We investigated changes in chlorophyll fluorescence from alternate leaf surfaces to assess the intraleaf light acclimation patterns in combination with natural variations in radiation, leaf angles, leaf mass per area (LMA), chlorophyll content (Chl) and leaf optical parameters. Measurements were conducted on bottom- and top-layer leaves of *Tilia cordata* Mill. (a shade-tolerant sub-canopy species, sampled at heights of 11 and 16 m) and *Populus tremula* L. (a light-demanding upper canopy species, sampled at canopy heights of 19 and 26 m). The upper canopy species *P. tremula* had a six times higher PSII quantum yield ($\Phi_{II}$) and ratio of open reaction centres ($qP$), and a two times higher LMA than *T. cordata*. These species-specific differences were also present when the leaves of both species were in similar light conditions. Leaf adaxial/abaxial fluorescence ratio was significantly larger in the case of more horizontal leaves. *Populus tremula* (more vertical leaves), had smaller differences in fluorescence parameters between alternate leaf sides compared with *T. cordata* (more horizontal leaves). However, optical properties on alternate leaf sides showed a larger difference for *P. tremula*. Intraspesifically, the measured optical parameters were better correlated with LMA than with leaf Chl. Species-specific differences in leaf anatomy appear to enhance the photosynthetic potential of leaf biochemistry by decreasing the interception of excess light in *P. tremula* and increasing the light absorptance in *T. cordata*. Our results indicate that intraleaf light absorption gradient, described here as leaf adaxial/abaxial side ratio of chlorophyll fluorescence, varies significantly with changes in leaf light environment in a multi-layer multi-species tree canopy. However, this variation cannot be described merely as a simple function of radiation, leaf angle, Chl or LMA, and species-specific differences in light acclimation strategies should also be considered.

**Keywords:** chlorophyll fluorescence, chloroplast acclimation, leaf inclination angle distribution, leaf optics, photochemical quenching, reflectance, transmittance, within-canopy variability.
Most plant models use the assumption of spherical leaf angle distribution for the leaf canopy, but this approach may cause an underestimation of vertically transmitted light (Stadt and Lieffers 2000), meaning that the calculations for the daily amount of photosynthesis are also biased (Sarlikioti et al. 2011).

Another assumption that is occasionally used in modeling exercises for estimating the photosynthetic performance of plants is that the non-destructive and timesaving fluorescence measurements of electron transport rate in the absence of photorespiration relate well to the quantum efficiency of photosynthesis measured gasometrically (Genty et al. 1989, Edwards and Baker 1993). However, numerous factors can change the shape of this relationship (Seaton and Walker 1990, Edwards and Baker 1993, Evans 1993, Maxwell and Johnson 2000, Tsuyama et al. 2003, van der Tol et al. 2009). Tsuyama et al. (2003) showed that the relationship between assimilation of CO_2 and fluorescence-based measurements of electron transport rate is affected the most by chlorophyll content and leaf structure. They proposed that the measurements of gas exchange and chlorophyll fluorescence detect signals from different populations of chloroplasts in a leaf. Already, Evans et al. (1993) proposed that fluorescence-based photosynthesis estimates from leaf adaxial side possibly provide underestimations, since the measuring beam does not penetrate deep enough into the leaf. Tsuyama et al. (2003), on the other hand, pointed out that the mere spatial restriction of the origin of the fluorescence measure as such is not enough to cause deviations from linearity in plots of quantum efficiency measured by fluorescence versus quantum efficiency of CO_2 but the non-linearity clearly depends on the chloroplasts at the illuminated surface.

Instead of using the common assumptions of spherical leaf angle distribution and homologous internal morphology of leaves, we investigate the natural variation in leaf angles, chlorophyll content, structure and optical traits of leaves resulting from the different light environments in a canopy vertical gradient and the corresponding leaf fluorescence from alternate leaf surfaces. Since ordinary chlorophyll fluorescence equipment measures photosynthetic properties within a relatively thin leaf layer, measurements from alternate leaf surfaces should reflect the intraleaf gradient of acclimation. We wanted to check a previously raised hypothesis, that the difference in fluorescence parameters measured from alternative surfaces of the leaf depends on the difference in average photon flux density at the two surfaces and should therefore be a function of leaf angle (Myers et al. 1997, Tsuyama et al. 2003). We emphasize the effects of two light gradients: (i) vertical light gradient within a canopy and (ii) light gradient inside a leaf between the abaxial and adaxial surfaces.

Materials and methods

Study area and canopy heights

Material for the study was collected from a deciduous mixed stand at Järvselja (58°22′N, 27°20′E, elevation 38–40 m), Estonia in July to August 1999. The canopy, with a total height of 27 m, had two distinct layers: the upper layer consisting mainly of dominating Populus tremula L. foliage (19–27 m) and a subcanopy layer of Tilia cordata Mill. (7–17 m). Leaves were mature and did not show visible signs of senescence. Trees were reached from permanent scaffoldings (height 25 m). Measurements were carried out for the top and bottom layers of both species (at heights of approximately 11 and 16 m for Tilia and 19 and 26 m for Populus ±1 m variation inside all height classes depending on branches accessible from the scaffolding).

Light conditions

Long-term light conditions at these four heights of canopy (11 and 16 m in Tilia, and 19 and 26 m in Populus foliage) were estimated by hemispherical photographic technique using Nikon Coolpix 900 digital camera equipped with a Nikon Fisheye Converter and analysis software Winscanopy 2.0 (Quebec, Canada). Light conditions were estimated using photographs taken at one time from above 10 leaves from each measured canopy layer (the same leaves that were used for fluorescence and pigment analysis). Direct radiation was calculated using analysis software from canopy gap fractions along solar tracks during 2-month intervals beginning from the summer solstice. Daily diffuse radiation was estimated from the proportion of visible sky, assuming a standard overcast sky model (Anderson 1966). As daily diffuse radiation was in good relation with direct radiation ($r = 0.85, P < 0.001$), canopy light conditions in present study were characterized by estimations of diffuse radiation, which showed less spatial and temporal variability than estimations of direct radiation.

Leaf angle distribution

The zenith angles of leaf blade to normal were measured using a protractor. One hundred leaves were measured in each canopy layer. For each foliage height, leaf inclination angle distribution was fitted using an ellipsoidal function, which is based on the assumption that the distribution of leaf angles in a canopy is identical to the angles of normal to small area elements on the surface of an ellipsoid (Campbell 1986). A single parameter, $x = b/a$, is required to describe the shape of the distribution; $b$ is the horizontal semi-axis of the ellipsoid, and $a$ is the vertical semi-axis. When $x = 1$, the ellipsoidal distribution becomes a spherical distribution, when $x > 1$, the ellipsoidal distribution becomes an oblate spheroid. First, theoretical leaf angle distributions were calculated for various $x$ values. To find the best theoretical approximation (and
respective $x$) the experimental data were fitted by minimizing the $\chi^2$ parameter. Inclination angles were measured also in the set of leaves used later in chlorophyll fluorescence and pigment measurements.

**Chlorophyll fluorescence**

Fluorescence yield from both leaf sides of at least 10 leaves from each canopy layer was measured in situ using a portable pulse-modulated fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). Minimum fluorescence yield ($F_0$) and maximal fluorescence yield ($F_m$) were measured after a darkening period of 10 min. PAM-2000 internal halogen actinic light source was switched on for 5 min on an intensity level of 1000 $\mu$mol m$^{-2}$ s$^{-1}$. Then fluorescence yield $F_v$, maximal ($F_m'$) and minimum fluorescence ($F_o'$) were measured.

Quantum yield of dark-adapted leaves ($F_v/F_m$) and light-adapted leaves ($\Phi_l$) was calculated from the equations of Genty et al. (1989). Photochemical quenching ($q_P$) and non-photochemical quenching (NPQ) were calculated according to Bilger and Björkman (1990). All fluorescence measurements were carried out between 9 AM and 7 PM (measuring period shortened with the shortening of natural daylight period). Measurements were randomized so that leaves from each layer were measured at various times of the day and on various days of the measurement period. The same protocol was used for measuring both leaf adaxial and abaxial sides. The time dependency of measured parameters was checked and temperature adjustment was applied to the fluorescence data according to Niinemets et al. (1999). As no significant correlations of fluorescence parameters with time or temperature were found (data not shown), the time and temperature dependency of fluorescence parameters was neglected in this study.

**Leaf mass per area**

After field measurements were finished, all leaves measured in situ were harvested and the area of each leaf was measured using a desk-scanner (ScanJet 4c, Hewlett Packard, Palo Alto, CA, USA) and an in-house computer program. Each leaf was dried to constant mass at 80 °C and leaf mass was measured for the determination of leaf mass per area (LMA) (g m$^{-2}$).

**Pigment analysis**

Leaf-discs (1 cm$^2$) were cut from every harvested leaf (at least 10 leaves of fluorescence measurements and 5 leaves of optics measurements from each canopy layer) and stored at $-18$ °C. Leaf chlorophyll was extracted in 80% aqueous acetone buffered with sodium phosphate (2.5 mM, pH 7.8), measured spectrophotometrically (S2000, Ocean Optics, Dunedin, FL, USA) and calculated according to the equation of Porra et al. (1989). A phosphate buffer was used to avoid the transformation of chlorophylls into pheophytins.

**Leaf optics**

Due to technical limitations, leaf optics were measured in another set of leaves in the year 2000. Five leaves at the same heights as the measurements of the previous year were collected. Leaf transmittance and reflectance spectra were measured with an integrating sphere (ISP-80-8-R, Ocean Optics) and fibre optic spectrophotometer (S2000, Ocean Optics). We used white reflectance standard (WS-2, Top Sensor Systems, Eerbeek, The Netherlands) for reflectance measurements. Although a wider range of spectra was measured and analysed, we have only shown reflectance parameters which were averaged from the wavelength range of 655–665 nm (red spectral region—the range of the strongest absorption by chlorophyll) and 550–560 nm (green spectral region) for the sake of clarity. Sample absorption at the same wavelength ranges was calculated as absorbance = $(1 – \text{reflectance} – \text{transmittance})$. Leaf spectral properties were measured from both leaf sides. For each leaf LMA and pigments content were determined as described above. Leaf mass per area and pigments content did not differ significantly between years (data not shown).

**Results**

**Light distribution and leaf characteristics**

In a joined canopy of *T. cordata* (shade tolerant) and *P. tremula* (light-demanding), relative diffuse radiation increased with increasing height ranging from 32% in the lower layer of *T. cordata* measured at 11 m to 68% in the upper layer of *P. tremula*, measured at 26 m (Figure 1a). However, light conditions between the two intermediate layers—the top layer of *T. cordata* at a height of 16 m and the bottom layer of *P. tremula* at 19 m—did not differ significantly (Figure 1a), probably due to the forest canopy structure. At a height of 16–19 m there was a leafless gap between the canopies of *P. tremula* and *T. cordata*.

Leaf mass per area and chlorophyll content per leaf area (Chl$_a$) were highest in the top layer of *P. tremula* and lowest in the bottom layer of *T. cordata*; however, within-species differences in LMA and Chl$_a$ between the two height levels were not statistically significant (Figure 1b, c). *Populus tremula* had two times higher LMA than *T. cordata* (Figure 1b).

When the leaf angle distribution of a wider selection of leaves ($N = 100$ in each canopy layer) was approximated with an ellipsoidal function, we found parameter $x$ decreasing with height in both species (Figure 2). In the upper layer of *P. tremula*, leaves were more pendant ($x = 0.72$), while in the lower layer of *P. tremula*, an almost spherical distribution of leaf inclination angles was detected ($x = 0.96$). Leaves of shade-tolerant *T. cordata* were in general more horizontal ($x = 4.8$ in the top layer and $x = 5.1$ in the bottom layer of *T. cordata*). The inclination angle of the smaller set of leaves used for chlorophyll fluorescence and
pigment measurements, was highest in the upper layer of *P. tremula* and lowest in the upper layer of *T. cordata* (Figure 2), similar significant difference between leaf angles of different species was found also in wider set of leaves (Kruskal–Wallis analysis of variance (ANOVA): *P* < 0.05), but in either of the datasets differences between two heights of single species were not statistically significant. One could expect the smallest leaf inclination angles in the bottom layer of *T. cordata*, as was the case in the larger set of leaves used for angle distribution calculations (fluorescence measurements were performed on a smaller set of leaves, explanation in Materials and methods, Figure 2). However, it appeared that the smaller random selection of leaves in the lowest canopy layer had slightly higher average inclination angles than those in the upper layer of *T. cordata* (Figure 2), but the difference was not statistically significant.

**Chlorophyll fluorescence**

Chlorophyll fluorescence parameters measured from the adaxial side of the leaf revealed significant differences between species. Quantum yield of light-adapted leaves (*Φ*<sub>II</sub>) and photochemical quenching coefficient (*q*<sub>P</sub>) were six times higher in the *P. tremula* than in the *T. cordata* canopy (Figure 1d–f). It is also important to note that these fluorescence parameters differed between canopy layers at a height of 16 m (the top layer of *T. cordata*) and at a height of 19 m (the bottom layer of *P. tremula*) (Figure 1d–f), where light conditions did not differ (Figure 1a), indicating that differences in *Φ*<sub>II</sub> and *q*<sub>P</sub> are species specific. Non-photochemical quenching had a maximal value in the top layer of *T. cordata* and a lowest value in the top layer of *P. tremula* (Figure 1f). Non-photochemical quenching also differed between top and bottom layer of the *T. cordata* canopy, but not in the canopy of *P. tremula*. Maximum photochemical efficiency of PSII (*F<sub>v</sub>/F<sub>m</sub>*) did not differ between species or different canopy layers.

**Leaf adaxial/abaxial side fluorescence**

We looked for differences between leaf adaxial and abaxial side measurements of chlorophyll fluorescence. We found that *Φ*<sub>II</sub> measured from leaf adaxial side was 1.6 times higher in
P. tremula and two times higher in T. cordata compared with abaxial side measurements, but the difference between species was not significant (Figure 3), nor did we find significant differences in adaxial/abaxial ΦII between measured canopy layers of either species. However, ΦII ratio of different leaf sides had a significant negative correlation with leaf angle when data from leaves of both species were pooled (Figure 3). The correlation remained significant for the P. tremula canopy, but was not significant in the T. cordata canopy (Figure 3). qP measured from leaf adaxial side was significantly larger than that measured from the abaxial side in both species and, like the ΦII ratio of different leaf sides, qP ratio was also negatively correlated with leaf angle (Figure 3). There was a significant species-specific difference, since the value of qP ratio was 1.5 times higher in T. cordata than in P. tremula (Figure 3). Non-photochemical quenching measured from leaf adaxial side was significantly larger than that measured from the abaxial side in the canopy of T. cordata, while in the canopy of P. tremula NPQ did not differ between alternate leaf sides (Figure 3). However, NPQ ratio of different leaf sides was clearly negatively correlated to leaf angle (Figure 3). If we looked at different species separately, the correlation remained significant in the canopy of P. tremula, but not in T. cordata (Figure 3). In the canopy of T. cordata NPQ ratio had a significant positive correlation with relative diffuse radiation ($r = 0.62, P < 0.001$) and LMA ($r = 0.52, P < 0.05$). No significant differences were found in adaxial/abaxial Fv/Fm when comparing different species. Our data did not reveal significant differences between different canopy layers of a single species when we looked at the adaxial/abaxial ratios of any fluorescence parameters. It was apparent that these fluorescence parameters (qP and ΦII) that had the strongest differences between species (Figure 1), also had the largest average adaxial/abaxial ratio (Figure 3).

Leaf optics

Spectral measurements showed that leaves of T. cordata absorbed more light than the leaves of P. tremula (Table 1), despite having a lower chlorophyll content (Figure 1c). When illuminated from the abaxial side, leaf absorbance was lower and reflectance was higher compared with the adaxial side in both species (Table 1). Differences between optical properties of adaxial and abaxial leaf surfaces were larger in P. tremula compared with T. cordata (Table 1).

Intraspecifically reflectance and absorptance were better related to LMA than Chl for both species and both wavelength regions (Table 2, Figure 4). However, chlorophyll content was clearly a better predictor for leaf optical properties when both species were pooled (Table 2, Figure 4). Chl and LMA were strongly correlated in both species (P. tremula $r = 0.79, P < 0.05$; T. cordata $r = 0.65, P < 0.05$). When both Chl and LMA were incorporated into multiple regression models, LMA still had a significant ($P < 0.05$) positive effect on leaf adaxial side absorbance in both wavelength regions (red and green) and within both species. Due to the strong effect of species on the relationship between leaf absorbance and LMA (Type III general linear model on leaf adaxial absorbance: species × LMA interaction $P < 0.005$), LMA, which was a good estimate for leaf optical parameters of individual species, was not suitable when species were pooled (Figure 4).

Discussion

Leaf adaxial/abaxial side fluorescence

Our results showed significant differences in leaf adaxial/abaxial side ratio of fluorescence parameters, referring to
varying intraleaf light acclimation profiles of chloroplasts at different canopy positions while the differences depended significantly on species. Similarly light adaption profile along canopy light availability gradient appeared to be strongly species dependent, since chlorophyll content, LMA and chlorophyll a fluorescence ($\Phi_{II}$ and $qP$) measured from leaf adaxial side appeared to differ mainly between species. Our findings, that $\Phi_{II}$ and $qP$ measured from leaf abaxial side were lower than for the adaxial side, indicated that chloroplasts near the abaxial surface were more shade adapted and had lower photosynthetic capacities than chloroplasts near the adaxial leaf surface of both species. Furthermore, as the ratio of adaxial/abaxial $\Phi_{II}$ and $qP$ of leaves varied through the forest canopy, the interpretation of chlorophyll a fluorescence signals that are usually measured on the adaxial sides of leaves becomes complicated. The origin of the chlorophyll fluorescence signal depends both on the depth of penetration and on the absorption of irradiance within the leaf and also on the emission and re-absorption of fluorescent light, and it changes with differences in the excitation wavelength (Vogelmann and Han 2000, Buschmann 2007, Peguero-Pina et al. 2009), chlorophyll content (Evans 1999, Vogelmann and Evans 2002) and leaf structure (Cui et al. 1991). As a result, chlorophyll fluorescence signals are derived mainly from the surface layers, since

| Table 1. Reflectance and absorptance measured from alternate leaf surfaces and the ratio between leaf adaxial and abaxial side reflectance and absorptance averaged from wavelength range of 655–665 nm (red spectral region) and 550–560 nm (green spectral region). Mean and SD are shown (N = 5). Asterisks denote a significant difference between leaf sides (Wilcoxon signed rank test: $P < 0.001$). Species with the same letter were not significantly different (Kruskal–Wallis ANOVA by ranks: $P > 0.05$). |
|----------------------------------|----------------------------------|----------------------------------|
| | Leaf adaxial side | Leaf abaxial side | Adaxial/abaxial |
| | Tilia cordata | Populus tremula | Tilia cordata | Populus tremula | Tilia cordata | Populus tremula |
| Green reflectance | 0.12 ± 0.008<sup>a</sup> | 0.13 ± 0.02<sup>b</sup> | 0.18 ± 0.01<sup>a</sup> | 0.24 ± 0.01<sup>b</sup> | 0.63 ± 0.05<sup>a</sup> | 0.54 ± 0.06<sup>b</sup> |
| Red reflectance | 0.05 ± 0.004<sup>a</sup> | 0.05 ± 0.006<sup>b</sup> | 0.12 ± 0.01<sup>a</sup> | 0.10 ± 0.01<sup>b</sup> | 0.48 ± 0.05<sup>a</sup> | 0.43 ± 0.07<sup>b</sup> |
| Green absorptance | 0.73 ± 0.04<sup>a</sup> | 0.72 ± 0.03<sup>b</sup> | 0.65 ± 0.04<sup>a</sup> | 0.61 ± 0.02<sup>b</sup> | 1.12 ± 0.02<sup>a</sup> | 1.18 ± 0.04<sup>b</sup> |
| Red absorptance | 0.92 ± 0.01<sup>a</sup> | 0.90 ± 0.009<sup>b</sup> | 0.86 ± 0.02<sup>a</sup> | 0.83 ± 0.01<sup>b</sup> | 1.07 ± 0.01<sup>a</sup> | 1.08 ± 0.02<sup>b</sup> |

| Table 2. Correlations between leaf optical parameters measured from leaf adaxial side (reflectance and absorptance averaged from wavelength range of 655–665 nm—red spectral region, and 550–560 nm—green spectral region) and LMA and chlorophyll content per leaf area (Chl<sub>s</sub>). Asterisks denote significant correlations where ***means $P < 0.001$, **$P < 0.01$ and *$P < 0.05$, ns means not significant values of $P$. |
|----------------------------------|----------------------------------|----------------------------------|
| | Tilia cordata | Populus tremula | Both species |
| | LMA | Chl<sub>s</sub> | LMA | Chl<sub>s</sub> | LMA | Chl<sub>s</sub> |
| Green reflectance | $-0.80^{***}$ | $-0.75^{***}$ | $-0.74^{***}$ | $-0.59^{**}$ | ns | $-0.37^{*}$ |
| Red reflectance | $-0.65^{**}$ | ns | $-0.47^{*}$ | ns | ns | ns |
| Green absorptance | $0.94^{***}$ | $0.80^{***}$ | $0.80^{***}$ | $0.66^{**}$ | ns | $0.68^{**}$ |
| Red absorptance | $0.86^{***}$ | $0.64^{**}$ | $0.72^{***}$ | $0.55^{*}$ | $-0.43^{**}$ | ns |

Figure 4. Relationships between leaf absorptance measured from leaf adaxial and abaxial sides (absorptance averaged from wavelength range of 655–665 nm—red spectral region, and 550–560 nm—green spectral region) and LMA and chlorophyll content per leaf area (Chl). Black dots representing T. cordata, empty dots P. tremula. Corresponding statistical parameters (mean, SD and correlation coefficients) are shown in Tables 1 and 2.
even high-light-grown leaves accumulate a significant proportion of low-light-acclimated chloroplasts due to leaf internal light gradient. When we further investigated the natural variation in acclimation profiles of chloroplasts (by measuring leaf adaxial/abaxial ratios of fluorescence) we found that differences in $\Phi_i$ and $qP$ measured from alternate leaf sides were smaller in more vertically oriented leaves. As leaf inclination angle controls the proportion of irradiance that strikes the upper and lower leaf surfaces, reducing excess light on the adaxial leaf surface and allowing more light to be utilized by chloroplasts located near the abaxial surface, more vertical leaf inclination angle indeed should reduce the adaxial/abaxial ratio of fluorescence parameters.

Traditionally, the leaf has been taken as a photosynthesizing unit and in several ecological plant or community models the fact that a leaf consists of a population of chloroplasts with variable properties resulting from intraleaf light gradient has been ignored (de Pury and Farquhar 1997, Kull 2002). Our results suggest the need for more extensive investigations on actual intraleaf light adaptation profiles of chloroplasts and their relationship to fluorescence parameters measured from adaxial side of leaf in order to avoid generating systematic errors where different correlative relationships are extrapolated over whole canopies or whole ecosystems. This is because differences in the light conditions of different sub-populations of chloroplasts can change the correlation between whole-leaf photosynthetic properties and the photosynthetic properties of individual chloroplasts near the leaf surface (Pequero-Pina et al. 2009). Thus, when comparing fluorescence signals derived from the surface layers with data that are not derived from the same sub-population of chloroplasts, for example, the whole-leaf gas-exchange measurements, some caution is needed. The origin of fluorescence signal becomes especially important in the light of recent attempts that are made with fluorescence imaging of whole plant (Baker 2008) or assessing terrestrial photosynthesis from space (Grace et al. 2007). Our results also indicate that the population of chloroplasts which are represented by fluorescence measurements might vary in multi-species systems, not merely because of different light intensity histories of single leaves, but also because of different within-leaf light-acclimation strategies of species (Uemura et al. 2006), which affect the steepness of leaf-internal light absorption gradient of leaves of different species.

Not all fluorescence parameters presented similar variations in adaxial/abaxial ratios within the forest canopy. Non-photochemical quenching measured from adaxial and abaxial surface did not differ in leaves of $P. tremula$, which was in accordance with our results that NPQ was also not related to vertical canopy light gradient in $P. tremula$. In the canopy of $T. cordata$, NPQ increased with increasing irradiance in the canopy light gradient, and we found a similar response to leaf internal light gradient. In the leaves of $T. cordata$, NPQ measured from the adaxial leaf surface was 1.5 times higher than that from the abaxial surface. Dark-adapted maximum quantum yield ($F_v/F_m$) did not differ between alternate leaf sides (data not shown), indicating that chloroplasts near adaxial and abaxial leaf surfaces were adequately acclimated to their respective light conditions. Our measurements suggest that, if we use fluorescence-based measurements of leaf surface for estimations of whole-leaf performance, more attention should be paid to possible errors in the fluorescence parameters that also have the strongest gradients in the plant canopy, or largest differences between species.

**Leaf optics**

Leaf optical properties are often used to describe the combined impact of leaf anatomy and biochemistry on light penetration through the leaf (Govaerts et al. 1996). Our measurements showed that both species had higher reflectance and lower absorptance when illuminated from leaf abaxial side, compared with adaxial side in both wavelengths: the difference between leaf sides was greater for $P. tremula$, which means that the change in leaf angle that allows a greater proportion of light to strike the abaxial surface results in a greater reduction in excess light through enhanced reflectance from the abaxial surface in $P. tremula$. In their multi-species synthesis Falster and Westoby (2003) showed that steeper leaf angles function more to reduce exposure to excess light, rather than to maximize carbon gain. Similarly, our fluorescence measurements revealed that the large differences in reflectance of alternate sides of $P. tremula$ leaves helped to counterbalance the effect of asymmetric internal anatomy of dorsiventral leaves by reducing the inhibitive effect of excess light on the abaxial side of the leaf and resulted in smaller differences in fluorescence parameters measured from leaf adaxial and abaxial surfaces. For different species the means of enhancing leaf reflectance may vary from changed epidermal structures at the leaf surface, such as leaf hairs (Ehleringer et al. 1976) or waxes (Cameron 1970), to different anatomical features, like a separation between the epidermis and mesophyll (Vogelmann 1993).

Light absorption gradient within a leaf depends strongly on leaf chlorophyll content and leaf anatomy, including the arrangement of chloroplasts (Brodersen and Vogelmann 2010). Based on the chlorophyll content, it would be expected that the leaves of $P. tremula$ and $T. cordata$ should have relatively similar absorptance, since, although the leaves of $T. cordata$ had a slightly lower chlorophyll content per leaf area, the difference was minimal. Indeed, we found that $P. tremula$ and $T. cordata$ had similar absorptances in the green spectral region when illuminated on the adaxial leaf surface. However, at a strongly absorbing wavelength (red spectral region) absorptance is unlikely to vary much with chlorophyll, which is...
consistent with our findings of a better correlation of chlorophyll with absorptance measured for the green spectral region. As we found a significant individual effect of LMA, and LMA × species interaction on leaf absorptance, we assumed that leaf structural parameters (for example, the arrangement of chloroplasts), which determine the light profile inside the leaf, can be best estimated by LMA for individual species (also reported by Souza and Válio 2003). As P. tremula had somewhat lower absorptance, but higher LMA, when compared with T. cordata, this means that leaf internal light gradient had to be less steep in leaves of P. tremula. This could influence the depth within the leaf, where the fluorescence signal is retrieved. The finding that P. tremula leaves somehow managed to more efficiently homogenize within-leaf light environment can be attributed to the sieve effect (Das et al. 1967), since the pigment distribution in leaves of P. tremula may be somewhat different from T. cordata. However, our optical measurements revealed that the differences between leaf sides were larger for P. tremula, while fluorescence parameters differed more between the alternate leaf sides of T. cordata. Therefore, the difference in optical properties between leaf sides did not cause all of the difference in fluorescence. It is possible that a fraction of the differences in leaf adaxial/abaxial fluorescence ratios can account for the specifics of fluorescence measurements, since, a less steep internal light gradient in P. tremula means that a larger fraction of fluorescence light is also reabsorbed on its way back to the measuring instruments. Buschmann (2007) has shown that chlorophyll fluorescence, which is generated deep within the mesophyll, would be partially re-absorbed (especially measurements made at 680 nm). This re-absorption is caused by the overlapping of the short-wavelength range of the chlorophyll fluorescence emission spectrum with the long-wavelength range of the chlorophyll absorption spectrum. As a result, our study indicates the need for more complex multi-species research on factors that could cause variations in the origin of the fluorescence signal in a dorsiventral leaf.

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Conflict of interest

None declared.

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