

A high-resolution portrait of the annual dynamics of photochemical and non-photochemical quenching in needles of *Pinus sylvestris*

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[Correction to article title, added after online publication 21 June 2011: "portray" is changed to "portrait".]

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Partitioning of excitation energy between photochemical quenching (PQ) and non-photochemical quenching (NPQ) processes is constantly adjusted in the leaf in order to preserve the photosynthetic energy balance. Adjustments in PQ and NPQ often result from a combination of different temporal components that can be simplified into reversible and sustained components. While reversible PQ and NPQ are relatively well understood, the controls behind the sustained components of PQ and NPQ, or the interaction between sustained and reversible NPQ, remain elusive. In this study, I used a full year of high-resolution chlorophyll fluorescence (ChlF) data obtained with a Monitoring-PAM fluorometer (Walz, Effeltrich, Germany) in needles of boreal *Pinus sylvestris* in situ to quantitatively analyse the dynamics and interaction between temporal components of NPQ and PQ and their control by the environment. To enable the estimation of sustained and reversible components of PQ and NPQ, a number of key ChlF parameters were reviewed and adapted to the analysis of long-term monitoring data. Overall, NPQ was drastically enhanced during winter via the accumulation of sustained NPQ in a process regulated by air temperature. Reversible NPQ retained some functionality even at temperatures well below zero and was not inhibited by the presence of sustained NPQ per se but by low temperatures alone. This suggests that temporal NPQ components co-operate in an additive rather than complementary fashion, conferring additional flexibility to the photoprotective role of NPQ. Finally, the potential of the sustained photochemical quenching parameter (qL_s) to track photoinhibition in situ was discussed.

Introduction

Photosynthetic energy input in leaves is directly proportional to the incident photosynthetic photon flux density (PPFD), but factors such as temperature, water availability or phenology largely control photosynthetic energy consumption. As a result, imbalances between energy absorption by photosynthetic pigments and energy consumption by the photosynthetic carbon reactions are common in leaves. Energy absorbed in

excess has been addressed in terms of excitation pressure (Huner et al. 1998) and associated to the formation of hazardous reactive oxygen species and photooxidative damage to the thylakoid membrane (Barber and Anderson 1992, Horton and Ruban 2005, Huner et al. 1998). Energy imbalances are often composed of independent temporal components. For example, a sudden sunfleck produces a rapid and short-lived energy imbalance in a shaded leaf, which may be superimposed to a more lasting energy imbalance caused

Abbreviations – A, leaf absorptance; α_{II} , relative cross-section area of PSII; ChlF, chlorophyll fluorescence; NPQ, non-photochemical quenching; PAM, pulse amplitude modulation; PQ, photochemical quenching.

by seasonal variation in light and temperature. Similarly, the acclimation of photosynthesis is equally made up of mechanisms with different temporal components. Yet, how do these components co-operate to cope with the multi-temporal dimension of energy imbalance?

Adjustment in the energy partitioning in photosystem II (PSII) between photochemical and non-photochemical processes is probably the acclimation mechanism presenting the highest temporal plasticity. These temporal dynamics can be best studied in evergreen species undergoing both diurnal and seasonal acclimation such as boreal Scots pine (*Pinus sylvestris*) (Ensminger et al. 2004, Ottander et al. 1995, Porcar-Castell et al. 2008a). When photosynthetic energy input exceeds its utilisation (i.e. energy is in excess), the plant acclimates by increasing the fraction of absorbed energy that is dissipated as heat in a process known as non-photochemical quenching (NPQ) (Bilger and Björkman 1990, Maxwell and Johnson 2000). NPQ involves mechanisms with different temporal kinetics. Temporal kinetics have been widely used as criteria to dissect total NPQ into components. A first NPQ component is rapidly reversible in the dark and has been referred as q_E , ΔpH -dependent NPQ, reversible NPQ, feedback de-excitation or flexible dissipation (Demmig-Adams and Adams 2006, Krause and Weis 1991). Reversible NPQ is known to involve the building of a transthylakoid ΔpH , the operation of the xanthophyll cycle, the protonation of PSII proteins and the presence of the SpbS protein (Demmig-Adams and Adams 2006, Eskling et al. 1997, Gilmore 1997, Horton et al. 1991, Krause and Weis 1991, Müller et al. 2001). A second component of NPQ does not reverse after a period of dark acclimation or during night and has been referred as q_L , sustained or inflexible NPQ (Demmig-Adams and Adams 2006, Krause and Weis 1991). Sustained NPQ has been associated to the overnight retention of zeaxanthin, the aggregation of light-harvesting proteins, the photoinhibition of reaction centres or the accumulation of specific families of proteins such as Elip or Ohps (Adams et al. 1994, Demmig-Adams and Adams 1996, 2006, Ensminger et al. 2004, Huner et al. 1998, Ivanov et al. 2003, Ottander et al. 1995). The phenomena of state transitions have also been addressed in terms of NPQ as q_T (Krause and Weis 1991), yet q_T does not involve any thermal quenching of excitation energy but 'quenching' by transfer from PSII to PSI, and it has not been considered to be of significance in higher plants (Müller et al. 2001, Niyogi 1999).

While the mechanisms behind the reversible component of NPQ and its environmental controls have been extensively studied and are well characterised (Demmig-Adams and Adams 2006, Gilmore et al. 1995, Gilmore 1997, Horton et al. 1991, Horton and Ruban

2005), the environmental controls behind the modulation of the sustained component of NPQ, or the interaction between sustained and reversible components of NPQ, remain elusive. Equally, the slow dynamics in photochemical quenching (PQ) capacity under natural conditions, for example associated with the accumulation of photoinhibited reaction centres or their recovery, have only been characterised at low temporal resolutions (Ensminger et al. 2004, Ottander et al. 1995, Porcar-Castell et al. 2008a, 2008b).

With the recent implementation of active chlorophyll fluorescence (ChlF)-monitoring instrumentation to field conditions (Porcar-Castell et al. 2008c), it is possible to follow the acclimation of photochemical and non-photochemical processes in PSII at a high temporal resolution, in situ, and over extensive periods of time.

The aim of this study was to quantitatively analyse the dynamics of and interaction between temporal components of non-photochemical quenching (NPQ) and photochemical quenching (PQ) capacities and to describe how these processes are modulated by light and temperature during one full year in needles of Scots pine. To enable the estimation of sustained and reversible components of NPQ and PQ, key ChlF parameters were reviewed and adapted to the analysis of long-term ChlF monitoring data.

Materials and methods

Long-term monitoring of ChlF in the field

ChlF was measured between 15 August 2008 and 15 August 2009 in needles of Scots pine trees (*P. sylvestris*) growing at SMEAR-II station in southern Finland and using a *MONITORING-PAM* Multi-Channel Chlorophyll Fluorometer (Walz, Effeltrich, Germany) (Porcar-Castell et al. 2008c). The *MONI-PAM* system uses modulated blue LED light to measure the fluorescence emitted from a leaf sample. Four emitter-detector units or *MONI-heads* were installed in two 47-year-old Scots pine trees (two *MONI-heads* per tree) with the help of custom-made aluminium supports that ensured that the same needles remained in place throughout the monitoring period (Fig. 1). Three to four pairs of needles were arranged in each *MONI-head* leaf clip. After a full year of measurements and for those *MONI-heads* where the needles were inserted from the side end with the Spectralon® plate (as shown in Fig. 1), signs of partial etiolation could be observed after 1 year in the leaf area immediately under the plate. This phenomenon was assumed to exert no significant effect on the fluorescence measurements that took place some 5–10 mm away along the needle length. Needles were selected



Fig. 1. A *MONI*-head measuring ChlF from Scots pine needles in a top canopy shoot. With the help of the custom-made supports, *MONI*-head and needles remain in exactly the same position relative to each other throughout the year.

from the top canopy and accessed with the help of a permanent scaffold tower. The *MONI-PAM* was connected to mains power using a 100 m cable and operated with WinControl® rev 3.13 from a computer located in a cabin 50 m from the tower. For each measuring point, the instrument recorded the instantaneous fluorescence (F), the maximal fluorescence (F'_m), as well as the incident PPFD, and air temperature. The *MONI-PAM* does not have a far red light source; therefore, minimum fluorescence (F_0) was estimated only during night. The intensity of the saturating light pulses was $4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, and the duration of the pulse was 0.8 s. Measuring light was switched off between measuring points, and two frequency settings were used to minimise the long-term effect of the extra light dosage to the needles: 10 min during the summer months and 30 min for the other months. At these settings, the average additional light dosage supplied by the saturating pulses was less than 3% of the daily integral quantum dose. Finally, the temperature sensitivity of the LED measuring light was corrected in the absolute fluorescence levels using data from a calibration experiment with a fluorescence standard. Absolute fluorescence levels in the calibration experiment were quite stable and increased only by 10%

when temperature decreased from 25 to -20°C , or by less than 5% when decreasing from 25 to 5°C .

Statistics

Significant differences in the variables between selected days in autumn, winter, spring and summer were detected by means of ANOVA and post hoc tests. Differences between two samples with a Student's *t*-test. Software used was SYSTAT 12 (Systat Software Inc, Chicago, IL).

Assumptions

(1) *Lake model assumption.* In a lake model, all the processes consuming excitation energy in the antennae of PSII compete for excitation from a common pigment pool. The lake model (Dau 1994, Joliot and Joliot 1964) is considered a good approximation for higher plants where connectivity between photosynthetic units is very high (Kramer et al. 2004, Lazar 1999). Based on a lake model, the fluorescence measured with a pulse amplitude modulation (PAM) fluorometer can be expressed

$$F = \beta I_{ML} A \alpha_{II} \frac{k_f}{k_f + k_D + k_{NPQ} + k_P} \quad (1)$$

where β is a parameter that depends on the fluorescence detector and the area under examination; I_{ML} the intensity of the modulated measuring light (constant); A the leaf absorptance; α_{II} the relative cross-section area of PSII or fraction of absorbed light captured by PSII; and k_f , k_D , k_{NPQ} and k_P the first-order rate constants of fluorescence, basal thermal energy dissipation, regulated thermal energy dissipation and photochemistry, respectively.

(2) To facilitate the analysis of sustained and reversible components of acclimation, k_{NPQ} was expressed

$$k_{NPQ} = k_{NPQ_S} + k_{NPQ_R} \quad (2)$$

where k_{NPQ_S} is the rate constant on sustained thermal dissipation (sustained NPQ) and k_{NPQ_R} the rate constant of reversible thermal dissipation (reversible NPQ). Night relaxation of reversible NPQ was used as criteria to separate between sustained or reversible NPQ. Similarly, the rate constant of photochemistry was expressed

$$k_P = k_{PSII} qL_T \quad (3)$$

where k_{PSII} is the intrinsic rate constant of photochemistry and qL_T the photochemical quenching parameter (Kramer et al. 2004), a factor that relates the current photochemical capacity (k_P) to the maximum or intrinsic capacity (k_{PSII}) as $qL_T = \frac{k_P}{k_{PSII}}$ and represents the fraction of functional and open reaction centres. Variations in photochemical capacity in PSII may occur

both in response to fast reduction/oxidation of the primary electron acceptor Q_A (open/closed reaction centres), or in response to slow processes affecting the operability of the reaction centres, such as photoinhibition (functional/non-functional reaction centres). Subsequently, qL_T was expressed

$$qL_T = qL_S qL_r \quad (4)$$

where qL_S is the sustained component of the photochemical quenching parameter (fraction of functional reaction centres) and qL_r its reversible component (fraction of open reaction centres). While both qL_S and qL_r may change concomitantly during daytime (although with different temporal kinetics), night adjustments in qL_T can be attributed to qL_S alone. In this study, I used the maximum night qL_T to discuss sustained changes in PQ.

(3) *Constant absorbance*. Annual chlorophyll contents for Scots pine trees in SMEAR-II Station range from 1 to 2 $\mu\text{mol Chl g}^{-1}$ DW (c. 400–800 $\mu\text{mol m}^{-2}$) (Porcar-Castell et al. 2008b), at which range changes in chlorophyll contents have been shown to exert only a very small effect on the measured Chf (Adams et al. 1990, Porcar-Castell et al. 2008a). We have also previously shown how the spring recovery in fluorescence levels precedes the increase in pigment concentrations in needles of Scots pine (Porcar-Castell et al. 2008c), indicating that the strong seasonal changes in absolute fluorescence levels cannot be explained

by changes in light absorption properties of the leaf related to chlorophyll content. As a first approximation, I assumed that seasonal changes in pigment contents in Scots pine needles did not significantly interfere with the measured fluorescence levels.

(4) *Absorption cross-section*. In the absence of a current understanding on how the relative absorption cross sections of PSII and PSI vary during the year in evergreen foliage, I assumed as a first approximation that the partitioning of excitation energy between PSII and PSI remained constant. Accordingly, any variation in excitation energy partitioning between photosystems would be embedded in the rate constant of sustained NPQ (k_{NPQ}), in a seasonal analogy to the short-term phenomena of state transitions (Müller et al. 2001, Niyogi 1999).

Adaptation of Chf parameters to the analysis of long-term Chf data

Seven core Chf parameters were defined based on Eqns 1–4 (Table 1) and used to derivate relative rate constants and quenching parameters (Table 2) or process yields (Table 3). See text in Tables 1–3 for further details on the derivation, original references and other clarifications.

For clarity reasons, PQ and NPQ are used as abbreviations of the phenomena of photochemical and non-photochemical quenching, while *PQ* and *NPQ* (in italics) are used to represent the respective fluorescence parameters.

Table 1. Estimation of core Chf parameters from monitoring data.

Parameter definition	Formulation	Eqn no.	Measuring protocol in long-term monitoring experiments
Reference minimal fluorescence	$F_{OR} = \beta I_{ML} A \alpha \frac{k_f}{k_f + k_D + k_{PSII}}$	5	Minimum fluorescence measured during night in leaves presenting no downregulation nor photoinhibition. One single reference for the whole monitoring period. F_{OR} was obtained from the night measuring point when the quantum yield of photochemistry registered the maximum for the whole monitoring experiment, around 0.83.
Reference maximal fluorescence	$F_{mR} = \beta I_{ML} A \alpha \frac{k_f}{k_f + k_D}$	6	Maximum fluorescence measured upon a saturating light pulse in leaves with no downregulation. One single reference for the whole monitoring period. F_{mR} was obtained from the night measuring point when the quantum yield of photochemistry registered the maximum for the whole monitoring experiment, around 0.83.
Night minimal fluorescence	$F_o = \beta I_{ML} A \alpha \frac{k_f}{k_f + k_D + k_{NPQs} + k_{PSII} qL_S}$	7	Minimum fluorescence measured during night when maximum night quantum yield of photochemistry was registered. A new F_o estimated every night.
Minimal fluorescence	$F'_o = \beta I_{ML} A \alpha \frac{k_f}{k_f + k_D + k_{NPQs} + k_{NPQr} + k_{PSII} qL_S}$	8	Minimum fluorescence measured in the dark, and when all primary electron acceptors are oxidised ($qL_r = 1$), other than F_o .
Night maximal fluorescence	$F_m = \beta I_{ML} A \alpha \frac{k_f}{k_f + k_D + k_{NPQs}}$	9	Maximum fluorescence measured upon a saturating light pulse and when maximum night quantum yield of photochemistry was registered. A new F_m estimated every night.
Maximal fluorescence	$F'_m = \beta I_{ML} A \alpha \frac{k_f}{k_f + k_D + k_{NPQs} + k_{NPQr}}$	10	Maximum fluorescence measured upon a saturating light pulse at any point in time.
Instantaneous fluorescence	$F = \beta I_{ML} A \alpha \frac{k_f}{k_f + k_D + k_{NPQs} + k_{NPQr} + k_{PSII} qL_S qL_r}$	11	Fluorescence measured at any other point in time

Table 2. Estimation of rate constants, relative rate constants and quenching parameters from monitoring data.

Parameter definition	Formulation	Eqn no.	Relative rate constant	Remarks
Rate constant of fluorescence	k_f	–	–	Constant
Rate constant of basal thermal energy dissipation	k_D	–	–	Constant
Intrinsic rate constant of photochemistry	$k_{PSII} = \left(\frac{F_{mR}}{F_{oR}} - 1\right)(k_f + k_D)$	12	$k'_{PSII} = \left(\frac{F_{mR}}{F_{oR}} - 1\right)$ $k'_{PSII} = PQ_{max}$	Represents the maximum PQ capacity under optimal conditions and it is assumed to remain constant throughout the monitoring experiment. The value of k_{PSII} depends on the selected reference F_{oR} and F_{mR} . Derived combining Eqns 5 and 6 (see also Kramer et al. 2004).
Rate constant of photochemistry	$k_P = \left(\frac{F_{mR}}{F} - \frac{F_{mR}}{F_m}\right)(k_f + k_D)$	13	$k'_P = \left(\frac{F_{mR}}{F} - \frac{F_{mR}}{F_m}\right)$ $k'_P = PQ$	Represents the actual PQ capacity. Note that the parameters PQ and NPQ have the same units (i.e. times $k_f + k_D$). Derived combining Eqns 10 and 11 and substituting k_{NPQ} by Eqn 14 (see also Laisk et al. 1997).
Rate constant of total regulated thermal energy dissipation	$k_{NPQ} = \left(\frac{F_{mR}}{F_m} - 1\right)(k_f + k_D)$	14	$k'_{NPQ} = \left(\frac{F_{mR}}{F_m} - 1\right)$ $k'_{NPQ} = NPQ$	Represents the total regulated thermal energy dissipation capacity. Where NPQ is the widely used NPQ parameter (Bilger and Björkman 1991). Derived by adding Eqns 15 and 16 (see also Laisk et al. 1997).
Rate constant of sustained thermal energy dissipation	$k_{NPQs} = \left(\frac{F_{mR}}{F_m} - 1\right)(k_f + k_D)$	15	$k'_{NPQs} = \left(\frac{F_{mR}}{F_m} - 1\right)$ $k'_{NPQs} = NPQ_s$	Represents the sustained component of k_{NPQ} . Derived by combining Eqns 6 and 9 (see also Maxwell and Johnson 2000, Porcar-Castell et al. 2008a).
Rate constant of reversible thermal energy dissipation	$k_{NPQr} = \left(\frac{F_{mR}}{F_m} - \frac{F_{mR}}{F_m}\right)(k_f + k_D)$	16	$k'_{NPQr} = \left(\frac{F_{mR}}{F_m} - \frac{F_{mR}}{F_m}\right)$ $k'_{NPQr} = NPQ_r$	Represents the reversible component of k_{NPQ} . Derived by combining Eqns 9 and 10 (see also Maxwell and Johnson 2000).
Total photochemical quenching	$qL_T = \frac{\left(\frac{1}{F} - \frac{1}{F_m}\right)}{\left(\frac{1}{F_{oR}} - \frac{1}{F_{mR}}\right)}$	17	–	Total photochemical quenching parameter based on a lake model. Denotes the fraction of open and functional reaction centres. Derived by combining Eqns 3, 12 and 13 (see also Havaux et al. 1991, Kramer et al. 2004).
Sustained photochemical quenching	$qL_s = \frac{\left(\frac{1}{F_o} - \frac{1}{F_m}\right)}{\left(\frac{1}{F_{oR}} - \frac{1}{F_{mR}}\right)}$	18	–	Represents the sustained component of qL_T . Denotes the fraction of functional reaction centres (non-photoinhibited). Note that F_o and F_m become F_o and F_m when measured in the absence of NPQ_r . Derived by combining Eqns 3, 4, 12 and 13 (see also Havaux et al. 1991, Kramer et al. 2004).
Reversible photochemical quenching	$qL_r = \frac{\left(\frac{1}{F} - \frac{1}{F_m}\right)}{\left(\frac{1}{F_o} - \frac{1}{F_m}\right)}$	19	–	Represents the reversible component of qL_T . Denotes the current fraction of open reaction centres. Derived by combining Eqns 4, 17 and 18 (see Kramer et al. (2004) for the original reference).

Results

Light and temperature data

The diurnal and annual pattern of variation in light and temperature were typical for the boreal climate of high latitudes, with long cold winters and short

mild summers (Fig. 2A), along with a marked annual variation in photoperiod and maximum light intensity during the day (Fig. 2B). Differences in temperature and light intensity between the start (August 2008) and the end of the experiment (August 2009) (Fig. 2A, B) were caused by inter-annual variability in cloudiness.

Table 3. Estimation of process yields from ChlF monitoring data.

Process yield	Formulation	Eqn no.	References
Yield of total NPQ	$\Phi_{NPQ} = \frac{F}{F_m} - \frac{F}{F_{mR}}$	20	Cailly et al. (1996), Laisk et al. (1997), Kornyejev and Hendrickson (2007)
Yield of sustained NPQ	$\Phi_{NPQs} = \frac{F}{F_m} - \frac{F}{F_{mR}}$	21	Porcar-Castell et al. (2008)
Yield of reversible NPQ	$\Phi_{NPQr} = \frac{F}{F_m} - \frac{F}{F_m}$	22	Laisk et al. (1997), Hendrickson et al. (2004), Kornyejev and Hendrickson (2007)
Yield of photochemistry	$\Phi_P = 1 - \frac{F}{F_m}$	23	Genty et al. (1989)
Yield of fluorescence and basal thermal energy dissipation	$\Phi_{f,D} = \frac{F}{F_m}$	24	Hendrickson et al. (2004), Kornyejev and Hendrickson (2007)

Raw fluorescence data

The annual variation in absolute fluorescence levels in Scots pine needles was characterised by the seasonal pattern in both instantaneous fluorescence (F) and maximal fluorescence (F'_m), with a strong decrease during autumn and subsequent recovery during late spring (Fig. 2C, D). In contrast, the diurnal pattern of variation in F and F'_m was different and changed also during the season: instantaneous fluorescence (F) increased during the daytime in summer and autumn (Fig. 2C), whereas F'_m decreased during the daytime and was at the maximum during the night. Conversely, both F and F'_m remained low and with little diurnal variation in winter and spring (Fig. 2C, D).

Annual variation in the rate constants

The diurnal and seasonal pattern of variation of F and F'_m shown in Fig. 2 was translated into variation in PQ and NPQ using the relative rate constant of photochemistry (k'_p) or PQ and the relative rate constant of regulated thermal energy dissipation (k'_{NPQ}) or NPQ , respectively (Table 2). Both NPQ and PQ presented seasonal and diurnal variations (Fig. 3). In the case of NPQ , the annual variation was dominated by the sustained component NPQ_s with a marked seasonal increase starting in November and reaching a maximum by the end of February, decreasing rapidly thereafter (Figs 3A and 4C). Comparatively, the reversible component NPQ_r was significantly smaller than the sustained component during winter and spring but larger during summer (Fig. 4). Furthermore, the maximum levels of NPQ registered throughout the year varied between 10 and 14 in different *MONI*-heads, whereas absolute maximum annual levels of the reversible NPQ_r did not exceed 4 and were typically around 2 in full sunlight (see e.g. Fig. 4C, E). The dynamics of PQ indicated an equally strong diurnal and seasonal variation pattern in photochemical quenching capacity in needles of Scots pine (Fig. 3B). PQ presented an important decrease

during daytime throughout the year, yet this decrease was less important during late autumn (Fig. 3B), when PPFD was very low (Fig. 2B). Seasonal changes in PQ (associated with qL_s or the fraction of functional reaction centres) presented a rapid and significant decrease during February (Figs 3B and 4G) and recovered towards summer.

Annual variation in quantum yields

The quantum yields of regulated thermal energy dissipation or NPQ (Φ_{NPQ}) and photochemistry (Φ_P) were inversely proportional both at the diurnal and seasonal scale (Fig. 5A, B). During summer and autumn, photochemistry was a dominant process in the energy partitioning in PSII and was significantly higher than that in winter and spring (Fig. 4H). The partitioning of excitation energy into NPQ was dominated by sustained NPQ during winter and spring (Fig. 4D, J) and by reversible NPQ during summer and autumn (Fig. 4F, J). However, the relationship between NPQ and photochemistry was not fully complementary, as the yield of other energy deactivation processes (i.e. mainly fluorescence and constitutive thermal energy dissipation) ($\Phi_{f,D}$) was not constant (Fig. 5C). Specifically, $\Phi_{f,D}$ was found to be significantly larger in summer and autumn compared with that in winter and spring (Fig. 4I).

Effect of light and temperature on photochemical capacity

The photochemical capacity in Scots pine needles, denoted by the relative rate constant of photochemistry (k'_p) or PQ , was found to be strongly dependent on the instantaneous light and temperature. Photochemical capacity decreased with increasing light intensities and the decrease was more pronounced the lower the temperatures were (Fig. 6A). Changes in PQ during night denoted variations in sustained photochemical quenching (qL_s) (Table 2), as $qL_r = 1$ in the dark. Dark levels of PQ were found to remain high until temperature

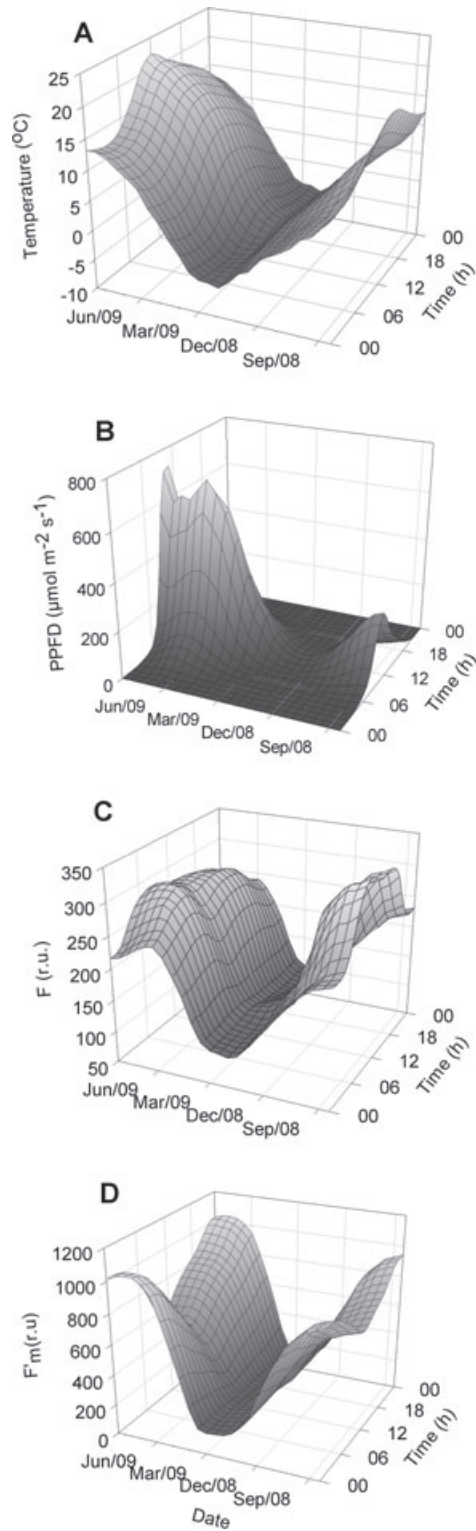


Fig. 2. Annual variation in temperature (A), PPFD (B), instantaneous fluorescence (C) and maximal fluorescence (D) in needles of Scots pine. Data correspond to a single *MONI*-head ($N = 1$).

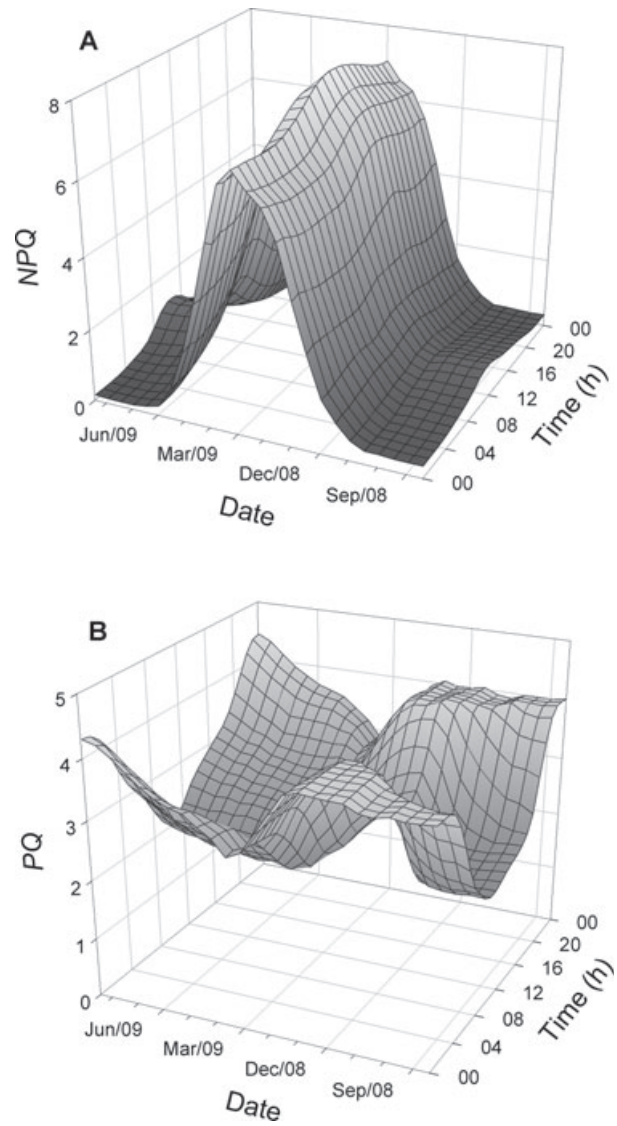


Fig. 3. Annual variation in NPQ capacity, expressed by *NPQ* (A), and in PQ capacity, expressed by *PQ* (B), in needles of Scots pine. Data correspond to a single *MONI*-head ($N = 1$), same as in Fig. 2.

decreased below zero (Fig. 6A). Further analysis of the temperature and light dependency of qL_s (Fig. 6B) showed that the sustained component of *PQ* tended to decrease with increasing mean daily PPFD, while the magnitude of the increase was found to be dependent on the mean daily temperature, i.e. while no decrease in qL_s was observed in warm sunny days, qL_s tended to decrease in cold days even under low light intensities. Finally, the slight increasing plateau present in both k_p' and qL_s at low temperatures and high irradiances corresponded to the points obtained during spring, when low temperatures combined with high PPFD were found.

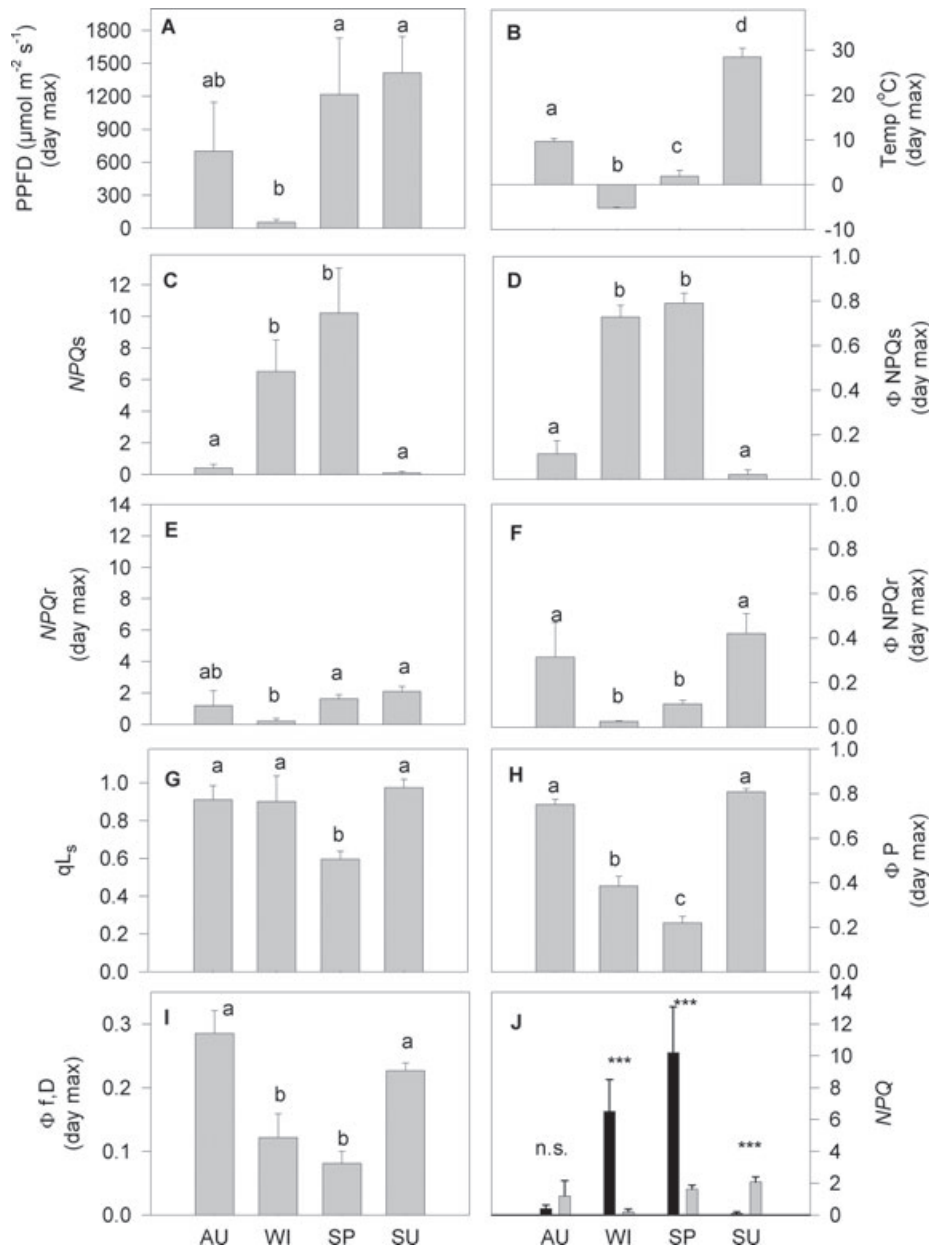


Fig. 4. Differences in specific variables among 4 days with clear skies in autumn (AU), winter (WI), spring (SP) and summer (SU). Bars correspond to means \pm standard deviation, with $N = 4$ (four *MONI*-heads). Because needles in each *MONI*-head experienced a slightly different diurnal pattern of PPFD, daily maximum levels in the variables were used in the analysis instead of selecting a particular time of the day. Daily maximum PPFD during the day (A); daily maximum temperature (B); sustained NPQ, NPQ_s (C); daily maximum quantum yield of sustained NPQ (Φ_{NPQ_s}) (D); daily maximum reversible NPQ, NPQ_r (E); daily maximum quantum yield of reversible NPQ (Φ_{NPQ_r}) (F); sustained photochemical quenching parameter qL_s (G); daily maximum quantum yield of photochemistry (Φ_P) (H); daily maximum quantum yield of fluorescence and constitutive thermal energy dissipation ($\Phi_{f,D}$) (I); and comparison between sustained (black columns) and reversible (gray columns) NPQ (J). Significant differences ($P < 0.05$) are denoted with different lower case letters or asterisks ($P < 0.001$).

Effect of light and temperature on NPQ capacity

The capacity of NPQ in Scots pine needles, denoted by NPQ , was found to be strongly dependent on the instantaneous light and temperature. NPQ increased

with light intensity and decreased with temperature (Fig. 7A). Interestingly, NPQ increased to a greater extent in response to PPFD the lower the temperatures were, but this tendency reversed and NPQ become less and less sensitive to PPFD under subfreezing temperatures.

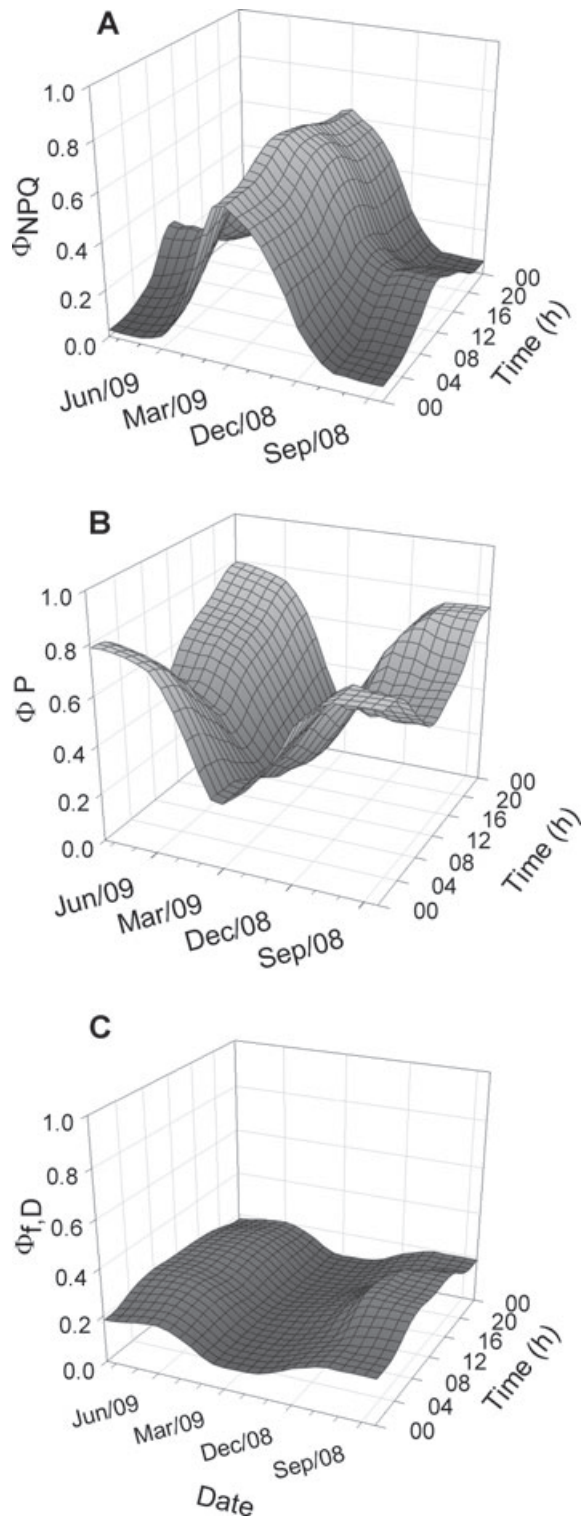


Fig. 5. Annual variation in the quantum yields of total NPQ Φ_{NPQ} (A), photochemistry Φ_P (B) and the combined quantum yield of fluorescence and constitutive thermal energy dissipation $\Phi_{f,D}$ (C) in needles of Scots pine. Data correspond to a single *MONI*-head ($N = 1$), same as in Fig. 2.

Also, a drastic increase in *NPQ* was observed at all light levels for temperatures below 7.5°C . The results shown in Fig. 7A represent the superimposed effect of sustained and reversible *NPQ*. As shown in Fig. 7B, the sensitivity of the sustained *NPQ* or *NPQ_s* to daily mean temperature and PPFD was analysed. The results showed how *NPQ_s* started to accumulate already when daily mean temperatures fell below *c.* 7.5°C (Figs 7B and 8A). Also, under cold weather, *NPQ_s* was dependent on the daily mean PPFD. Thus, for the same temperatures, *NPQ_s* was larger in spring (higher PPFD) compared with autumn (lower PPFD). Finally, the temperature dependency of the sustained and reversible components of *NPQ* was compared as shown in Fig. 8. The results indicate that while sustained *NPQ* started to accumulate once mean daily temperatures went below a threshold (in this case around 5°C), the capacity of the reversible component of *NPQ* (represented in Fig. 8B by the maximum *NPQ_r* registered at each temperature) decreased rapidly when temperatures went below 0°C . At temperatures above zero, the maximum capacity for reversible *NPQ* tended to gradually decrease with an increase in temperature.

Discussion

The deployment of long-term field PAM ChlF monitoring instrumentation allowed for the first time to present a high-resolution portrayal of the annual dynamics in PQ and *NPQ* in needles of Scots pine in situ. Because of the high temporal resolution and coverage of the data, the interaction between different temporal components of PQ and *NPQ* or their controls by light and temperature could be evaluated. The maximum capacity of *NPQ* was found to be drastically enhanced during winter being up to three times higher than that in summer, via the accumulation of sustained *NPQ* and in a process closely regulated by air temperature. In turn, reversible *NPQ* was found to retain some functionality in needles of Scots pine even at temperatures well below zero and was not inhibited by the presence of sustained *NPQ*. These results confirm that sustained and reversible *NPQ* components co-operate in an additive rather than complementary way, conferring further flexibility to the photoprotective role of *NPQ* to cope with the multi-temporal dimension of the energy imbalances present in the leaf under a wide range of environmental conditions. Furthermore, it was shown that sustained *NPQ* alone was the cause behind the observed decrease in quantum yield of photochemistry in Scots pine needles during autumn and winter, while both sustained *NPQ* and sustained reversible *NPQ* components co-operate in an additive rather than complementary way, conferring further flexibility to the photoprotective role of *NPQ* to cope with the multi-temporal dimension of the energy imbalances present in the leaf under a wide range of environmental conditions. Furthermore, it was shown that sustained *NPQ* alone was the cause behind the observed decrease in quantum yield of photochemistry during early spring,

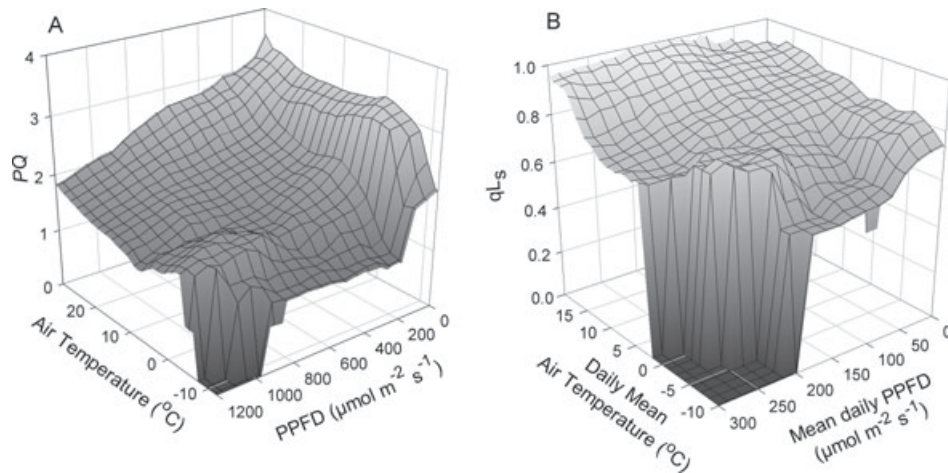


Fig. 6. Instantaneous and seasonal modulation of photochemical quenching capacity by light and temperature. (A) Instantaneous relationship between PQ , air temperature and PPFD throughout the course of a year in needles of Scots pine; (B) seasonal relationship between the sustained photochemical quenching parameter qL_s , daily mean air temperature and daily mean PPFD. Zero surface points at freezing temperatures and high irradiances denote absence of data. Data correspond to a single *MONI*-head ($N = 1$), same as in Fig. 2.

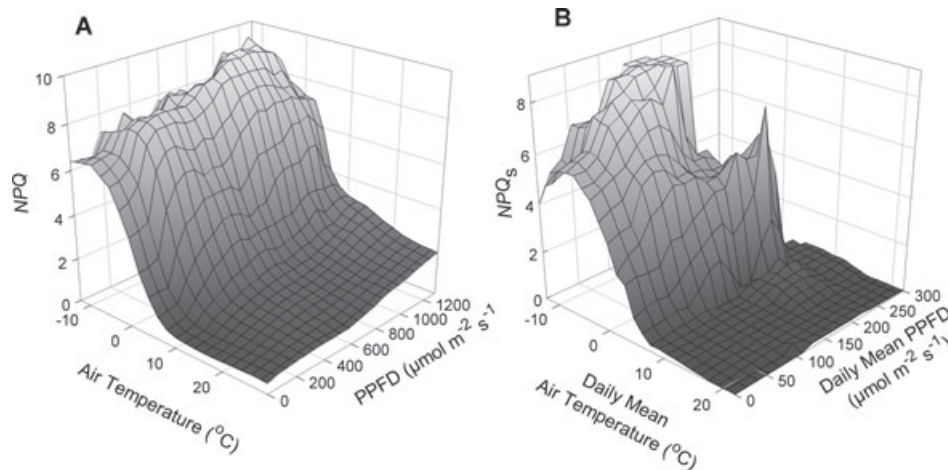


Fig. 7. Instantaneous and seasonal modulation of non-photochemical capacity by light and temperature. (A) Instantaneous relationship between NPQ , air temperature and PPFD throughout the course of a year in needles of Scots pine; (B) seasonal relationship between sustained NPQ , daily mean air temperature and daily mean PPFD. Zero surface points at freezing temperatures and high irradiances denote absence of data. Data correspond to a single *MONI*-head ($N = 1$), same as in Fig. 2.

when low temperatures and high irradiances might have enhanced net photoinhibition.

Annual dynamics in maximum quantum yield of photochemistry

Photosynthetic gas exchange decreases radically in boreal conifers in response to freezing temperatures (Kolari et al. 2007, Öquist 1983) and stops when water freezes extracellularly a few degrees below zero (Öquist 1983). Simultaneously, PSII has been found to be strongly downregulated and photoinhibited during

winter in needles of boreal conifers as indicated by a sustained depression in the maximum quantum yield of photochemistry (F_v/F_m) (Ensminger et al. 2004, Lundmark et al. 1998, Ottander and Öquist 1991, Ottander et al. 1995, Porcar-Castell et al. 2008a, Strand and Lundmark 1987). Winter depression in F_v/F_m has been partly attributed to the photoinhibition of reaction centres, indicated by a reduction in the levels of D1 PSII reaction centre protein (Ensminger et al. 2004, Ottander et al. 1995) or by a reduction in the relative rate constant of photochemistry (Porcar-Castell et al. 2008a, 2008b), as well as to the presence of sustained NPQ, indicated

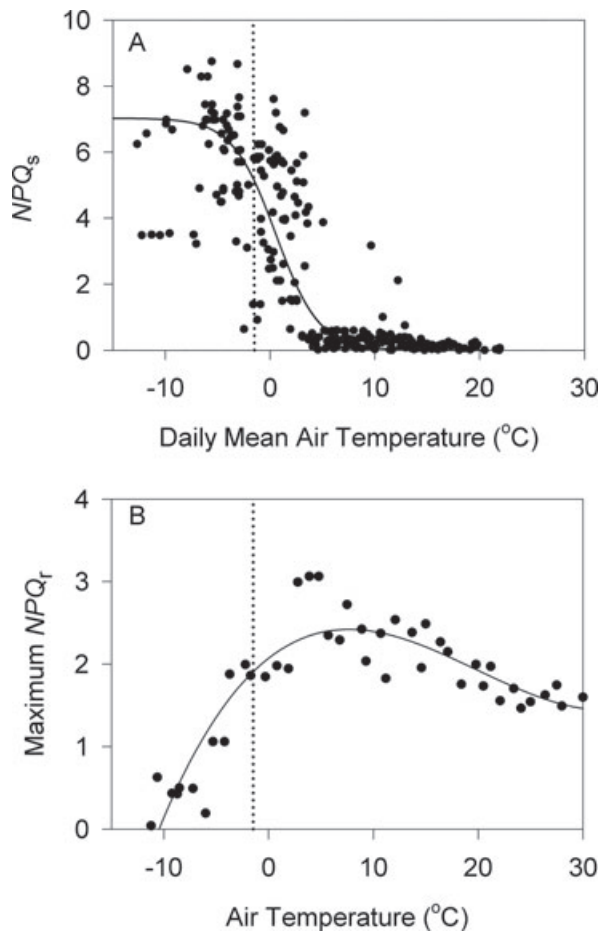


Fig. 8. Effect of temperature on the adjustment of different components of NPQ. (A) Relationship between sustained NPQ_s and daily mean temperature throughout the course of the year in needles of Scots pine. (B) Relationship between maximum reversible NPQ_r and instantaneous air temperature, estimated as the maximum reversible NPQ registered at each temperature (i.e. the NPQ_r value at 10°C represents the maximum NPQ_r registered throughout the year for temperatures between 9.5 and 10.5°C). Data correspond to a single *MONI*-head ($N = 1$), same as in Fig. 2.

by a sustained increase and retention of zeaxanthin (Ensminger et al. 2004, Ottander et al. 1995, Porcar-Castell et al. 2008b), or an increase in the sustained component of NPQ (Porcar-Castell et al. 2008a, 2008b). The results presented here clearly demonstrate that the reduction in maximum quantum yield of PSII observed during autumn and winter (Fig. 3H) is caused by the increased capacity of sustained NPQ alone as indicated by NPQ_s (Fig. 3C), while both sustained NPQ (Fig. 3C) and photoinhibition of reaction centres (Fig. 3G) participate in the further decrease in maximum quantum yield of PSII undergone during spring (Fig. 3H). The dynamics of these processes and their modulation by light and temperature are discussed next.

Annual dynamics in NPQ

The maximum capacity of NPQ was found to increase drastically during winter compared with summer (Figs 2A and 3), with total NPQ values during winter of up to 14, reflecting the strong downregulation undergone in needles of Scots pine during winter. These high NPQ levels are consistent with our previous observations in overwintering Scots pine (Porcar-Castell et al. 2008a, 2008b), where the sustained component of NPQ during Spring was around 12. However, these NPQ levels are substantially higher than those widely found in the literature, i.e. between 0.5 and 3.5 (Maxwell and Johnson 2000). This is not surprising because studies reporting NPQ have been so far biased towards reversible NPQ because of the logistic challenges of estimating sustained NPQ in deeply downregulated leaves (Adams et al. 1994). Interestingly, the enhancement in NPQ during winter (represented by the parameter NPQ) was found to overcompensate for the reduction in PQ (represented by the parameter PQ) (Fig. 2). While the sum of NPQ and PQ have been found to remain relatively constant in response to a number of stresses and in a number of species (Laisk et al. 1997), this does not appear to be the case in overwintering Scots pine, where the sum $NPQ + PQ$ increased towards winter because of the large increase in NPQ. This increase suppressed the fluorescence yield during winter (Figs 1C and 4C) and could explain the widely observed seasonal decrease in F_0 or F levels in boreal or temperate evergreens during winter (Ottander et al. 1995, Porcar-Castell et al. 2008a, 2008b, Soukupová et al. 2008).

The drastic increase in sustained NPQ in overwintering Scots pine needles may be the result of different processes. Aggregation of light harvesting complexes of photosystem II (LHCII) has been found to take place during winter in needles of Scots pine (Ottander et al. 1995) and to enhance NPQ (Horton et al. 1991, Pascal et al. 2005, Tang et al. 2007) by creating new quenching sites or promoting heat dissipation within the aggregate by already existing quenching sites (Van Oort et al. 2007). These observations are consistent with the radical increase in sustained NPQ reported here during winter in Scots pine needles. Alternatively, part of the observed winter increase in sustained NPQ could also be explained by a decrease in the absorption cross-section area of PSII α_{II} , in a seasonal analogy to the fast process of state transitions (qT). Further research will be needed to reveal the relative role of the processes behind the observed increase in NPQ capacity in overwintering Scots pine needles.

The interaction of light and temperature with NPQ was examined as shown in Figs 6 and 7. Incident PPFD

was shown to increase reversible NPQ or qE to a different extent depending on temperature. Interestingly, although zeaxanthin formation and the qE mechanism are thought to be strongly inhibited at low temperatures (Bilger and Björkman 1991, Demmig-Adams et al. 1989, Eskling et al. 1997), traces of reversible NPQ activity were still found down to temperatures as low as -10°C (Fig. 7B). On the other hand, the accumulation of sustained NPQ started at temperatures above those that strongly inhibited reversible NPQ (Fig. 7). During the first cold autumn days, NPQ increased during the morning hours in response to illumination (reversible NPQ) but at sunset part of this NPQ would not reverse and pre-dawn levels of NPQ (sustained NPQ) were higher the following day. The contrary took place during the first warm days in spring, and intermediate situations were present in response to slight warm or cold spells. But, are reversible and sustained NPQ complementary? Do they follow a mechanism by which reversible NPQ becomes sustained NPQ and vice versa?

While the temperature dependencies of sustained and reversible NPQ (Fig. 7) are consistent with a complementary conversion of reversible into sustained NPQ on decreasing temperatures and the other way around when temperatures increase, the overall capacity of reversible NPQ was not inhibited by the presence of sustained NPQ per se (Fig. 4C, E), rather by low temperatures alone (Fig. 7B). These observations suggest that reversible and sustained NPQ might constitute at least partly independent mechanisms that are additive rather than complementary. Studying the interaction between sustained and reversible NPQ in response to different sources of stress, other than freezing temperatures, might help in clarifying this interaction. Overall, the accumulation of sustained NPQ at temperatures higher than those at which reversible NPQ is strongly inhibited, combined with the residual dynamics of reversible NPQ at below zero temperatures, confer a very high flexibility and environmental plasticity to NPQ. Close co-operation of sustained and reversible NPQ would promote effective acclimation to the multi-temporal energy imbalances undergone by Scots pine needles under the rigorous boreal environment.

The sustained photochemical quenching parameter qL_s as a proxy of photoinhibition?

The slow dynamics in photochemical capacity in Scots pine needles were followed using a modified form of the quenching parameter qL (Kramer et al. 2004) in order to account only for the sustained changes in PQ (Table 2, Eqn 18). The quenching parameter qL and its temporal variants presented in this study (Table 2, Eqns 17–19)

are based on a lake model assumption. Kramer et al. (2004) compared the performance of different organisational models (lake, separate units or connected units) and concluded that estimating the fraction of open reaction centres (with Q_A oxidised) using the lake model assumption or a connected units model with a more realistic value for the probability of exciton transfer between photosynthetic units (Joliot and Joliot 1964, Kramer et al. 2004, Lazar 1999) yielded very similar results. To the best of my knowledge, seasonal changes in connectivity between PSII units have not been quantitatively characterised before, let alone in overwintering evergreens. In the absence of this knowledge, the possibility of a severe decrease in connectivity in overwintering foliage cannot be ruled out. A severe decrease in connectivity would result in underestimation of the fraction of functional reaction centres by qL_s (see Fig. 1 in Kramer et al. 2004). The dynamics in connectivity in evergreen foliage and its implications on qL_s deserve thus further research.

The parameter qL_s (Table 2) is a relative form of the parameter $1/F_o - 1/F_m$, which has been used as a measure of the rate constant of energy trapping or photochemistry in dark acclimated leaves (Baker and Oxborough 2004, Havaux et al. 1991, Matsubara and Chow 2004), shown to be linearly proportional to the fraction of functional reaction centres as measured by repetitive single-turnover flashes (Lee et al. 1999, 2001). In its relative form, qL_s denotes the proportion of functional reaction centres relative to a reference state (obtained when night maximum quantum yield of photochemistry or F_v/F_m is maximum, c. 0.83). Thus, a value of qL_s of 0.5 denotes that approximately 50% of the reaction centres that were functional during the reference period are non-functional (or photoinhibited).

Following the commonly accepted model of photoinhibition, net photoinhibition is the result of differences between the rate of photoinhibition and the rate of recovery (Aro et al. 1993, Tsonev and Hikosaka 2003), where the rate of photoinhibition is proportional to PPFD (Tyystjärvi and Aro 1996) and the rate of recovery is strongly inhibited at low temperatures (Krause 1994, Tyystjärvi 2008). From this model, it follows that net accumulation of photodamaged reaction centres should be observed with increasing PPFD especially at lower temperatures. The sustained decrease in PQ observed here in Scots pine needles, during spring (Fig. 2B), when irradiances were increasing while temperatures were still low, and the observed light and temperature dependency of qL_s (Fig. 5B) are in full agreement with this model.

Overall, keeping in mind the potential interactions between connectivity among PSII units and the lake model assumption used in qL_s , the parameter qL_s (Table 2) provides an informative and useful proxy of

the fraction of functional reaction centres, which can be easily monitored in the field using ChlF.

Final remarks

Combination of long-term ChlF measurements with analysis of PQ and NPQ, using the parameters *PQ* and *NPQ*, respectively, opens up a new range of study possibilities towards the understanding of the acclimation of photosynthetic to the environment. In particular, the high temporal resolution and coverage of long-term ChlF monitoring data may facilitate the development of mechanistic models of the light reactions of photosynthesis and their posterior implementation to the analysis of instantaneous fluorescence data, a milestone towards the interpretation of remotely sensed ChlF (Malenovký et al. 2009, Meroni et al. 2009).

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