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Seasonal acclimation of photosystem II in *Pinus sylvestris*. I. Estimating the rate constants of sustained thermal energy dissipation and photochemistry

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Summary Acclimation of the partitioning of absorbed light energy in Photosystem II (PSII) between photochemical and non-photochemical processes includes short-term adjustments that are rapidly reversed in the dark and seasonal acclimation processes that are unaffected by dark acclimation. Thus, by using dark-acclimated leaves to study the seasonal acclimation of PSII, the confounding effect of short-term adjustments is eliminated. The maximum quantum yield of photochemistry, estimated by chlorophyll fluorescence analysis as F_v/F_m, where $F_v = (F_m - F_o)$, and F_m and F_o are maximum and minimum chlorophyll fluorescence, respectively, has been widely used to follow the seasonal acclimation of PSII, because it is measured in dark-acclimated leaves. Seasonal changes in F_v/F_m can be caused by adjustments in either the photochemical capacity in PSII, or the capacity of thermal dissipation in PSII, or both. However, there is a lack of chlorophyll fluorescence parameters that can distinguish between these processes. In this study, we introduce two new parameters: the rate constants of sustained thermal energy dissipation (k_{NPQ}) and of photochemistry (k_P). We estimated k_{NPQ} and k_P from dark-acclimated F_o and F_m measured during spring recovery of photosynthesis in Scots pine (*Pinus sylvestris* L.) trees. We suggest that k_{NPQ} and k_P be used to study the mechanisms underlying the observed seasonal acclimation in PSII, because these parameters provide quantitative data that complement and extend F_v/F_m measurements.

Keywords: chlorophyll concentration, chlorophyll fluorescence, energy partitioning, Scots pine.

Introduction

Acclimation of photosynthetic light reactions to the environment occur on different timescales. Short-term adjustments, which occur at a timescale of seconds to minutes, involve the de-epoxidation of xanthophyll-cycle pigments and protonation of photosystem II (PSII) proteins (Müller et al. 2001, Horton et al. 2005). These adjustments modulate the energy partitioning between photochemistry and thermal energy dissipation, and are rapidly reversed in the dark (Krause and Weis 1991, Müller et al. 2001). In contrast, long-term or seasonal acclimation processes involve adjustments in leaf chlorophyll and carotenoid concentrations, or in the amounts of specific PSII proteins (Öquist and Huner 2003, Ensminger et al. 2006). Seasonal acclimation processes modulate not only the energy partitioning between photochemistry and thermal energy dissipation but also light absorption in PSII. Unlike short-term adjustments, seasonal acclimation in PSII occurs on a timescale of days to weeks, and is not readily reversible in the dark. Therefore, dark-acclimated leaves facilitate study of the seasonal acclimation of PSII by removing the confounding effect of the short-term adjustments.

The maximum quantum yield of photochemistry can be estimated from chlorophyll fluorescence measurements as F_v/F_m, where $F_v = (F_m - F_o)$, and F_m and F_o are maximum and minimum chlorophyll fluorescence in dark-acclimated leaves, respectively (Kitajima and Butler 1975). The F_v/F_m ratio has been widely used to follow the seasonal acclimation of PSII, because it is based on data obtained exclusively from dark-acclimated leaves (Adams and Demmig-Adams 1994, Ottander et al. 1995, Ensminger et al. 2004, Slot et al. 2005). Changes in F_v/F_m indicate either downregulation or recovery of the photosynthetic light reactions. Changes in F_v/F_m can be caused by adjustments in either the photochemical capacity in PSII, or the capacity of thermal dissipation in PSII, or both.

In the model proposed by Kitajima and Butler (1975), increasing thermal dissipation in PSII decreases both F_o and F_m. In contrast, inactivation or damage to reaction centers, and any other process impairing the photochemical utilization of exci-

tation energy in PSII, increase only F_o. Comparison of the patterns of change in F_o and F_m has been used to provide a qualitative assessment of sustained thermal dissipation of excitation energy or inactivation of PSII reaction centers (Krause 1988, Barber et al. 1989, Demmig-Adams et al. 1989, Dau 1994, Ottander et al. 1995, Verhoeven et al. 1996, Yamane et al. 1997); however, no parameters have been identified that can provide a quantitative analysis of such data.

In this paper we introduce two new fluorescence parameters, both obtained from measurements of F_o and F_m in dark-acclimated leaves, to quantitatively estimate the seasonal changes in the rate constants of sustained thermal energy dissipation (k_{NPQ}) and photochemistry (k_P). The new parameters account for how seasonal changes in leaf chlorophyll concentration may affect light absorptance. We tested the new parameters with chlorophyll fluorescence data obtained from field measurements in overwintering Scots pine (*Pinus sylvestris* L.) trees.

Materials and methods

Theoretical framework

Excitation energy in PSII can be partitioned into the following main processes: chlorophyll fluorescence (k_f); constitutive thermal energy dissipation (k_D); photochemistry (k_P); and regulated thermal energy dissipation (k_{NPQ}), where k denotes the respective rate constants (s^{-1}) (Kramer et al. 2004). Regulated thermal dissipation originates from short-term adjustments or seasonal acclimation mechanisms in PSII that lead to non-radiative decay of excitation energy. As described by Kornyeyev and Hendrickson (2007), no differences in the rate constants between populations of PSII with open/functional or closed/damaged reaction centers are considered. Furthermore, k_P represents the overall photochemical rate constant of a mixed population of PSII. This rate differs from the bimolecular rate constant of photochemistry for PSII (k_{PSII}) (Shinkarev and Govindjee 1993), which represents the maximum photochemical capacity when all the primary quinone acceptors are oxidized and the fraction of photoinhibited reaction centers is minimal. Photoinhibited or damaged reaction centers can be considered comparable to closed centers in that neither is able to perform linear electron transport. We expressed the rate constant of photochemistry (k_P) as $k_P = k_{PSII}\,[Q_A][RC]$, where $[Q_A]$ is the fraction of open reaction centers with the primary acceptor Q_A in an oxidized state (Kitajima and Butler 1975, Porcar-Castell et al. 2006, Kornyeyev and Hendrickson 2007) and [RC] is the functional fraction of PSII reaction centers (i.e., undamaged). In practice, both $[Q_A]$ and [RC] affect photochemistry in the same way; however, their kinetics differ in that $[Q_A]$ rapidly recovers in dark-acclimated leaves $[Q_A] = 1$, because the primary electron acceptors are re-oxidized in the dark, whereas [RC] does not readily recover in the dark in photoinhibited leaves [RC] < 1, because recovery of damaged reaction centers requires de novo synthesis of proteins (Kanervo et al. 2005).

Following the approach of Kitajima and Butler (1975), the yield of chlorophyll fluorescence can be expressed as:

$$\Phi F = \frac{k_f}{k_f + k_D + k_{NPQ} + k_{PSII}[Q_A][RC]} = \frac{k_f}{k_f + k_D + k_{NPQ} + k_P} \quad (1)$$

where k_f, k_D, k_{NPQ} and k_P represent the overall capacity of each of these processes in the PSII population under examination.

Because short-term adjustments in PSII are reversible in the dark, regulative heat dissipation in dark-acclimated leaves will include only the sustained component. Thus, in dark-acclimated leaves, k_{NPQ} represents the rate constant of sustained thermal dissipation. Changes in k_{NPQ} reflect the seasonal adjustment in the capacity for sustained thermal dissipation. Changes in sustained thermal dissipation may be a result of acclimation in the de-epoxidation status of xanthophyll-cycle pigments (Ottander et al. 1995, Ensminger et al. 2004), structural changes in the thylakoid membrane that enhance thermal dissipation (Ottander et al. 1995), changes in the fraction of thermal dissipation by nonfunctional reaction centers (Lee et al. 2001, Hendrickson et al. 2005, Sveshnikov et al. 2006, Kornyeyev and Hendrickson 2007), and any other mechanism affecting the seasonal thermal dissipation in PSII. Conversely, changes in k_P will reflect seasonal changes in [RC] or the fraction of functional RCs, because $[Q_A] = 1$ in dark-acclimated leaves.

Estimating seasonal changes in the rate constants of photochemistry and regulated thermal energy dissipation

Fluorescence intensity F as detected by a chlorophyll fluorometer can be expressed as:

$$F = \beta I_{MB} A a \frac{k_f}{k_f + k_D + k_{NPQ} + k_P} \quad (2)$$

where β is a proportionality constant that depends on the fluorometer detector, I_{MB} is the constant radiation of the pulse-modulated measuring light, A is leaf absorptance and a is the fraction of absorbed light that is captured by PSII.

In the absence of down-regulation, when the maximum photochemical yield F_v/F_m is typically around 0.84 (Schreiber 1986), we can assume that the rate constant of sustained thermal dissipation equals zero ($k_{NPQ} = 0$), and after application of a saturating light-pulse that completely reduces the primary electron acceptor Q_A, $[Q_A] = 0$, the rate constant of photochemistry will also tend to zero ($k_P = 0$). And maximum chlorophyll fluorescence, F_{ms}, can be expressed as:

$$F_{ms} = \beta I_{MB} A_s a_s \frac{k_f}{k_f + k_D} \quad (3)$$

where the 's' subscript indicates summer values that are used to estimate the seasonal acclimation of k_P and k_{NPQ}.

Conversely, if there is sustained down-regulation of PSII, as in boreal Scots pine under severe winter conditions (Ottander et al. 1995, Ensminger et al. 2004), the rate constant of sustained thermal dissipation k_{NPQ} does not decrease to zero after a period of dark acclimation, and F_m can be expressed as:

$$F_m = \beta I_{MB} A a \frac{k_f}{k_f + k_D + k_{NPQ}} \quad (4)$$

Similarly, under the same conditions, F_o measured by a weak measuring light that does not reduce the primary electron acceptor Q_A, $[Q_A] = 1$, can be expressed as:

$$F_o = \beta I_{MB} A a \frac{k_f}{k_f + k_D + k_{NPQ} + k_P} \quad (5)$$

where $k_P = k_{PSII}[RC]$. Finally, the rate constant of sustained thermal dissipation k_{NPQ} can be estimated by combining Equations 3 and 4:

$$k_{NPQ} = \left(\frac{F_{ms}}{F_m} \frac{A a}{A_s a_s} - 1 \right)(k_f + k_D) \quad (6)$$

By combining Equations 4 and 5, and substituting k_{NPQ} from Equation 6, k_P can be estimated as:

$$k_P = k_{PSII}[RC] = \left(\frac{F_{ms}}{F_o} - \frac{F_{ms}}{F_m} \right) \frac{A a}{A_s a_s} (k_f + k_D) \quad (7)$$

Our objective was to investigate not the seasonal variations in the absolute values of the rate constants k_{NPQ} and k_P (s^{-1}), but their relative changes and seasonal patterns. Therefore we denote k'_{NPQ} and k'_P as the parameters representing the seasonal changes in the rate constants of sustained thermal dissipation and photochemistry relative to the sum of k_f and k_D, simplified as $k_f + k_D = 1$. In addition, we assumed that k_f and k_D remain constant.

Seasonal quantum yields and energy partitioning

The seasonal adjustments in energy partitioning in PSII can be estimated from the yield equation of each of the energy consuming processes as:

$$\Phi_P = \left(\frac{k'_P}{k'_P + k'_{NPQ} + 1} \right) = \left(\frac{F_m - F_o}{F_m} \right) \quad (8)$$

$$\Phi_{NPQ} = \left(\frac{k'_{NPQ}}{k'_P + k'_{NPQ} + 1} \right) = \left(\frac{F_o}{F_m} - \frac{F_o}{F_{ms}} \right) \frac{A_s a_s}{A a} \quad (9)$$

$$\Phi_{f,D} = \left(\frac{1}{k'_P + k'_{NPQ} + 1} \right) = \left(\frac{F_o}{F_{ms}} \right) \frac{A_s a_s}{A a} \quad (10)$$

where the fluorescence parameters result from substituting k'_P and k'_{NPQ} in Equations 6 and 7.

Estimating light absorption from total chlorophyll concentration

We assumed that light is chiefly absorbed by chlorophyll and that carotenoids play only a minor role (Bassi and Caffarri 2000). Therefore, if ε (m^2 μmol^{-1}) is the light extinction coefficient for the absorption of PAR by chlorophyll inside the leaf, A can be estimated from leaf chlorophyll (Chl) concentration (μmol m^{-2} projected leaf area), according to Parson and Nagarajan (2003), as:

$$A = 1 - 10^{-\varepsilon Chl} \quad (11)$$

Monitoring chlorophyll fluorescence

Chlorophyll fluorescence of three 45-year-old Scots pine trees (*P. sylvestris*), growing at SMEAR-II station (Station for Measuring Forest-Ecosystem-Atmosphere Relations) in southern Finland (61°51′ N, 24°17′ E, 181 m elevation), was monitored from winter 2003 to summer 2003. Three branches from the third whorl, counted from the treetop, were selected from each tree. The branches, about 12 m above ground, were accessed from a permanent scaffold. On each sampling date, one measurement per branch and tree was performed ($n = 9$).

At noon, two randomly selected pairs of needles were dark acclimated on each branch for 2 h using dark-acclimation clips (Hansatech, U.K.). Subsequently, F_o and F_m were measured with a portable chlorophyll fluorometer (FMS-2, Hansatech) at ambient temperature. The distance and angle between needles and optical parts of the fluorometer as well as the properties of the measuring light were kept constant throughout the measurements.

Monitoring chlorophyll concentration

Two pairs of needles were collected on each sampling date from each of the three branches and combined per tree ($n = 3$). Samples were immediately frozen in liquid nitrogen and stored at −80 °C. Subsequently, needle samples were ground in liquid nitrogen, freeze-dried and extracted with 100% acetone buffered with $NaHCO_3$ for 2 h at 4 °C. Pigments were separated by high-performance liquid chromatography (HPLC) with a reversed-phase C-18 column (Knaur, Berlin), as described by Ensminger et al. (2001). Dry-mass-based pigment data were converted to a leaf area basis using previously calculated specific leaf area values for Scots pine at SMEAR II (Palmroth and Hari 2001).

Micrometeorological data

Air temperature and photosynthetic active radiation (PAR) were measured at a tower at SMEAR II and averaged to yield 30-min values. Temperature was measured at 8.4 m above ground with Pt100 sensors shielded from direct radiation, and PAR was measured above the canopy with a Li-Cor LI-190 SZ sensor placed 18 m above ground.

Estimating light extinction coefficients

Minimum and maximum chlorophyll fluorescence, and leaf chlorophyll concentrations were measured over the summer in 20 Scots pine seedlings growing in a field near SMEAR II (Porcar-Castell et al. 2008*a*). The seedlings had been artificially exposed to one of four light environments during the winter and spring by growing them under gray (neutral density) shading net, which induced changes in needle chlorophyll concentrations. On each measuring date, chlorophyll concentration was determined from pooled samples obtained for each treatment, and chlorophyll fluorescence was measured for each of the five trees (n = 5) per treatment, as described above.

We selected those treatments and days when F_v/F_m ranged from 0.86 to 0.88 to compare physiologically similar needles. According to Equation 3, changes in F_m can be caused only by changes in A, assuming that a remains constant. Because the correlation between F_v/F_m and F_m was not significant (P = 0.52), and because all measurement settings were kept constant, we assumed that differences in F_m were attributable solely to changes in A. Subsequently, differences in F_m were plotted against A (calculated with Equation 8), and ε was estimated as the value when the correlation between F_m and A had a zero intercept and a slope equal to 1.

Results and discussion

Seasonal changes in the maximum quantum yield of photochemistry, and minimum and maximum chlorophyll fluorescence

The maximum photochemical yield of PSII remained below 0.4 until mid-April (Figure 1a), which is typical for boreal conifers during winter (Ottander et al. 1995, Ensminger et al. 2004). After mid-April, F_v/F_m started to recover as spring temperatures increased (data not shown) and reached summer values of 0.84 by the end of May, indicating recovery of photochemical capacity and the absence of sustained thermal dissipation in PSII.

Absolute F_o and F_m values are influenced by the amount of chlorophyll in the sample under examination. In our experiment, leaf chlorophyll concentrations ranged from 202 μmol m^{-2} in January to 428 μmol m^{-2} in June. Yet, differences in the pattern of F_o relative to the pattern of F_m cannot be attributed to changes in A or chlorophyll concentrations because A remained constant during the few seconds when F_o and F_m were measured. Additionally, the measurement error was minimized by keeping the measuring settings and the leaf area constant, as indicated by the relatively low standard errors for the F_o and F_m measurements (Figure 1b).

During spring recovery, the seasonal patterns of F_o and F_m in Scots pine revealed three main phases (Figure 1b). From mid-January until mid-April, both F_o and F_m remained relatively low (Figure 1, Phase I), indicating sustained thermal dissipation in PSII, because thermal dissipation reduces both F_o and F_m (Kitajima and Butler 1975, Demmig-Adams et al. 1989). However, the qualitative analysis of the spring recovery

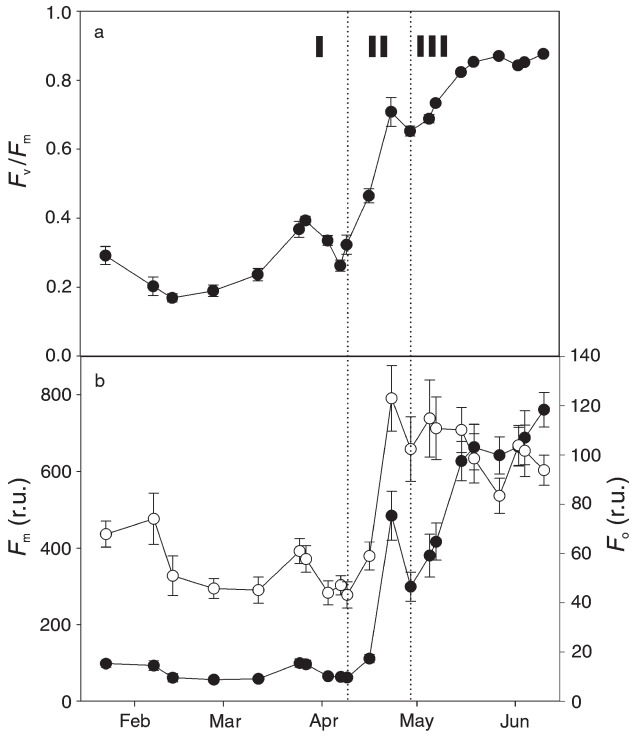

Figure 1. Seasonal changes in the maximum quantum yield of (a) photochemistry (F_v/F_m); and (b) minimum (F_o; ○) and maximum (F_m; ●) chlorophyll fluorescence. Dashed vertical lines separate the three main phases (I, II, III) during the spring recovery of PSII activity. Each value is a mean ± SE (n = 9).

in PSII activity shown in Figure 1 does not reveal whether the photochemical capacity was also inhibited during Phase I, or what the relative effects of photochemical and non-photochemical processes were on the observed decrease in F_v/F_m (Figure 1a). A second phase (Figure 1, Phase II) began during the second half of April, when both F_o and F_m rapidly increased, indicating that the sustained thermal dissipation in PSII had relaxed. Finally, Phase III of the spring recovery of PSII activity (Figure 1) began during May, when F_m values continued to increase while F_o values remained constant or decreased (e.g., May 27). The additional increase in F_m during Phase III indicates that the levels of sustained thermal dissipation had relaxed further. Furthermore, the finding that F_o did not increase with F_m suggests a simultaneous increase in photochemical capacity, which would counteract the effect of a decrease in sustained thermal dissipation in F_o, explaining the pattern of change in F_o during Phase III. Changes in F_o have previously been related to damage/recovery of the PSII reaction center (RC) (Krause 1988, Yamane et al. 1997), or to structural changes in PSII affecting the migration of excitation energy to the RC or the transfer of excitation energy between RCs (Schreiber and Armond 1978, Krause and Weis 1984). Similarly, the increase in F_o during Phase III (Figure 1b) would be consistent with spring recovery of damaged PSII RCs or structural rearrangements in PSII favoring the transfer of excitation energy to the RCs, which may occur during

spring recovery in boreal Scots pine (Ottander et al. 1995, Ensminger et al. 2004, Sveshnikov et al. 2006).

Seasonal changes in the rate constants of photochemistry and regulated thermal energy dissipation

Seasonal changes in the maximum quantum yield of photochemistry, Φ_{Pmax} or F_v/F_m, can be caused by changes in photochemical capacity (represented by k_P), or by changes in the capacity for thermal dissipation (represented by k_{NPQ}). Seasonal changes in the rate constant for sustained thermal dissipation (k'_{NPQ}) and the rate constant for photochemistry (k'_P), relative to the sum of k_D and k_f (Figure 2), indicate that low values of F_v/F_m until mid-April were caused by high sustained thermal dissipation (high k'_{NPQ}) combined with low photochemical capacity (low k'_P). Similarly, the analysis revealed that the rapid increase in F_v/F_m (Figure 1a, Phase II), during the second half of April was caused by a tenfold decrease in the rate constant for sustained thermal dissipation (represented by k'_{NPQ}) (Figure 2, Phase II). The subsequent slow increase in F_v/F_m during May and June (Figure 1a, Phase III) corresponded to a further decrease in the k'_{NPQ} together with an increase in k'_P (Figure 2, Phase III). These results support the qualitative analysis of F_o and F_m shown in Figure 1, with the difference that k'_{NPQ} and k'_P provide a quantitative estimate of the seasonal acclimation of PSII, thus complementing and extending the F_v/F_m measurements.

Seasonal acclimation of energy partitioning in PSII

Seasonal acclimation of energy partitioning in PSII can be estimated with k'_P and k'_{NPQ} (Figure 3). Using Equations 9 and 10, the yield of non-photochemical processes (Kramer et al. 2004) could be partitioned between sustained thermal dissipation (Φ_{NPQ}) and the combination of fluorescence and constitutive thermal dissipation ($\Phi_{f,D}$). The energy partitioning presented in Figure 3 depicts the effect of the seasonal acclimation only and does not reflect how the energy is partitioned during the day in response to the diurnal short-term adjustments in PSII (Porcar-Castell et al. 2006, 2008*b*).

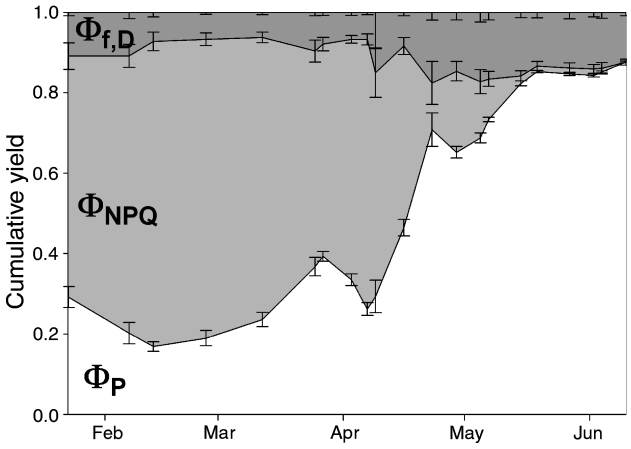

Figure 3. Seasonal changes in the partitioning of absorbed light energy in PSII, between fluorescence and constitutive thermal dissipation ($\Phi_{f,D}$), sustained thermal dissipation (Φ_{NPQ}) and photochemistry (Φ_P). Yields were obtained with Equations 8–10. Error bars represent SE, $n = 9$.

Integrating changes in light absorptance

The light extinction coefficient of the leaf (ε), which was estimated by analyzing needles differing in chlorophyll concen-

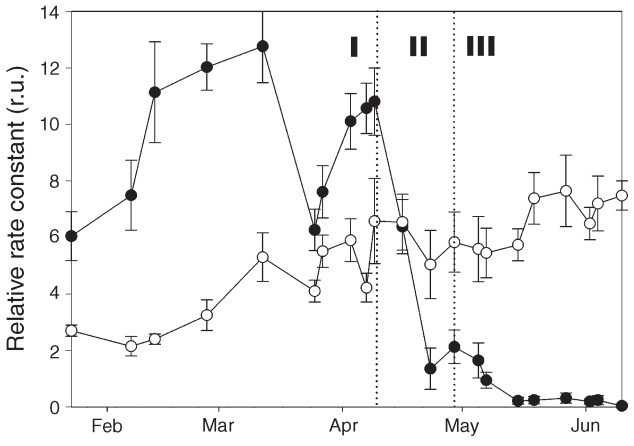

Figure 2. Seasonal changes in the rate constant of thermal dissipation (k'_{NPQ}) (●) and the rate constant of photochemistry (k'_P) (○), relative to the sum of k_f and k_D (Equations 6 and 7). Dashed vertical lines separate the three main phases (I, II, III) during the spring recovery of PSII activity (see text for details). Error bars represent SE, $n = 9$.

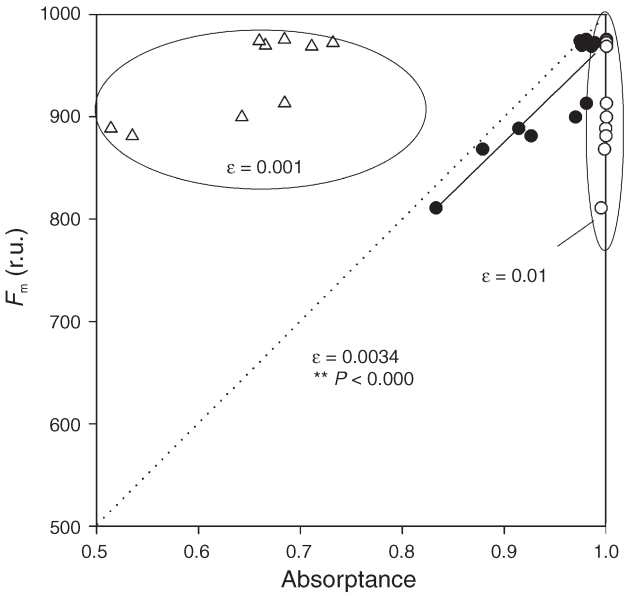

Figure 4. Estimation of the leaf light extinction coefficient (ε). Data were obtained from non-stressed Scots pine seedlings. Values correspond with mean maximum chlorophyll fluorescence (F_m) obtained from five trees; leaf absorptance was estimated based on needle chlorophyll concentration (Equation 8). The light extinction coefficient was estimated as the value for the correlation between F_m and A yielding a zero intercept and a slope equal to 1. Correlations for smaller (△), larger (○) and estimated (●) ε values are shown.

tration but having similar physiological status (i.e., F_v/F_m between 0.86 and 0.88), was 0.0034 m^2 μmol^{-1} (Figure 4). The value is comparable to the value of 0.003 m^2 μmol^{-1} obtained for spinach leaves at 450 nm (Vogelmann and Evans 2002). Leaf morphology may affect leaf optical properties (Johnson et al. 2005) including ε. However, because we measured only fully developed needles, we assumed that leaf morphology and ε remained constant during the monitoring period.

We compared Scots pine needles with differing chlorophyll concentrations but similar maximum quantum yields of photochemistry (Figure 5a). As expected, differences in chlorophyll concentration similarly influenced F_m (Figure 5c) and leaf absorptance (Figure 5b). However, the estimated values of k'_{NPQ} and k'_P were not significantly correlated with leaf chlorophyll concentration (Figures 5d and 5e). We conclude that Equations 6 and 7 successfully corrected for the effect that changes in leaf chlorophyll concentration have on chlorophyll fluorescence measurements, and subsequently on k'_{NPQ} and k'_P.

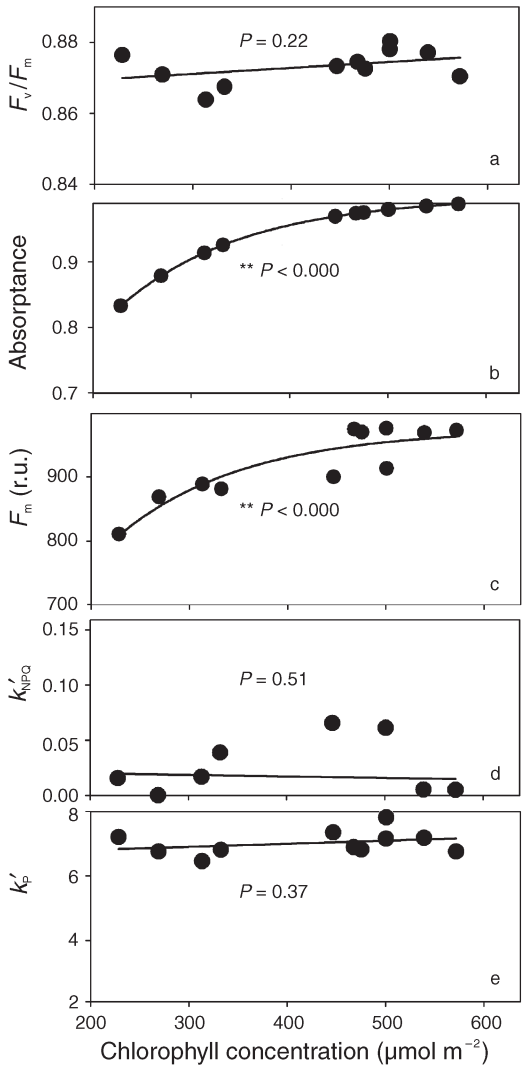

Figure 5. Chlorophyll concentration and biophysical properties of needles of non-stressed Scots pine trees: (a) maximum quantum yield of photochemistry, F_v/F_m; (b) estimated needle absorptance; (c) maximum chlorophyll fluorescence, F_m; and (d) estimated sustained rate constant of thermal dissipation and (e) photochemistry, relative to k_f and k_D. Each value in panels a, c, d and e reflects the mean value of five trees. The *P* values indicate the significance of (a, d, e) the slope in the linear models and of (b, c) the two parameters of a single rectangular hyperbola.

Seasonal changes in leaf chlorophyll concentration will affect light absorption differently, depending on the optical thickness of the leaf. Saturation in light absorption in optically thick leaves (i.e., high chlorophyll concentrations or high extinction coefficients; Equation 11) may prevent changes in chlorophyll concentration from significantly affecting light absorption. Therefore, when monitoring optically thick leaves or when chlorophyll concentrations remain constant, the absorptance terms A and A_s in Equations 6 and 7 can be omitted.

Assumptions and interpretations

We assumed that the partitioning of absorbed light energy between PSII and PSI remained constant and that $a = 0.5$ during spring recovery. To our knowledge, it is not known to what extent, if any, the absorption cross section of PSII relative to PSI (i.e., parameter a) changes over the seasons under natural conditions. Therefore, seasonal changes in a deserve further research and, if required, should be entered into Equations 6 and 7.

Seasonal changes in k_P can be associated with changes in [RC], or the proportion of functional RCs (Equation 1). However, the connectivity between photosynthetic units affects the correlation between [RC] and the actual proportion of functional RCs (Kramer et al. 2004). Different amounts of excitation energy can be transferred between PSII units with closed/damaged RCs to units with open/functional RCs depending on the connectivity. With zero connectivity between PSII units, [RC] will reflect the actual fraction of functional RCs, whereas with perfect connectivity—a "lake model"—this relationship becomes curvilinear (Joliot and Joliot 1964, Kramer et al. 2004). Therefore, seasonal changes in the connectivity between photosynthetic units need to be considered if the fraction of functional RCs is to be estimated from [RC].

Application

The rate constants for sustained thermal dissipation (k'_{NPQ}) and photochemistry (k'_P), relative to k_f and k_D, were used to analyze quantitatively the seasonal acclimation of photochemical and non-photochemical processes. The new parameters facilitated study of the seasonal acclimation of PSII by chlorophyll fluorescence, because until now most of the current chlorophyll fluorescence parameters were developed to study rapid adjustments in PSII rather than seasonal acclimation. For example k'_{NPQ} and k'_P can be used to link chlorophyll fluorescence data with seasonal physiological and biochemical adjustments in PSII (Porcar-Castell et al. 2008*a*). In conclusion, we propose that k'_{NPQ} and k'_P be used to study the mechanisms underlying the observed seasonal acclimation of PSII in the field because they complement and extend F_v/F_m measurements. Parame-

ters k'_{NPQ} and k'_P may be of particular interest to ecophysiologists studying seasonal processes.

Acknowledgments

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