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Abstract

This laboratory experiment tested the ability of the spectral index called 'area under curve normalised to maximal band depth' (ANMB) to track dynamic changes in the xanthophyll cycle of Norway spruce (*Picea abies* (L.) Karsten) needles. Four-year-old spruce seedlings were gradually acclimated to different photosynthetic photon flux densities (PPFDs) and air temperature regimes. The measurements were conducted at the end of each acclimation period lasting for 11 days. A significant decline in the chlorophylls to carotenoids ratio and the increase of the amount of xanthophyll cycle pigments indicated a higher need for carotenoid-mediated photoprotection in spruce leaves acclimated to high PPFD conditions. Similarly, the photochemical reflectance index (PRI) changed from positive to negative values after changing light conditions from low to high intensity as a consequence of the increase in carotenoid content. Systematic responses of PRI to the de-epoxidation state of xanthophyll cycle pigments (DEPS) were, however, observed only during high temperature treatments and after the exposition of needles to high irradiance. The ANMB index computed from needle reflectance between 507 and 556 nm was able to track dynamic changes in DEPS without any influence induced by changing the content of leaf photosynthetic pigments (chlorophylls, carotenoids).

Keywords

Diurnal course, growth chambers, photosynthetic pigments composition, *Picea abies*, reflectance continuum removal, xanthophyll cycle

Disciplines

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**Reflectance continuum removal spectral index tracking the xanthophyll cycle
photoprotective reactions in Norway spruce needles**

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ABSTRACT

This laboratory experiment tested the ability of the spectral index called Area under curve Normalized to Maximal Band depth (ANMB) to track dynamic changes in the xanthophyll cycle of Norway spruce (*Picea abies*) needles. Four-year old spruce seedlings were gradually acclimated to different photosynthetic photon flux densities (PPFDs) and air temperature regimes. The measurements were carried out at the end of each acclimation period lasting for 11 days. A significant decline in the chlorophylls to carotenoids ratio and the increase of the amount of xanthophyll cycle pigments indicated a higher need for carotenoid-mediated photoprotection in spruce leaves acclimated to high PPFD conditions. Similarly, the Photochemical Reflectance Index (PRI) changed from positive to negative values after changing light conditions from low to high intensity as a consequence of the increase in carotenoid content. Systematic responses of PRI to the de-epoxidized state of xanthophyll cycle pigments (DEPS) were, however, observed only during high temperature treatments and after the exposition of needles to high irradiance. The ANMB index computed from needle

reflectance between 507 and 556 nm was able to track dynamic changes in DEPS without any influence induced by changing the content of leaf photosynthetic pigments (chlorophylls, carotenoids).

Key words: *Picea abies*, growth chambers, reflectance continuum removal, xanthophyll cycle, diurnal course, photosynthetic pigments composition

INTRODUCTION

Spatio-temporal estimation of plant photosynthesis at a global scale is required for comprehensive understanding of the terrestrial carbon cycle and the determination of CO₂ uptake by plants (Baldocchi 2003). Carbon flux measurements of eddy-covariance towers have a local character, but can be scaled up to larger areas using specific remote sensing (RS) techniques. A traditional RS approach assessing the vegetation photosynthetic activity via estimation of green plant biomass indicated a close relationship between the Normalized Difference Vegetation Index (NDVI) (Tucker 1979) and the fraction of photosynthetically active radiation (fPAR) absorbed by green vegetation (Myneni and Williams 1994). Spaceborne measurement assessing the amount of fPAR used for photosynthetic processes is, nevertheless, still a challenging task (Grace *et al.* 2007). The Photochemical Reflectance Index (PRI) was applied in several studies to estimate the actual photosynthetic efficiency and other related variables of green vegetation (Garbulsky *et al.* 2011). PRI is a normalized narrowband vegetation index conventionally calculated as $(R_{531} - R_{570}) / (R_{531} + R_{570})$ (Gamon *et al.* 1992), where R is the leaf reflectance at a noted wavelength. Changes in leaf PRI were found to follow dynamic reactions of the xanthophyll cycle that regulates photosynthesis via thermal dissipation of excessive light energy (Demmig-Adams *et al.* 1999).

The width of denotation and the strength of particular physiological signals influencing leaf reflectance are driven by the irradiation, but they are also influenced by differences in leaf anatomy and morphology (Nichol *et al.* 2002). The reflectance change at 531 nm, which is used as a dynamic indicative component in PRI, integrates several processes accompanying the photoprotection reactions. Among them, the xanthophyll cycle pigment interconversion has been identified as a process with a significant impact on the reflectance at around 525 nm. An influence of conformational changes in pigment-binding proteins, which indicates the engagement of xanthophyll cycle pigments to photoprotection, emerges at the wavelengths

above 531 nm (Bilger *et al.* 1989; Gamon *et al.* 1990; Peñuelas *et al.* 1995; Gamon *et al.* 1997).

The link between PRI and the plant physiological status through an understanding of photochemical quenching and non-photochemical quenching processes was investigated by Peñuelas *et al.* (1995), Dobrowski *et al.* (2005), Nichol *et al.* (2006) and Ripullone *et al.* (2011). The relationship suggested between PRI and photosynthetic efficiency was shown to be inconsistent due to the changes in foliar pigment content and also canopy architecture (Barton and North 2001; Filella *et al.* 2004; Hilker *et al.* 2008b; Suarez *et al.* 2008). The degradation of chlorophylls generally reduces PRI as a result of the increase in reflectance at 570 nm relative to that at 531 nm (Moran *et al.* 2000; Sims and Gamon 2002; Nakaji *et al.* 2006). Hernandez-Clemente (2011) proposed to avoid this issue by using instead a reflectance at around 512 nm as the referential waveband. Finally, Hilker *et al.* (2010; 2009) demonstrated that PRI can sufficiently indicate the plant physiological status even at the canopy level after correcting differences in viewing angles, which minimized a negative influence of canopy structure on the physiological signal embedded in the reflectance at 531 nm.

Realizing that the photoprotective physiological signal is observable within several wavelengths of a broad blue-green spectral region, the objective of our experiment was to test a new spectrometric indicator of leaf xanthophyll reactions based on the reflectance continuum removal transformation (Broge and Leblanc 2001; Kokaly and Clark 1999). Continuum removal was applied to the spruce needle reflectance within the region of the xanthophyll cycle denotation between 507 – 556 nm. Leaf reflectance was measured in an integrating sphere equipped with a collimated light source. This standard and reproducible measurement set-up ensured stable and accurate reflectance determinations (Gamon and Surfus 1999). Investigated Norway spruce seedlings were exposed to irradiance intensities simulating cloudy and sunny days with moderate and high temperatures to test the robustness of a newly proposed optical indicator towards distinct environmental conditions. During the experimental treatments the plant material was placed into growth chambers to ensure steady environmental conditions. This type of experimental set-up allowed us to evaluate leaf reflectance responses to various physiological states corresponding with particular combinations of air temperature and light intensities. The sensitivity assessment of both optical indices, i.e. PRI and new continuum removal based optical index, to the

photoprotective xanthophyll transformation was supported by the fluorescence measurements of a quantum yield of photosystem II photochemistry (Φ_{PSII}) (Genty *et al.* 1989).

MATERIALS AND METHODS

Plant material and experimental design

Four-year old Norway spruce (*Picea abies* (L.) Karsten) seedlings were gradually acclimated to three regimes differing in environmental conditions inside two growth chambers (HB 1014, Bioline-Heraeus, Hanau, Germany). The duration of each acclimation regime was 11 days. The daily courses of particular microclimatic parameters for the individual acclimation regimes are shown in Fig. 1.

The seedlings of both chambers were initially acclimated to a low photosynthetic photon flux density (PPFD) irradiance and moderate air temperature (T_{air}) (LI-LT acclimation regime) with maximum PPFD at ‘midday’ of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and T_{air} in the range of 15–25°C. In both chambers PPFD was then increased to reach a ‘midday’ maximum of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (HI regime). In one chamber, the daily course of T_{air} remained unchanged (HI-LT regime), whereas in the second growth chamber the daily course of T_{air} ranged from 20–35°C, resulting in a high irradiance and high air temperature (HI-HT) acclimation regime. For the last acclimation regime the irradiation conditions of both chambers remained high (HI), whereas the temperature was increased. In the first growth chamber, low T_{air} (LT) was increased to high T_{air} (i.e. resulting in HI-HT regime), whereas T_{air} in the second growth chamber was increased even further to 25–40°C (HI-HT2 regime). The plants of both growth chambers were sufficiently watered (200 ml per plant/day). Relative air humidity was kept between 50 and 65% in all treatments. Daily increases in PPFDs for LI regimes were set to 10, 100, 160 and $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, and to 25, 300, 500 and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for HI regimes (Fig. 1). Due to the horizontal heterogeneity of the illumination inside the growth chambers, the incident PPFDs for the measured shoots varied up to 10%, when compared to the computed average PPFDs.

All experiments were carried out on current and one-year old needles collected from the two uppermost whorls of the seedlings, where the incident irradiance was ensured to reach the predefined PPFD levels (see Fig. 1). Prior to each measurement cycle, the spruce seedlings were acclimated to the actual environmental conditions for at least half an hour. The

measurements were conducted at predawn (before the light in the chambers was turned on at 8:30 and 10:00 am), in the morning (around 10:30 - 13:30), at noon (around 14:00 - 17:00), and in the afternoon (around 17:30 - 20:30) of simulated daily courses. Each measuring cycle consisted of needle reflectance determination, needle collection for analysis of photosynthetic pigment content and the fluorescence measurement. The needle samples were collected from five trees of each growth chamber, and two reflectance measurements were carried out per sample. The samples were kept in growth chamber conditions during the whole measurement. To avoid misrepresentation of DEPS acquisitions due to the reflectance measurement, needles lined up in the carrier were kept inside the growth chamber for 10 minutes at the required irradiance for pre-adaptation. Their reflectance was measured from the illuminated side. The very same needles were used for the xanthophyll analysis performed with an HPLC system and spectrometric assessment of chlorophylls and carotenoids. After scanning the needle sample for gap fraction estimation, it was illuminated once again for additional 5 minutes. The light-adapted needles were stored in liquid nitrogen. The full measurement procedure, starting from needle detachment until freezing needle samples in liquid nitrogen, took a total of about 20 minutes. Since our previous laboratory tests showed no significant change in DEPS of spruce needles within the first 30 minutes after the shoot detachment (Štroch, unpublished data), this should be an acceptable processing time without a significant influence on the leaf xanthophyll composition. Finally, the chlorophyll *a* fluorescence parameters were measured at the same spot of each marked tree throughout the whole experiment.

Reflectance measurements

The leaf reflectance of the spruce needles sampled was measured according to the method of Daughtry et al. (1989) modified by Malenovský et al. (2006a). The LI-1800 spectroradiometer (Li-Cor Inc., Lincoln, NE, USA) coupled with an integrating sphere LI-1800-12 (Li-Cor, USA) was used to acquire the spruce needle reflectance measurements between 400 and 1100 nm, with a wavelength interval of 1 nm. The directional-hemispherical reflectance of the sampled needles was computed based on the following equation:

$$R = \frac{R_{TOTAL} / R_{REF}}{1 - GF} , \quad (Eq. 1)$$

where R_{TOTAL} is the flux of radiation reflected from the sample in reflectance mode, R_{REF} is the flux of radiation reflected from a BaSO_4 reference standard in reference mode and GF is the gap fraction between the sample needles illuminated in the reflectance mode. Needles tied up in a sample holder were scanned using a double lamp digital scanner to compute the sample (GF) (Mesarch *et al.* 1999). GF was obtained via image processing, determined as the number of pixels of the total gap area between the illuminated needles divided by the number of pixels of the total (illuminated) area measured.

Concept of the ANMB₅₀₇₋₅₅₆ index

Continuum removal is a mathematical, reflectance normalizing transformation (Clark and Roush 1984; Kokaly and Clark 1999; Broge and Leblanc 2001) that allows the comparison of individual absorption features based on their common baseline. Continuum removal enhances reflectance bands by correcting apparent shifts in the band maximum caused by wavelength dependent scattering. The concept of a newly tested continuum removal based optical index called Area under curve Normalized to Maximum Band Depth (ANMB) has been described in detail in the peer-reviewed article by Malenovsky *et al.* (2006b). The continuum removal transformation was applied to the measured needle directional-hemispherical reflectance between 507 and 556 nm to isolate the reflectance signal affected by the xanthophyll cycle. A spectral interval between 507 and 556 nm was selected to include two known spectral features related to the interconversion of xanthophyll cycle pigments: i) a subtle absorbance between 505 and 515 nm (Bilger *et al.* 1989) and ii) a broader reflectance change at around 531 nm with two contributing components located at 526 and 545 nm (Gamon *et al.* 1997). The wavelength extent of the xanthophyll-induced reflectance change is difficult to assess due to a specific boundary of particular pigments on the binding proteins, and dynamic changes in the chemical environment within the leaves (Ustin *et al.* 2006).

In our experiment, the continuum removal transformation was applied to the needle reflectance according to Kokaly and Clark (1999). The reflectance at a particular wavelength was normalized to the value of the same wavelength, which is located on a straight line interpolated linearly between the reflectance values at 505 and 556 nm. Then, the Area Under Curve ($\text{AUC}_{507-556}$) was computed as the integration of the area under the continuum-removed reflectance according to the equation:

$$AUC_{507-556} = \frac{1}{2} \sum_{i=1}^{n-1} (\lambda_{i+1} - \lambda_i) (R_{CR(\lambda_{i+1})} + R_{CR(\lambda_i)}), \quad (\text{Eq. 2})$$

where $R_{CR(\lambda_i)}$ and $R_{CR(\lambda_{i+1})}$ are the continuum-removed reflectance values of the spectral bands λ_i and λ_{i+1} in the wavelengths i and $i+1$ located within the selected spectral interval, and n is the number of spectral bands, which is equal to 50 for the given spectral interval of 507 – 556 nm and the resolution of 1 nm. Finally, $ANMB_{507-556}$ was computed as the ratio of $AUC_{507-556}$ and a maximal band depth of the continuum-removed reflectance between 507-556 nm ($MBD_{507-556}$):

$$ANMB_{507-556} = \frac{AUC_{507-556}}{MBD_{507-556}}. \quad (\text{Eq. 3})$$

The purpose of normalization by $MBD_{507-556}$ was to emphasize changes in the reflectance continuum-removed spectral bands adjacent to that in which $MBD_{507-556}$ is located.

Analysis of photosynthetic pigments

The contents of foliar pigments, i.e. the total chlorophylls (Chl $a+b$) and total carotenoids (Car $x+c$), and also the Chl a/b and Chl $a+b$ /Car $x+c$ ratios were measured with the spectrophotometer UV/VIS 550 (Unicam, Cambridge, England) from supernatant obtained after the centrifugation (for 3 min at 480 g) of pigment extracts in 80% acetone with a small amount of $MgCO_3$. The contents of needle pigments were determined according to equations by Lichtenthaler (1987). Chl $a+b$ and Car $x+c$ contents were expressed per needle projection area that was estimated from digital images of the needles analyzed. The needle images were recorded with a standard table scanner and their projections were estimated using the Cernota software (Kalina and Slovák 2004).

The content of xanthophyll cycle pigments, i.e. antheraxanthin (A), violaxanthin (V), and zeaxanthin (Z), was estimated by the gradient reversed-phase HPLC (TSP Analytical, Kent City, USA) (Kurasová *et al.* 2003). The conversion factors for contents of the individual carotenoids were used according to Färber and Jahns (1998). The conversion state of the xanthophyll cycle pigments (de-epoxidation state; DEPS) was calculated as:

$$DEPS = (A + Z) / (V + A + Z) \quad (\text{Gilmore and Björkman 1994}). \quad (\text{Eq. 4})$$

Measurements of quantum yield of photosystem II photochemistry

The quantum yield of photosystem II photochemistry (Φ_{PSII}) was measured directly on shoots of the trees under the actual PPFD and T_{air} conditions using a portable chlorophyll fluorometer (PAM-2000, Heinz Walz, Effeltrich, Germany). The measurements were carried out after at least 30 min of exposure to the given growth conditions in the morning, at noon and in the afternoon (i.e. under PPFDs of 100 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for LI plants and 300 and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for HI plants). The corresponding Φ_{PSII} was calculated according to Genty et al. (1989):

$$\Phi_{\text{PSII}} = (F_{\text{M}}' - F_{\text{S}})/F_{\text{M}}', \quad (\text{Eq. 5})$$

where F_{S} is the steady-state fluorescence and F_{M}' is the maximum fluorescence emission obtained after the application of the saturating light pulse lasting for 0.8 s and producing incident PPFD $> 3500 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Data analysis

Statistical differences between the means of leaf pigment characteristics were tested using a two-sample F-test for variances, followed by a Student's t-test computed at level of significance $P < 0.05$. The Student's t-test was applied based on the results of the F-test, assuming either equal or unequal variances. A nonparametric Mann–Whitney U-test was used to compare datasets with uneven numbers of elements. The determination coefficient (R^2) was computed to express the variation percentage of a dependent variable explained by an established regression with the independent variable. The significance of the statistical model was tested at probability levels $P < 0.05$, $P < 0.01$ and $P < 0.001$, using the analysis of variance (ANOVA). All data analyses and statistical tests were carried out with the mathematical-statistical software R (R Development Core Team 2010).

RESULTS AND DISCUSSION

Changes in photosynthetic pigments composition during acclimation regimes

Throughout all the experiments a needle morphological parameter called specific leaf area (SLA), which is defined as the ratio of leaf area to dry weight, was invariant (Fig. 2A). We, therefore, assume that all the needles in our experiment had comparable geometrical proportions (in particular needle thickness). Contrary to SLA, the content of photosynthetic

pigments varied between the acclimation regimes. Increases in incoming irradiation during LT treatment favored the synthesis of chlorophylls, whereas the transition of plants to HT conditions promoted the degradation of foliar chlorophylls (Fig. 2B). Although the Chl $a+b$ increase in the case of acclimation to HI-LT and also the Chl $a+b$ decrease in the case of acclimation to HI-HT are insignificant, the Chl $a+b$ difference between LT and HT treatments is significant at $P < 0.05$ (Student's t -test). A temperature increase also led to a chlorophyll degradation in the HI-LT acclimated plants. Chl $a+b$ for the HI-HT2 acclimation regime seems to be slightly higher when compared to the HI-HT plants at the end of the acclimation. However, this difference is not statistically significant.

A clear overview of the foliar pigment composition is offered by the pigment ratios in Fig. 3. The response of the pigment composition in spruce needles upon acclimation to the HI regime corresponds with the typical reactions of plants exposed to enhanced irradiances. As the light-harvesting complexes possess quite a low Chl a/b ratio, this ratio can be used as an indirect indicator of the size of photosystem II (PSII) light-harvesting complexes (LHCII) (van Amerongen and van Grondelle 2001). Chl a/b is higher in spruce needles acclimated to the HI regime than to the LI regime irrespective of the temperature (Fig. 3A). Still, the difference in Chl a/b of needles acclimated to LI and HI conditions was found to be statistically insignificant. Just a slight increase in the Chl a/b ratio may indicate a negligible LHCII reduction, but one has to keep in mind that the Chl a/b ratio is also influenced by the change in the PSII/photosystem I (PSI) stoichiometry. According to Thayer and Björkman (1992) Chl a/b is higher in PSI than in PSII. An increase of the PSII/PSI ratio typically observed in the case of high intensity light acclimation tends to decrease Chl a/b , acting against the effect of the LHCII reduction. (když se nad tím krutopřísně pozastavím, chceme ukázat nebo se zastavujeme nad tím proč jsou pigmenty jaké jsou, konkrétní hypotézu netestujeme – myslím podání rozložení pigmentů v rostlině; extra bota to snad není) -> Toto si musis rozhodnout sam nebo s Otikem, ja nejsem odbornik na LHCII ani PSII/PSI, etc.

A more reliable indicator of the LHCII apportionment is, therefore, the Chl $a+b$ /Car $x+c$ ratio. In comparison to the reaction centers, the LHCII proteins show a high Chl $a+b$ /Car $x+c$ ratio (Sarijeva *et al.* 2007). Consequently, a significant decrease in the Chl $a+b$ /Car $x+c$ ratio (Fig. 3B) indicates a rapid LHCII reduction upon acclimation to the HI regime, which is not observable within the Chl a/b ratio changes. On one hand, decreases of Chl $a+b$ /Car $x+c$ in the HI-LT and HI-HT2 treatments are associated with an increase in the Car $x+c$ content per unit needle area (Fig. 2C). This observation indicates the synthesis of PSII reaction centers,

which, as discussed previously, complicates the usage of Chl *a/b* as the indicator of LHCII size. On the other hand, a decrease of Chl *a+b/Car x+c* in the case of the HI-HT treatment in chamber two is result of Chl *a+b* decline (Fig. 2C). Chl *a/b* of these plants remained unchanged (Fig. 3A).

The observed decrease of the Chl *a+b/Car x+c* ratio after an 11-day exposure to HI indicates an enhanced need for carotenoid-mediated photoprotection under the HI regime (Behera *et al.* 2002). A dissipation of excess light energy in Norway spruce needles is efficiently mediated by transformation of the xanthophyll cycle pigments (Štroch *et al.* 2008) that can be non-destructively observed using PRI (Peñuelas *et al.* 1995). The amount of xanthophyll cycle pigments rose significantly upon acclimation to the HI regime (Fig. 3C), demonstrating an increasing demand for thermal dissipation of excessive light energy occurring under HI conditions. The observed increase of the VAZ content accompanied by the reduction of LHCII after HI acclimation is in agreement with previously published results by Demmig-Adams and Adams (1996), Kurasová *et al.* (2002) or Lichtenthaler *et al.* (2007).

After the acclimation to LI-LT conditions, spruce needles started the light period with DEPS between 30-40 % (Fig. 4), while at the end of the HI acclimation, predawn DEPS raised significantly to 40-55 % ($P < 0.05$, Student's *t*-test). As expected, the maximal DEPS was always observed during the midday period under the most severe illumination intensity (DEPS of 85-90 % during the HI regime), when plants had the highest demand of the xanthophyll-mediated photoprotection. Such high DEPS values are in accordance with results by Kurasová *et al.* (2003) showing very efficient V de-epoxidation in the photosynthetic apparatus of Norway spruces when compared to other plant species.

Dynamic temporal changes in PRI and ANMB₅₀₇₋₅₅₆

Daily courses of PRI measured during all treatments are shown in Fig. 5. Based on findings of Peñuelas *et al.* (1995), a rapid reduction in PRI can be expected after the exposition of evergreen plants to excessive irradiation. Comparison of the results in Fig. 5 and Fig. 4, suggests, however, a rather low correlation of PRI with xanthophyll cycle pigment changes induced by irradiation changes. PRI remained almost invariant under the low light intensities in both growth chambers. One can observe midday PRI decline only under the HI-HT and HI-HT2 regimes and also a systematic shift from positive to negative values after the acclimation to high irradiation treatments (Fig. 5). These are the only two significant changes observed in

PRI values measured ($P < 0.05$, Mann–Whitney U-test). According to Garrity et al. (2011), PRI responds not only to the actual content of carotenoids, driving particularly the spectral response at 531 nm, but to some extent it is also influenced by chlorophyll absorption at 570 nm. Since needle Chl $a+b$ increased after acclimation to the HI-LT regime in the first growth chamber, but decreased after acclimation to the HI-HT regime in the second chamber (Fig. 2B), this shift cannot be attributed solely to the varying Chl $a+b$ content as published previously by Moran et al. (2000) or by Sims and Gamon (2002). The observed negative PRI most likely results from a decrease in Chl $a+b$ / Car $x+c$ ratio (compare Fig. 3BC and Fig. 5), as reported by Stylinsky et al. (2002) and also by Guo et al. (2006) for various plant species. Nevertheless, it is important to note that the PRI of plants acclimated to HI-HT2 conditions, after Chl $a+b$ / Car $x+c$ reaching minimum value, remained comparable with the PRI of other HI treatments.

Fig. 6 shows the daily courses and the statistically significant changes in ANMB₅₀₇₋₅₅₆ ($P < 0.05$, Mann–Whitney U-test) for all the irradiation-temperature regimes tested. During the simulated daytime periods, the dynamics of mean ANMB₅₀₇₋₅₅₆ closely followed changes in light intensity, irrespective of the air temperature inside the growth chambers. ANMB₅₀₇₋₅₅₆ decreased from morning until midday and subsequently increased in the afternoon as a consequence of changing photon absorption within the examined spectral region. Fig. 7 demonstrates changes observed in the shape of the reflectance signal between 507 and 556 nm in relation to the leaf xanthophyll de-epoxidation. To illustrate the change, the MBD₅₀₇₋₅₅₆ normalized continuum-removed reflectance (Fig. 7B) of two needle samples (the first one with DEPS ~ 50% and the second one with DEPS ~ 85%) was subtracted from the same needle reflectance transformation of a sample with DEPS ~ 30% (serving as a baseline). Fig. 7C shows a large negative difference in the MBD₅₀₇₋₅₅₆ normalized continuum-removed reflectance with a local maximum at around 526 nm and a smaller but steady positive difference between 535 and 550 nm, both of them getting larger with an increasing xanthophyll DEPS. This result is in line with the finding by Gamon et al. (1997) that the 526-nm component correlates more strongly with the xanthophyll pigment conversion compared to the 545-nm component. However, Gamon et al. (1997) also noted that both components are functionally interconnected and should not be treated separately, as the 526-nm component occurs only in the presence of the 545-nm component. Although these findings favor our choice of the 507 – 556 nm reflectance as a suitable interval for ANMB computation it is important to mention that also other similar wavelength intervals, including the spectral

changes induced by the violaxanthin deepoxidation, can potentially provide comparable results.

A closer investigation of the measured needle spectral signatures revealed that a GF between the needles presented at the sample port of the integrating sphere does, to a certain extent, influence the reflectance amplitude, and also the values of the parameter ANMB₅₀₇₋₅₅₆. Studies focused on measuring optical properties of conifer leaves advise to keep the GF between 0.3 and 0.5 (e.g. Mesarch et al., 1999). Unfortunately, due to the limited amount of available plant material (only six spruce seedlings could be placed in one growth chamber), the GF size of needle samples measured in our experiment was slightly higher, ranging from 0.45 to 0.6. A statistically significant linear regression was found between the GF size and ANMB₅₀₇₋₅₅₆ for the group of samples with DEPS between 40 and 55% ($R^2 = 0.53$, $P < 0.001$, $n = 42$; data not shown). Although this outcome suggests a negative dependency of ANMB₅₀₇₋₅₅₆ on GF, this dependency is non-systematically distributed across the measurements of all irradiation-temperature treatments. Computing a mean of the repetitive spectral measurements and the use of the boxplot representation minimize the impact of the non-systematic GF influence in our leaf reflectance measurements. Considering this fact, we are confident that the temporal responses of mean ANMB₅₀₇₋₅₅₆ (Fig. 6) do not reflect changes in GF but follow daily changes in DEPS of the xanthophyll cycle pigments (Fig. 4). (krutopřísny recenzent namítne že údaj R² neříká nic o směrnici) -> směrnice je zminena: a negative dependency of ANMB₅₀₇₋₅₅₆ on GF.

Statistical relationships of vegetation indexes with DEPS and Φ_{PSII}

A statistically significant linear regression established between PRI and DEPS ($R^2 = 0.34$, $P < 0.01$, Fig. 8A) implies that PRI is able to explain about 34% of the DEPS variability. Compared to this result, a stronger mutual correlation was revealed between ANMB₅₀₇₋₅₅₆ and DEPS. The linear regression testing the statistical dependency of ANMB₅₀₇₋₅₅₆ on corresponding DEPS values resulted in $R^2 = 0.64$ ($P < 0.001$, Fig. 8B) in the case of 24 mean index values, and $R^2 = 0.53$ ($P < 0.001$) when tested for single spectral measurements (data not shown). The ANMB₅₀₇₋₅₅₆-DEPS statistical dependency is weakened mainly by the afternoon measurements of the second day, when DEPS was persistently high in both chambers (Fig. 4), whereas ANMB₅₀₇₋₅₅₆ already started to relax (Fig. 6). In spite of this, ANMB₅₀₇₋₅₅₆ exhibits the ability to follow DEPS independently of the size of the VAZ pool

and Chl *a+b*/Car *x+c* ratio, which both varied significantly among needles acclimated to different microclimatic conditions (Fig. 3). Also, a varying needle chlorophyll content (Fig. 2B) does not seem to affect the ability of ANMB₅₀₇₋₅₅₆ to capture the dynamics of the xanthophyll cycle pigments. The regression between ANMB₅₀₇₋₅₅₆ and DEPS is strong across samples that vary considerably in content of these photosynthetic pigments.

Investigation of the changes in the quantum yield of photosystem II photochemistry (Φ_{PSII}) (Fig. 9) revealed similar relationships for both spectral indices (Fig. 10). Statistically significant linear regressions with $R^2 = 0.57$ and $R^2 = 0.48$ ($P < 0.001$) were established for PRI and ANMB₅₀₇₋₅₅₆, respectively. These results suggest a lower sensibility of both optical indices to Φ_{PSII} , especially for low Φ_{PSII} values (Fig. 10). In the case of PRI, the index remained low at the end of the acclimation to the HI-HT2 regime, which corresponds with Φ_{PSII} of about 60%. The same Φ_{PSII} was observed at noon for HI-LT acclimated plants, but without a noticeable decrease in PRI (compare Fig. 9 and Fig. 5). Busch *et al.* (2009) also reported a weak relationship between PRI and Φ_{PSII} measured for Jack pine (*Pinus Banksiana*) seedlings. They observed Φ_{PSII} being related mainly to the changes in temperature, whereas PRI followed changes in light intensity. In our experiment, PRI seems to respond rather to increased temperature stress, which may demonstrate a low capacity of evergreen conifers from the cold regions to adjust their temperature optimum for photosynthesis (Way and Sage 2008).

Although the ANMB₅₀₇₋₅₅₆ dynamic can follow temporal changes in DEPS, its dependency on Φ_{PSII} is weaker and less consistent (Fig. 10B). This result points out the necessity to examine responses of ANMB₅₀₇₋₅₅₆ to more complex changes in the photosynthesis performance. A high water vapour pressure deficit induces stomata closure which, consequently, reduces the CO₂ diffusion into the leaf interior and limits plant photosynthetic processes (e.g. Štroch *et al.* 2010). Since the relative air humidity of our experiments was kept stable (between 50 – 65 %), the behavior of ANMB₅₀₇₋₅₅₆ under natural conditions, where the relative air humidity can drop even lower and the incidence of solar irradiation can reach intensities higher than 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, still has to be investigated. Therefore, future studies should be focused on plants that manage and regulate even more excessive irradiation. Secondly, the ability of ANMB₅₀₇₋₅₅₆ to track dynamic transformations of the xanthophyll cycle pigments needs to be tested for plant species of both photosynthetic pathways (C3 and C4 plants), growing in their natural environment. Since several recent studies demonstrated a negative influence of the complex canopy structure (e.g. a conifer forest) on the performance of PRI (Hilker *et al.* 2008a; Suarez

et al. 2008; Hernandez-Clemente *et al.* 2011), the sensitivity of the ANMB₅₀₇₋₅₅₆ index to canopy architecture needs to be investigated before its application to airborne and spaceborne remote sensing data.

CONCLUSION

This study demonstrates the potential of the ANMB₅₀₇₋₅₅₆ reflectance continuum removal index to indicate the actual status of the xanthophyll-based photochemical protective processes in plants. Norway spruce needles react to increasing light intensity and temperature by rapid changes in their photosynthetic pigment composition. This acclimation is accompanied by large adjustments in the content of chlorophylls and carotenoids and, in particular, of xanthophylls. ANMB₅₀₇₋₅₅₆ was able to follow the dynamic changes in the depoxidation state of the xanthophyll pigments, while neither being affected by temperature increase nor by content alterations in needle chlorophylls and carotenoids. PRI demonstrated a similar dynamic behavior as ANMB₅₀₇₋₅₅₆, but only under the combined irradiation-temperature stress conditions. Although our statistical test revealed a significant relationship of photoprotective xanthophyll reactions and ANMB₅₀₇₋₅₅₆ computed from the leaf reflectance of Norway spruce needles, it still needs to be verified by follow-up laboratory or field experiments carried out with other plant species. If approved, it might become a perspective spectral indicator for remote sensing, especially for airborne/spaceborne imaging spectroscopy observations of the vegetation canopy light use efficiency.

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REFERENCES

- Baldocchi DD (2003) Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. *Global Change Biology* **9**, 479-492.
- Barton CVM, North PRJ (2001) Remote sensing of canopy light use efficiency using the photochemical reflectance index - Model and sensitivity analysis. *Remote Sensing of Environment* **78**, 264-273.
- Behera RK, Mishra PC, Choudhury NK (2002) High irradiance and water stress induce alterations in pigment composition and chloroplast activities of primary wheat leaves. *Journal of Plant Physiology* **159**, 967-973.
- Bilger W, Björkman O, Thayer SS (1989) Light-induced spectral absorbance changes in relation to photosynthesis and the epoxidation state of xanthophyll cycle components in cotton leaves. *Plant Physiology* **91**, 542-551.
- Broge NH, Leblanc E (2001) Comparing prediction power and stability of broadband and hyperspectral vegetation indices for estimation of green leaf area index and canopy chlorophyll density. *Remote Sensing of Environment* **76**, 156-172.
- Busch F, Huner NPA, Ensminger I (2009) Biochemical constraints 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.
- Clark RN, Roush TL (1984) Reflectance spectroscopy - quantitative analysis techniques for remote-sensing applications. *Journal of Geophysical Research* **89**, 6329-6340.
- Daughtry CST, Ranson KJ, Biehl LL (1989) A new technique to measure the spectral properties of conifer needles. *Remote Sensing of Environment* **27**, 81-91.
- Demmig-Adams B, Adams WW (1996) Xanthophyll cycle and light stress in nature: Uniform response to excess direct sunlight among higher plant species. *Planta* **198**, 460-470.

Demmig-Adams B, Adams WW, Ebbert V, Logan BA (1999) Ecophysiology of the xanthophyll cycle. In 'Photochemistry of Carotenoids' pp. 245-269. (Kluwer Academic Publ: Dordrecht)

Dobrowski SZ, Pushnik JC, Zarco-Tejada PJ, Ustin SL (2005) Simple reflectance indices track heat and water stress-induced changes in steady-state chlorophyll fluorescence at the canopy scale. *Remote Sensing of Environment* **97**, 403-414.

Färber A, Jahns P (1998) The xanthophyll cycle of higher plants: influence of antenna size and membrane organization. *Biochimica Et Biophysica Acta-Bioenergetics* **1363**, 47-58.

Filella I, Peñuelas J, Llorens L, Estiarte M (2004) Reflectance assessment of seasonal and annual changes in biomass and CO₂ uptake of a Mediterranean shrubland submitted to experimental warming and drought. *Remote Sensing of Environment* **90**, 308-318.

Gamon JA, Field CB, Bilger W, Björkman O, Fredeen AL, Peñuelas J (1990) Remote sensing of the xanthophyll cycle and chlorophyll fluorescence in sunflower leaves and canopies. *Oecologia* **85**, 1-7.

Gamon JA, Peñuelas J, Field CB (1992) A NarrowWaveband Spectral Index That Tracks Diurnal Changes in Photosynthetic Efficiency. *Remote Sensing of Environment* **41**, 35-44.

Gamon JA, Serrano L, Surfus JS (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, Surfus JS (1999) Assessing leaf pigment content and activity with a reflectometer. *New Phytologist* **143**, 105-117.

Garbulsky MF, Peñuelas J, Gamon J, Inoue Y, Filella 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, Vierling 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 JM, 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.

Gilmore AM, Björkman O (1994) Adenine nucleotides and the xanthophyll cycle in leaves. II. Comparison of the effects of CO₂- and temperature-limited photosynthesis on photosystem II fluorescence quenching, the adenylate energy charge and violaxanthin de-epoxidation in cotton. *Planta* **192**, 537-544.

Grace J, Nichol C, Disney M, Lewis P, Quaife T, Bowyer P (2007) Can we measure terrestrial photosynthesis from space directly, using spectral reflectance and fluorescence? *Global Change Biology* **13**, 1484-1497.

Guo JM, Trotter CM, Newton PCD (2006) Initial observations of increased requirements for light-energy dissipation in ryegrass (*Lolium perenne*) when source/sink ratios become high at a naturally grazed Free Air CO₂ Enrichment (FACE) site. *Functional Plant Biology* **33**, 1045-1053.

Hernandez-Clemente R, Navarro-Cerrillo RM, Suarez L, Morales F, Zarco-Tejada PJ (2011) Assessing structural effects on PRI for stress detection in conifer forests. *Remote Sensing of Environment* **115**, 2360-2375.

Hilker T, Coops NC, Hall FG, Black TA, Wulder MA, Nesic Z, Krishnan P (2008a) Separating physiologically and directionally induced changes in PRI using BRDF models. *Remote Sensing of Environment* **112**, 2777-2788.

Hilker T, Coops NC, Schwalm CR, Jassal RS, Black TA, Krishnan P (2008b) Effects of mutual shading of tree crowns on prediction of photosynthetic light-use efficiency in a coastal Douglas-fir forest. *Tree Physiology* **28**, 825-834.

- Hilker T, Hall FG, *et al.* (2010) Remote sensing of photosynthetic light-use efficiency across two forested biomes: Spatial scaling. *Remote Sensing of Environment* **114**, 2863-2874.
- Hilker T, Nesic Z, Coops NC, Lessard D (2009) A New, Automated, Multiangular Radiometer Instrument · for Tower-Based Observations of Canopy Reflectance (AMSPEC II). *Instrumentation Science & Technology* **38**, 319-340.
- Kalina J, Slovák V (2004) The inexpensive tool for the determination of projected leaf area. *Ekologia-Bratislava* **23**, 163-167.
- Kokaly RF, Clark RN (1999) Spectroscopic determination of leaf biochemistry using band-depth analysis of absorption features and stepwise multiple linear regression. *Remote Sensing of Environment* **67**, 267-287.
- Kurasová I, Čajánek M, Kalina J, Urban O, Špunda V (2002) Characterization of acclimation of *Hordeum vulgare* to high irradiation based on different responses of photosynthetic activity and pigment composition. *Photosynthesis Research* **72**, 71-83.
- Kurasová I, Kalina J, Urban O, Štroch M, Špunda V (2003) Acclimation of two distinct plant species, spring barley and Norway spruce, to combined effect of various irradiance and CO₂ concentration during cultivation in controlled environment. *Photosynthetica* **41**, 513-523.
- Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**, 350-382.
- Lichtenthaler HK, Ac A, Marek MV, Kalina J, Urban O (2007) Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology and Biochemistry* **45**, 577-588.
- Malenovský Z, Albrechtová J, Lhotáková Z, Zurita-Milla R, Clevers J, Schaepman ME, Cudlín P (2006a) Applicability of the PROSPECT model for Norway spruce needles. *International Journal of Remote Sensing* **27**, 5315-5340.

- Malenovský Z, Ufer CM, Lhotáková Z, Clevers JGPW, Schaepman ME, Albrechtová J, Cudlín P (2006b) A new hyperspectral index for chlorophyll estimation of a forest canopy: Area under curve Normalized to Maximal Band depth between 650-725 nm. *EARSel eProceedings* **5**, 161-172.
- Mesarch MA, Walter-Shea EA, Asner GP, Middleton EM, Chan SS (1999) A revised measurement methodology for conifer needles spectral optical properties: Evaluating the influence of gaps between elements. *Remote Sensing of Environment* **68**, 177-192.
- Moran JA, Mitchell AK, Goodmanson G, Stockburger KA (2000) Differentiation among effects of nitrogen fertilization treatments on conifer seedlings by foliar reflectance: a comparison of methods. *Tree Physiology* **20**, 1113-1120.
- Myneni RB, Williams DL (1994) On the Relationship between FAPAR and NDVI *Remote Sensing of Environment* **49**, 200-211.
- Nakaji T, Oguma H, Fujinuma 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.
- Nichol CJ, Lloyd J, Shibistova O, Arneth A, Roser C, Knohl A, Matsubara S, Grace J (2002) Remote sensing of photosynthetic-light-use efficiency of a Siberian boreal forest. *Tellus Series B-Chemical and Physical Meteorology* **54**, 677-687.
- Nichol CJ, Rascher U, Matsubara S, Osmond B (2006) Assessing photosynthetic efficiency in an experimental mangrove canopy using remote sensing and chlorophyll fluorescence. *Trees-Structure and Function* **20**, 9-15.
- Peñuelas J, Filella I, Gamon JA (1995) Assessment of photosynthetic radiation-use efficiency with spectral reflectance. *New Phytologist* **131**, 291-296.

R Development Core Team (2010) R: A language and environment for statistical computing.
R Foundation for Statistical Computing. *Vienna, Austria*. ISBN 3-900051-07-0.

Ripullone F, Rivelli AR, Baraldi R, Guarini R, Guerrieri R, Magnani F, Penuelas J, Raddi S,
Borghetti M (2011) Effectiveness of the photochemical reflectance index to track
photosynthetic activity over a range of forest tree species and plant water statuses. *Functional
Plant Biology* **38**, 177-186.

Sarijeva G, Knapp M, Lichtenthater HK (2007) Differences in photosynthetic activity,
chlorophyll and carotenoid levels, and in chlorophyll fluorescence parameters in green sun
and shade leaves of Ginkgo and Fagus. *Journal of Plant Physiology* **164**, 950-955.

Sims DA, Gamon JA (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.

Stylinski CD, Gamon JA, Oechel WC (2002) Seasonal patterns of reflectance indices,
carotenoid pigments and photosynthesis of evergreen chaparral species. *Oecologia* **131**, 366-
374.

Suarez L, Zarco-Tejada PJ, Sepulcre-Canto G, Perez-Priego O, Miller JR, Jimenez-Munoz JC,
Sobrino J (2008) Assessing canopy PRI for water stress detection with diurnal airborne
imagery. *Remote Sensing of Environment* **112**, 560-575.

Štroch M, Kuldová K, Kalina J, Špunda V (2008) Dynamics of the xanthophyll cycle and
non-radiative dissipation of absorbed light energy during exposure of Norway spruce to high
irradiance. *Journal of Plant Physiology* **165**, 612-622.

Štroch M, Vrábl D, Podolinská J, Kalina J, Urban O, Špunda V (2010) Acclimation of
Norway spruce photosynthetic apparatus to the combined effect of high irradiance and
temperature. *Journal of Plant Physiology* **167**, 597-605.

Thayer SS, Björkman O (1992) Carotenoid distribution and deepoxidation in thylakoid pigment-protein complexes from cotton leaves and bundle-sheath cells of maize. *Photosynthesis Research* **33**, 213-225.

Tucker CJ (1979) Red and photographic infrared linear combinations for monitoring vegetation *Remote Sensing of Environment* **8**, 127-150.

Ustin SL, Asner GP, Gamon JA, Huemmrich KF, Jacquemoud S, Schaepman M, Zarco-Tejada P, Ieee (2006) Retrieval of Quantitative and Qualitative Information about Plant Pigment Systems from High Resolution Spectroscopy. In '2006 Ieee International Geoscience and Remote Sensing Symposium, Vols 1-8' pp. 1996-1999. (Ieee: New York)

van Amerongen H, van Grondelle R (2001) Understanding the energy transfer function of LHCII, the major light-harvesting complex of green plants. *Journal of Physical Chemistry B* **105**, 604-617.

Way DA, Sage RF (2008) Elevated growth temperatures reduce the carbon gain of black spruce [*Picea mariana* (Mill.) BSP]. *Global Change Biology* **14**, 624-636.

Fig. 1: Diurnal courses of microclimatic conditions in both growth chambers. Photosynthetic photon flux density (PPFD) and air temperature (T_{air}) were combined in LI-LT, HI-LT, HI-HT and HI-HT2 treatments. The daily course of relative air humidity (RH_{air}) remained stable (50-65%) throughout the whole experiment. A black solid line indicates the real time schedule of the regime in chamber one, whereas a grey dashed line indicates the regime in chamber two.

Fig. 2: (A) Specific leaf area (SLA), (B) total content of chlorophylls (Chl $a+b$) and (C) the content of carotenoids (Car $x+c$) measured at the end of the nighttime period (i.e. after 12 h of darkness) for needles acclimated to four irradiation-temperature regimes (LI-LT, HI-LT, HI-HT, HI-HT2). The left-hand side column represents measurements in growth chamber one, the right-hand side column in chamber two. Means (columns) and standard deviations (bars) are presented (n=5). Data followed by the same letter indicate statistically insignificant differences ($P < 0.05$; Student's t-test).

Fig. 3: (A) Ratio of chlorophyll *a* to chlorophyll *b* (Chl *a/b*), (B) ratio of total chlorophylls to total carotenoids (Chl *a+b*/Car *x+c*) and (C) the content of xanthophyll cycle pigments per total chlorophyll content (VAZ/Chl *a+b*) estimated for the particular acclimation regimes. Needles were sampled at the end of the nighttime period on the last day of acclimation to the given regime. The left-hand side column shows measurements of chamber one, the right-hand side column measurements of chamber two. Means (columns) and standard deviations (bars) are presented (n=5). Data followed by the same letter indicate statistically insignificant differences ($P < 0.05$; Student's *t*-test).

Fig. 4: The de-epoxidation state of the xanthophyll cycle pigments [DEPS = $(Z+A)/(V+A+Z)*100$] determined in the daily course at the end of the particular acclimation regimes. (A) data of chamber one, (B) data of chamber two. Means (columns) and standard deviations (bars) are presented (n=5) for needle samples examined per treatment and time of the day. Data followed by the same letter indicate statistically insignificant differences ($P < 0.05$; Student's t-test).

Fig. 5: Daily courses of the Photochemical Reflectance Index (PRI) computed from the database of 205 measured reflectance spectra. Needles were sampled during a last simulated daytime period of acclimation to the given regime (LI-LT, HI-LT, HI-HT, and HI-HT2). The left-hand side column presents daily courses of PRI for measurements in chamber one, the right-hand side column shows PRI measurements in chamber two. Central lines represent the medians, boxes represent 50% of the data, and whiskers represent the minimum and maximum values. Dark dots connected with the straight line represent an average of 7-10 values measured during each cycle. Data followed by the same letter indicate statistically insignificant differences ($P < 0.05$; Mann–Whitney U-test).

Pozn: v textu se odvolavame na rozdíly významné, ale někde jsou hodnoty takové, že významný rozdíl neukazují, např komora 1 LT-LT poledne vs komota 1 HI-LT tma – je tam jedna až pár hodnot, které by mohly výstup zpochybnit, ale pro více jak 90 procent údajů to neplatí

-> pokud je potřeba udelej změnu v textu taká by to odpovídalo grafu!!!

Fig. 6: Daily course of ANMB₅₀₇₋₅₅₆ computed from the database of 205 measured reflectance spectra. Needles were sampled during the last simulated daytime period of acclimation to the given regime (LI-LT, HI-LT, HI-HT, and HI-HT2). The left-hand side column presents daily courses of ANMB₅₀₇₋₅₅₆ for measurements in chamber one, the right-hand side column shows measurements in chamber two. Central lines represent the medians, boxes represent 50% of the data, and whiskers represent the minimum and maximum values. Dark dots connected with the straight line represent an average of 7-10 values measured during each cycle. Data followed by the same letter indicate statistically insignificant differences ($P < 0.05$; Mann–Whitney U-test).

Pozn: nicneříkající statistika, není zdůvodněno v textu proč byla počítána, co říká

-> zmíním v textu srovnávací test, předpokládám že všechny P pro Mann–Whitney U-test jsou < 0.05 ne $> ???!!!$... potřeba prekontrolovat v celém textu i pro T-test!!!

Fig 7. A) Reflectance signatures measured for needle samples of the same gap fraction size (0.51) at three DEPS levels (30, 50 and 85%), B) continuum-removed reflectance normalized to the maximum band depth (MBD) between 507 and 556 nm for the same reflectance signatures, C) MBD normalized continuum-removed reflectance of needle samples with DEPS equal to 50% and 85% subtracted from the same reflectance transformation of a sample with DEPS equal to 30% (calculated from the curves of Fig. 7B).

Fig. 8: Dependency between (A) mean values of PRI and DEPS ($n = 24$), and (B) mean values of ANMB₅₀₇₋₅₅₆ and DEPS ($n = 24$). Average values and standard deviation of both vegetation indices and DEPS are calculated from 5-10 measurements acquired during daily courses of each treatment.

Fig. 9: Daily changes in quantum yield of photosystem II photochemistry (Φ_{PSII}) for plants acclimated to the particular LI-LT, HI-LT, HI-HT and HI-HT2 regime. (A) measurements of chamber one, (B) measurements of chamber two. Values are means $\pm$ SD for 5 needle samples examined per acclimation regime at the given time of the day. Data followed by the same letter indicate statistically insignificant differences ($P < 0.05$; Student's t-test).

Fig. 10: Linear regression between quantum yield of photosystem II photochemistry (Φ_{PSII}) and A) PRI, and B) ANMB₅₀₇₋₅₅₆ calculated from directional-hemispherical reflectance of spruce needles acclimated to the LI-LT, HI-LT, HI-HT and HI-HT2 regimes (n=18). Average values and standard deviation of both vegetation indices and Φ_{PSII} are calculated from 5-10 measurements acquired for each treatment.