq1	1.92 ± 0.81	1877.4 ± 1686.5	0.90 ± 0.18	0.988	(1998)
		npq2	3.23 ± 0.35	1156.2 ± 207.4	1.32 ± 0.11	0.997	
Begonia× erythrophylla	SS	LL	1.27 ± 0.04	388.3 ± 14.1	3.51 ± 0.36	0.992	Burritt and
0 2 1 2		LL/HL	1.77 ± 0.05	467.3 ± 15.8	2.95 ± 0.23	0.997	Mackenzie
		HL	2.83 ± 0.17	618.3 ± 38.6	2.82 ± 0.32	0.995	(2003)
Hedera canariensis	SS	LL	3.61 ± 0.04	382.6 ± 6.3	2.90 ± 0.13	0.999	Bilger and
		LL+DTT	1.14 ± 0.18	656.2 ± 218.9	1.09 ± 0.20	0.987	Björkman
		HL	5.34 ± 0.33	761.1 ± 59.6	2.05 ± 0.24	0.996	(1990)
		HL+DTT	1.48 ± 0.05	437.0 ± 27.8	2.20 ± 0.26	0.994	× ,
Quercus petraea	SS	Dense	2.23 ± 0.03	158.2 ± 6.35	1.83 ± 0.13	0.998	Rodríguez-
~ 1		Edge	5.55 ± 0.11	423.2 ± 13.3	2.04 ± 0.10	0.999	Calcerrada et al. (2007)

		Zostera marina	SS SS RLC5 RLC10 RLC40	LL HL HL HL HL	$\begin{array}{c} 0.87 \pm 0.15 \\ 3.12 \pm 0.25 \\ 1.36 \pm 0.06 \\ 2.34 \pm 0.17 \\ 3.37 \pm 0.15 \end{array}$	$\begin{array}{c} 797.3 \pm 302.0 \\ 400.5 \pm 65.3 \\ 276.9 \pm 26.9 \\ 399.0 \pm 60.7 \\ 360.7 \pm 30.4 \end{array}$	$\begin{array}{c} 1.01 \pm 0.14 \\ 1.30 \pm 0.16 \\ 1.39 \pm 0.17 \\ 1.02 \pm 0.11 \\ 1.45 \pm 0.12 \end{array}$	0.995 0.995 0.997 0.997 0.998	Ralph and Gademann (2005)
	Bryophyta (mosses)	Eurhynchium crassinervium Pogonatum urnigerum Polytrichum juniperinum Tortula (Syntrichia) ruralis Racomitrium aquaticum Trichocolea tomentella	SS		$5.79 \pm 0.58 \\ 5.45 \pm 0.48 \\ 5.90 \pm 0.34 \\ 12.23 \pm 0.42 \\ 26.27 \pm 3.23 \\ 1.45 \pm 0.06$	$\begin{array}{c} 237.9 \pm 34.7 \\ 584.7 \pm 65.2 \\ 689.3 \pm 34.3 \\ 458.6 \pm 16.5 \\ 830.8 \pm 92.8 \\ 68.7 \pm 4.5 \end{array}$	$\begin{array}{c} 1.53 \pm 0.12 \\ 1.79 \pm 0.15 \\ 2.68 \pm 0.17 \\ 2.61 \pm 0.15 \\ 2.33 \pm 0.18 \\ 2.36 \pm 0.33 \end{array}$	0.998 0.998 0.998 0.999 0.999 0.999	Marschall and Proctor (2004)
	Chlorophyta (green algae)	Chara corallina	SS		1.67 ± 0.04	34.0 ± 0.5	6.80 ± 0.73	0.993	Krupenina and Bulychev (2007)
		Chlamydomonas reinhardtii	SS	wt npq5	$\begin{array}{c} 1.83 \pm 1.46 \\ 0.19 \pm 0.01 \end{array}$	$\begin{array}{c} 1010.0 \pm 1660.0 \\ 56.2 \pm 3.58 \end{array}$	$\begin{array}{c} 0.81 \pm 0.15 \\ 3.66 \pm 0.74 \end{array}$	0.992 0.986	Elrad et al. (2002)
		Picochlorum sp.	SS		1.88 ± 0.29	410.2 ± 142.0	1.06 ± 0.20	0.975	Dimier et al. (2007a)
	Eustigmatophyceae	Nannochloropsis oculata	RLC10		1.01 ± 0.06	224.5 ± 31.7	1.01 ± 0.08	0.998	Cosgrove and Borowitzka (2006)
DD-DT	Bacillariophycea (diatoms)	Chaetoceros socialis Skeletonema marinoi Thalassiosira rotula	SS		$\begin{array}{c} 5.71 \pm 0.08 \\ 1.27 \pm 0.03 \\ 1.14 \pm 0.18 \end{array}$	$\begin{array}{c} 149.4 \pm 4.8 \\ 233.0 \pm 9.3 \\ 198.9 \pm 89.8 \end{array}$	$\begin{array}{c} 1.88 \pm 0.10 \\ 1.95 \pm 0.13 \\ 0.89 \pm 0.23 \end{array}$	0.999 0.998 0.977	Dimier et al. (2007b)
		Fragilariopsis cylindrus	SS	5 μmol m ⁻² s ⁻¹ 65 μmol m ⁻² s ⁻¹ 125 μmol m ⁻² s ⁻¹	$\begin{array}{c} 1.00 \pm 0.18 \\ 0.74 \pm 0.08 \\ 0.59 \pm 0.12 \end{array}$	$\begin{array}{c} 637.5 \pm 266.1 \\ 1023.1 \pm 233.6 \\ 1024.5 \pm 540.6 \end{array}$	$\begin{array}{c} 1.08 \pm 0.23 \\ 1.05 \pm 0.9 \\ 0.94 \pm 0.13 \end{array}$	0.966 0.996 0.986	Kropuenske et al. (2009)
		Phaeodactylum tricornutum	SS		2.30 ± 0.10	367.6 ± 24.1	2.59 ± 0.45	0.984	Lavaud et al.

		Skeletonema costatum Thalassiosira. oceanica Thalassiosira pseudonana Thalassiosira weissflogii			$\begin{array}{c} 2.77 \pm 0.75 \\ 0.88 \pm 0.13 \\ 1.05 \pm 0.04 \\ 1.58 \pm 0.03 \end{array}$	$2825.9 \pm 1569.0 \\ 859.4 \pm 282.2 \\ 276.8 \pm 18.4 \\ 227.1 \pm 8.3$	$\begin{array}{c} 0.86 \pm 0.08 \\ 0.95 \pm 0.13 \\ 1.96 \pm 0.22 \\ 2.70 \pm 0.22 \end{array}$	0.998 0.992 0.993 0.996	(2007)
		Phaeodactylum tricornutum	SS	IL CL	$\begin{array}{c} 9.30 \pm 0.10 \\ 2.16 \pm 0.08 \end{array}$	288.5 ± 6.2 334.4 ± 17.2	$\begin{array}{c} 2.77 \pm 0.16 \\ 3.22 \pm 0.71 \end{array}$	0.999 0.980	Lavaud et al. (2002)
		Navicula phyllepta	RLC10 RLC30 RLC60 SS		$\begin{array}{c} 0.19 \pm 0.06 \\ 0.54 \pm 0.07 \\ 0.91 \pm 54.5 \\ 2.90 \pm 0.57 \end{array}$	$\begin{array}{c} 1057.6 \pm 731.3 \\ 972.2 \pm 370.1 \\ 450.0 \pm 54.5 \\ 1152.8 \pm 463.0 \end{array}$	$\begin{array}{c} 1.01 \pm 0.36 \\ 0.73 \pm 0.11 \\ 1.55 \pm 0.28 \\ 1.10 \pm 0.26 \end{array}$	0.976 0.997 0.990 0.989	Perkins et al. (2006)
		Nitzschia palea	SS	LL HL	$\begin{array}{c} 3.48 \pm 0.17 \\ 5.62 \pm 0.24 \end{array}$	$\frac{115.8 \pm 12.2}{399.9 \pm 27.1}$	$\begin{array}{c} 1.38 \pm 0.15 \\ 1.58 \pm 0.08 \end{array}$	0.991 0.999	Cruz and Serôdio (2008)
	Dinophyta (dinoflagellates)	Symbiodinium sp.	RLC5	25 °C 33 °C 37 °C 38 °C	$\begin{array}{c} 0.15 \pm 0.01 \\ 0.53 \pm 0.05 \\ 1.20 \pm 0.05 \\ 0.37 \pm 0.01 \end{array}$	$\begin{array}{c} 143.3 \pm 10.5 \\ 115.8 \pm 22.5 \\ 77.3 \pm 6.7 \\ 39.9 \pm 2.2 \end{array}$	$\begin{array}{c} 2.39 \pm 0.32 \\ 1.21 \pm 22.5 \\ 1.18 \pm 0.08 \\ 1.66 \pm 0.17 \end{array}$	0.996 0.994 0.998 0.995	Ralph et al. (2001)
	Haptophyta	Phaeocystis antarctica	SS	5 μmol m ⁻² s ⁻¹ 65 μmol m ⁻² s ⁻¹ 125 μmol m ⁻² s ⁻¹	$\begin{array}{c} 0.27 \pm 0.03 \\ 1.49 \pm 1.01 \\ 0.84 \pm 0.13 \end{array}$	$\begin{array}{c} 426.7 \pm 49.7 \\ 2953.0 \pm 3309.0 \\ 755.1 \pm 175.0 \end{array}$	$\begin{array}{c} 1.93 \pm 0.29 \\ 1.07 \pm 0.27 \\ 1.94 \pm 0.56 \end{array}$	0.990 0.985 0.986	Kropuenske et al. (2009)
Composite		Microphytobenthos	RLC50 N-SLC		$\begin{array}{c} 4.09 \pm 0.12 \\ 3.72 \pm 0.13 \end{array}$	$\begin{array}{c} 349.2 \pm 17.8 \\ 546.2 \pm 35.5 \end{array}$	$\begin{array}{c} 2.39 \pm 0.26 \\ 1.49 \pm 0.10 \end{array}$	0.994 0.996	Herlory et al. (2007)
		Phytoplankton	SS	No mixing Mixing	$\begin{array}{c} 5.07 \pm 0.52 \\ 1.85 \pm 0.09 \end{array}$	$546.6 \pm 85.5 \\ 446.4 \pm 21.9$	$\begin{array}{c} 1.42 \pm 0.13 \\ 2.73 \pm 0.24 \end{array}$	0.998 0.997	Kromkamp et al. (2008)

Table 4. Summary of the meta-analysis of the results of the fitting of the model to published

860 NPQ vs. E curves (listed in Table 3). Mean values \pm one standard error.

861

Model parameters

XC type	NPQ _m	E_{50}	n
VAZ	3.30 ± 0.49	926.7 ± 135.1	1.77 ± 0.13
DD-DT	1.88 ± 0.41	657.9 ± 145.9	1.60 ± 0.14
Mixed	3.68 ± 0.67	472.1 ± 47.3	2.01 ± 0.33
All	2.89 ± 0.35	824.3 ± 99.2	1.73 ± 0.09

Figure legends

865 866 Fig. 1. Fitting of Eq. 2 to light-response curves of NPQ measured in the plant A. thaliana grown 867 under low (LL), moderate (ML) and high light (HL). a. Light-response curves (data points), 868 fitted model (lines), and estimates of model parameters. b. Residuals of the model fitting. 869 870 Fig. 2. Fitting of Eq. 2 to light-response curves of NPQ measured in the diatom N. palea grown 871 under low (LL), moderate (ML) and high light (HL). a. Light-response curves (data points), 872 fitted model (lines), and estimates of model parameters. b. Residuals of the model fitting. 873 874 Fig. 3. Comparison between the light-response curves of NPQ and of ETR of A. thaliana grown 875 under low (LL), moderate (ML) and high light (HL). Estimates of the parameters of the model 876 of Eilers and Peeters (1988) fitted to the ETR vs. E curves are presented. Estimates of the 877 parameters of the curves fitted to the NPQ vs. E curves are presented in Table 2. 878 879 Fig. 4. Comparison between the light-response curves of NPQ and ETR of N. palea grown 880 under low (LL), moderate (ML) and high light (HL). Estimates of the parameters of the model 881 of Eilers and Peeters (1988) fitted to the ETR vs. E curves are presented. Estimates of the 882 parameters of the curves fitted to the NPQ vs. E curves are presented in Table 2. 883 884 Fig. 5. Relationship between the parameter E_{50} of Eq. 2 fitted to the NPQ vs. E curves and the 885 parameter E_k of the Eilers and Peeters (1988) model fitted to the ETR vs. E curves, for the plant 886 A. thaliana (circles) and the diatom N. palea (squares) grown under high (white), moderate 887 (gray) and low light (black). 888 889 Fig. 6. Comparison between the ratios E_{50}/E_k (a) and NPQ(E_k)/NPQ_m (b) as calculated for the 890 published (excluding cases with impaired XC operation) and experimental (this study) light-

- response curves of NPQ and ETR in VAZ and DD-DT types of XC. Bars represent one standard
- error, boxes represent the lower (25%) and upper (75%) quartiles, and the thin and thick
- horizontal lines inside the boxes represent the mean and the median, respectively.









908 Figure 5



911 Figure 6

