Zeaxanthin-independent energy quenching and alternative electron sinks cause a decoupling of the relationship between the photochemical reflectance index (PRI) and photosynthesis in an evergreen conifer during spring

Emmanuelle Fréchette¹,², Christopher Y. S. Wong¹,³, Laura Verena Junker¹,², Christine Yao-Yun Chang¹,² and Ingo Ensminger¹,²,³,*

¹ Department of Biology, University of Toronto at Mississauga, 3359 Mississauga Road, ON, Canada
² Graduate Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada
³ Graduate Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada

* To whom correspondence should be addressed, E-mail: ingo.ensminger@utoronto.ca

Received 29 April 2015; Revised 26 August 2015; Accepted 28 August 2015

Editor: Susan von Caemmerer

Abstract

In evergreen conifers, the winter down-regulation of photosynthesis and its recovery during spring are the result of a reorganization of the chloroplast and adjustments of energy-quenching mechanisms. These phenological changes may remain undetected by remote sensing, as conifers retain green foliage during periods of photosynthetic down-regulation. The aim was to assess if the timing of the spring recovery of photosynthesis and energy-quenching characteristics are accurately monitored by the photochemical reflectance index (PRI) in the evergreen conifer Pinus strobus. The recovery of photosynthesis was studied using chlorophyll fluorescence, leaf gas exchange, leaf spectral reflectance, and photosynthetic pigment measurements. To assess if climate change might affect the recovery of photosynthesis, seedlings were exposed to cold spring conditions or warm spring conditions with elevated temperature. An early spring decoupling of the relationship between photosynthesis and PRI in both treatments was observed. This was caused by differences between the timing of the recovery of photosynthesis and the timing of carotenoid and chlorophyll pool size adjustments which are the main factors controlling PRI during spring. It was also demonstrated that zeaxanthin-independent NPQ mechanisms undetected by PRI further contributed to the early spring decoupling of the PRI-LUE relationship. An important mechanism undetected by PRI seems to involve increased electron

Abbreviations: A, CO₂ assimilation (in μmol CO₂ m⁻² s⁻¹); ATP, adenosine triphosphate; α, needle absorptance; Car, total carotenoids (in mmol mol⁻¹ Chl); CET, cyclic electron transport rate (in μmol electrons m⁻² s⁻¹); Chl, total chlorophylls (in μmol g⁻¹ fresh weight); DEPS, de-epoxidation status of the xanthophyll cycle (in mol mol⁻¹); ETRₚ, electron transport rate of PSII (in μmol electrons m⁻² s⁻¹); ETRₛ, fraction of light directed to PSII; ETRₛ, electron transport rate of PSI (in μmol electrons m⁻² s⁻¹); F₁₋₂, minimal fluorescence of light-adapted needles; F₆₋₇, maximum fluorescence of dark-adapted needles; F₆₋₇, maximum quantum yield of PSII; F₆₋₇, maximum fluorescence of light-adapted needles; Fₛ, minimal fluorescence of light-adapted needles; LUEₚ, light-use efficiency of photosynthesis; LUEₛ, light-use efficiency of CO₂ assimilation (in μmol CO₂ mol⁻¹ quanta); NPQ, non-photochemical quenching; P, P₇₀₀ signal; Pₛ, difference between fully reduced and fully oxidized P₇₀₀; Pₛ, maximum change of the P₇₀₀ signal upon application of SP; Pₛ, minimal P₇₀₀ signal; P₆₀₀, photosynthetic photon flux density (in μmol quanta m⁻² s⁻¹); PRI, photochemical reflectance index; ΔPRI, difference between PRI of dark-adapted needles and PRI measured at 2,000 μmol quanta m⁻² s⁻¹; PS, photosystem; PTOX, plastid terminal oxidase; SP, saturating pulse; Sp, cold spring treatment seedlings; Sp, warm spring treatment seedlings; Su, summer-acclimated seedlings; Φₑₑₑ, energy quenching by fluorescence and dissipated constitutively; Φₑₑₑ, fraction of overall P₇₀₀ that cannot be oxidized by a saturation pulse due to a lack of electron acceptors; Φₑₑₑ, fraction of overall P₇₀₀ oxidized due to a lack of electron donors; Φₑₑₑ, proportion of light absorbed by PSI antenna and thermally dissipated via xanthophyll-regulated NPQ; Φₑₑₑ, effective quantum yield of PRI; Φₑₑₑ, effective quantum yield of PSII of light-adapted needles; 1–qP, excitation pressure of PSII; VAZ, total xanthophyll cycle pigments (in mmol mol⁻¹ Chl); Wi, winter-acclimated seedlings.

© The Author 2015. Published by Oxford University Press on behalf of the Society for Experimental Biology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
transport around photosystem I, which was a significant energy sink during the entire spring transition, particularly in needles exposed to a combination of high light and cold temperatures.

**Key words:** Climate change, Eastern white pine (*Pinus strobus*), leaf pigments, light-use efficiency of photosynthesis, photochemical and non-photochemical quenching, photochemical reflectance index, spring recovery of photosynthesis, xanthophyll cycle.

**Introduction**

Recent increases in global temperature are causing large-scale changes in carbon cycling of the conifer-dominated northern forest ecosystems (Piao et al., 2008; Zhang et al., 2013; IPCC, 2014). Since photosynthesis is highly variable in space and time, its assessment with high spatial and temporal resolution is essential accurately to assess the actual and projected effects of global climate change on the carbon budget of these forests. Remote sensing can provide data across a range of spatial scales and has become an important source of information for modelling global carbon cycling. In particular, the photochemical reflectance index (PRI), a parameter that can be derived from remotely sensed spectral reflectance data, has recently received considerable attention for its ability to detect changes in the photosynthetic efficiency of leaves, canopies, and entire ecosystems (Garbulsky et al., 2011).

The biological basis of PRI is its capacity to detect the spectral signature of pigments involved in the dissipation of excess light and, hence, the efficiency of photosynthesis. To ensure optimum plant performance in ever-changing light environments, leaves constantly acclimate to balance the amount of light absorbed and their requirements for energy utilization (Ensminger et al., 2006). While it is advantageous to maximize the absorption of light energy to fuel photosynthesis, the absorption of excess light that exceeds the capacity of photosynthesis can produce harmful reactive oxygen species (Barber and Andersson, 1992; Hüner et al., 1998; Niyogi, 2000). Plants use different strategies to maintain a balance between the energy they absorb and the energy utilized for photochemistry. One strategy is to regulate light absorption capacity by, for example, chloroplast relocation (Kodama et al., 2008). Another strategy is the regulation of light-use efficiency (LUE), i.e. the partitioning of absorbed light energy between photochemical and non-photochemical pathways (Demming-Adams and Adams, 2006; Ensminger et al., 2006). Evergreen conifers retain most of their chlorophyll content throughout the year and adjust LUE by various mechanisms in response to environmental conditions and depending on the season. During summer, the harmless removal of excess light, referred to as non-photochemical quenching (NPQ), is mediated by xanthophyll cycle pigments. Excess light energy rapidly induces the de-epoxidation of xanthophyll cycle pigments, i.e. the conversion of violaxanthin into the energy-quenching antheraxanthin and zeaxanthin (Niyogi et al., 2005; Demming-Adams and Adams, 2006; Verhoeven, 2014). Changes in the de-epoxidation status of xanthophyll cycle pigments (DEPS) affect leaf spectral reflectance at 531 nm. This change in reflectance amplitude at 531 nm is used to derive PRI by comparing it to the xanthophyll-insensitive band at 570 nm (Gamon et al., 1992, 1997; Peñuelas et al., 1995). During winter, however, the xanthophyll cycle is arrested in a state primed for sustained energy quenching where zeaxanthin is retained, chlorophylls are partially degraded, photosystem (PS) II core units are reorganized (Ensminger et al., 2006; Demmig-Adams and Adams, 2006; Verhoeven, 2014) and photoprotective pigments such as lutein and β-carotene are up-regulated (Adams and Demmig-Adams, 1994; Ottander et al., 1995; Filella et al., 2009; Verhoeven, 2014). These long-term, slowly reversible pigment adjustments are associated with this sustained mode of NPQ (Demmig-Adams and Adams, 2006).

Both short- and long-term pigment adjustments lead to changes in leaf spectral properties. These changes can be detected through leaf spectral reflectance measurements and have been used to measure PRI. In evergreen conifers, PRI was reported to be a good proxy for xanthophyll cycle dynamics over the course of a day (Nakaji et al., 2006; Harris et al., 2014), although there are reports that PRI has limitations to reflect the diurnal variation of xanthophyll de-epoxidation under excessive light (Kováč et al., 2013). Recent studies have also reported good correlations between PRI and LUE over longer timescales, e.g. seasons (Garbulsky et al., 2011). It was suggested that PRI varies not only as a consequence of dynamic changes in xanthophyll pigments, but also as a result of long-term adjustments of pigment pools that allow plants to acclimate to changing environmental conditions throughout the year (Sims and Gamon, 2002; Garrity et al., 2011; Wong and Gamon, 2015a, b; Hmimina et al., 2015). Besides the adjustment of pigment pools, it is also important to note that, on the canopy scale, changes in leaf area, foli- age clumping, and the distribution of shadow fraction affect the PRI signal that is detected from canopy spectral measurements (Hilker et al., 2008, 2010). Long-term adjustments of chlorophyll and carotenoid pool sizes can be observed during spring in conifers, when photosynthesis recovers from winter stress (Ensminger et al., 2004, 2008). These adjustments were shown to be a considerable source of PRI variation during the spring transition (Busch et al., 2009; Porcar-Castell et al., 2012; Wong and Gamon, 2015a, b). Over the course of the year, Wong and Gamon (2015a) observed that PRI variation in lodgepole and ponderosa pine (*Pinus contorta* D. and *P. ponderosa*) correlated better with the ratio of carotenoids per chlorophyll than xanthophyll cycle activity. In boreal Scots pine (*P. sylvestris* L.), Porcar-Castell et al. (2012) came to the same conclusion and reported that PRI was a good
proxy for LUE during most of the year except during severe cold stress in the early spring. Similarly, Busch et al. (2009) observed that PRI could not explain variations in the quantum yield of PSII of Jack pine (P. banksiana Lamb.) seedlings during spring, when excess energy was predominantly dissipated via sustained quenching. The inability of the PRI to accurately detect changes in LUE during the winter–spring transition reflects the reorganization of the chloroplast during spring, that involves replacement of sustained NPQ by the rapidly reversible, flexible energy dissipation via the xanthophyll cycle (Demmg-Adams and Adams, 2006; Zarter et al., 2006; Ensminger et al., 2008).

In addition to sustained quenching, several zeaxanthin-independent NPQ mechanisms and alternative electron sinks might contribute to a discrepancy in the PRI–LUE relationship in conifers throughout the year (Busch et al., 2009; Porcar-Castell et al., 2012), although there is no direct evidence yet. NPQ mechanisms might include PSII and PSI reaction centre quenching (Öquist and Hüner, 2003), quenching of singlet excited chlorophyll by carotenoid pigments (Trebst, 2003; Telfer, 2005; Jahns and Holzwarth, 2012), and quenching via the lutein epoxide cycle (Matsubara et al., 2012). Alternative electron sinks can include plastid terminal oxidase (PTOX)-mediated electron transport to oxygen (Savitch et al., 2010) or photorepiration (Takahashi and Badger, 2011). Cyclic electron transport around PSI can also contribute significantly to the removal of excess energy during winter and early spring (Ivanov et al., 2002). Recently it was suggested that the provision of ATP produced from cyclic electron transport maintains chloroplast integrity during chilling stress (Huang et al., 2010) and supports the recovery from chilling stress. Furthermore, it has been established that the stoichiometry of the two photosystems adjusts to balance electron transport under cold conditions (Ivanov et al., 2001; Ensminger et al., 2004). Because some of these processes have the potential to adjust LUE downstream of PSII, they will remain undetected by PRI. Assessing the contribution of zeaxanthin-independent mechanisms to NPQ during the spring recovery of photosynthesis can, therefore, reveal sources of the decoupling observed in the PRI–LUE relationship during that season (Busch et al., 2009; Porcar-Castell et al., 2012; Wong and Gamon, 2015b).

In the boreal zone, the spring recovery of photosynthesis is largely driven by air temperature, which determines the physiological state of the chloroplast (Ensminger et al., 2004). This includes the excitation pressure on PSII and PSI, energy partitioning between the photosystems, and the requirement for excess energy dissipation. The phenology of these events and, in turn, the PRI–LUE relationship, are likely to change as spring temperatures increase in the future (IPCC, 2014). For instance, the effects of warmer spring conditions on the transition from sustained NPQ to energy-dependent NPQ are uncertain, but will most likely affect the PRI–LUE relationship during that transitory period.

In the present study, the photosynthetic recovery of winter-acclimated Eastern white pine (Pinus strobus L.) seedlings exposed to cold or warm simulated spring conditions in controlled growth environments was followed in order (i) to investigate the effect of spring temperature on the transition from the sustained quenching mode of NPQ to energy-dependent quenching in conifer needles; (ii) to determine whether the spring timing of seasonal carotenoid and chlorophyll pool size adjustments, and thus of PRI recovery, are consistent with the recovery of LUE; and (iii) to identify zeaxanthin-independent NPQ mechanisms that contribute to the decoupling of the PRI–LUE relationship during the winter–spring transition.

### Materials and methods

#### Plant material and growth conditions

Three-year-old Eastern white pine (P. strobus L.) seedlings were obtained in April 2011 and 2013 from a local nursery (Somerville Seedlings, Everett, Ontario, Canada), planted in a mixture of sand and sphagnum peat moss (1:3 v/v) and fertilized with 28:10:10 mineral fertilizer (Miracle-Gro, Scotts, Marysville, OH, USA). Seedlings were kept outside in an experimental garden at the University of Toronto at Mississauga (ON, Canada) until transfer to environmental growth chambers (Biochambers, Winnipeg, Canada) during December. The seedlings were acclimated for 6 weeks to simulated winter conditions (2–5 °C day/night; 8 h photoperiod at 400 μmol quanta m⁻² s⁻¹). Winter-acclimated seedlings (Wi) were then shifted for 36 d to either a cold spring (Sp₀) or a warm spring (Sp₉) treatment. The temperature in Sp₀ was set to 10/5 °C (day/night) and in Sp₉ it was set to 15/10 °C (day/night) and the photoperiod was 12 h in both spring treatments (Table 1). The light intensity was constantly monitored with a PAR sensor mounted at the top of the seedling canopy and maintained at an intensity of 1,400 μmol quanta m⁻² s⁻¹ (Table 1). Incident sunlight under fluctuating natural conditions may reach light intensities well above 1,800 μmol quanta m⁻² s⁻¹. However, preliminary experiments showed that a constant light intensity higher than 1,500 μmol quanta m⁻² s⁻¹ over the full course of the photoperiod caused severe photodamage in our seedlings and light intensity in our growth chambers was therefore set to values not exceeding 1,400 μmol quanta m⁻² s⁻¹. Wi seedlings were sampled and measured 1 d prior to transfer to spring conditions (day 0). Subsequent sampling and measurements of spring plants was done after transfer to spring conditions on days 1, 3, 6, 12, 18, 24, and 36 of the experiment. Measurements and samples of summer-acclimated needles were obtained from summer seedlings (Su) that had been kept outdoors in the experimental garden and were then acclimated for 6 weeks to simulated summer conditions (22/15 °C day/night; 14 h photoperiod at 1,400 μmol quanta m⁻² s⁻¹ light intensity). All measurements and needle sampling were performed on previous year needles of the topmost portion of the leader shoot. All data were obtained from two independent experiments performed in 2012 and 2014 using identical settings and protocols. In order to minimize any chamber effects further, the seedlings were rotated between chambers every 2 weeks.

#### Table 1. Overview of growth conditions within the chambers during the spring simulation

| Experimental treatment | Air temperature (°C; day/night) | Photoperiod (h) | Light intensity (μmol m⁻² s⁻¹) |
|------------------------|---------------------------------|-----------------|-------------------------------|
| Wi, winter             | 2/−5                            | 8               | 400                           |
| Sp₀, cold spring       | 10/5                            | 12              | 1,400                         |
| Sp₉, warm spring       | 15/10                           | 12              | 1,400                         |
| Su, summer             | 22/15                           | 14              | 1,400                         |
Chlorophyll fluorescence measurements

At each time point, chlorophyll-fluorescence measurements were performed using a Dual-PAM-100 (Walz, Effeltrich, Germany). Measurements were done on bundles of 10–15 needles that were aligned in parallel to form a single layer of needles in the leaf clip holder of the Dual-PAM-100. A saturating light pulse (SP) was applied to dark-adapted (pre-dawn) needles for the determination of \( F_o \) and \( F_m \) (minimal and maximum fluorescence). Maximum quantum yield of PSII \((F_o/F_m)\) was calculated according to Genty et al. (1989):

\[
F_o = \left( \frac{F_m - F_s}{F_m} \right)
\]

The needles were then exposed to a sequence of 2.5-min intervals with actinic light of increasing intensity (0–2,000 \( \mu \)mol quanta \( m^{-2} s^{-1} \)), each step followed by a 400 ms saturating pulse (SP) of 10,000 \( \mu \)mol quanta \( m^{-2} s^{-1} \) for the determination of \( F_m \) (maximum fluorescence of light-adapted needles), and a weak pulse of far-red light for determination of \( F_a \) (minimal fluorescence of light-adapted needles). Energy partitioning parameters were calculated according to Hendrickson et al. (2004). The effective quantum yield of PSII of light-adapted needles \((\Phi_{\text{PSII}})\) reflects the proportion of light absorbed by PSII which is used for photochemistry and was calculated as:

\[
\Phi_{\text{PSII}} = 1 - \frac{F_s}{F_m}
\]

where \( F_s \) is the yield of fluorescence of a light-adapted sample. The proportion of light that is absorbed by PSII antenna and thermally dissipated via xanthophyll-regulated NPQ \((\Phi_{\text{NPQ}})\) was calculated as:

\[
\Phi_{\text{NPQ}} = \frac{F_s - F_i}{F_m - F_i}
\]

The energy quenched by fluorescence and dissipated constitutively \((\Phi_{\text{FD}})\) was calculated as:

\[
\Phi_{\text{FD}} = \frac{F_i}{F_m}
\]

The electron transport rate of PSII \(\text{ETR}_{\text{II}}\), in \( \mu \)mol electron \( m^{-2} s^{-1} \) was calculated according to Genty et al. (1989):

\[
\text{ETR}_{\text{II}} = \Phi_{\text{PSII}} \times \text{PPFD} \times \alpha \times d_I
\]

where PPFD is the applied light intensity \((\mu \text{mol quanta } m^{-2} s^{-1})\), \( \alpha \) is the absorbance, i.e. the fraction of incident light absorbed by leaves, and \( d_I \) the fraction of light directed to PSII. Values of \( \alpha \) were calculated as \( \alpha = 1 - \text{transmittance–reflectance} \). Given the thickness of pine needles, transmittance was assumed to be 0. However, it should be noted that a small proportion of light can be transmitted through conifer needles (Lukeš et al., 2013). Reflectance was measured with a Unispec-SC spectrometer over the 400–700 nm wavelength range (Huang et al., 2012). Values of \( d_I \) were calculated using the ratio of \( \Phi_{\text{FD}}/\Phi_{\text{FD}} \) (see below) at low light intensity (60\( \mu \)mol quanta \( m^{-2} s^{-1} \)), where CET is assumed to be absent and \( \text{ETR}_{\text{II}} = \text{ETR}_{I} \) (Huang et al., 2012).

The excitation pressure of PSII \((1-qP)\) was calculated as:

\[
1-qP = 1 - \frac{F_o - F_s}{F_m - F_o}
\]

To assess energy partitioning characteristics at a diurnal timescale, light response curves were measured on day 0 as well as on day 12 of the experiment. A dark-adapted bundle of needles was exposed to 10-min steps of increasing actinic light intensity (0–2,000 \( \mu \)mol quanta \( m^{-2} s^{-1} \)). At each light step, \( \Phi_{\text{PSII}}, \Phi_{\text{NPQ}}, \) and \( \Phi_{\text{FD}} \) were recorded.

PSI absorbance measurements

Absorbance changes of the reaction centre chlorophyll of PSI \((P700)\) were assessed simultaneously with chlorophyll fluorescence measurements using a Dual-PAM-100. The P700 signal \((P)\) was calculated as the difference between the 875 nm and 830 nm transmittance signals. Firstly, P700 oxidation was transiently induced by applying a SP after far-red pre-illumination of dark-adapted needles. Briefly, after the SP, the minimal P700 signal was measured to capture a state of full P700 reduction. The difference between the fully reduced and fully oxidized states is denoted \( P_m \). Secondly, actinic illumination was applied with the same actinic light intensities and SPs used for fluorescence. Upon application of each maximum change of the P700 signal \((P_o)\) was determined. Each SP was followed by a 1 s dark interval for the full reduction of P700 and determination of the minimal P700 signal \((P_o)\).

The three types of quantum yields of energy conversion in PSI were assessed according to Klughammer and Schreiber (1994) and calculated according to Pfündel et al. (2008). The effective quantum yield of PSI \((\Phi_{\text{PSI}})\) in the light was calculated as:

\[
\Phi_{\text{PSI}} = \frac{P_m - P}{P_m}
\]

The fraction of overall P700 that is oxidized in a given state due to a lack of electron donors (donor side limitation; \( \Phi_{\text{ND}} \)), was calculated as:

\[
\Phi_{\text{ND}} = \frac{P - P_o}{P_m}
\]

The fraction of overall P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of electron acceptors (acceptor side limitation, \( \Phi_{\text{NA}} \)), was calculated as:

\[
\Phi_{\text{NA}} = \frac{P_m - P^*}{P_m}
\]

Analogous to \( \text{ETR}_{\text{II}} \), the electron transport rate of PSI \((\text{ETR}_{I})\, \text{in } \mu \text{mol electron } m^{-2} s^{-1} \) was calculated as:

\[
\text{ETR}_{I} = \Phi_{\text{PSI}} \times \text{PPFD} \times \alpha \times d_I
\]

where \( d_I \) was calculated as \( d_I = 1 - d_{II} \) (Huang et al., 2012). Cyclic electron transport \((\text{CET}\), in \( \mu \)mol electron \( m^{-2} s^{-1} \)) was calculated according to Huang et al. (2012) as:

\[
\text{CET} = \text{ETR}_{I} - \text{ETR}_{\text{II}}
\]

Photosynthetic gas exchange measurements

To assess variations in photosynthetic activity at a seasonal timescale, photosynthetic gas exchange was measured at each time point (GFS-3000, Walz, Effeltrich, Germany). Measurements started 2 h after the lights were turned on inside the growth chambers. A bundle of attached needles was oriented to form a flat plane and inserted in the leaf cuvette. CO\(_2\) concentration in the cuvette was set to 400 ppm, temperature was set to growth temperature (Table 1) and humidity was set at 60% RH. Net CO\(_2\) assimilation \((A\), in \( \mu \)mol CO\(_2\) \( m^{-2} s^{-1} \)) was measured at growth light intensity (1,400 \( \mu \)mol quanta \( m^{-2} s^{-1} \)) once steady-state assimilation was achieved. Immediately after the measurements, needles were detached from the seedling and measured for surface area using the WinSEEDLE software.
package (Regent Instruments Inc., Québec, Canada). The light-use efficiency of CO₂ assimilation (LUEₐ, in mol CO₂ mol⁻¹ quanta) was calculated as:

\[ \text{LUE}_\text{A} = \frac{\text{Assimilation}}{\text{PPFD}} \]  

(12)

To assess variations in photosynthetic activity at a diurnal time-scale, light response curves were measured on days 0 and 12 in both treatments. A fully dark-adapted bundle of needles was exposed to a sequence of eight 10-min light steps of increasing actinic light intensity (0–2,000 μmol quanta m⁻² s⁻¹). Measurement and cuvette conditions were identical to those used for assessing variation of photosynthetic activity at a seasonal time-scale (see above). At each light step, A was measured and LUEₐ was calculated.

Spectral reflectance measurements

Seasonal variations in PRI were assessed from leaf spectral reflectance measurements using a Unispec-SC spectrometer (UNI007, PP Systems, Haverhill, MA, USA) equipped with an internal tungsten halogen light source. The spectrometer was connected to a bifurcated fibre-optic (UNI400) and a leaf clip (UNI500) maintaining the fibre-optic on the needle surface at a fixed angle of 60° relative to needle axis (2 mm diameter spot size). Leaf bidirectional reflectance was computed by dividing reflected irradiance by the radiance obtained from a white reflectance standard (Spectralon, Labsphere, North Sutton, NH, USA) taken immediately before each leaf measurement. Dark current instrument noise was subtracted from white standard and leaf radiance measurements. Reflectance was measured on needles of the topmost part of the leader shoot. Needles were in bundles of approximately 10–15 needles arranged in parallel to form a single layer flat plane. The integration time was set to 10 ms and 40 scans were averaged for each measurement followed by interpolation of the ~3.3 nm resolution output of the spectrometer to 1 nm bandwidths using the software Multispec v. 5.1.0 (Purdue University, Indiana, USA). Finally, PRI was calculated according to Peñuelas et al. (1995):

\[ PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}} \]  

(13)

where \( R_{531} \) and \( R_{570} \) represent leaf reflectance at 531 and 570 nm, respectively. In order to compare seasonal PRI variation, pre-dawn PRI measurements were used to separate the effects of long-term and short-term pigment variations on PRI (Gamon and Berry, 2012).

To assess the effect of short-term xanthophyll pigment variations on PRI and infer the magnitude of diurnal PRI variation light response curves were measured on days 0 and 12. A bundle of needles was set up in the leaf clip and exposed to eight 10-min light steps of increasing actinic light intensity (0–2,000 μmol quanta m⁻² s⁻¹) using the Unispec-SC internal light source. Three scans were averaged at each light step. To assess the range of diurnal PRI variation, ΔPRI was calculated as the difference between PRI of dark-adapted needles and PRI measured at 2,000 μmol quanta m⁻² s⁻¹ (Gamon and Berry, 2012).

Statistical analyses

The effects of treatments on individual parameters at each time point were estimated using mixed model analysis of variance (ANOVA). For all statistical analyses the data from two experiments run on two different years were pooled together. Year was included as a random effect in the mixed model to account for the two replicate experiments. The analysis was performed using the difflmsmeans function in the lmerTest package, using R version 3.1.1 (R Core Team, 2014).

In order to evaluate the strength of the relationship between PRI and physiological parameters, R² values were obtained from linear regressions with log-transformed variables and the slope was considered significantly different from zero when P < 0.05. Regressions were performed using GraphPad Prism 6 software version 6.05 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Seasonal variation in energy partitioning of PSII and PSI

Winter-acclimated seedlings (Wi) were assessed on day 0 of the experiment and then transferred to simulated spring growth conditions. Following transfer to spring conditions, a clear response of energy partitioning to longer photoperiod and warmer temperature was observed. In both spring treatments, the recovery of photosynthesis reached a steady state by day 12 of the experiment (Fig. 1A). By that time, the effective quantum yield of PSII (ΦPSII) was approximately twice as high in the warm spring treatment (SPw) as in the cold spring treatment (SPc). After photosynthetic recovery, 60% of absorbed energy in SPc was dissipated via xanthophyll-regulated NPO (ΦNPO) and 40% via fluorescence or dissipated
constitutively ($\Phi_{f,D}$; Fig. 1B, C). In Spw, however, the majority (75%) of NPQ was facilitated by $\Phi_{NPQ}$ (Fig. 1B). In Wi seedlings, more than 90% of all light energy absorbed was dissipated via $\Phi_{f,D}$, but after 18 d of exposure to spring conditions this value had declined to approximately 40% in Sp$_C$ and 20% in Sp$_W$ (Fig. 1C).

In addition to measurements of energy partitioning in PSII, energy conversion in PSI was assessed (Fig. 1D, E, F). Upon transfer to spring conditions, the effective quantum yield of PSI ($\Phi_{PSI}$) quickly recovered, with a faster rate in Sp$_W$ compared with Sp$_C$ (Fig. 1D). However, by day 18 of the experiment, $\Phi_{PSI}$ was higher in Sp$_C$ and remained so until the end of the experiment. During that period, quantum yield in Sp$_C$ was five times higher in PSI compared with PSII. By contrast, in Sp$_W$ seedlings, the quantum yield of PSI was only 1.5 times higher than in PSII (Fig. 1A, D). In both spring treatments, the fraction of overall P700 oxidized due to a lack of electron donors ($\Phi_{ND}$) transiently increased on the first day of the experiment, and then gradually declined in the following days (Fig. 1E). $\Phi_{ND}$ stabilized to a steady state by day 18 of the experiment to values significantly higher in Sp$_C$ than in Sp$_W$. In both treatments, the fraction of overall P700 that cannot be oxidized by a saturation pulse due to a lack of electron acceptors ($\Phi_{NA}$) dropped on the first day of exposure to spring conditions (Fig. 1F). In Sp$_C$, $\Phi_{NA}$ continued to decrease until the end of the experiment. By contrast, $\Phi_{NA}$ in Sp$_W$ increased after day 1, until it reached a plateau by day 12 and then remained significantly higher than in Sp$_C$ until the end of the experiment.

Acclimation to Wi conditions resulted in high $d_I$ values (0.88 ± 0.02) compared with $d_{II}$ (0.12 ± 0.02). Exposure to both

---

**Fig. 1.** Time-course of energy partitioning characteristics of *P. strobus* needles exposed to cold and warm spring treatments. (A) $\Phi_{PSII}$, fraction of absorbed light at PSII used for photochemistry; (B) $\Phi_{NPQ}$, fraction of absorbed light at PSII dissipated via xanthophyll-regulated thermal dissipation; (C) $\Phi_{f,D}$, sum of fluorescence and constitutive thermal dissipation at PSII; (D) $\Phi_{PSI}$, effective photochemical quantum yield of PSI; (E) $\Phi_{ND}$, fraction of P700 oxidized due to a lack of electron donors; and (F) $\Phi_{NA}$, fraction of P700 that cannot be oxidized by a saturation pulse due to a lack of electron acceptors. Measurements were taken at 1400 µmol quanta m$^{-2}$ s$^{-1}$ light intensity. Each data point represents $n$=10–11 seedlings ±SE and asterisks indicate significant differences ($\alpha$ < 0.05) between treatments at a given time point.
spring treatments resulted in a gradual increase in $d_{II}$ and a decline in $d_{I}$ as the experiment progressed, with generally higher $d_{I}$ values in SpC than in SpW (Table 2). Throughout the experiment, the electron transport rate of PSI (ETR$_{I}$) was higher than the electron transport rate of PSII (ETR$_{II}$) in both spring treatments (Fig. 2A, B). The highest ETR$_{I}$ values were observed in SpC between days 12 and 24 of the experiment. Cyclic electron transport (CET) was significantly higher in SpC than in SpW from day 12 of the experiment, with CET approximately 300% higher in SpC than in SpW during the second half of the experiment (Fig. 2C).

**Photosynthetic recovery and seasonal changes in PRI**

Consistent with the pattern observed in energy partitioning (Fig. 1), a recovery of photosynthetic activity was observed over the 36 d of our experiment (Fig. 3). By the end of the experiment, the maximum quantum yield of PSII ($F_v/F_m$) had recovered from a value of 0.13 in Wi seedlings (day 0) to values of 0.46 in SpC and 0.70 in SpW (Fig. 3A). From day 3, $F_v/F_m$ was significantly higher in SpW than in SpC. In both treatments, the excitation pressure of PSII ($1-qP$) declined from day 1 of the experiment until day 18, but then recovered considerably until day 36 of the experiment (Fig. 3B). Values of $1-qP$ were significantly higher in SpC during most of the experiment. In both treatments, the light-use efficiency of CO$_2$ assimilation (LUE$_A$) recovered quickly during the first 12 d of the spring treatment, with a faster rate of recovery in SpW than in SpC (Fig. 3C). LUE$_A$ was consistently higher in SpW compared with SpC (Fig. 3C). Under winter conditions, a PRI value of $-0.168$ was recorded. In SpC, PRI slightly declined until day 6 of the experiment and then increased again by day 12 and remained at winter-like values until the end of the experiment (Fig. 3D). In SpW, PRI was also relatively stable for the first 12 d of the experiment, with most of its recovery occurring between days 12 and 24 of the experiment, and with final values of approximately $-0.05$. After day 24, PRI was significantly higher in SpW than in SpC.

**Seasonal changes in foliar pigment content**

The transfer of seedlings from winter to spring conditions resulted in a clear response of photosynthetic pigment composition in both treatments (Fig. 4). Chlorophyll (Chl) FW$^{-1}$ remained stable until day 12 of the experiment, but recovered to summer levels after day 18 (Fig. 4A). By the end of the experiment, the pool of Chl had increased by 30% in SpC and by 53% in SpW. In both treatments, Chl $a/b$ increased

## Table 2. Fraction of light absorbed by PSII and PSI ($d_{II}$ and $d_{I}$) and absorptance ($\alpha$) of P. strobus needles exposed to winter, cold spring, warm spring, and summer treatments (n=11 seedlings ±SE)

| Day         | $d_{II}$ ± SE | $d_{I}$ ± SE | $\alpha$ ± SE |
|-------------|---------------|--------------|---------------|
| Winter      | 0.12 ± 0.02   | 0.88 ± 0.02  | 0.87 ± 0.01   |
| Cold spring |               |              |               |
| 1           | 0.12 ± 0.01   | 0.88 ± 0.01  | 0.86 ± 0.01   |
| 3           | 0.19 ± 0.03   | 0.81 ± 0.03  | 0.88 ± 0.01   |
| 6           | 0.15 ± 0.02   | 0.85 ± 0.02  | 0.88 ± 0.01   |
| 12          | 0.25 ± 0.02   | 0.75 ± 0.02  | 0.87 ± 0.01   |
| 18          | 0.20 ± 0.02   | 0.80 ± 0.02  | 0.88 ± 0.01   |
| 24          | 0.18 ± 0.02   | 0.82 ± 0.02  | 0.87 ± 0.01   |
| 36          | 0.22 ± 0.02   | 0.78 ± 0.02  | 0.86 ± 0.01   |
| Warm spring |               |              |               |
| 1           | 0.13 ± 0.02   | 0.87 ± 0.02  | 0.88 ± 0.01   |
| 3           | 0.19 ± 0.02   | 0.81 ± 0.02  | 0.89 ± 0.01   |
| 6           | 0.27 ± 0.03   | 0.73 ± 0.03  | 0.87 ± 0.01   |
| 12          | 0.34 ± 0.03   | 0.66 ± 0.03  | 0.88 ± 0.004  |
| 18          | 0.37 ± 0.03   | 0.63 ± 0.03  | 0.87 ± 0.004  |
| 24          | 0.44 ± 0.03   | 0.56 ± 0.03  | 0.87 ± 0.01   |
| 36          | 0.44 ± 0.02   | 0.55 ± 0.02  | 0.86 ± 0.01   |
| Summer      | 0.53 ± 0.01   | 0.47 ± 0.01  | 0.85 ± 0.01   |

---

Fig. 2. Time-course of (A) electron transport rate of PSI; (B) electron transport rate of PSII; and (C) cyclic electron transport rate of P. strobus needles exposed to cold and warm spring treatments. Measurements were taken at 1400 µmol quanta m$^{-2}$s$^{-1}$ light intensity. Each data point represents n=10–11 seedlings ±SE and asterisks indicate significant differences ($\alpha$ <0.05) between treatments at a given time point.
over the course of the experiment and, as of day 12, higher Chl a/b was observed in SpW than in SpC (Fig. 4B). By contrast, carotenoid (Car) Chl–1 peaked in SpC on day 18 of the experiment, and remained significantly higher in SpC than in SpW until day 36 (Fig. 4C). Xanthophyll cycle pigments (V AZ) Chl–1 remained fairly stable over the course of the experiment in SpC but declined in SpW seedlings after day 3 (Fig. 4D). The amount of zeaxanthin Chl–1 decreased over the first 6 d of the experiment in both treatments, increased again until day 24 in SpC, and was significantly higher in SpC than in SpW for most of the experiment (Fig. 4E). By day 36, zeaxanthin had decreased by 44% in SpC and by 66% in SpW compared with Wi seedlings on day 0. A similar trend was observed for the de-epoxidation status of the xanthophyll cycle (DEPS; Fig. 4F), which was higher in SpC compared with SpW throughout most of the experiment. For instance, by day 36 DEPS was 0.75 and 0.61 mol mol−1 in SpC and SpW, respectively. In both treatments, the amount of β-carotene Chl–1 also declined throughout the experiment. Most of the β-carotene Chl–1 was lost after day 24 of the experiment, with 21% of the winter β-carotene pool lost in SpC, and 38% lost in SpW (Fig. 4G). At any time during the experiment, the amount of β-carotene did not significantly differ between treatments. Lutein Chl–1 in SpC initially declined on the first days of the experiment, and then increased until day 18, before it decreased again until the end of the experiment. In SpW, lutein Chl–1 decreased consistently throughout the experiment (Fig. 4H). From day 18, significantly higher levels of lutein were observed in SpC compared with SpW.

Variation in the relationships between PRI and physiological parameters during the winter–spring transition

The relationship between PRI and energy partitioning varied depending on exposure time to the treatments (Fig. 5). For all energy partitioning parameters, no relationship with PRI was observed during days 0–3 of the experiment, but significant relationships for days 6–36 and/or the entire experiment (Fig. 5). Similar trends were observed for DEPS, where samples from days 0–3 were clearly separated from samples taken between days 6–36 (Fig. 6E, F). By contrast, relationships between PRI and Chl FW–1 or Car Chl–1 were significant for all time periods (Fig. 6A–D).

Response of photosynthesis to short-term variations in light intensity of seedlings acclimated to winter, spring or summer conditions

After 12 d of acclimation to simulated spring conditions, the seedlings exhibited different responses to increasing light intensity compared with winter conditions (Fig. 7). Assimilation increased from almost 0 μmol CO2 m–2 s–1 in Wi seedlings to approximately 3 μmol CO2 m–2 s–1 in SpC and 7 μmol CO2 m–2 s–1 in SpW at 2,000 μmol quanta m–2 s–1 light intensity (Fig. 7A). Assimilation in SpC and SpW had recovered 31% and 76% of the capacity observed in Su at 2,000 μmol quanta m–2 s–1 light intensity. The higher maximum rate of assimilation in spring seedlings was accompanied by lower light compensation points compared with Wi (122.7 ± 42.9 μmol...
Decoupling of the PRI signal and photosynthesis during spring

quanta m\(^{-2}\)s\(^{-1}\)), with 80.7 ± 23.1 μmol quanta m\(^{-2}\)s\(^{-1}\) in Sp\(_C\) and 14.2 ± 8.6 μmol quanta m\(^{-2}\)s\(^{-1}\) in Sp\(_W\). Compared with winter, LUE\(_A\) was slightly higher in Sp\(_C\), while LUE\(_A\) in Sp\(_W\) was only slightly lower than Su values (Fig. 7B). Assessing the changes in energy partitioning via chlorophyll-fluorescence revealed a different pattern. The short-term light response of \(\Phi_{\text{PSII}}\) in Sp\(_W\) was very close to Su values, but considerably higher \(\Phi_{\text{NPQ}}\) and lower \(\Phi_{\text{f,D}}\) values were observed over the full range of light intensities in Sp\(_W\) (Fig. 7C, D, E). By contrast, Sp\(_C\) seedlings showed \(\Phi_{\text{NPQ}}\) values similar to Wi, but considerably higher \(\Phi_{\text{PSII}}\) and lower \(\Phi_{\text{f,D}}\). PRI was still very close to winter values in both spring treatments, which had recovered approximately 18% of their Su values (Fig. 7F). The range of diurnal PRI variation differed between treatments, with a ΔPRI of –0.033 in Su, a ΔPRI of –0.008 in Sp\(_C\), a ΔPRI of –0.022 in Sp\(_W\), and a ΔPRI of –0.0032 in Wi.

**Discussion**

Conifers undergo a reorganization of components of the chloroplast during winter stress

The winter conditions simulated in our experiment induced the complete down-regulation of photosynthesis in pine
seedlings (Fig. 3C) including a 30% reduction of the chlorophyll that was present in summer-acclimated needles (Fig. 4A). Light absorption by chlorophylls remaining in the winter-acclimated needles resulted in high excitation pressure of PSII, as indicated by $1-qP$ values close to 1 (Fig. 3B).

Low $F_v/F_m$ values observed under winter conditions indicated that the chlorophyll pigments were retained in a quenched, photoprotected state (Ottander et al., 1995; Ensminger et al., 2004). Under winter conditions, approximately 90% of all energy absorbed by pine needles was dissipated thermally via...
sustained NPQ (Fig. 1C) as reported in winter-acclimated *P. strobus* trees (Verhoeven, 2013). This sustained capacity for NPQ was facilitated by xanthophyll cycle pigments maintained in a highly de-epoxidized state compared with the summer seedlings (Fig. 4D, F). Accumulation of xanthophylls was also accompanied by larger amounts of lutein and β-carotene, reflecting an increased capacity for triplet Chl quenching (Fig. 4G, H) that has been observed in several other overwintering conifer species (Adams and Demmig-Adams, 1994; Ottander et al., 1995, Sveshnikov et al., 2006). This winter state concurred with highly negative PRI values of approximately –0.2 (Fig. 3D), which is comparable to PRI values reported in winter down-regulated pine species (Wong and Gamon, 2015a, b).

Although the functionality of both photosystems was impaired under winter conditions, PSI was less affected than PSII (Table 2; Fig. 1, 2). While PSII electron transport was suppressed almost completely (Fig. 2A), approximately 50% of the capacity observed in summer needles was preserved in PSI (Fig. 2B). This is in accordance with
Ivanov et al. (2001) who observed that PSI has a higher level of resistance to winter stress compared with PSII. The repression of linear electron transport downstream of PSII was accompanied by marked donor-side limitation of PSI, as indicated by high $\Phi_{\text{ND}}$ values (Fig. 1E). Interestingly, $\Phi_{\text{NA}}$ in winter-acclimated needles was the same as that observed during summer, suggesting a retention of the pool size of PSI electron acceptors during winter-acclimation, possibly as a strategy to facilitate rapid recovery of carbon fixation in the early spring. The observed imbalance between electron transport at PSII and PSI points to enhanced cyclic electron transport around PSI (Johnson, 2011). This indicates that, in winter-acclimated seedlings, electron flow around PSI plays a considerable role in the removal of excess light. Oxidized P700 can efficiently quench chlorophyll fluorescence (Öquist and Hüner, 2003); thus, it is concluded that PSI was probably an important quencher of absorbed light in our winter-acclimated seedlings.

It is important to note that, in this study, needle absorptance ($\alpha$) was calculated with the assumption that needle transmittance is zero. It was shown recently that a small proportion of light (<5% of the visible spectrum) can be transmitted through conifer needles (Lukeš et al., 2013). The absorptance values presented in Table 2 might, therefore, slightly overestimate the true $\alpha$, which can potentially result in a minor overestimation of ETR. However, the error is minimal and equally affects $\text{ETR}_{\text{II}}$ and $\text{ETR}_{\text{I}}$ and the overall trends observed in our data are not affected. It is recommended that future studies include measurements of reflectance and transmittance to facilitate the estimation of leaf absorptance and the calculation of $\alpha$ and ETR.

Fig. 7. The response to light of P. strobus needles acclimated to winter, cold spring, warm spring or summer conditions for (A) photosynthetic CO$_2$ assimilation; (B) LUE$_{\text{A}}$, light-use efficiency of CO$_2$ assimilation; (C) $\Phi_{\text{PSII}}$, fraction of absorbed light used for photochemistry; (D) $\Phi_{\text{NPQ}}$, fraction of light quenched via xanthophyll-regulated thermal dissipation; (E) $\Phi_{\text{f,D}}$, sum of fluorescence and constitutive thermal dissipation; and (F) PRI. Each data point represents $n=6$–8 seedlings ±SE.
Different rates of recovery cause decoupling of PRI and LUE during the early stages of spring

Immediately after transfer to our spring treatments, a decrease of the excitation pressure of PSII was observed (1–qP, Fig. 3B), reflecting the role of air temperature in restoring the redox state of the chloroplast (Demmig-Adams and Adams, 2006; Ensminger et al., 2008). In both spring treatments, the maximum quantum yield of PSII recovered rapidly, indicating a fast reorganization of the photosynthetic apparatus under warmer conditions. A considerable recovery of assimilation was also observed within the first days of exposure to spring treatments (Fig. 3C). A concomitant decrease in zeaxanthin pools and a decrease in the de-epoxidation state of the xanthophyll cycle (Fig. 4E, F) were also observed, both indicating a decreased requirement for photoprotection. In P. sylvestris, Ensminger et al. (2008) also reported the rapid recovery of photosynthetic capacity, along with the rapid relaxation of DEPS, during the first few days of exposure to a simulated spring treatment. In our experiment, the quick decline in zeaxanthin Chl$^-$ and DEPS occurred concomitantly with the transition from sustained quenching to dynamic quenching mediated by xanthophyll cycle pigments (Fig. 1B, C).

Interestingly, under warm spring conditions, it took more than 18 d before PRI recovered from winter stress while, under cold spring conditions, no PRI recovery was observed. Similarly, levels of Chl FW$^{-1}$ and Car Chl$^-$ did not recover from winter stress until after day 12 (Fig. 4A, C). In the field, boreal spring conditions are characterized by large day-to-day variations in air temperature, and photosynthesis recovers quickly and opportunistically with increasing air temperature. Conversely, photosynthesis can rapidly revert back to a down-regulated state with the occurrence of cold episodes (Ensminger et al., 2004, 2008). Maintaining large pools of carotenoids in needles after photosynthetic recovery reflects a strategy that allows for rapid photoprotection during sudden low temperature episodes. On the other hand, the delay in Chl synthesis during the early stages of photosynthetic recovery prevents the absorption of excess light and photo-oxidative stress. This is supported by the fact that the timing of Car down-regulation and Chl up-regulation coincided with the occurrence of the maximum rates of photosynthesis after day 12 of the experiment (Figs 3, 4). A mismatch in the timing of the spring recovery was also reported by Wong and Gamon (2015b). They observed that the recovery of electron transport, LUE, and the epoxidation status of the xanthophyll cycle (EPS) occurred approximately 2–3 weeks earlier than the recovery of PRI in P. ponderosa and P. contorta seedlings exposed to natural spring conditions. These differences in the timing of recoveries indicate that, during the early stages of photosynthetic recovery, xanthophyll cycle dynamics are not the main factor controlling PRI. PRI therefore insufficiently detects variations in LUE during that time.

At a seasonal time-scale, PRI is mostly controlled by carotenoid and chlorophyll pool sizes

Previous studies on boreal pine species have reported a strong contribution of pigment pools on PRI. For instance, Porcar-Castell et al. (2012) reported that variations in the pool size of xanthophyll cycle pigments over the year control the dynamics of PRI in P. sylvestris, while Wong and Gamon (2015a, b) reported a combined effect of all carotenoid pigments in P. ponderosa and P. contorta. Our experiment revealed that PRI and DEPS did not correlate well during the early stages of spring recovery (days 0–3), when NPQ was predominantly facilitated via the sustained quenching mode. By contrast, PRI correlated consistently with Chl and Car pigment pool sizes throughout the spring simulation, regardless of the degree of photosynthetic recovery (Fig. 6A–D). Furthermore, Chl and Car were responsible for a large proportion of the PRI variation observed. The range of PRI variation between winter and summer seedlings was 0.22 while the largest range of PRI variation observed at a diurnal scale was only 0.033 (Fig. 7F). Accordingly, the magnitude of variation in PRI due to long-term pigment pool adjustments was more than six times higher than the magnitude caused by short-term changes in xanthophyll cycle pigments. This ratio suggests that, on an annual scale, the PRI signal reflects the small contribution of xanthophyll cycle dynamics superimposed on the much larger effects of seasonal pigment adjustments. Thus, the adjustments in pigment pools conceal the diurnal variations in PRI, especially during the winter–spring transition from sustained NPQ to energy-dependent NPQ.

Alternative energy sinks contribute to the early spring discrepancy in the PRI–LUE relationship

During the spring transition from sustained to reversible zeaxanthin-dependent NPQ, adjustments in NPQ may occur independently of xanthophyll pigment conversions and decouple PRI from NPQ. This was observed during the entire duration of the spring simulation, where sustained quenching was retained to some extent in both the cold and warm treatments and particularly during the first days of exposure to spring conditions. What causes the delay of the release of sustained quenching? Sustained NPQ or the thermal dissipation of excess energy is achieved through structural reorganization of PSII. These changes include the aggregation of LHClI as a major energy dissipation pathway (Horton et al., 1991, 2005; Ottander et al., 1995). Unlike the xanthophyll-regulated LHClI aggregation associated with energy-dependent quenching (Horton et al., 2005; Ruban et al., 2012), this cold-induced reorganization reflects a zeaxanthin-independent quenching mechanism which is not detected by PRI. In addition to LHClI aggregation, another zeaxanthin-independent electron sink that remains undetected by PRI is the diversion of excess energy to PSI-driven cyclic electron transport. In P. strobus seedlings, the recovery of PSII function was generally slowed down and impaired in the cold spring treatment. PSII electron transport in the cold spring treatment recovered to values approximately half of that observed in the warm spring treatment and at a slower recovery rate (Fig. 2A). Interestingly, PSI electron transport in the cold spring treatment greatly surpassed the amount recorded in the warm treatment during the second half of the experiment (Fig. 2B). Ivanov et al. (2001) suggested that PSI photochemistry in
early spring supplies the ATP required to maintain the integrity of chloroplasts while supporting recovery from winter stress. In this experiment, enhanced PSI-driven electron transport coincided with a high excitation pressure of PSII (Figs 2C, 3B). High light and low temperature in our cold spring treatment imposed increased excitation pressure and probably photodamage to the D1 protein of PSII. Because de novo synthesis of the D1 protein requires a large amount of ATP (Murata et al., 2007), cyclic electron flow was proposed to be an essential component in the recovery of PSII under conditions of impaired linear electron transport (Huang et al., 2010). This may indicate that, in the cold spring treatment, a larger fraction of absorbed energy was invested in ATP production via PSI-driven electron transport, rather than being dissipated as heat via the xanthophyll cycle as was the case in the warm spring treatment.

Under conditions of cold stress, both PSII and PSI may be important non-radiative quenchers through charge recombination events (Lunde et al., 2000; Öquist and Hüner, 2003). In addition, Savitch et al. (2010) suggested that PTOX-mediated electron transport to oxygen is a major electron sink during winter. However, such measurements were outside the scope of this experiment, but these energy sinks probably contribute to NPQ during the winter–spring transition without apparent effect on PRI. This early spring decoupling of PRI and LUE seems to result in the overestimation of LUE (Fig. 5A, B), as reported by Porcar-Castell et al. (2012) in P. sylvestris during early spring, when foliage was down-regulated due to severe cold stress.

Conclusions

Our results demonstrate an early spring decoupling in the PRI–LUE relationship caused by differences between the timing of the recovery of photosynthesis and the timing of carotenoid and chlorophyll pigment pool size adjustments which are the main controlling factors of PRI during spring. It is also demonstrated that zeaxanthin-independent NPQ mechanisms undetected by PRI further contributed to this decoupling. One of the main mechanisms contributing to the decoupling of the PRI–LUE relationship probably involves PSI-driven electron transport, which appears to be a significant energy sink during the entire spring transition, particularly in needles exposed to a combination of high light and cold temperatures.

Future studies should also aim to validate the mechanisms identified here on mature trees and in natural systems where additional causes of PRI variation, such as illumination angle or canopy structure, are likely to impose additional complexity to the signal detected from leaf reflectance measurements.

Acknowledgements

Financial support from NSERC and the Canada Foundation for Innovation to IE is gratefully acknowledged. EF acknowledges the receipt of a Graduate Student Research Award from the University of Toronto Center for Global Change Science and PhD funding from the Department of Cell and Systems Biology at the University of Toronto and Ontario Graduate Scholarships. The authors are also grateful to Christopher Juliao and Daniel Marsden for their help and assistance in the laboratory.

References

Adams III WW, Demmig-Adams B. 1994. Carotenoid composition and down-regulation of photosystem II in three conifer species during the winter. Physiologia Plantarum 92, 461–458.

Barber J, Andersson B. 1992. Too much of a good thing: light can be bad for photosynthesis. Trends in Biochemical Sciences 17, 61–66.

Busch F, Hüner NPA, Ensminger I. 2009. Biochemical constrains limit the potential of the photochemical reflectance index as a predictor of effective quantum efficiency of photosynthesis during the winter spring transition in Jack pine seedlings. Functional Plant Biology 36, 1016–1026.

Demmig-Adams B, Adams III WW. 2006. Photo-protection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytologist 172, 11–21.

Ensminger I, Svishenkiv D, Campbell D, Funk C, Jansson S, Lloyd J, Shibiotsava O, Öquist G. 2004. Intermittent low temperatures constrain spring recovery of photosynthesis in boreal Scots pines forests. Global Change Biology 10, 995–1008.

Ensminger I, Busch F, Humer N. 2006. Photostasis and cold acclimation: sensing low temperature through photosynthesis. Physiologia Plantarum 128, 28–44.

Ensminger I, Schmidt L, Lloyd J. 2008. Soil temperature and intermittent frost modulate the rate of recovery of photosynthesis in Scots pine under simulated spring conditions. New Phytologist 177, 428–442.

Fiellia I, Porcar-Castell A, Murné-Bosch S, Bäck J, Garbulsky M, Peñuelas J. 2009. PRI assessment of long-term changes in carotenoids/chlorophyll ratio and short-term changes in de-epoxidation state of the xanthophyll cycle. International Journal of Remote Sensing 30, 4443–4455.

Gamon JA, Peñuelas J, Field CB. 1992. A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. Remote Sensing of Environment 41, 35–44.

Gamon J, Serrano L, Sufans J. 1997. The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. Oecologia 112, 492–501.

Gamon JA, Berry JA. 2012. Facultative and constitutive pigment effects on the Photochemical Reflectance Index (PRI) in sun and shade conifer needles. Israel Journal of Plant Sciences 60, 85–95.

Garbulsky MF, Peñuelas J, Gamon J, Inoue Y, Fiellia I. 2011. The photochemical reflectance index (PRI) and the remote sensing of leaf, canopy and ecosystem radiation use efficiencies: a review and meta-analysis. Remote Sensing of Environment 115, 281–297.

Garrity SR, Eitel JUH, Verling LA. 2011. Disentangling the relationships between plant pigments and the photochemical reflectance index reveals a new approach for remote estimation of carotenoid content. Remote Sensing of Environment 115, 628–635.

Genty B, Briantais J-M, Baker NR. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990, 87–92.

Harris A, Gamon J, Pastorello G, Wong C. 2014. Retrieval of the photochemical reflectance index for assessing xanthophyll cycle activity: a comparison of near-surface optical sensors. Biogeosciences 11, 6277–6292.

Hendrickson L, Furbank R, Chow W. 2004. A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. Physiologia Plantarum 8, 73–81.

Hilker T, Coops N, Hall F, Black T, Wulder M, Nescic Z, Krishnan P. 2008. Separating physiologically and directionally induced changes in PRI using BRDF models. Remote Sensing of Environment 112, 2777–2788.

Hilker T, Hall F, Coops N, Lyapustin A, Wang Y, Nescic Z, Grant N, Black A, Wulder M, Klijn N. 2010. Remote sensing of photosynthetic light-use efficiency across two forested biomes: spatial scaling. Remote Sensing of Environment 114, 2863–2874.

Hrimina G, Merlier E, Dufrêne E, Soudani K. 2015. Deconvolution of pigment and physiologically related photochemical reflectance index variability at the canopy scale over an entire growing season. Plant, Cell and Environment (in press).
Horton P, Ruban A, Rees D, Pascal AA, Noctor G, Young AJ. 1991. Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCl chlorophyll–protein complex. FEBS Letters 292, 1–4.

Horton P, Wentworth M, Ruban A. 2005. Control of the light harvesting function of chloroplast membranes: the LHCl-aggregation model for non-photochemical quenching. FEBS Letters 579, 4201–4206.

Huang W, Zhang S-B, Cao K-F. 2010. Stimulation of cyclic electron flow during recovery after chilling-induced photoinhibition of PSI. Plant and Cell Physiology 51, 1922–1928.

Huang W, Yang S-J, Zhang S-B, Zhang J-L, Cao K-F. 2012. Cyclic electron flow plays an important role in photoprotection for the resurrection plant Paraboea rufescens under drought stress. Planta 235, 819–828.

Hüner N, Öquist G, Sarhan F. 1998. Energy balance and acclimation to light and cold. Trends in Plant Science 3, 224–230.

IPCC. 2014. Climate change 2014: impacts, adaptation, and vulnerability. Part B: Regional aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Barros VR, Field CB, Dokken DJ, Mastrandrea MD, Mach KJ, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, and White LL (eds.). Cambridge University Press: Cambridge, UK, and New York, USA.

Ivanov A, Sane P, Zeinalov Y, Malmberg G, Gardeström P, Huner N, Öquist G. 2001. Photosynthetic electron transport adjustments in overwintering Scots pine (Pinus sylvestris L.). Planta 213, 575–585.

Ivanov A, Sane P, Zeinalov Y, Simidjiev I, Huner N, Öquist G. 2002. Seasonal responses of photosynthetic electron transport in Scots pine (Pinus sylvestris L.) studied by thermoluminescence. Planta 215, 457–465.

Jahns P, Holsworth A. 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochimica et Biophysica Acta 1817, 182–193.

Johnson G. 2011. Physiology of PSI cyclic electron transport in higher plants. Biochimica et Biophysica Acta 1807, 384–389.

Klughammer C, Schreiber U. 1994. An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via P700*-absorbance changes at 830 nm. Planta 192, 261–268.

Kodama Y, Tsuboi H, Kagawa T, Wada M. 2008. Low temperature-induced chloroplast relocation mediated by a blue light receptor, phototropin 2, in fern gametophytes. Journal of Plant Research 4, 521–528.

Kováč D, Malenovsky Z, Urban O, Spunda V, Kalina J, Ac A, Kaplan V, Hanus J. 2013. Response of green reflectance continuum removal index to the xanthophyll de-epoxidation cycle in Norwegian spruce needles. Journal of Experimental Botany 64, 1817–1827.

Lukeš P, Steberle P, Rautiainen M, Möttus M, Vanhatalo K. 2013. Optical properties of leaves and needles for boreal tree species in Europe. Remote Sensing Letters 4, 667–676.

Lunde C, Jensen PE, Haldrup A, Knootzel J, Scheller HV. 2000. The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408, 613–615.

Matsubara S, Forster B, Waterman M, Robinson S, Pogson B, Gunning B, Osmond B. 2012. From ecophysiology to phenomics: some implications of photoprotection and shade–sun acclimation in situ for dynamics of thylakoids in vitro. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 3503–3514.

Murata N, Takahashi S, Nishiyama Y, Allahverdiev S. 2007. Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta–Bioenergetics 1767, 414–421.

Namkai T, Oguma H, Fujimura Y. 2006. Seasonal changes in the relationship between photochemical reflectance index and photosynthetic light use efficiency of Japanese larch needles. International Journal of Remote Sensing 27, 493–509.

Niyogi KK. 2000. Safety valves for photosynthesis. Current Opinion in Plant Biology 3, 455–460.

Niyogi K, Li X-P, Rosenberg V, Jung H-S. 2005. Is PsbS the site of non-photochemical quenching in photosynthesis? Journal of Experimental Botany 56, 375–382.

Öquist G, Huner NP. 2003. Photosynthesis of overwintering evergreen plants. Annual Review of Plant Biology 54, 329–355.

Otander C, Campbell D, Öquist G. 1995. Seasonal changes in photosystem II organisation and pigment composition in Pinus sylvestris. Pflanze 197, 176–183.

Penuelas J, Fillella I, Gaman J. 1995. Assessment of photosynthetic radiation-use efficiency with spectral reflectance. New Phytologist 131, 291–296.

Pfündel E, Klughammer C Schreiber U. 2008. Monitoring the effects of reduced PS II antenna size on quantum yields of photosystems I and II using the Dual-PAM-100 measuring system. PAM Application Notes 1, 21–24.

Piao S, Ciais P, Friedlindstein P, Peylin P, Reichstein M, Luyssaert S, Margolis H, Fang J, Barr A, Chen A. 2008. Net carbon dioxide losses of northern ecosystems in response to autumn warming. Nature 451, 49–52.

Porcar-Castells A, Garcia-Plazaola J, Nichol C, Kolar P, Olascoaga B, Kuusinen N, Fernández-Marin B, Pulkkinen M, Juurola E, Nikinmaa E. 2012. Physiology of the seasonal relationship between the photochemical reflectance index and photosynthetic light use efficiency. Oecologia 170, 313–323.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Ruban A, Johnson M, Duffy C. 2012. The protective molecular switch in the photosystem II antenna. Biochimica et Biophysica Acta–Bioenergetics 1817, 167–181.

Savitch L, Ivanov A, Kroli M, Sprott D, Öquist G, Huner N. 2010. Regulation of energy partitioning and alternative electron transport pathways during cold acclimation of lodgepole pine is oxygen dependent. Plant and Cell Physiology 51, 1555–1570.

Sims D, Gaman J. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sensing of Environment 81, 337–354.

Svobhnikov D, Ensminger I, Ivanov AG. 2006. Excitation energy partitioning and quenching during cold acclimation in Scots pine. Tree Physiology 26, 325–336.

Takahashi S, Badger M. 2011. Photoprotection in plants: a new light on photosystem II damage. Trends in Plant Science 16, 53–60.

Telfer A. 2005. Too much light? How β-carotene protects the photosystem II reaction centre. Photochemical & Photobiological Sciences 4, 950–956.

Trebast A. 2003. Function of beta-carotene and tocopherol in photosystem II. Zeitschrift für Naturforschung. C, Journal of Biosciences 58, 609–620.

Verhoeven A. 2013. Recovery kinetics of photochemical efficiency in winter stressed conifers: the effects of growth light environment, extent of winter stress and species. Physiologia Plantarum 147, 147–158.

Verhoeven A. 2014. Sustained energy dissipation in winter evergreens. New Phytologist 201, 57–65.

Verhoeven A, Osmolak A, Morales P, Crow J. 2009. Seasonal changes in abundance and phosphorylation status of photosynthetic proteins in eastern white pine and balsam fir. Tree Physiology 29, 361–374.

Wong CY, Gaman JA. 2015a. Three causes of variation in the photochemical reflectance index (PRI) in evergreen conifers. New Phytologist 206, 187–195.

Wong CY, Gaman JA. 2015b. The photochemical reflectance index provides an optical indicator of spring photosynthetic activation in evergreen conifers. New Phytologist 206, 196–208.

Zarter CR, Demming-Adams B, Ebbert V, Adamska I, Adams WW. 2006. Photosynthetic capacity and light harvesting efficiency during the winter-to-spring transition in subalpine conifers. New Phytologist 172, 283–292.

Zhang X, Gurney KR, Peylin P, Chevallier F, Law RM, Patra PK, Rayner PJ, Rödenbeck C, Krol M. 2013. On the variation of regional CO₂ exchange over temperate and boreal North America. Global Biogeochemical Cycles 27, 991–1000.