Kováč, Daniel; Veselá, Barbora; Klem, Karel; [et al.]. «Correction of PRI for carotenoid pigment pools improves photosynthesis estimation across different irradiance and temperature conditions». Remote sensing of environment, Vol. 244 (July 2020), art. 111834. DOI 10.1016/j.rse.2020.111834

Correction of PRI for carotenoid pigment pools improves photosynthesis estimation across different irradiance and temperature conditions

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Abstract

We studied the influence of changing carotenoid pigments on the sensitivity of the photochemical reflectance index (PRI) to photosynthesis dynamics. The goal of the measurements was to examine how the introduction of ΔPRI into the working dataset can improve the estimation of photosynthesis. Spectral and photosynthetic characteristics of European beech and Norway spruce saplings were periodically measured in growth chambers with an adjustable irradiance and temperature. Patterns of environmental changes inside the growth chambers were created by periodic changes in irradiance and temperature. Four general irradiance periods lasting 10-12 days each were established. Introduced irradiance regimes varied in the sum of daily irradiance and amplitude of irradiance changes. Temperature was changed with more complex patterns to induce changes in xanthophyll cycle pigments at various time scales within these regimes. Our measurements confirmed the PRI linkage to photosynthetic light use efficiency (LUE). However, the strength of this connection was found to be dependent on changing pigment concentrations, specifically on the change in the ratio of chlorophylls to carotenoids. Furthermore, a negative interference in photosynthesis estimation from PRI was recorded if the temperature was lowered overnight to 12° C. The differential PRI (ΔPRI), calculated as the simple difference between the PRI value measured during the daytime period and in early morning (PRI_0), revealed a decreased effect from pigments and cold temperature on LUE estimation. The regression analysis among all measured data identified an increased association between PRI and LUE following the introduction of ΔPRI from R^2 = 0.26 to 0.69 in beech and from R^2 = 0.61 to 0.77 in spruce data. The analyses showed that both leaf carotenoid concentrations and the conversion state of xanthophyll cycle pigments played a significant role in determining PRI and PRI_0 values and that the accurate assessment of these pigments in PRI across multiple levels of stress from irradiance and temperature might improve estimations of LUE through ΔPRI. In our data, ΔPRI appeared to be a good measure of photosynthesis, the dynamics of which differed between beech and spruce saplings upon switching temperatures.

KEYWORDS: photochemical reflectance index, proximal sensing, irradiance, temperature, carotenoids, xanthophyll cycle, light use efficiency

1 | INTRODUCTION

The usefulness of a photochemical reflectance index (PRI) approach to link plant photosynthetic CO_2 uptake efficiency in changing light (light use efficiency, LUE) and remotely sensed data has been widely reported (Garbulsky et al., 2008; Peñuelas et al., 2011). PRI is based on our understanding of the photoprotective role of xanthophyll cycle pigments within plants. When plants absorb more light energy than can be used by chlorophyll to produce glucose, excessive light energy is either transferred to xanthophyll molecules and emitted as heat or emitted as fluorescence (Gamon et al., 1992; Rascher et al., 2009). Variations in xanthophyll cycle pigment concentrations and conversions produce reflectance changes at a wavelength of 531 nm. Comparing this reflectance with a reference wavelength (typically 570 nm) can be used to detect stress (Gamon et al., 1997, 1992; Peñuelas et al., 1995). Examination of empirical work has determined a fundamental relationship between plant photochemistry and PRI that is based on the responsiveness of photosynthesis to irradiance.

The variability of factors that may affect canopy-measured PRI can produce confusion in interpreting PRI, in addition to the desirable effects introduced by changing pigments. In field applications, sun–canopy–sensor geometry can exert a strong effect on the resulting PRI values, as light fields may vary in complex ways with canopy structure (Middleton et al., 2009; Sims et al., 2006). Wu et al. (2015) examined crops and found the structural dependence of PRI on leaf area index (LAI). In addition, these authors proposed a solution for removal of the structural signal, and their results imply that LAI change as a consequence of a change in the illumination angle can impact the measured PRI of the canopy. The results reported by Wu et al. (2015) and Gitelson et al. (2017) suggest that canopy structure-related LAI interferences should be considered when evaluating PRI responses between canopies with lower LAI.

The foliar ratio of chlorophylls to carotenoids (Chl*a+b*/Car*x+c*) has a strong impact on the observed PRI variability (Filella et al., 2009). Decreasing Chl*a+b*/Car*x+c* may be related to photosynthesis downregulation in stressed plants, but the changes in pigments may also cause impairment of the PRI–LUE relationship over the long term. Attempts have been made to eliminate the Chl*a+b*/Car*x+c* variability in the measured signal by deconvoluting the influence of pigments. Gamon and Surfus (1999) have shown an opportunity to detract the extent of xanthophyll cycle pigment conversions by subtracting measured PRI value from the starting PRI value in introduced ΔPRI ($\Delta PRI = PRI_0 - PRI$). Following this work, Gamon and Berry (2012) characterized differences in ΔPRI between sun and shade leaves as induced with pigment changes. This study has shown that PRI_0 represents a PRI state to which PRI values can be related if the comparison of diurnal changes and plant groups is required. Ideally, PRI_0 is measured at low irradiance on leaves with inactivated protective functions to isolate the slow changing component of PRI most likely related to Chl*a+b*/Car*x+c* and the concentration of lutein pigments. Magney et al. (2016) determined that the ΔPRI does respond to diurnal physiological changes resulting from changes in VPD, air temperature, and stomatal conductance and suggested that these changes may be dependent on the observed pigment dynamics. Nitrogen availability affecting amounts of chlorophylls was considered a primary driver of ΔPRI sensitivity in this study, defining the slope of the observed dependency. ΔPRI yields better correlations in nutrient-deficient plots, thereby indicating the importance of carotenoid levels in observed relationships. There are several good examples showing the opportunity to better estimate LUE from PRI measured at the top of the canopy, if the value of PRI is corrected to the morning PRI_0 (Hmimina et al., 2015, 2014; Ripullone et al., 2011; Soudani et al., 2014). The presented studies often involve drought stress in the experimental design as the main factor driving photosynthesis declines and the magnitude of PRI response (Magney et al., 2016). We intend to further study the basics of the improved relationship between photosynthetic LUE and ΔPRI, and the desirable role of changing pigments in developing these relationships under conditions involving induced changes in irradiance and temperature.

Differences in the sensitivity of photosynthetic processes to light and temperature stress among species suggest the occurrence of interactive effects on photosynthetic pigments. An insufficient understanding of the associated reflectance signature in relation to the measure of photosynthesis is consequential (Ollinger, 2011; Sims and Gamon, 2002). The co-variation of environmental drivers in the upper canopy suggests that leaves in the upper canopy are often exposed to greater stress originating from exposure of leaves to direct light (Niinemets and Valladares, 2004). Upper canopy leaves may suffer from a variety of additional stresses, among which temperature stress may play a particularly substantial role (Williams et al., 1996). The upper canopy crown, usually accounted for in spectrometric measurements, may represent a substantial portion of the canopy involved in the observed gas exchange (Coops et al., 2017). However, the functional trait attributes of plants may not be the only features able to explain changes in PRI in a given environment. It may also be necessary to account for the PRI variations with changing Chl*a+b*/Car*x+c* that determines the constitutive (slow-changing) component of the PRI. It has been suggested that the finer definition of the facultative process (fast-changing with xanthophyll cycle) can help to detect LUE and responses to summer stress, such as heat and drought (Gamon and Bond, 2013). Our previous development provided elementary knowledge of PRI changes in relation to dynamic fluctuations of photosynthetically active radiation (PAR) and temperature (Kováč et al., 2018). This study suggests large improvement in estimating LUE from ΔPRI, with measurements of PRI_0 that correspond to the xanthophyll cycle pigment conversion state in the dark. Changes in PRI–LUE and ΔPRI–LUE relationships have yet to be investigated with dynamic irradiance increases and temperature changes occurring on a daily scale.

We aimed to study further the sensitivity of both PRI and ΔPRI to decreasing and increasing photosynthesis in fluctuating environments. In this study, we focused on observations of the effect of mutual interactions among the plant pigments and overall canopy LAI on the developing relationship between PRI and LUE in changing temperature conditions. Understanding the aspects of LUE estimations using PRI would improve the applicability of PRI measurements in remote-sensing applications. We aimed to overcome the canopy structural effect on measured PRI by fixing the illumination–observation setup of the measurement by measuring canopies within the closed environment of a growth chamber. We thus compared responses in photosynthesis, pigments and PRI of tree species with distinctive sensitivities to low and high temperatures. Norway spruce trees, which prefer cold regions and higher altitudes, were compared with European beech trees, which show tolerance to higher temperatures and grow at lower altitudes. We established four regimes that varied in their daily sum of irradiance income even as the trees were undergoing similar periodic changes in temperature on a daily scale. We measured how the changes in pigments within these regimes affected the observed PRI–LUE relationship and examined the role of established pigment concentrations in the developing sensitivity of PRI and ΔPRI to LUE.

2 | MATERIALS AND METHODS

2.1 | Setup of the experiment

For the study, we selected two tree species with different habitat preferences: European beech *(Fagus sylvatica)* and Norway spruce *(Picea abies)*. These species are widespread within the Czech Republic and the Central European region, with a distribution generally occurring according to their contrasting climate and temperature demands. Seedlings of the selected tree species aged 3–4 years old (0.5–0.6 m tall) were grown in pots in the garden of the Global Change Research Institute from early spring until the new leaves or needles were well developed. Thereafter, the trees were moved to the experimental setting of growth chambers FS-SI-4600 (Photon Systems Instruments, Drásov, Czech Republic), where they were processed for reflectance data acquisition, foliar photosynthetic pigments estimation and photosynthesis measurements under changing conditions of irradiance (PAR) and temperature. The initial acclimation period lasting 1 week (photoperiod 14 hours, irradiance 100 µmol m^{-2} s^{-1}, day/night temperatures set to 23/18°C and relative air humidity of 65%/80%) was followed with individual regimes that differed in the sum of daily irradiance income, daily amplitude of irradiance changes and temperature dynamics. Four periods of 10–12 days were established that varied in daily irradiance amplitudes of 300–600–1200 (IRR1), 600–900–1500 (IRR2), 900–1200–1500 (IRR3), and 300–600–900 (IRR4) µmol m^{-2} s^{-1}. Detailed descriptions of irradiance and temperature changes according to time of day within each regime are provided in the attached research data. The second day of each irradiance period IRR1–IRR4 was established as a control day to measure plant responses to what can be termed "standard" temperature changes following the irradiance fluctuations. The last 2 days of each irradiance period were devoted to measuring parameters in fluctuating temperatures. The first day of this 2-day measurement period started with the same temperature as on the previous day. The temperature was then experimentally lowered at midday to 16 °C. This decreasing temperature was started after the trees had been heated for half an hour during the midday period of highest PAR. The temperature decrease to 16 °C was gradual over a period of 2 h (12:00 to 14:00). Temperature was lowered compared to previous days as well as during the dark (night) period to 12°C. Cold period temperatures remained the same for each irradiance regime. Heating after a cold period was started during the morning of the following day. During that following day, the temperature was increased compared to the previous control days while the irradiance level remained unchanged. Ranges of the temperature change on normal days of IRR1–IRR4 were 20–26° C, 24–30° C, 25–35° C, and 19–24° C.

In the morning of each measuring day before the lights in the growth chambers were switched on, the leaves and needles of beech and spruce saplings were collected for the estimation of foliar photosynthetic pigments (Chl*a+b*, Car*x+c*) and individual carotenoid concentrations (antheraxanthin

(A), violaxanthin (V), zeaxanthin (Z)). Concentrations of chlorophylls and carotenoids were estimated spectrophotometrically using a Specord 500 spectrophotometer (Analytik Jena, Jena, Germany). Pigment concentrations in pigment extracts have been estimated from absorbance curves using Lichtenthaler equations (1987). Amounts of xanthophyll cycle pigments were estimated by the HPLC method (Kurasová et al., 2003), and the conversion state of the xanthophyll cycle pigments (i.e., de-epoxidation state, or DEPS) was calculated according to Gilmore and Björkman (1994) as DEPS = (A + Z) / (V + A + Z). The maximum quantum yield of photosystem II photochemistry (Fv/Fm) was estimated from measurements using a PAM-2500 portable chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany) for dark adapted leaves. The Fv/Fm was calculated as Fv/Fm = (Fm − F_0)/Fm, in which F_0 and Fm represent the minimum (F_0) and maximum (Fm) chlorophyll fluorescence of darkness-adapted leaves.

Reflectance factors of canopies created by four saplings of either beech (in July-August) or spruce (September-October) species were measured in the growth chambers using a custom-made system for measuring reflectance that is based on two JAZ spectrometers (Ocean Optics, Dunedin, FL, USA). The spectrometric system is used for measurements in the dual field of view configuration. The trees that were measured with a spectrometer were not moved throughout the measurement period. Each JAZ unit measures data in spectral range between 340 and 1025 nm in 2048 channels with a spectral resolution (FWHM) of 1 nm for each channel. The reflectance estimation was based on comparisons between the radiant flux reflected from the measured canopy and incident (reference) irradiance at each measuring wavelength similar to Gamon et al. (2015). Optimization of the measurement system for measurements in growth chambers was performed using a spectralon panel reflecting a down-welling radiant flux at PAR of 1500 µmol m^{-2} s^{-1}. In addition, the radiance data estimation accounts for the optimization of the signal measured using each unit to integration time to strengthen the signal-to-noise ratio, conversion of the measured digital photon count signal to radiometric units, and dark current spectrum correction of this signal. All the supporting information for the calculations of reflectance factors from the measured data using the current establishment of the spectrometer, optical fibres (2-meter-long QP600-UV-VIS optical fibres), and cosine reduction (CC-3-UV-T) was estimated prior to initiating the measurements. The correction data were loaded into the measuring system (on a desktop computer attached to the spectrometer), which processed the measured data automatically while saving as text files the basic outputs from each data measurement. The spectrometric method used for measuring reflectance factors is described in greater detail by Kováč et al. (2018). The system setup ensured automatic data acquisition, starting in the morning and continuing at a 1-minute frequency throughout the day. The computer connected to the spectrometers via the USB port controlled simultaneous measurements by both spectrometers. The LED Fyto-Panels (Photon Systems Instruments) that create the light environment inside the growth chambers (Fig. 1) also served as the light source for measuring canopy reflectance factors. The linear change in the light intensity produced by the Fyto-Panels enabled the measurement of canopy reflectance factors without impacting the illumination directions and shadow fractions of vegetation while changing the irradiance. As a result, we could attribute the variability in reflectance to pigment changes in the measured leaves rather than to changes in canopy shadows as a consequence of changing illumination angles. The sampled area covering four tree crowns was ca. 44 cm in diameter, which was provided by the optical-fibre tip's 23° field of view and the 1-metre distance between the trees and measuring optics. Examples of measured reflectance curves are shown in Fig. 1. PRI was calculated following Gamon et al. (1997) as PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$ from reflectance at wavelengths of 530.37, 530.73, 531.08, 531.44, 531.79 nm (average used to estimate the value of R_{531}), and 570.31, 570.67, and 571 nm (R_{570}). Development of PRI during the day period on control days within each IRR regime is shown in Fig. 2. The average of the PRI data in the final 5 minutes of the initial low-PAR period (under 100 µmol m^{-2} s^{-1}) was taken as a PRI_0 value because a small rise in PRI was always observed in response to activating the photosynthesis functions in the morning. Based on the PRI_0 value, a differential PRI was later calculated as $\Delta PRI = PRI_0 - PRI$ (Gamon and Berry, 2012) for each PRI acquisition during the day.

The main focus of the gas-exchange measurement was to estimate the actual photosynthesis rate (*A*), stomatal conductance (*Gs*), and transpiration (*Tr*). Measurements were collected five times per day at PAR set at 300, 600, 900, 1200, or 1500 μmol m^{-2} s^{-1} and at temperatures that were adjusted accordingly. A portable photosynthesis system LI-6400 XT (LI-COR Biosciences, Lincoln, NE, USA) was used to measure the photosynthetic dynamics of leaves in the upper crown. Leaves were measured inside an LI-6400-02B leaf assimilation chamber. The light use efficiency (LUE) was calculated as LUE = *A*/PAR. Within each IRR regime, gas exchange and chlorophyll fluorescence were initially measured on the second day, referred to as the control day, and later on two consecutive days with induced cooling of trees towards the end of each irradiance regime. The actual quantum yield of photosystem II photochemistry (Φ_{PSII}) was calculated from PAM-2500 estimated values of steady state fluorescence (*F*s) and light-adapted maximum fluorescence (*F*m′) as Φ_{PSII} = (*F*m′ − *F*s)/*F*m′ (Genty et al., 1989) for each gas exchange data acquisition. Similarly, non-photochemical fluorescence quenching (NPQ) was calculated as NPQ = (*F*m − *F*m′)/*F*m′. Photochemical fluorescence quenching (qP) was calculated as qP = (*F*m′ − *F*s)/(*F*m′ − F_0′) from *F*s, *F*m′ and light-adapted minimum fluorescence (F_0′). A database was created of spectrometric and physiology data measured under irradiances ranging from 300–1500 μmol m^{-2} s^{-1} and temperature ranging from 14–35° C. The core data are available online.

2.2 | Data analysis

All analyses of the collected data were performed using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). To visualize and quantitatively summarize the multivariate covariation of optical variables (PRI and ΔPRI), major vectors of environmental factors, leaf pigment concentrations, and physiology measures, we performed a principal component analysis (PCA). Principal component 1 and 2 scores were plotted in a PCA biplot. PCA analysis and visualization were conducted using the R “factoextra” and “ggplot2” packages. The data for the individual parameters were tested for normality using the Kolmogorov–Smirnov test. Tukey’s HSD post hoc ($p < 0.05$) multiple range test was performed using the HSD.test function in the “agricolae” package to evaluate differences between individual treatments. Data for the Tukey analyses are displayed in bar plot figures. A logarithmic regression model was used to study the relationships among PRI, ΔPRI, and LUE at probability levels $p < 0.05$, $p < 0.01$, and $p < 0.001$. Scores of regression analyses among the data within the individual IRR regimes and between all data are shown in Table 1, and fitted logarithmic equations for pairs of data are shown in Table 2. Dependency graphs between these key variables are displayed in Fig. 8.

3 | RESULTS

3.1 | Relationships among environmental, optical, and physiological variables

Principal component 1 (PC1) explained 37.6% of the total variation in European beech and 38.9% of the total variation in Norway spruce data (Fig. 3). The functional traits responded significantly to PAR. PAR determines the positions of individual values on the variable factor map, with individual data points measured at low irradiances situated towards the bottom, negative part of the axis, whereas data measured under high irradiances (1500 µmol m^{-2} s^{-1}) are situated towards the opposite end of the graph. Even though trait responses to PAR showed general trends that were common to both species, responses for both species were positioned on a gradient ranging from those that did not respond significantly (with a closer link to PC2) to those that displayed high PAR trait plasticity. The analysis showed that PAR differences were a common factor in determining the distribution of Φ_{PSII}, qP, LUE, and ΔPRI on the variable factor map. High PAR values produce low photochemical quenching and LUE values and extremely negative ΔPRI values. The impact of PAR on values of PRI and NPQ was still significant, but the relationship among PAR, PRI, and NPQ was weaker than expected (calculated from research data). PRI was the only variable with a link to PC1, which showed a strong correlation to Chl*a+b*/Car*x+c* belonging to PC2 with a coefficient of correlation (*R*) equal to 0.89 ($p < 0.001$) in the beech data set and 0.58 ($p < 0.001$) in the spruce data (calculated from research data). The lower LAI of the beech canopy produced deeper amplitudes of PRI (Fig. 2) and resulted in a higher correlation between Chl*a+b*/Car*x+c* and PRI.

Principal component 2 (PC2) explained 23.8% of the total PCA variation in European beech and 25.2% of that in Norway spruce data (Fig. 3). The main factors *A*, *Tr*, and *Gs* were placed in the lower and upper quadrants of the PCA biplots in beech and spruce, respectively staying in contrast to the position of temperature vector. This difference in directionality of *A*, *Tr* and *Gs* vectors is likely associated with species specific sensitivity of photosynthesis to temperature variations. The opposite temperature effects on the regulation of photosynthesis were reflected also in the directionality of the adjacent Chl*a+b*/Car*x+c* and PRI vectors.

3.2 | Factors affecting the development of PRI and ΔPRI

The two principal components in the PCA plots comparing early morning (before 8:00) values of selected parameters to midday values (Fig. 4) explained approximately 64% of variability in the tested data. The dependencies in the graphs could be rotated according to significance of predictors, but the basic relationships among the measured data were similar between the species. Key relationships among midday values of PAR, Φ_{PSII}, LUE and ΔPRI were similar to those identified among all the data in Fig. 3. NPQ showed a link to midday PRI, which was itself correlated with the Chl*a+b*/Car*x+c* (including VAZ/Chl*a+b*) ratio. The PRI showed a progressive decline (Fig. 7) that corresponded rather to a decreasing Chl*a+b*/Car*x+c* (Fig. 6). The third set of correlations in these PCA graphs was formed by variable vectors of *A*, predawn DEPS and *F*v/*F*m. These connections placed *F*v/*F*m and predawn DEPS into the role of photosynthesis predictor.

Chl*a+b*/Car*x+c* plays an important role in determining both PRI_0 and midday PRI values. However, sharp differences in predawn DEPS (Fig. 6) with a significant correlation with differences in ΔPRI suggested that xanthophyll cycle pigment de-epoxidation was very important in the development of ΔPRI. Among those factors affecting ΔPRI development, PAR was found to be a very important factor that affects the magnitude of the response (Fig. 4). Although PRI_0 showed a weaker association with morning DEPS (Fig. 4), the correlation between PRI_0 and predawn DEPS was nevertheless significant (research data), thus confirming the importance of xanthophyll cycle pigments in the observed trends. Shifts in the PRI_0–DEPS relationship might be influenced by Chl*a+b*/Car*x+c* modulating PRI value.

3.3 | PRI dynamics that are consequential to photosynthesis

Differences in the regulation of photosynthesis with temperature are most apparent from comparisons between photosynthesis data that are measured midday (Fig. 5). Based on the observed differences, relationships were developed, as shown in Fig.4. The PRI response was to a certain extent sensitive to dynamic day-to-day changes in photosynthesis induced by temperature (Fig. 7), thus indicating the utility of PRI for estimating dynamics of photosynthesis in fluctuating temperatures. However, sensitivity to temperature-induced changes in photosynthesis did not occur within irradiance regimes IRR1 and IRR2 with progressive declines in Chl*a+b*/Car*x+c* (Fig. 6). The decreasing PRI in beech during cold periods (second measuring days) in the IRR regimes (Fig. 7) corresponded to declining photosynthesis under high-light and low-temperature conditions (Fig. 5). The further decrease in PRI on the following day that was in contrast to the rising A might be related first to the increased predawn DEPS and diminished PRI_0 under low-temperature conditions, consequently also resulting in a lower PRI at midday (Figs. 6 and 7). The observed trend of progressively decreasing PRI with Chl*a+b*/Car*x+c* was also observed for the spruce data set. Based on these observations we assume that the decreasing PRI with Chl*a+b*/Car*x+c* and cold stress was a limiting factor in enhancing the PRI response to LUE.

In contrast, ΔPRI showed an increase and a lower magnitude in spruce that were consistent with the rise in photosynthesis upon the reduced midday temperature on measurement day 2 for each IRR regime (Fig. 7). This sensitivity was the result of the increasing PRI values in spruce (Fig. 7), whereas a less pronounced increase in PRI was observed in beech that was consistent with the reduced photosynthesis, as shown in Fig. 9. This opposite reaction in ΔPRI in beech and spruce observed in the final two measurement days for each IRR period suggested an overall increased sensitivity of ΔPRI to photosynthesis (Figs. 5 and 7).

The inconclusive results of first-day measurements for each IRR regime might indicate possible minor limitations of the applicability of ΔPRI. The low ΔPRI consistent with the high Chl*a+b*/Car*x+c* on the initial day of IRR1 (Fig. 6) disrupted the observed ΔPRI–LUE dependency in the spruce data. The high ΔPRI amplitude in beech during the first measurement days for IRR2 and IRR3 resulted from a sudden PAR increase. Closer connections between photosynthesis and ΔPRI were observed towards the end of the measurements as a consequence of the diminishing Chl*a+b*/Car*x+c*. In addition to the observed general trends, we should highlight the lowered ΔPRI in response to the higher LUE and *A* under later, low-irradiance conditions of IRR4 that greatly strengthened the overall sensitivity of ΔPRI to LUE (Figs. 5 and 7). This observation thus favours ΔPRI for estimating photosynthesis responses.

3.4 | Role of xanthophyll cycle pigments in the sensitivity of ΔPRI to LUE

Predawn DEPS was increased under conditions of higher irradiance stress and coincided with the decreasing Chl*a+b*/Car*x+c* ratio and imposition of stress due to low night temperature (Figs. 4 and 6). The dynamics of this response occurring on a day-to-day basis showed the rapid response of xanthophyll cycle pigments to stress conditions. Nevertheless, DEPS value established during night period is considered an important input requirement for accurately determining PRI_0. Consequently precisely estimated ΔPRI enables to differentiate between unstressed and stress conditions.

Predawn DEPS in spruce needles on the second day of the measurements within each IRR, which marked the beginning of the period of dynamic temperature change, was lowered compared with the values in beech and to the predawn DEPS in spruce on the first control day of the high-irradiance regimes (Fig. 6). This phenomenon might be attributed to higher LAI of spruce trees, thereby providing them with a greater capacity to manage excessive light in a given environment. The differences in predawn DEPS were equally matched in PRI_0 values (Fig. 7). A small difference between PRI and PRI_0 thus indicated increased photosynthesis rates in cold temperatures under conditions of high light (Fig. 5). By contrast, cold night temperatures produced a high predawn DEPS, which in combination with a high midday drop in PRI under high temperatures resulted in a large ΔPRI, which is consistent with a drop in photosynthesis in spruce species. The reverse dynamics in pigments and PRI were observed between these two days in beech (Fig. 7), concurrent with the photosynthesis dynamics (Fig. 5).

3.5 | Regression analyses of PRI and ΔPRI relationships to LUE

Among the observed relationships, and considering the stratifications between irradiance regimes (IRR1–IRR4), the strongest relationships between PRI and LUE were observed in the dynamic light environments IRR1, IRR2, and IRR4 with a changing light intensity from low to high (Table 1). The R^2 values for the relationship between LUE and PRI under these conditions were often are as large as 0.8 (Table 1). The overall relationship between LUE and PRI was impacted by the decreasing Chl*a+b*/Car*x+c*, with a greater effect observed in the beech data set. By introducing ΔPRI, the overall R^2 for the assessment of LUE was raised from R^2 = 0.26 to 0.69 in beech and from R^2 = 0.61 to 0.77 in spruce (Fig. 8). ΔPRI failed to improve the LUE assessment in the highest irradiance period, with irradiances between 900 and 1500 μmol m^{-2} s^{-1} (IRR3, Table 1). High carotenoid levels served as a basis for the effective assessment of LUE from ΔPRI in the following regime of low irradiance (IRR4) with imposed low stress from irradiances in the 300–900 μmol m^{-2} s^{-1} range. The accuracy of the LUE assessment using ΔPRI in this period rose by 0.1 to R^2 = 0.91 and R^2 = 0.77 in the beech and spruce data sets, respectively.

4 | DISCUSSION

The advantage of using ΔPRI over applying simply measured PRI for the purpose of extracting LUE has been examined in this study. The presented work confirmed that the de-epoxidation cycle of leaf pigments involved in NPQ has a general effect of decreasing the reflectance magnitude at wavelengths around 531 nm (Gamon et al., 1990) with increasing light. The dynamics of PRI in our experimental regimes of heating and cooling furthermore indicated that PRI shows an ability to track photosynthesis changes in environments of fluctuating temperature. According to the measured data, the PRI-LUE connection may be consequential to the connection between LUE and ΦPSII, as has been previously suggested (Nichol et al., 2006; Rahimzadeh-Bajgiran et al., 2012). Although the relationship of PRI to photosynthesis beyond the ΦPSII-PRI connection may be constrained by factors related to excessive energy-consuming processes that are not involved in either xanthophyll cycle or carbon assimilation, such as photorespiration (altered Mehler reaction and nitrate reduction) or cyclic electron transport of photosystem I (PSI) (Fréchette et al., 2015; Magney et al., 2017; Porcar-Castell et al., 2008), our measurements confirm the negative interference from the slowly changing Chl*a+b*/Car*x+c* ratio on photosynthesis estimation using PRI. The dependency of PRI on Chl*a+b*/Car*x+c* deteriorates the relationship between PRI and LUE over the season, as previously shown by many authors (Fréchette et al., 2016; Hmimina et al., 2014; Sims and Gamon, 2002). The R^2 for the PRI–LUE relationship from various plant functional types is often reported to be below 0.60, as reviewed by Garbulsky et al. (2011). We were able to reach this level of connection between PRI and LUE in the spruce data set; a lower R^2 of 0.26 in this relationship was estimated in beech data set, which we consider to be a consequence of the lower canopy LAI of the selected beech trees resulting in higher PRI amplitudes, as similar changes in Chl*a+b*/Car*x+c* have been estimated between species (Fig. 6).

There are many examples in the literature showing a negative interference from Chl*a+b*/Car*x+c* in LUE estimation, and several studies have suggested tactics to minimize the interfering effect of Chl*a+b*/Car*x+c*. The correction procedure may also be dependent on the application type. A detailed analysis of measurements at changing observation angles and changing sun illumination angles at grown canopy sites demonstrated the necessity to address dynamic reflectance changes in the canopy structure and differences in the Chl*a+b*/Car*x+c* ratio between sunlit and shaded canopy portions (Hilker et al., 2008). This research addressed the necessity to create a deconvoluting algorithm to estimate LUE at each unique ecosystem station by taking into account only those PRI measurements taken at certain measurement geometries from unattended spectral systems (Hilker et al., 2010). In the improved approach to estimate LUE that resulted from this work, the Chl*a+b*/Car*x+c* ratio has become regarded as an important variable indicative of canopy stress that must be accounted for – and corrected – when interpreting PRI data. A group of researchers is proposing to deconvolute xanthophyll-related signals by applying transformation via continuum removal of the reflectance signature within the 500- to 600-nm range (Kováč et al., 2013, 2012; Woodgate et al., 2019) to minimize the impact of Chl*a+b*/Car*x+c* on the measurement outputs. Hernández-Clemente et al. (2011) used the 512-nm band as a reference when estimating DEPS and basic physiology measures from airborne data over boreal forests. The strong normalization effect of red-edge bands around 680 nm for evaluating the variability of the "light-exposed" PRI has been reported in airborne data applications (Zarco-Tejada et al., 2013) and when processing satellite images (Drolet et al., 2008). Ongoing research continues to show that deconvoluting the early morning (predawn) state of pigments can be used to reduce the impact of pigment changes on measurement outputs in applications designed to measure plant stress and hence LUE (Hmimina et al., 2015; Liu et al., 2013; Ripullone et al., 2011).

A single PRI (PRI and PRI_0) value taken during the day is sensitive to bulk changes in Chl*a+b*/Car*x+c* throughout the season (Garrity et al., 2011). Continuous lowering of Chl*a+b*/Car*x+c* has been observed in our data within each irradiance period IRR1–IRR4 (Fig. 6), thereby producing variability in PRI (Fig. 7). This drop in PRI has been partly helpful in determining the reduction in photosynthesis during regimes IRR1–IRR3, as the decreasing LUE is associated with increasing irradiance stress, as also reported by Peñuelas et al. (1995). We recognized a large interference from Chl*a+b*/Car*x+c* when data from low-irradiance IRR4 were included in the overall data analysis. This interference in LUE estimation was removed with ΔPRI. Within the individual regimes, the relationship between PRI and LUE was dependent on the observed change in xanthophyll cycle pigments following changes in irradiance during the daytime period (Fig. 8, Table 1). The highest LUE-PRI connection has been achieved with induced daily low to high PRI changes under conditions simulating irradiance increases from low to high intensity. These conditions are similar to those used by Gamon and Surfus (1999) in their pioneering study examining ΔPRI changes with DEPS. The observed trends suggest that Chl*a+b*/Car*x+c* may be unambiguous in data across longer periods if the constitutive pigment impact on PRI is efficiently discarded. Estimations of pigments at different time scales may be indirectly indicative of plant stress because leaf optical properties are directly impacted by the pigment composition (Peñuelas and Fillela, 1998; Wong and Gamon, 2015).

Deconvolution of PRI into slow-changing and fast-changing components according to Gamon and Berry (2012), assuming that the “dark” component of PRI (PRI_0) is also changing between days with night retention of de-epoxided xanthophyll cycle forms (Kováč et al., 2018), narrows the LUE estimation from PRI. It should be noted that our PRI measurements were based on spectral sampling at nadir observations and under constant light fields so that the directional effects associated with changes in illumination angles (Hernandez-Clemente et al., 2016) were small. This approach helped to narrow the external factors that impact PRI, but even after removing structural effects from LAI, the PRI–LUE correlation was still not strong. The ΔPRI improved the sensitivity of the measured signal to LUE across multiple conditions. The variations in LUE-PRI and LUE-ΔPRI relationships were evaluated upon observing temporal dynamics in species, yielding contrasting sensitivity to temperature changes (Fig. 5). The correction procedure unravelled the effects of Chl*a+b*/Car*x+c* and cold temperature on LUE estimation, two main factors among three common factors impacting PRI as shown by Wong and Gamon (2015). Although the spectral shift due to cold temperature was first reported as an albedo shift related to changes in the physical properties of leaves in very cold temperatures (Wong and Gamon, 2015), we detected low temperature interference on the LUE estimation from PRI at 12° C. The shift was apparent by the decreased PRI_0 at 12° C and decreased PRI measured on the days following the application of cold stress (Fig. 7). In the context of our measurements, the shift appears to be only partly related to the high DEPS.

A predictive pattern of decreasing PRI towards midday was typically observed in our testing data set when the temperature increase followed the PAR increase (Fig. 2). By comparing dynamic PRI changes in response to experimental temperature lowering (Fig. 9), we endeavoured to identify the change in PRI in response to the increased photosynthetic efficiency in spruce and to low temperature stress in beech trees. It is well known that the observed PRI dynamic changes within a matter of minutes to hours result from the synergy between xanthophyll cycle pigment conversions and management of the energetic carriers ATP and NADPH under changing conditions (Nichol et al., 2000), thereby producing signal variation with photosynthesis efficiency. We detected an increase in PRI in spruce in response to enhanced photosynthesis reactions (Fig. 9). As a consequence of enhancing photo protection in beech, a less-pronounced increase in PRI was measured (Fig. 9), resulting in a decreased PRI compared with the photosynthetically more efficient spruce. Similar PRI responses to the photosynthesis changes induced in the diurnal frame have been reported by Gamon et al. (1992), who examined differences in PRI between control, nutrient-stressed and water-stressed sunflower leaves. In our experiment, feedback between PRI and photosynthetic reactions was detected, which greatly improved the accuracy of ΔPRI in tracking dynamic photosynthetic changes with induced temperature variations.

However, the studies of ΔPRI do not involve variations in this signal caused by the canopy structure, which may render leaf traits difficult to discern (Knyazikhin et al., 2012). Magney et al. (2016) postulated that ΔPRI affords limited improvements in decoupling the structural (LAI) effect from the raw PRI signal, and thereby, PRI_0 is primarily sensitive to variability in pigment content and should not be used to correct for changes in LAI. For efficient disentangling of the desirable effect in ΔPRI to match its value with photosynthesis, an accurate assessment of PRI_0 that is consistent with the dynamics in DEPS was required (Figs. 6 and 7). Particularly, PRI_0 estimation on the second measurement days for each irradiance regime is considered a crucial factor for development of the observed LUE-ΔPRI. However, examining aspects of PRI_0 and ΔPRI estimation on these days revealed importance of LAI in the observed relationships, resulting from determinations of sapling capacity to manage the available light (Ellsworth and Reich, 1993). Assessment of the PRI_0 signal is based on the dependency of PRI on the de-epoxidation state of xanthophyll cycle pigments (Gamon et al., 1990), and night-time levels of DEPS are often influenced by temperature and induced stress (Demmig-Adams and Adams, 1996; Gilmore and Björkman, 1995). The causality of the relationship between ΔPRI and LUE appears to be a consequence of the differences in canopies LAI between species and levels of imposed stress. The results highlight the importance of estimating PRI_0 that matches DEPS and a highly complex nature of the mechanisms involved in remote assessments of photosynthesis change, underscoring the need for very precise data.

By concept, ΔPRI appears to be a suitable tool for observing the dynamics of changes within small areas of vegetated surfaces throughout day periods to monitor short-term variations in leaf pigments. Further assuming the field application of presented ΔPRI concept, difficulties may arise when isolating PRI_0 in early morning field reflectance measurements. The errors in estimation may originate from the cosine response of the diffusers used and from the relative variation in the measured signal of the total down-welling radiance flux. Variations in measured incoming radiation flux are caused by deviations in the measurement of direct and diffuse components of the down-welling flux passing through the diffusers (Pacheco-Labrador et al., 2019). The fraction of diffuse irradiance is usually low in remote-sensing applications if the cloudy data are discarded, but the contribution of diffuse irradiance can be large in the case of automated proximal sensing comparing data on a large temporal scale and including measurements acquired at low and high sun elevations (Gamon et al., 2015; Pacheco-Labrador and Martín, 2015). Estimating PRI_0 and PRI from reflectance data may be challenging due to the unknown directional distribution of the diffuse irradiance. Most applications have been set up for PRI_0 from measurements that have occurred in a period around 9 a.m. when the solar zenith is around or greater than 40° (Liu et al., 2013; Magney et al., 2016) and the sun is potentially yielding a higher intensity. This phenomenon may be considered a consequence of the observed cosine response in the measured data. Improvements in instrumentation for the measurements may also be required to reproduce data with higher standards.

The results highlight a great diversity of parameters to which PRI measurements may refer in beech and spruce forests. They also show that PRI is highly comparable to indicators based on relative units such as Φ_{PSII}, LUE, or the Chl*a+b*/Car*x+c* ratio, and that assessment of LUE can be improved with the use of ΔPRI, which uses the balance between PRI and PRI_0. The involvement of protection mechanisms that are not involved in any of the NPQ mechanisms may be a reason for the weakened correlation between ΔPRI and NPQ, but the weaker PRI–NPQ connection is often a result of the Chl*a+b*/Car*x+c* adjustments to light conditions (Stylinski et al., 2002). It can be presumed that the strong association between variables is a result of applying artificial light, which minimizes fluctuations of PRI, Φ_{PSII}, NPQ, and LUE in the data set, as reported by Sukhov and Sukhova (2018). For the practical problem of field remote sensing, the results show that the application of PRI can be effective for investigating photosynthesis in dynamic irradiance and temperature regimes if the pigments are sufficiently disentangled in the signal. The presented work may serve as premise for data processing if interfering factors in spectral measurements in the field are efficiently reduced. However, measurements of control plants that have not been affected by stressors are often necessary to establish the dependency of optic parameters on observed factors (Damm et al., 2018; Sukhova and Sukhov, 2018).

CONCLUSION

The results showed that photosynthesis estimation using PRI over periods longer than days may be limited by changing dynamics of the foliar Chl*a+b*/Car*x+c* ratio with stress from irradiance. The extent of the Chl*a+b*/Car*x+c* limitation in our measurements was dependent on the leaf area index of the measured trees; we recorded greater interference from Chl*a+b*/Car*x+c* in beech seedlings with a lower LAI. An improved assessment of dynamic changes in photosynthetic activity induced with changes in irradiance and temperature was achieved using ΔPRI. The measurements also showed the importance of foliage and environmental factors for the observed dynamics in PRI. The estimation of ΔPRI was based on measuring PRI_0 under low irradiance. Temperature was considered a factor that induces changes in xanthophyll cycle pigment DEPS observed in PRI. The improved approach as proposed by introducing ΔPRI may deconvolute part of the interference in the PRI signal. The limitations of the ΔPRI may be related to changes in this signal with sudden increases in irradiance when evaluating canopies with a lower LAI. Low foliar amounts of xanthophyll cycle pigments may render a low amplitude of ΔPRI, which was a minor limiting factor when evaluating our spruce dataset. Conversely, on days starting with low temperature, ΔPRI was effective in disentangling the negative effect caused by low temperature on the photosynthesis estimation. Other stresses and less predictive patterns of environmental changes may exist in the field; the mechanism should be further tested under various conditions.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education, Youth, and Sports of the Czech Republic within the National Infrastructure for Carbon Observations-CzeCOS (No. LM2015061) and SustES – Adaptation strategies for sustainable ecosystem services and food security under adverse environmental conditions (CZ.02.1.01/0.0/0.0/16_019/0000797). JP also acknowledges funding from European Research Council Synergy grant ERC-SyG-2013-610028 IMBALANCE-P.

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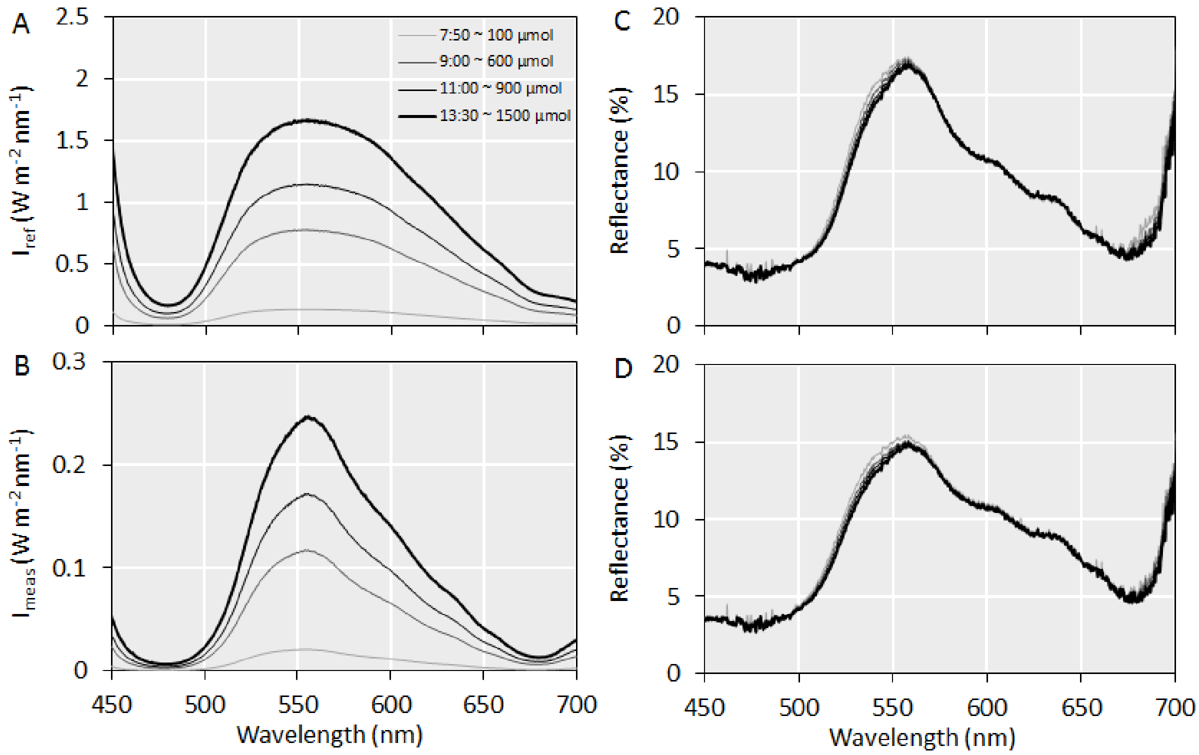

Fig. 1 (A) Spectral intensity emitted by white LED panels (I_{ref}) in growth chambers under four photosynthetically active radiation (PAR) intensities: 100, 600, 900, and 1500 µmol m^{-2} s^{-1}; (B)

spectral intensity reflected from spruce saplings under corresponding PAR intensities (I_{meas}); reflectance spectra of European beech (C) and Norway spruce (D) saplings under corresponding irradiances in one day during the IRR2 regime.

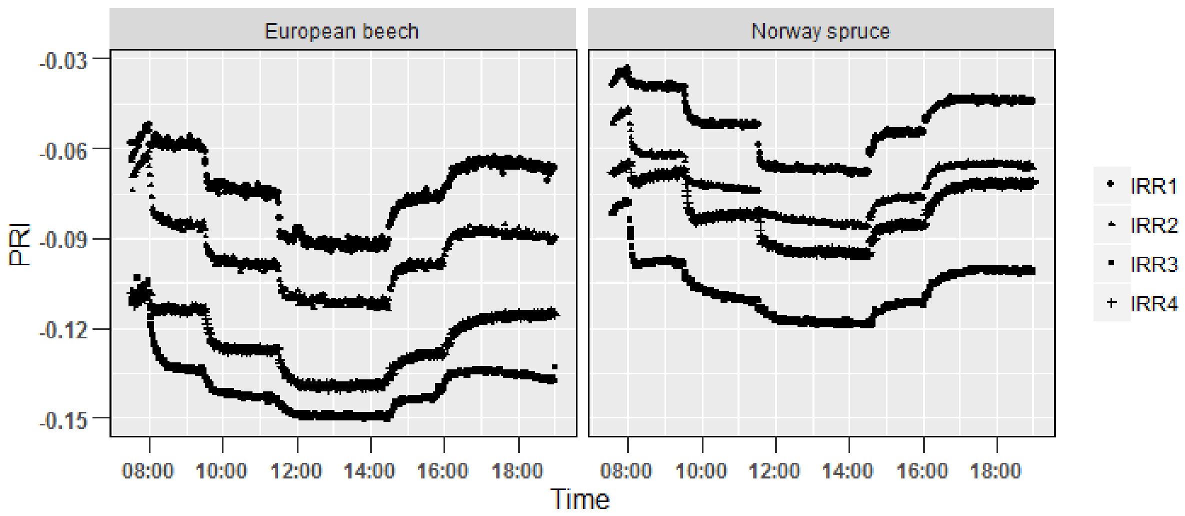

Fig. 2 PRI dynamics on the control (normal) day of each irradiance regime (IRR1, IRR2, IRR3, IRR4) as measured for beech and spruce canopies.

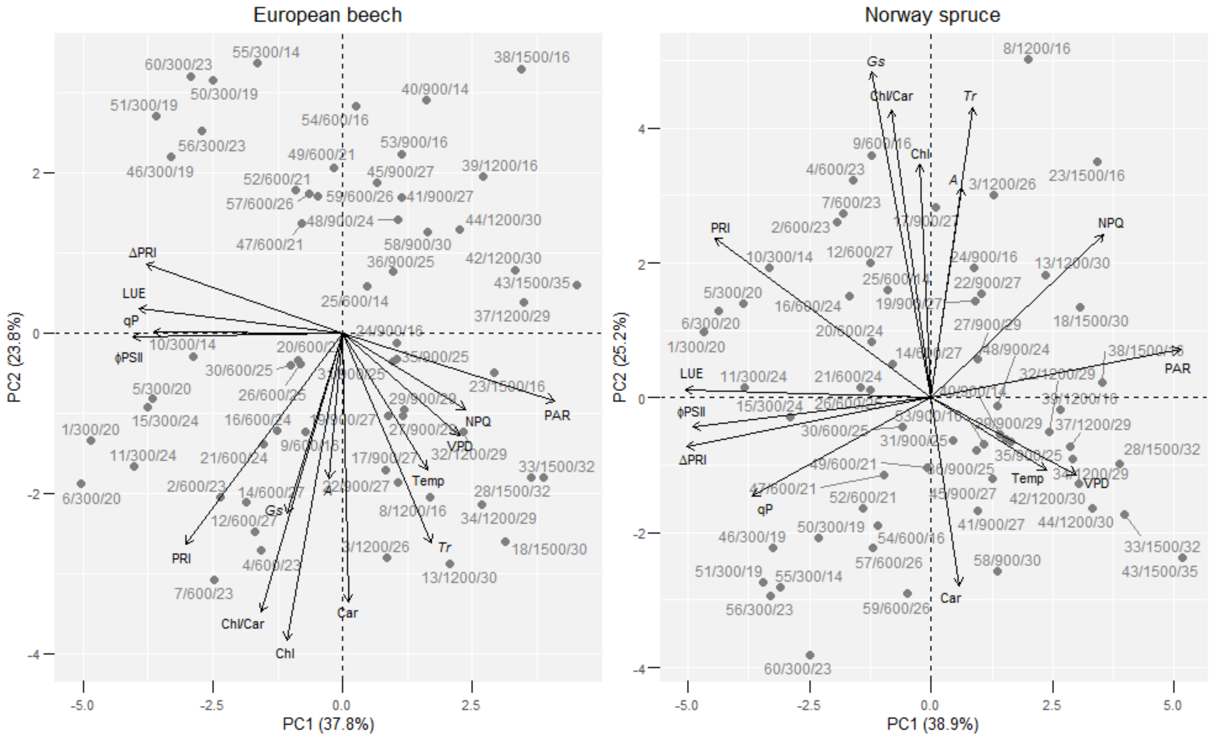

Fig. 3 Biplot of variables and individuals measured showing interactions among spectral information (PRI and ΔPRI), environmental conditions (PAR, Temp, VPD) inside growth chambers, and gas exchange (*A*, *Gs*, *Tr*, LUE), active fluorescence (Φ_{PSII}, NPQ, qP), and foliar pigment (Chl, Car, Chl /Car) characteristics of the examined vegetation. Individual data points are labelled to indicate the number

of measurements taken/irradiance/temperature. A group of 5 measurements represent data collected on one day of measurement. A total of 60 values from 12 measurement days in leaves of spruce and beech trees are shown.

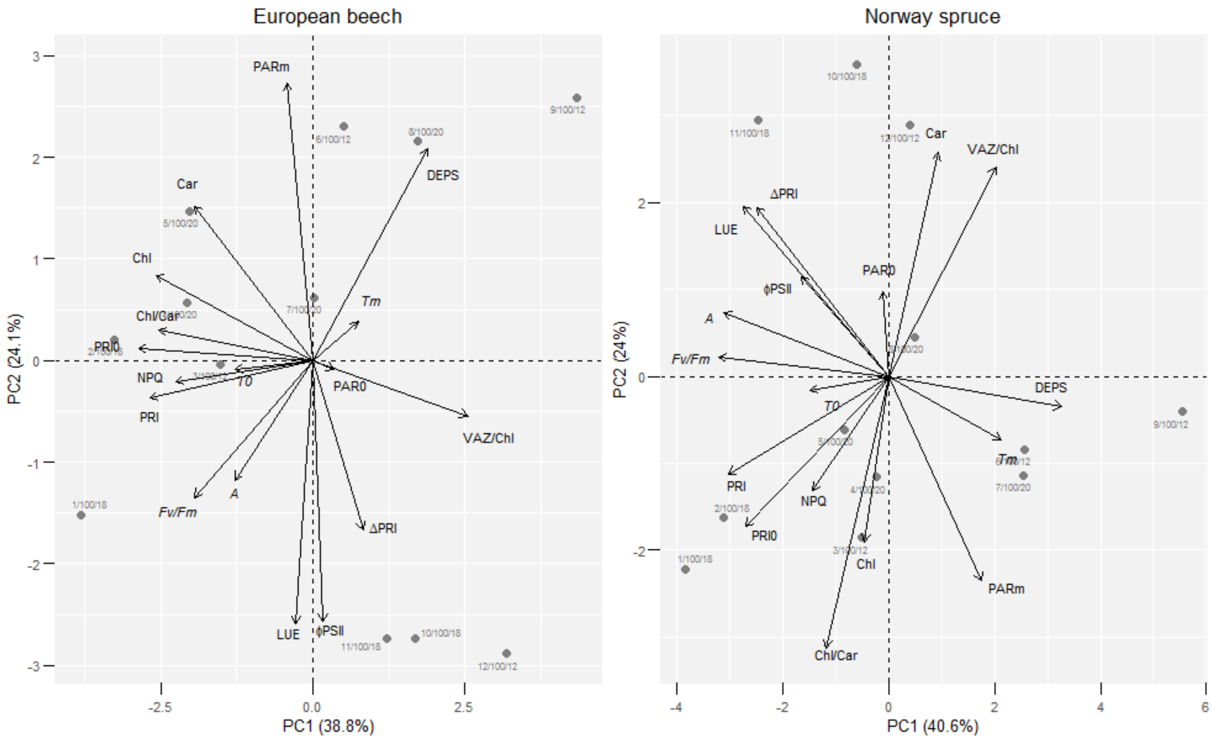

Fig. 4 Biplots of variables and individuals for measurement days (n = 12) of beech and spruce trees showing interactions among foliar pigments (Chl/Car, VAZ/Chl, predawn DEPS), spectral information (PRI_0, midday PRI, ΔPRI), environmental conditions (PAR0, midday PARm, overnight *T*0, and midday

temperature, *T*m), and key physiology measures estimated both predawn (*F*v/*F*m) and during midday (*A*, LUE, Φ_{PSII}, NPQ). Individual data points are labelled with the measurement day number/irradiance at dawn/night temperature.

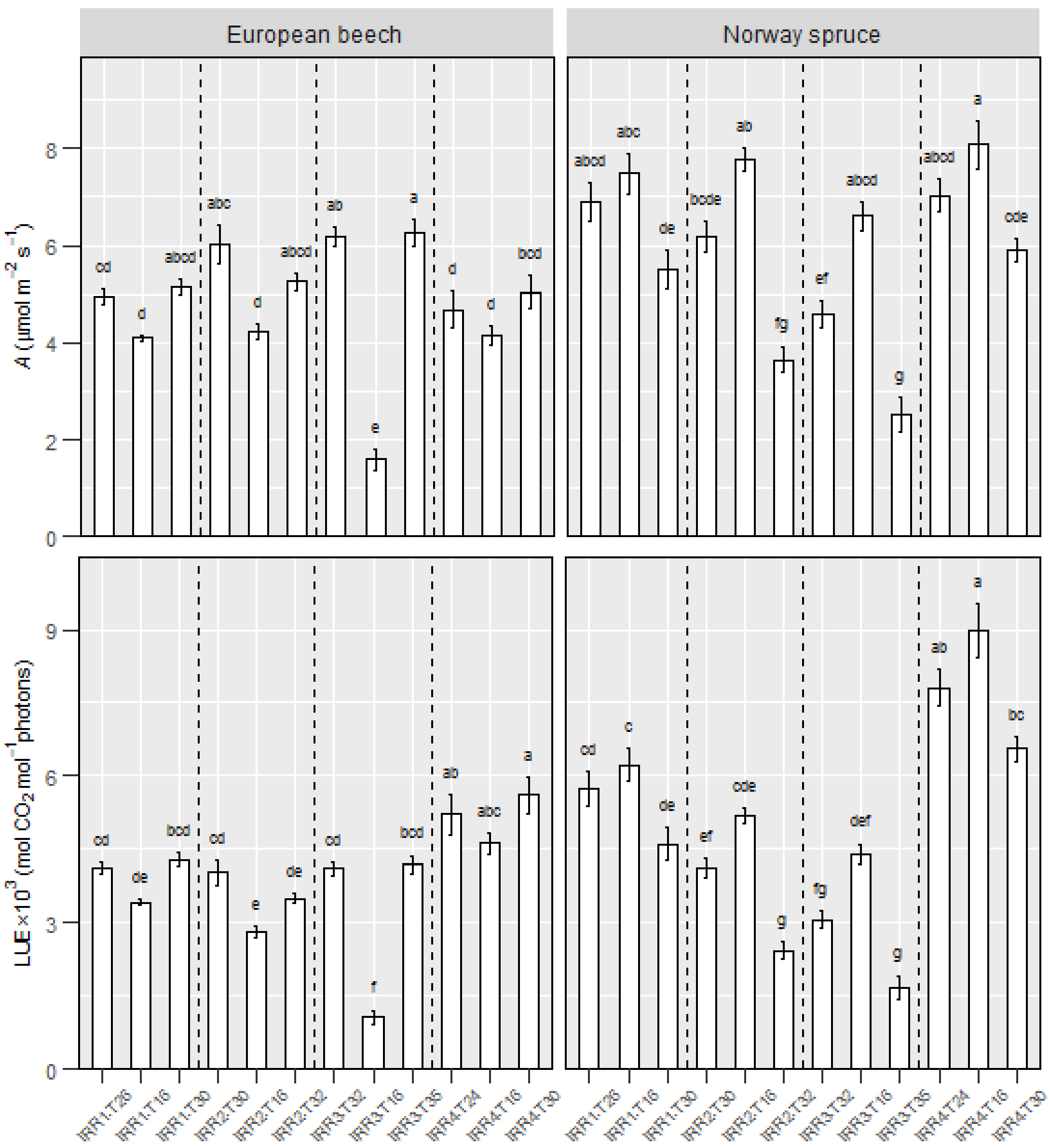

Fig. 5 Photosynthetic capacity (*A*) and light use efficiency (LUE) in leaves of beech and spruce trees at midday of measurement days under four irradiance regimes (IRR1–IRR4). IRR period label and midday temperature indicate irradiance-and-temperature conditions during the measurements. Means and standard deviations (error bars) of the measurements are presented (n = 6). Letters indicate homogeneous groups (p < 0.05; Tukey's post hoc test). Different letters above the bars indicate statistically significant differences between means.

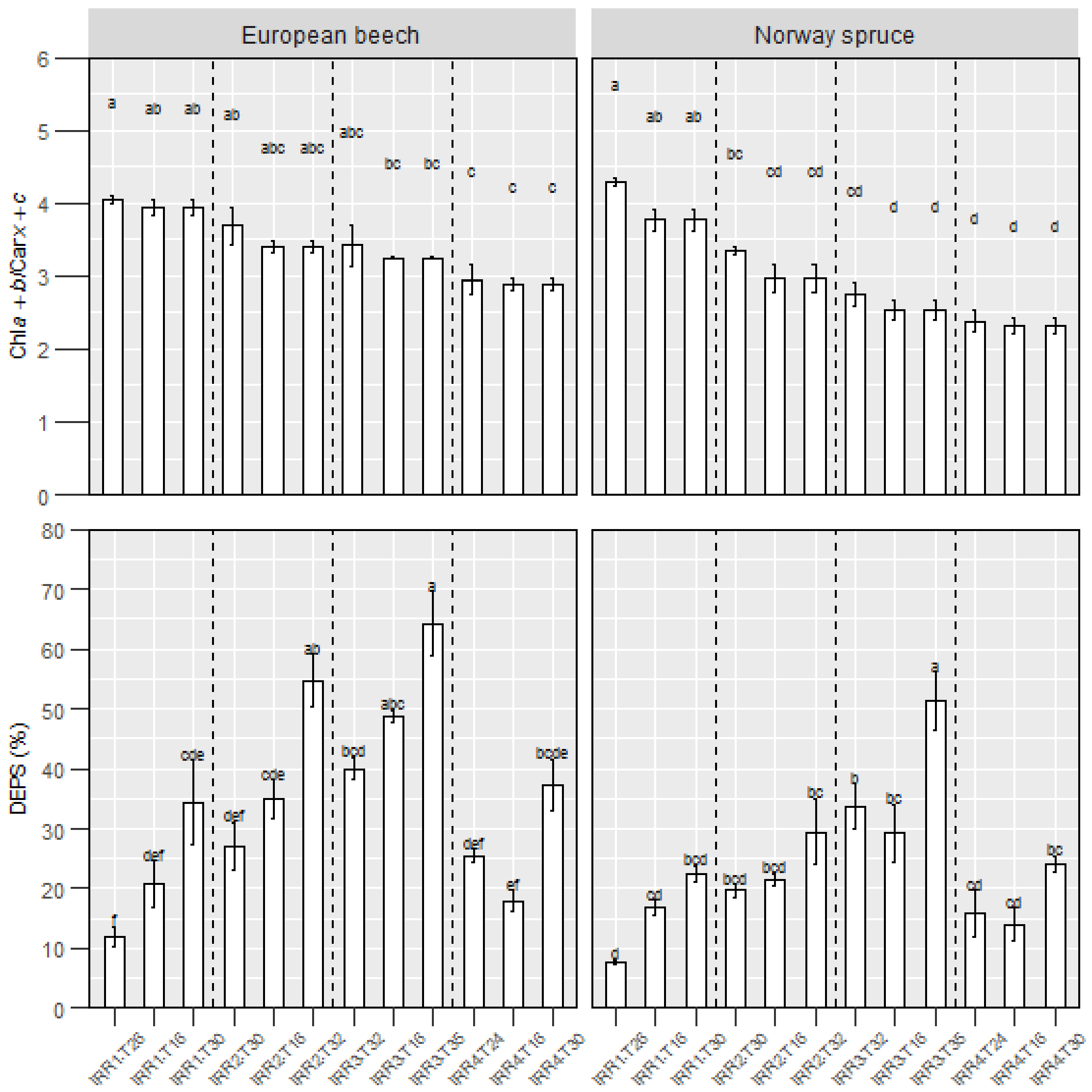

Fig. 6 Ratio of chlorophylls to carotenoids and de-epoxidation state of xanthophyll cycle pigments (DEPS) estimated for leaves of darkness-acclimated beech and spruce trees on days of physiological measurements. Means and standard deviations (error bars) of the measurements are presented (n = 3). Letters indicate homogeneous groups ($p < 0.05$; Tukey's post hoc test). Different letters above the bars indicate statistically significant differences between the means.

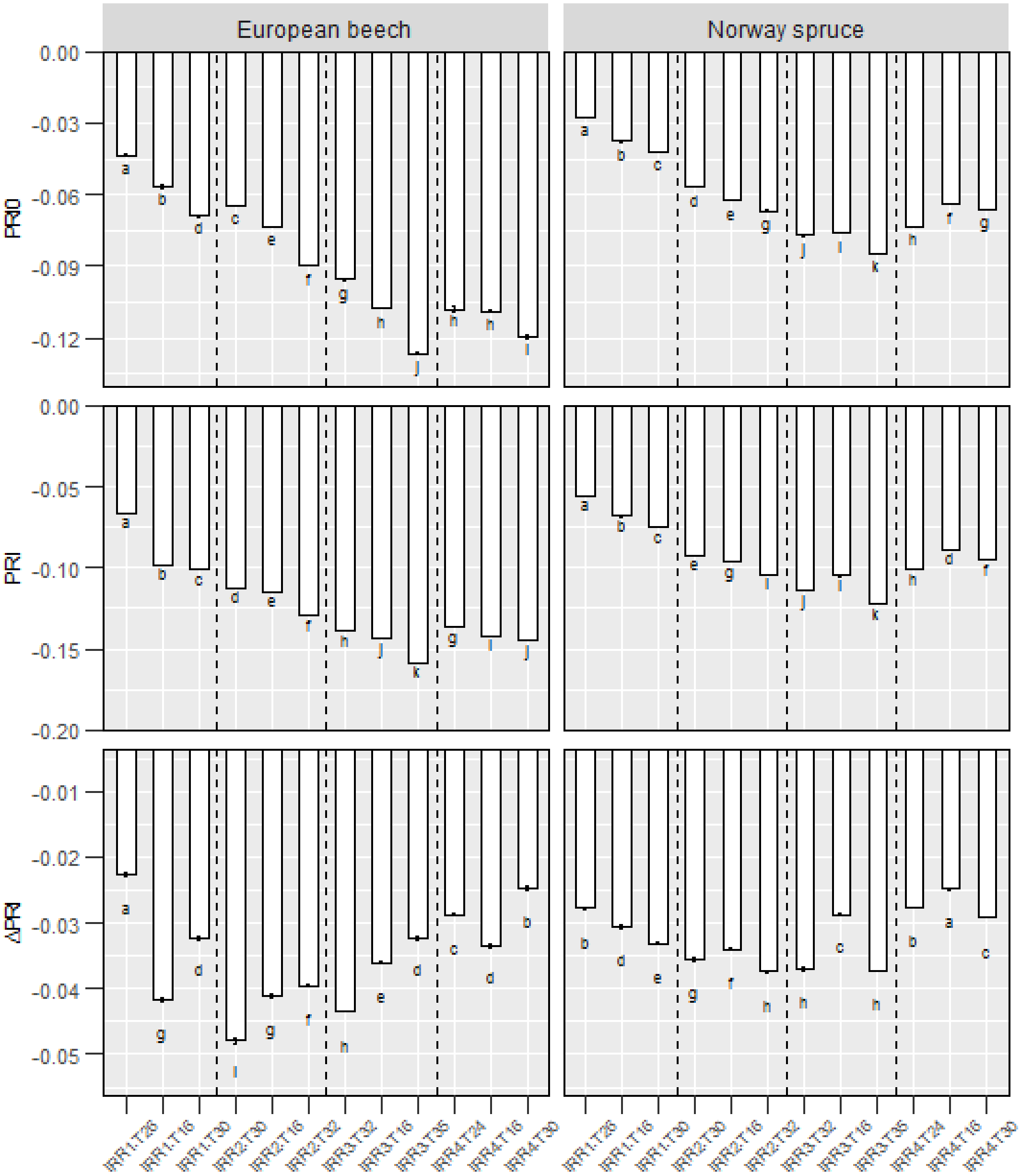

Fig. 7 Development of early morning PRI_0, midday PRI, and differential ΔPRI in changing midday temperature conditions under four irradiance regimes (IRR1–IRR4), separated by broken lines. Letters indicate homogeneous groups ($p < 0.05$; $n = 6$; Tukey's post hoc test).

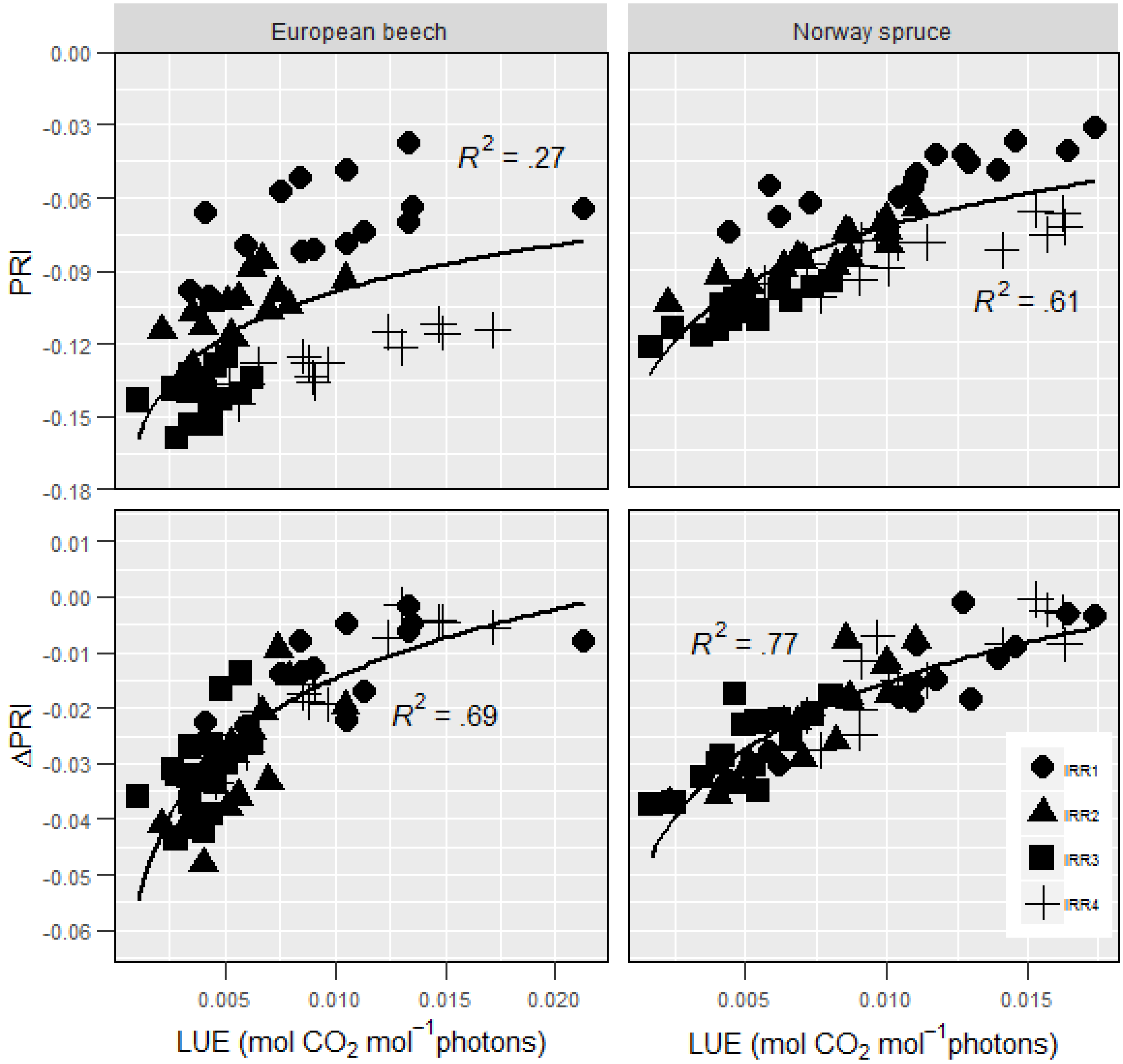

Fig. 8 Relationships between LUE and PRI (ΔPRI) in all data measured during all four irradiance regimes (IRR1–IRR4). Coefficients of determination for overall relationships between variables are displayed in the figure (*n* = 60).

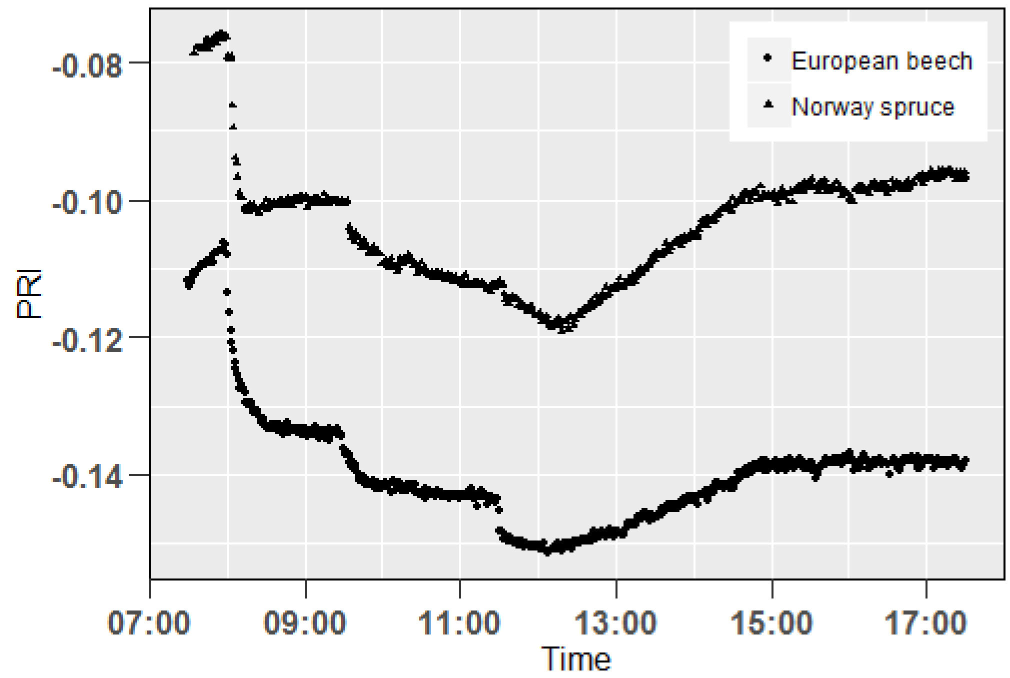

Fig. 9 PRI dynamics on a measurement day with midday cooling to 16 °C during the highest-irradiance regime, IRR3. Physiology measurements and optical data to report PRI during the midday period were taken at 14:00. Cooling of trees started at 12:00.

Table 1 Coefficients of determination (R^2) for PRI-LUE and ΔPRI-LUE relationships in beech and spruce saplings as observed in Fig. 8. Relationships estimated for data pairs estimated in four individual IRR regimes (*n* = 15) and among all measured data (*n* = 60). The significance of the results was tested at levels **p* < 0.05, ***p* < 0.01, and ****p* < 0.001; ns indicates a non-significant result

IRR regime	European beech		Norway spruce	
	PRI-LUE	ΔPRI-LUE	PRI-LUE	ΔPRI-LUE
IRR1	0.30*	0.68***	0.79***	0.81***
IRR2	0.36*	0.54***	0.77***	0.74***
IRR3	0.07^{ns}	0.20^{ns}	0.71***	0.53***
IRR4	0.82***	0.91***	0.62***	0.77***
Total	0.27***	0.69***	0.61***	0.77***

Table 2 Fitting logarithmic equations for PRI-LUE and ΔPRI-LUE relationships in beech and spruce saplings as observed in Fig. 8. Coefficients of determination for these relationships are shown in table 1. Equations were estimated for data pairs estimated in four individual IRR regimes (*n* = 15) and among all measured data (*n* = 60)

IRR regime	European beech		Norway spruce	
	PRI-LUE	ΔPRI-LUE	PRI-LUE	ΔPRI-LUE
IRR1	y=0.0191ln(x)+0.0202	y=0.0181ln(x)+0.0701	y=0.0267ln(x)+0.0702	y=0.0221ln(x)+0.0852
IRR2	y=0.0177ln(x)+0.0128	y=0.0197ln(x)+0.0738	y=0.0229ln(x)+0.0296	y=0.0198ln(x)+0.0764
IRR3	y=0.0057ln(x)+0.1091	y=0.0087ln(x)+0.0176	y=0.0164ln(x)+0.0188	y=0.0119ln(x)+0.0362
IRR4	y=0.0230ln(x)+0.0191	y=0.0232ln(x)+0.0931	y=0.0255ln(x)+0.0344	y=0.0249ln(x)+0.0991
Total	y=0.0027ln(x)+0.0259	y=0.0179ln(x)+0.0678	y=0.0339ln(x)+0.0836	y=0.0175ln(x)+0.0655