

Physiology of the seasonal relationship between the photochemical reflectance index and photosynthetic light use efficiency

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Abstract The photochemical reflectance index (PRI) is regarded as a promising proxy to track the dynamics of photosynthetic light use efficiency (LUE) via remote sensing. The implementation of this approach requires the relationship between PRI and LUE to scale not only in space but also in time. The short-term relationship between PRI and LUE is well known and is based on the regulative process of non-photochemical quenching (NPQ), but at the seasonal timescale the mechanisms behind the relationship remain unclear. We examined to what extent sustained forms of NPQ, photoinhibition of reaction centres, seasonal changes in leaf pigment concentrations, or adjustments in the capacity of alternative energy sinks affect the seasonal relationship between PRI and LUE during the year in needles of boreal Scots pine. PRI and NPQ were highly correlated during most of the year but decoupled in early spring when the foliage was deeply downregulated. This phenomenon was attributed to differences in the physiological mechanisms controlling the seasonal dynamics of PRI and NPQ. Seasonal adjustments in the pool size of the xanthophyll cycle pigments, on a chlorophyll basis,

controlled the dynamics of PRI, whereas the xanthophyll de-epoxidation status and other xanthophyll-independent mechanisms controlled the dynamics of NPQ at the seasonal timescale. We conclude that the PRI leads to an underestimation of NPQ, and consequently overestimation of LUE, under conditions of severe stress in overwintering Scots pine, and most likely also in species experiencing severe drought. This severe stress-induced decoupling may challenge the implementation of the PRI approach.

Keywords Acclimation · Fluorescence · NPQ · Photosynthesis · Remote sensing

Introduction

The acclimation of photosynthesis generates a number of optical signals that can be used to follow the dynamics of photosynthesis from a distance. Optical data has been regarded as a very promising tool to facilitate the upscaling of photosynthesis from airborne or satellite platforms (Gamon et al. 2006; Grace et al. 2007; Hilker et al. 2008a). Plants use different approaches to adjust their photosynthetic performance during the year: they regulate light absorption capacity (adjusting leaf chlorophyll concentration at the leaf-level, or total leaf area at the plant-level), they regulate the partitioning of absorbed light energy between photochemical and non-photochemical pathways with the subsequent modulation in light use efficiency (LUE), or they use combinations of both (Huner et al. 1998). For example, annual dynamics in gross primary production (GPP) of deciduous biomes are largely controlled by the seasonality in light absorption capacity (linked to budburst and senescence), whereas annual dynamics in GPP of evergreen biomes are largely controlled

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by changes in LUE. For this reason, greenness indexes such as the normalized difference vegetation index (NDVI) have proved especially useful in tracking the annual dynamics of photosynthesis of deciduous forest ecosystems (Xiao et al. 2004; Sjöström et al. 2009), but fail to capture the annual dynamics of photosynthesis of biomes dominated with evergreen vegetation (Garbulska et al. 2008). In this context, the photochemical reflectance index (PRI) has been proposed as an optical proxy of light use efficiency, that is expected to facilitate the estimation and upscaling of GPP of evergreen forest ecosystems (Grace et al. 2007; Garbulska et al. 2010; Coops et al. 2010; Goerner et al. 2010). PRI has been extensively tested as proxy of LUE at various spatial (leaf to landscape) and temporal (seconds to months) scales (Gamon et al. 1992, 1997; Peñuelas et al. 1995; Stylinski et al. 2002; Nichol et al. 2002; Rahman et al. 2004; Guo and Trotter 2004; Drolet et al. 2005; Sims et al. 2006; Nakaji et al. 2006; Peguero-Pina et al. 2008; Garbulska et al. 2008; Goerner et al. 2009). At the landscape level, the PRI has been derived using data from the moderate resolution imaging spectroradiometer (MODIS) on board the AQUA and TERRA satellites and implemented to the estimation of intra and interannual dynamics in LUE (Rahman et al. 2004; Drolet et al. 2005; Garbulska et al. 2008; Goerner et al. 2009). This approach requires that the relationship between PRI and LUE scales up across space and time. However, the relationship between PRI and LUE is affected by a number of physical and physiological factors that constrain the implementation of a generic model for the estimation of LUE from PRI. Physical factors such as canopy structure, shadow fraction, sun-target-sensor geometry, or background properties all affect the relationship between PRI and LUE (Barton and North 2001; Hilker et al. 2008b; Goerner et al. 2010). In turn, a number of physiological factors may also affect the relationship between PRI and LUE, especially at the seasonal timescale which is critical for remote sensing applications.

At the diurnal timescale, the mechanistic relationship between PRI and LUE is based on the optical signal generated during a regulatory process, by which excitation energy absorbed in excess is harmlessly dissipated as heat. This process results in the effective reduction in LUE and has been widely associated with (and used as a synonym of) the non-photochemical quenching (NPQ) of the chlorophyll fluorescence signal (Bilger and Björkman 1990; Gamon et al. 1992). This type of NPQ is termed reversible, flexible, energy- or ΔpH -dependent NPQ (Demmig-Adams and Adams 2006; Porcar-Castell 2011). Reversible NPQ is induced by the protonation of key proteins in the thylakoid membrane and the de-epoxidation of the xanthophyll cycle pigment violaxanthin to zeaxanthin under excess light (Müller et al. 2001). The resulting structural and biochemical changes not only promote the thermal dissipation of

excitation energy but also alter the leaf reflectance around 531 nm, the signal exploited by the PRI (Gamon et al. 1992; Peñuelas et al. 1995; Gamon and Surfus 1999). Consequently, a strong functional relationship has been found between PRI and reversible NPQ at the diurnal timescale (Evain et al. 2004; Raddi et al. 2005; Nichol et al. 2006). In turn, sustained NPQ operates at the seasonal timescale (Demmig-Adams and Adams 2006; Porcar-Castell 2011) and has been associated to the overnight retention of zeaxanthin, the aggregation of light-harvesting proteins, the photoinhibition of reaction centres, or the accumulation of Elip or Ohps proteins (Ottander et al. 1995; Demmig-Adams and Adams 1996; Huner et al. 1998; Ivanov et al. 2003; Ensminger et al. 2004). However, the relationship between PRI and sustained NPQ at the seasonal timescale remains unknown. Similarly, the mechanisms behind the seasonal relationship between PRI and LUE still remain unclear.

Apart from the operation of the xanthophyll cycle pigments, the PRI is also affected by the leaf chlorophyll and carotenoid concentrations, among species (Peñuelas et al. 1995; Garrity et al. 2011), within the canopy (Gamon et al. 1997), or in response to the seasonal acclimation in pigment pools (Stylinski et al. 2002; Nakaji et al. 2006; Filella et al. 2009). Therefore, seasonal changes in pigment contents may influence the seasonal relationship between PRI and LUE. In fact, decoupling between PRI and LUE was reported in the deciduous Japanese larch during senescence when strong adjustments in pigments contents took place (Nakaji et al. 2006). In addition, the presence of sustained forms of NPQ has been found to interact with the relationship between PRI and photochemistry during spring recovery in overwintering Jack pine needles (Busch et al. 2009). Furthermore, seasonal changes in the quantum efficiency of photosystem II (PSII), i.e. the light use efficiency at the level of PSII, may be caused not only by adjustments in non-photochemical quenching capacity (NPQ), but also in photochemical quenching capacity (PQ), or in both (Krause and Weis 1991; Porcar-Castell 2011). Since the PRI is expected to track NPQ but not PQ, seasonal changes in PQ caused by photoinhibition of reaction centres, defined as damage of the reaction centre D1 protein requiring de novo protein synthesis (Aro et al. 1993), might also decouple PRI from LUE at the seasonal timescale. Finally, seasonal changes in the capacity of alternative sinks of ATP and NADPH, such as photorespiration (Ögren 1984), might also decouple the seasonal relationship between PRI and LUE.

The aim of this study was to investigate the physiological mechanisms behind the seasonal relationship between PRI and LUE in evergreen foliage, by assessing the effect that presence of sustained NPQ, photoinhibition of reaction centres, seasonal changes in pigment concentrations, or adjustments in the capacity of alternative energy sinks have

in the seasonal relationship between the PRI and LUE. We followed these processes during the course of a year in needles of boreal Scots pine undergoing strong seasonal acclimation of photosynthesis.

Materials and methods

Data were collected from the top crowns of three 47 year-old Scots pine trees (*Pinus sylvestris* L.) growing at SMEAR-II Station in southern Finland (61°51'N, 24°18'E) between 24 February 2009 and 18 February 2010. Four branches were marked in each tree for repetitive sampling purposes. Youngest fully developed needles were used throughout the experiment. Because the focus of this study was on seasonal processes, most of the measurements were carried out during night time to avoid interference from diurnal acclimation processes, repeating them occasionally at midday. Four needle pairs were detached and optical measurements were immediately carried out before freezing the needles in liquid nitrogen (4 branch replicates \times 3 tree replicates). Needles from the branch replicates were eventually pooled together in each tree for further pigment analysis.

Photosynthetic photon flux density (PPFD) and air temperature were measured at 1-min intervals above the canopy with a quantum sensor (Li-Cor LI-190; Lincoln, NE, USA), and with ventilated Pt100 sensors shielded from direct radiation, respectively. Resulting data was averaged to yield 30 min.

For fluorescence measurements, needles were arranged in a dark-acclimation clip (Hansatech, UK) so that the full measuring area of the clip (c. 0.5 cm²) was completely filled with needles. Dark-acclimation clips were used only to ensure that the same leaf area was measured time after time. The operating efficiency of photosystem II (ϕ_p) was immediately probed from the needles using a FMS-2 portable fluorometer (Hansatech) and by supplying a saturating light pulse ($>4,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) of 0.8 s duration. Current (F) and maximal fluorescence in the light (F'_m) were registered and used to estimate ϕ_p (Genty et al. 1989), as

$$\phi_p = 1 - \frac{F}{F'_m} \quad (1)$$

During night time measurements, F'_m was assumed to be F_m (maximal fluorescence) and F to be F_o (minimal fluorescence) (Porcar-Castell et al. 2008a), and the maximum quantum efficiency of photochemistry ($\phi_{p\text{max}}$) (Genty et al. 1989) was estimated as

$$\phi_{p\text{max}} = 1 - \frac{F_o}{F_m} \quad (2)$$

In order to estimate the seasonal changes in NPQ and PQ, we selected a reference minimal and maximal fluorescence, F_{oR} and F_{mR} from the summer night with highest $\phi_{p\text{max}}$, when NPQ and photoinhibition of reaction centres can be assumed to be zero (Porcar-Castell 2011). We have previously shown that annual changes in chlorophyll contents undergone in Scots pine needles do not significantly interfere with the estimation of PQ and NPQ (Porcar-Castell et al. 2008a). In addition, the spring recovery in fluorescence levels in boreal Scots pine precedes that of chlorophyll contents by some weeks (Porcar-Castell et al. 2008b), and therefore seasonal changes in chlorophyll contents can be assumed not to interfere with the estimation of NPQ and PQ in boreal Scots pine needles. The parameter *NPQ* (in italics to differentiate it from the process itself), was estimated as (Bilger and Björkman 1990; Porcar-Castell 2011)

$$NPQ = \left(\frac{F_{mR}}{F'_m} - 1 \right) \quad (3)$$

In this form, *NPQ* includes both sustained and reversible components of NPQ and denotes the total capacity for non-photochemical quenching of excitation energy. When estimated from night time data ($F'_m = F_m$) *NPQ* included only the sustained component. Similarly, the parameter *PQ* was estimated as (Laisk et al. 1997; Porcar-Castell 2011)

$$PQ = \left(\frac{F_{mR}}{F} - \frac{F_{mR}}{F'_m} \right) \quad (4)$$

In this form, *PQ* includes both the sustained and reversible components of PQ, sustained in response to photoinhibition of reaction centres, and reversible in response to the redox state of the primary quinone acceptors (Porcar-Castell 2011). When estimated from night data ($F = F_o$ and $F'_m = F_m$), *PQ* included only the sustained component, denoting the photochemical quenching capacity in the presence of photoinhibition of reaction centres. We use *PQ* instead of the photochemical quenching parameter qL (Kramer et al. 2004; Porcar-Castell 2011) because *PQ* facilitates the quantitative comparison with *NPQ* as they have the same relative units.

A spectroradiometer (FieldSpec-HH; ASD, Boulder, CO, USA) equipped with a fibre optic and plant probe (ASD) were used to measure the reflectance spectra. Spot size was 10 mm, sampling interval was 1.6 nm, and spectral resolution 3.5 nm. In order to measure the leaf reflectance from exactly the same sample and immediately after fluorescence, we used a modified dark-acclimation clip (Hansatech) for the measurements. The external diameter of the dark-acclimation clip fitted perfectly with the front measuring window of the plant probe so that the measuring clip could be easily and repeatedly placed in exactly the same position time after time. At the start of the

measurements, and following every four samples, a calibrated Spectralon® reference panel was placed in the dark-acclimation clip and a dark current and white reference measurement were registered. Integration time was 540 ms, and, to avoid accumulation of NPQ during measurements, spectra were averaged over 5 measurements only. Typically, the third spectral average was stable and the sample leaf reflectance was recorded in less than 10 s. The photochemical reflectance index (PRI) was estimated as

$$\text{PRI}_n = \frac{\rho_n - \rho_{570}}{\rho_n + \rho_{570}} \quad (5)$$

where ρ_{570} is the reference reflectance at 570 nm and ρ_n the signal reflectance at the wavelength n . If not stated otherwise, the general form of the PRI with the signal reference band $n = 531$ nm was used in the calculations. In addition, the alternative forms PRI_{525} , PRI_{539} and PRI_{545} were estimated (Gamon et al. 1992; Peñuelas et al. 1995).

Frozen needle material was manually ground in liquid nitrogen and lyophilised. Ca. 50 mg of freeze dried material was suspended with 0.7 mL of 99.8 % acetone, mixed in vortex, and extracted for 2 h at 4 °C. Extracts were centrifuged for 15 min at 15,000 rpm and 4 °C. Supernatant was collected and the pellet was re-suspended with 0.7 mL acetone, mixed in vortex and extracted for 15 min in an ultrasound bath. The latter process was repeated once more and extracts were stored at –80 °C. Pigment separation was performed by HPLC with a reverse phase C18 column (Waters Spherisorb ODS1, 4.6 × 250 mm; MA, USA), following the method of García-Plazaola and Becerril (1999), after García-Plazaola and Becerril (2001). Identification and quantification was carried out with a photodiode array detector. The de-epoxidation state of the xanthophyll cycle pigments (DEPS) was estimated as

$$\text{DEPS} = \frac{Z + 0.5A}{V + A + Z} \quad (6)$$

where V , A , and Z are leaf concentrations (mol g⁻¹ DW) of the xanthophyll cycle pigments violaxanthin, antheraxanthin and zeaxanthin, respectively.

LUE was estimated using data from three shoot chambers installed in the top crowns of Scots pine trees growing in the same stand (Hari et al. 1999). The chambers were made of acrylic plastic, had a volume of 1 dm³, and remained open most of the time exposing the pine shoot to the ambient conditions. During measurements, the chambers were automatically closed for 1 min, 40–70 times a day. PPFD and temperature, as well as transpiration and assimilation rates were determined on a projected leaf area basis. Instantaneous LUE was calculated as:

$$\text{LUE} = \frac{A + R}{\text{PPFD}} \quad (7)$$

where A is the net photosynthetic assimilation in (μmol CO₂ m⁻² s⁻¹) and R is the respiration (μmol CO₂ m⁻² s⁻¹). The respiration term was estimated from night data using a linear regression model against temperature and assuming that daytime respiration followed the same temperature response. The regression model was recalculated for each measurement point using night data from a 2-week window centred around each sampling date. Estimation of LUE is strongly affected by the instantaneous response of photosynthesis to light and temperature. However, since we wanted to compare LUE to night PRI and fluorescence data (displaying only seasonal variation), we computed a normalised LUE estimate (LUE_n) in an attempt to capture the seasonal fluctuations in LUE and smooth out the variation caused by the instantaneous response of photosynthesis to light and temperature. Accordingly, only morning (before 1200 hours) and low PPFD (0–400 μmol m⁻² s⁻¹) points were used to restrict LUE to low light and low vapour pressure deficit conditions, respectively. In addition, LUE obtained this way were averaged over a 5-day window around the sampling point to compute LUE_n.

Seasonal time-series of biological variables present often high collinearity complicating the estimation of statistical models. A preliminary principal component analysis of our data indicated that eight out of nine variables were highly represented in the first principal component, denoting high collinearity (data not shown). Therefore, since the purpose of the present study was to provide a first exploration on the factors behind the seasonal development of the PRI signal rather than produce a statistical model that would explain the PRI, we chose to use simple Pearson's correlation to evaluate relationships between variables at the seasonal domain, instead of linear regression models.

Results

The seasonal pattern of variation in light and temperature during the course of the experiment was typical of the boreal climate (Fig. 1). All physiological and photosynthetic parameters displayed a marked seasonal variation (Fig. 2). In addition, NPQ (Fig. 2a), PQ (Fig. 2c), ϕ_P (Fig. 2e), PRI (Fig. 2g) and DEPS (Fig. 2f) displayed variable degrees of diurnal variation. No diurnal variation was observed in leaf chlorophyll concentration (Fig. 2b), xanthophyll cycle pool on a chlorophyll basis (Fig. 2d) or carotenoid pool on a chlorophyll basis (not shown, but of equal pattern to that in Fig. 2d). The sustained component of the parameter NPQ (Fig. 2a, filled circles) was several times higher during winter and early spring compared to summer, reaching values of up to 10 on 18 February 2010

Fig. 1 Seasonal variation in daily maximum photosynthetic photon flux density (PPFD) (grey line) and daily mean temperature (black line) during the course of the experiment. Sampling dates are marked with vertical dotted lines

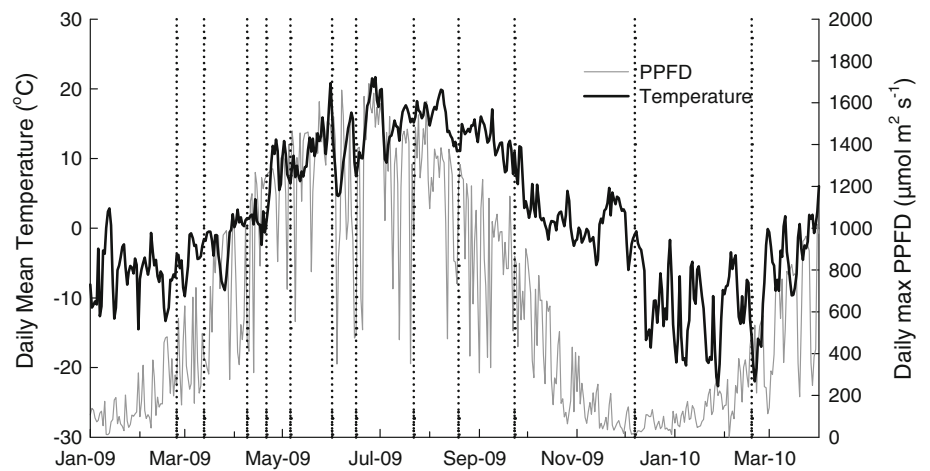


Fig. 2 Seasonal and daily variation of key variables. Seasonal variation is represented by the night data (filled circles), and diurnal variation by the differences between night and day data (open circles) for each sampling date. Non-photochemical quenching parameter (a), needle total chlorophyll concentration (b), photochemical quenching parameter (c), total xanthophyll cycle pigments per chlorophyll (d), operating quantum efficiency of photochemistry ϕ_P (open circles), or maximum quantum efficiency of photochemistry ϕ_{Pmax} (closed circles) (e), de-epoxidation state of the xanthophyll cycle pigments (f), photochemical reflectance index (g), and normalised photosynthetic light use efficiency estimated as described in the “Materials and methods” (h). Points represent means of three biological replicates ($n = 3$) and SE (shown only when larger than symbols). Except for LUE_n , where they represent averages over n temporal replicates obtained from a shoot chamber

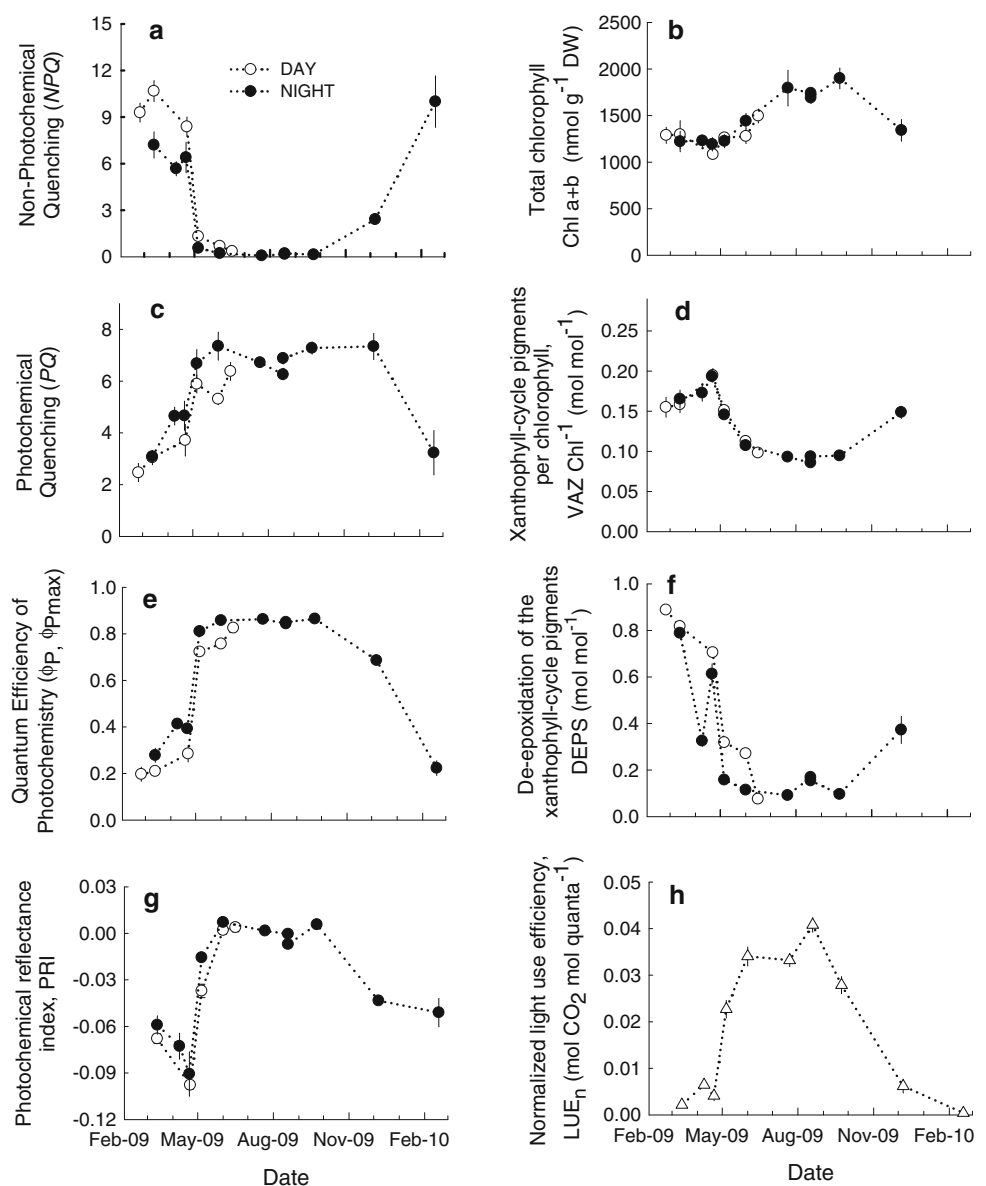


Table 1 Pearson correlation matrix showing the correlation coefficient (r) between variables

	PRI	NPQ	PQ	Φ_p	Total Car	Chl a + b	Total VAZ	DEPS	Car Chl ⁻¹	VAZ Chl ⁻¹
PRI	1									
NPQ	-0.894	1								
PQ	0.767	-0.906	1							
Φ_p	0.915	-0.987	0.931	1						
Total Car	-0.597	0.478	-0.276	-0.467	1					
Chl a + b	0.733	-0.632	0.63	0.689	-0.08	1				
Total VAZ	-0.925	0.786	-0.589	-0.803	0.808	-0.59	1			
DEPS	-0.856	0.945	-0.896	-0.953	0.382	-0.666	0.744	1		
Car Chl ⁻¹	-0.917	0.768	-0.684	-0.812	0.522	-0.886	0.87	0.754	1	
VAZ Chl ⁻¹	-0.952	0.809	-0.699	-0.849	0.538	-0.865	0.908	0.8	0.99	1

$n = 15$, observations represented means of three biological replicates

(Fig. 2a, closed circles). In contrast, maximum values of PQ during summer were around 6 and decreased to almost 2 during daytime on 24 February 2009 (Fig. 2c). Interestingly, PQ remained still high in early December even though temperatures had already been well below zero (Fig. 1). The PRI was relatively low during winter but decreased even further during April concomitantly with a peak in the VAZChl⁻¹ (Fig. 2d) before increasing to the summer levels. DEPS was found to remain high overnight during winter and spring (Fig. 2f), except for a decrease in night retention of DEPS registered at the beginning of April, after several days of cloudy weather and noon temperatures above zero (Fig. 1).

Interestingly, NPQ and PRI were found to correlate best with different biochemical variables at the seasonal timescale (Table 1; Fig. 3). NPQ was strongly correlated with DEPS (Fig. 3a), and only one point deviated from the trend which corresponded with the sudden decrease in night retention of DEPS during early April (Fig. 2f), when NPQ did not decrease proportionally, comparatively NPQ did not correlate so well with the total pool of xanthophyll cycle pigments on a chlorophyll basis (VAZ Chl⁻¹) (Fig. 3b). In contrast, PRI was strongly correlated with VAZ Chl⁻¹ (Fig. 3d), whereas correlation between PRI and DEPS was less remarkable (Fig. 3c). No significant differences in the slopes of the correlation coefficients were found when using day or night observations, therefore all points were treated together (NPQ vs. DEPS: $t = 1.09$, $df = 12$, $P = 0.30$; PRI vs. DEPS: $t = 0.04$, $df = 11$, $P = 0.97$; NPQ vs. VAZ Chl⁻¹: $t = 0.78$, $df = 12$, $P = 0.49$; PRI vs. VAZ Chl⁻¹: $t = 1.46$, $df = 11$, $P = 0.17$).

PRI and NPQ were found to be linearly and strongly correlated during most of the year (Fig. 4). However, the linear relationship broke down consistently during early spring both in March 2009 and again in February 2010 (Fig. 4, triangles), when PRI did not vary together with NPQ . When comparing the correlation between different

spectral forms of the PRI and NPQ (Table 2), the classic PRI₅₃₁ (using the 531 nm as signal band) was found to correlate best with the seasonal variation in NPQ when excluding the February and March points, but PRI₅₄₅ was the form that correlated better with NPQ during February and March or when plotting the full annual correlation.

Both NPQ and PQ were found to be strongly correlated with the quantum efficiency of photochemistry (ϕ_p) (Table 1), with a particularly strong correlation between NPQ and ϕ_p . In turn, NPQ and PQ were also negatively correlated but not fully complementary at the seasonal timescale, with NPQ increasing to a larger extent relative to the decrease in PQ (Fig. 2a, c). The annual variation in the maximum quantum efficiency of photochemistry ϕ_{pmax} was positively correlated with that of the LUE_n (Fig. 5a). The correlation was however undermined by the lack of correlation during summer, when LUE_n varied independently of the maximum quantum efficiency of photochemistry ϕ_{pmax} (Fig. 5a). In addition, the data point obtained during December fell out the main trend, i.e. maximum quantum efficiency of photochemistry was still rather high while LUE_n was remarkably low. A strong positive and significant correlation was found between the PRI and the LUE_n at the seasonal timescale ($r = 0.88$) ($t = 4.719$, $df = 8$, $P = 0.002$) (Fig. 5c), however, no significant correlation between variables was found when examining the two clusters of points separately, i.e. growing season points with LUE_n > 0.02 ($t = -0.719$, $df = 3$, $P = 0.524$), or cold season points with LUE_n < 0.01 ($t = -0.699$, $df = 3$, $P = 0.535$) (Fig. 5c).

Discussion

PRI and NPQ (estimated via the parameter NPQ) remained strongly correlated during most of the year in the presence of both sustained and reversible forms of NPQ (Fig. 4a), but the

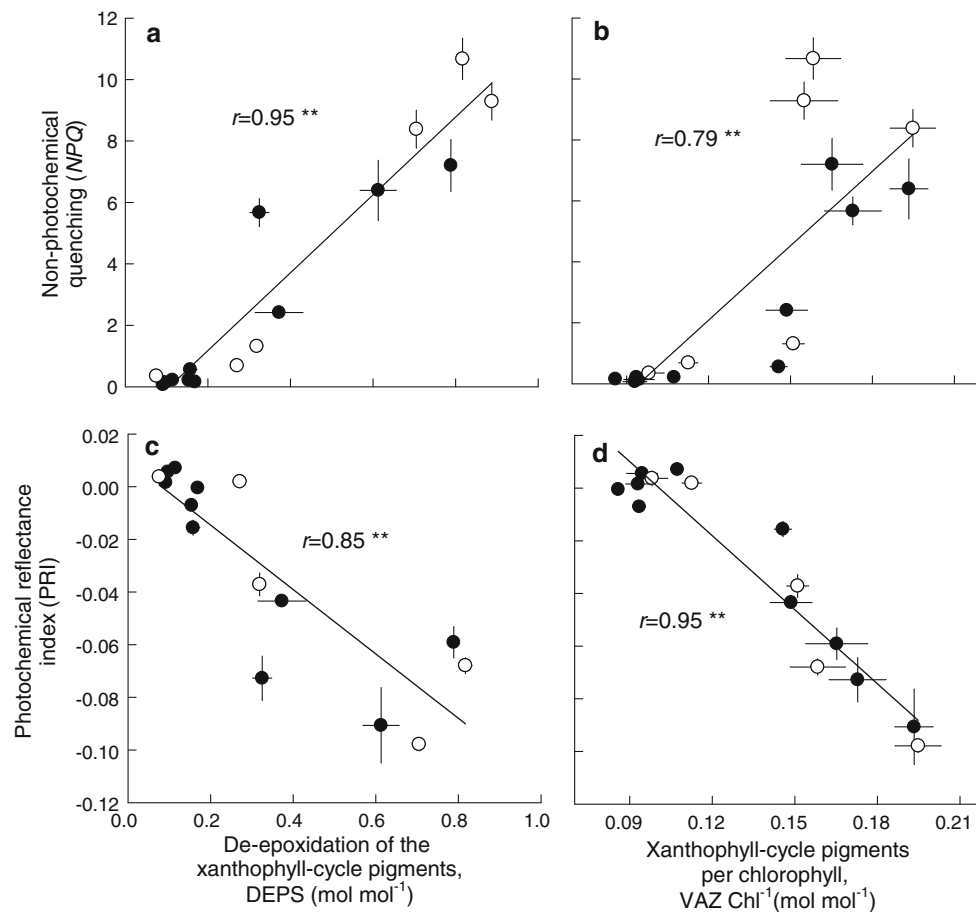


Fig. 3 Seasonal correlation between key biochemical variables and the parameter *NPQ* or *PRI*. Data points were obtained during night (filled circles) or day (empty circles). Correlation between the state of de-epoxidation of the xanthophyll cycle pigments and *NPQ* (a), or *PRI*

(c), and between xanthophyll cycle pigments on a chlorophyll basis and *NPQ* (b), or *PRI* (d). Points represent means of three biological replicates ($n = 3$) and SE (shown only when larger than symbols). Lines were fitted for clarity reasons. $^{**}P < 0.01$

relationship consistently broke down for the early spring months in 2009 and again in 2010 (Fig. 4a) when the photosystems were deeply downregulated. The decoupling is attributed both to differences in the controlling factors behind the *NPQ* and *PRI* signals at the seasonal timescale, as well as to the presence of xanthophyll cycle-independent forms of *NPQ* during winter. *NPQ* was strongly correlated with *DEPS* and to a much lesser extent with the total pool of xanthophyll cycle pigments on a chlorophyll basis (*VAZ Chl*⁻¹), while the opposite was true for the *PRI* (Fig. 3). These results strongly support the hypothesis that the *PRI* signal is controlled by adjustments in carotenoid and chlorophyll pools rather than *DEPS* at the seasonal timescale, where the marginal diurnal *PRI* variation caused by reversible *NPQ* is superimposed on the signal (Sims et al. 2006).

As a result, adjustments in *NPQ* that take place independently of the pool of xanthophyll cycle pigments will decouple *PRI* from *NPQ*, as was the case for the deeply downregulated needles of boreal Scots pine reported here (Fig. 4a). When this decoupling takes place, *PRI*

underestimates *NPQ* and consequently *LUE* would be overestimated. *NPQ* and *PRI* have also been found to decouple in a similar fashion after a period of severe drought in a canopy of Kermes oak (*Quercus coccifera* L.) where *NPQ* remained high while *PRI* had relaxed (Peguero-Pina et al. 2008), which suggests that the phenomena of seasonal decoupling between *PRI* and *NPQ* reported here may appear in response to different sources of stress. In addition, the relationship between *PRI* and *LUE* was also found to break down during years with severe drought in chaparral species (Sims et al. 2006), where the *PRI* was found to remain relatively high compared to *LUE*, further supporting the overestimation effect caused by decoupling between *PRI* and *NPQ* under severe stress.

Plants tend to increase the capacity for *NPQ* by increasing the sizes of their xanthophyll cycle pool pigments (Demmig-Adams and Adams 1996; Demmig-Adams 1998) thus, even though *PRI* would be controlled by *VAZ Chl*⁻¹ instead of *DEPS*, it should still be found to correlate well with *NPQ*. However, under severe stress conditions,

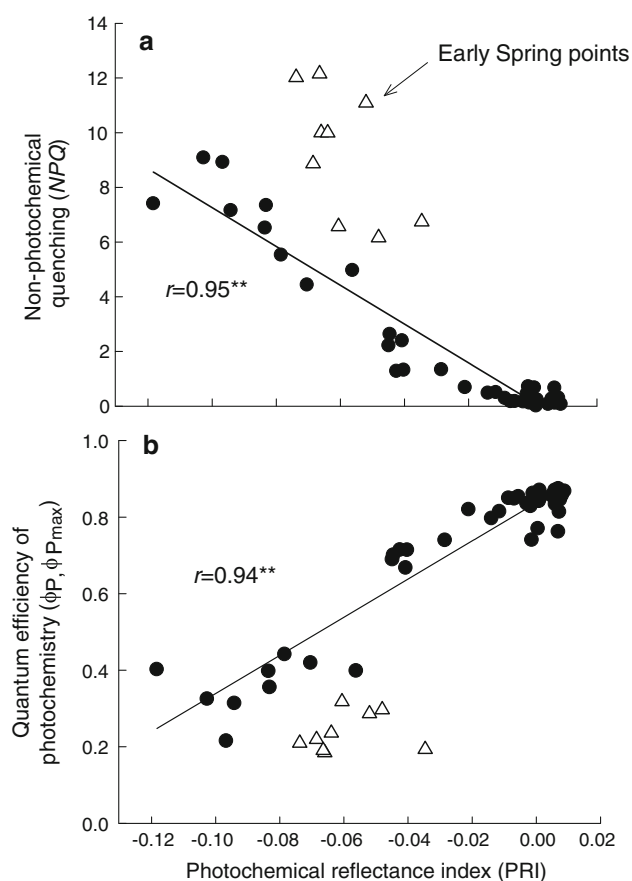


Fig. 4 Seasonal correlation between PRI and the parameter *NPQ* (a), or between PRI and the quantum yield of photochemistry (b) in needles of boreal Scots pine. Points represent means over four branch replicates per tree. Lines were fitted for clarity reasons excluding early spring points. ** $P < 0.01$

Table 2 Seasonal correlation coefficients (r) between the parameter *NPQ* and different spectral forms of PRI in Scots pine needles

	PRI _{525–570}	PRI _{531–570}	PRI _{539–570}	PRI _{545–570}
<i>NPQ</i> (excluding early spring points)	0.93	<i>0.93</i>	0.93	0.9
<i>NPQ</i> (early spring points only)	0.49	<i>0.69</i>	0.81	0.82
<i>NPQ</i> all data	0.78	<i>0.83</i>	0.86	0.86

The standard form of PRI is highlighted in italic

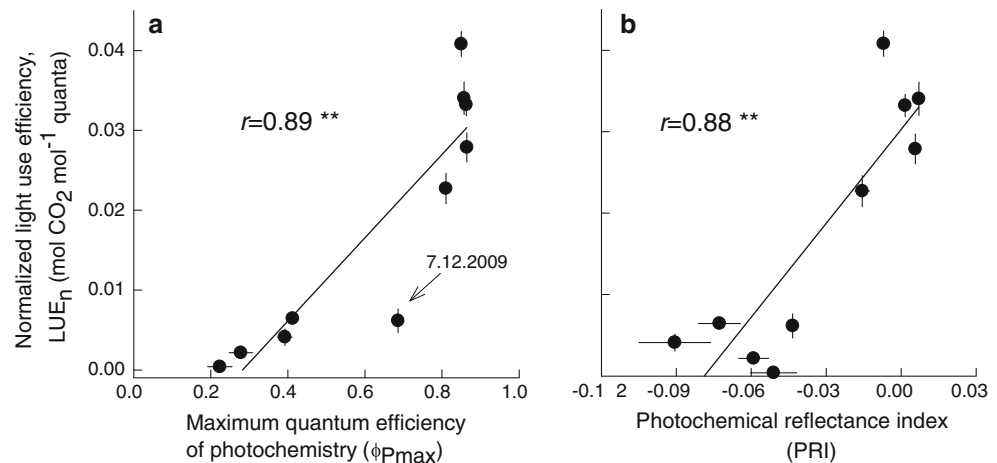
NPQ can be enhanced by mechanisms that do not directly involve the xanthophyll cycle pigments (Demmig-Adams and Adams 2006; Busch et al. 2009; Porcar-Castell 2011). Even if the strong correlation between NPQ and DEPS suggests that DEPS alone would control NPQ at the seasonal timescale (Fig. 3a), the data obtained during early April confirm the participation of xanthophyll cycle-independent processes in the generation of sustained NPQ,

when night retention of DEPS decreased drastically after a short period of mild weather (Fig. 2f) but NPQ was not proportionally affected (Fig. 2a). This is consistent with the winter aggregation of light harvesting complexes which enhance the capacity for thermal energy dissipation in overwintering Scots pine needles (Ottander et al. 1995; Ensminger et al. 2004; Porcar-Castell et al. 2008b). Interestingly, the annual correlation between NPQ and PRI improved when using PRI₅₄₅ instead of PRI₅₃₁ (Table 2); in particular, PRI₅₄₅ was much better correlated with NPQ during the early spring decoupling than PRI₅₃₁. PRI₅₄₅ has been associated with the rapid conformational changes induced by protonation of key thylakoid membrane proteins during reversible NPQ (Gamon et al. 1997). Similarly, it is tempting to suggest that PRI₅₄₅ could capture the structural changes in the thylakoid membrane associated with the aggregation of light-harvesting complexes and accumulation of sustained NPQ in overwintering evergreens better than PRI₅₃₁. Overall, zeaxanthin-independent forms of NPQ, as for example the winter aggregation of light-harvesting complexes, break down the linearity of the relationship between PRI and NPQ by further enhancing NPQ without apparent effect on PRI. The potential of other forms of PRI such as PRI₅₄₅ to track these changes should be better evaluated.

Next, even if PRI would be able to perfectly track NPQ, seasonal processes such as photoinhibition of reaction centres (Aro et al. 1993; Porcar-Castell 2011) might also decouple PRI from LUE if variations in NPQ and PQ are not complementary, because photoinhibition of reaction centres might decrease the quantum efficiency of PSII (ϕ_P) independently of NPQ, being invisible to the PRI. We found that PQ and NPQ were not fully complementary at the seasonal timescale, but since NPQ processes dominated the annual variability in ϕ_P in Scots pine needles (Table 1), we conclude that the effect of the photoinhibition in decoupling PRI from ϕ_P is marginal in needles of boreal Scots pine.

Finally, even if PRI would be able to perfectly track ϕ_P , seasonal changes in the capacity of factors such as cyclic electron transport routes through photosystem I (Shikanai 2007), alternative metabolic pathways for photosynthetic ATP and NADPH (Harbinson et al. 1990), or photorespiration (Ögren 1984), may all decouple the seasonal correlation between the quantum efficiency of PSII (ϕ_P) and the light use efficiency of photosynthesis (LUE). To evaluate the significance of these potential decouplers at a seasonal timescale, we analysed the relationship between the night ϕ_P or maximum quantum efficiency of photochemistry and a 5-day average LUE normalized for morning and low-light data, LUE_n (Fig. 5a). As a whole, a reasonable correlation was found between ϕ_P and LUE_n (Fig. 5a), yet there was no apparent correlation during summer time.

Fig. 5 Seasonal correlation between normalized light use efficiency (LUE_n) and the maximum quantum efficiency of photochemistry (a), or between LUE_n and the photochemical reflectance index (b). Points represent means of three biological replicates ($n = 3$) and SE (shown only when larger than symbols), or over n temporal replicates obtained from a shoot chamber in the case of LUE_n . Lines were fitted for clarity reasons



Bearing in mind the intrinsic limitations of estimating LUE from field measurements, the lack of correlation during summer between LUE_n and ϕ_P is consistent with the activity of alternative energy sinks. In fact, it could be expected that processes such as photorespiration would play a role during summer but not during the cold months, because photorespiration, or the oxygenation reaction of RuBP, is more inhibited at low temperatures than its carboxylation (Ögren 1984). Overall, seasonal adjustments in the capacity of alternative energy sinks, other than CO_2 assimilation, added noise to the annual relationship between PRI and LUE during the growing season in boreal Scots pine needles. The lack of correlation between LUE and ϕ_{Pmax} obtained in December (Fig. 5a) indicates differences in the dynamics of acclimation of light and carbon reactions of photosynthesis to the boreal environment. Carbon reactions are inhibited by low temperatures at the beginning of winter in Scots pine (Kolari et al. 2007), while light reactions remain relatively active in Scots pine needles during the dark boreal winter days (see high PQ during December in Fig. 2c denoting absence of photoinhibition) (Porcar-Castell 2011).

We conclude that the PRI underestimates NPQ at the seasonal timescale when the foliage is strongly down-regulated in response to low-temperature stress, and most likely also to severe drought, resulting in overestimation of LUE. This severe stress-induced decoupling may challenge the implementation of the PRI approach. In addition, the annual dynamics in the capacity of alternative energy sinks further complicate the seasonal relationship between PRI and LUE by adjusting LUE downstream from the light reactions that generate the optical signals. Overall, although the PRI was able to successfully register the winter–summer–winter transition in LUE, estimating the dynamics of LUE from PRI data at a finer resolution will probably require the integration of other sources of optical data, such as greenness indexes or passive chlorophyll

fluorescence (Meroni et al. 2009). In particular, the high correlation between PRI and NPQ throughout the year reported here (excluding periods of severe stress) could be used to facilitate the interpretation of the passive fluorescence signal (F) by constraining the non-photochemical quenching effect on F using the PRI.

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