Near-distance imaging spectroscopy investigating chlorophyll fluorescence and photosynthetic activity of grassland in the daily course

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Abstract. Detection of grassland canopy chlorophyll fluorescence (Chl-F) conducted with an imaging spectroradiometer provided evidence of potential remote sensing estimation of steady-state Chl-F (Chl-F*). Daily near-nadir views of extremely high spatial resolution hyperspectral images were acquired from a distance of 4 m for temperate montane grassland in the Czech Republic. Simultaneously, measurements of Chl-F and total chlorophyll content (Chl-a+b) were made on a single leaf at ground level were collected. A specifically designed ‘shade removal’ experiment revealed the influence of dynamic physiological plant processes on hyperspectral reflectance of three wavelengths: 532, 686 and 740 nm. Based on this information, the vegetation indexes R686/R630, R740/R800 and PRI calculated as (R532−R750)/(R532+R750) were tested for statistical significance with directly measured Chl-F parameters (maximum fluorescence yield, Fm/Fm′; steady-state chlorophyll fluorescence, Chl-Fs and actual quantum yield, Φs). The grassland species under investigation were: Festuca rubra agg. (L.), Hieracium sp., Plantago sp., Nardus stricta (L.) and Jacea pseudophrygia (C.A. Meyer). The coefficients of determination (R2) for best-fit relationships between PRI-Φs and PRI-Chl-Fs, measured in the daily course, show a high variability of 0.23–0.78 and 0.20–0.65, respectively. Similarly, R2 for the R686/R630-Φs and R686/R630-Chl-Fs relationships varied between 0.20–0.73 and 0.41–0.70, respectively. The highest average R2 values were found between PRI and Chl-a+b (0.63) and R686/R630 and Chl-a+b (0.72). The ratio R740/R800 did not yield a statistically significant relation with Chl-F parameters.

Additional keywords: actual fluorescence yield, chlorophyll fluorescence, grassland ecosystem, hyperspectral remote sensing, vegetation indexes.

Introduction

One of the most important key players in ongoing global climate change (IPCC 2007) is the global productivity of the terrestrial ecosystem (Running et al. 1999). Recent evidence suggests decreasing carbon sink capacity of the biosphere at local scale up to global scale (Canadell et al. 2007). For this reason, the remote sensing scientific community is reinforcing efforts to capture spatial and temporal dynamics in photosynthetic processes and carbon cycling of the terrestrial vegetation using air- and spaceborne image data (e.g. Grace et al. 2007). For example, the remotely sensed estimation of the light use efficiency (LUE, calculated as CO2 assimilation rate per incident photosynthetic photon flux density), regarded as an important parameter for the estimation of global carbon assimilation (Monteith 1977), is expected to greatly improve the accuracy of the global productivity estimation (e.g. Ahl et al. 2004).

The significant relations between remotely sensed reflectance signals and rapid physiological processes in plants have been found at spectral regions of three wavelengths: 530, 690 and 740 nm (Gamon et al. 1990; Zarco-Tejada et al. 2000a, 2000b). The reflectance changes in the region around 531 nm were attributed to the photo-protective reaction forming the zeaxanthin pigment and affecting the LUE (Gamon et al. 1992), but more recently also to the chlorophyll fluorescence (Chl-F) parameters, such as non-photochemical quenching (NPQ) and actual quantum yield (Φs) of PSII (Evain et al. 2004). The reflectance changes at the wavelengths of 690 nm and 740 nm were related mainly to Chl-F emissions originating
from PSII (Zarco-Tejada et al. 2000a). The changes in zeaxanthin concentration were related to the reflectance intensity at 531 nm by means of the physiological (also referred to as plant or photochemical) reflectance index (PRI), calculated as $(R_{670} - R_{690})/(R_{670} + R_{690})$, where $R_{670}$ is the reflectance at 570 or 550 nm (Gamon et al. 1992; Gamon and Surius 1999). The use of this vegetation index has been verified by strong leaf level experimental evidence. It is possible to estimate the vegetation LUE from spectrometric leaf and to some extent, from canopy measurements in daily and seasonal courses, when applied to several plant species growing in various environmental conditions (Grace et al. 2007; Suarez et al. 2008). Nevertheless, as shown in scenarios modelled by Barton and North (2001) using canopy-level radiative transfer models and in further experimental studies (Filella et al. 2004), the application of remotely sensed PRI as an actual measure of photosynthesis or LUE (e.g. Drolet et al. 2005) is far from being operational, owing to high sensitivity to the non-physiological factors and other features commonly associated with the remote sensing acquisitions (e.g. angular anisotropy of the vegetation canopy reflectance, sun-target-sensor geometry, spectral mixing with a non-photosynthetic biomass, atmospheric effects). Recently, PRI has been shown to be a robust proxy of LUE only when photochemical efficiency of PSII is well coordinated with carbon assimilation (Guo and Trotter 2004, 2006; Inoue and Penuelas 2006).

Numerous studies provided evidence for a possible detection of the Chl-F signal at 690 and 740 nm wavelengths from the leaf (e.g. Buschmann et al. 1994), but also canopy reflectance measurements (Gamon and Surius 1999; Zarco-Tejada et al. 2000a, 2000b, 2002, 2003; Dobrowski et al. 2005). For instance, the ratio of reflectance at 690 nm and at 630 nm ($R_{690}/R_{630}$), designed to track changes in steady-state Chl-F (Chl-$F_{s}$), was identified as a useful indicator of early stress reactions of plants (Zarco-Tejada et al. 2000b). Similarly, the vegetation indices (VIs), such as $R_{695}/R_{430}$ and $R_{695}/R_{760}$, are statistically linked to the early vegetation stress reactions (Carter 1994), however, there is no known causal physiological explanation for these relations. Recently, Dobrowski et al. (2005) used a small canopy experiment to investigate a non-invasive detection of early heat-stress reactions of plants. It revealed a statistically significant correlation between Chl-$F_{s}$ and VIs $R_{740}/R_{630}$ and $R_{690}/R_{600}$, whereas a correlation of low significance was found in the case of PRI.

It has been suggested that the Chl-$F_{s}$ signal is capable of tracking changes in the photosynthesis performance induced by actual heat and water stress influences (Flexas et al. 2000, 2002; Dobrowski et al. 2005). A recent seasonal study by Soukupová et al. (2008) showed that Chl-$F_{s}$ can potentially be an accurate phenological indicator of the beginning and end of a photosynthetically active period of overwintering evergreen temperate plants. Further, Moya et al. (2004) found that relative Chl-F yield, retrieved passively at oxygen atmospheric absorption line (760 nm) using a method of Frauenhofer line discriminator principle, may indicate the stress reactions of maize plants. Still, plant physiologists consider Chl-$F_{s}$ as being rather a general non-specific signal integrating information on several photosynthetic processes (Papageorgiou and Govindjee 2004).

‘Process-related’ remote sensing techniques focus on monitoring of physiological plant processes using aircraft and/or satellite sensors. Several airborne/ground experimental campaigns, e.g. the European Space Agency SEN2FLEX campaign in 2005, have been aiming their estimation of LUE and Chl-$F_{s}$ at vegetation reflectance (Zarco-Tejada et al. 2000b; Grace et al. 2007). Despite this, canopy measurements conducted in the daily course at very high spatial resolution are necessary to reveal the complexity of the Chl-$F_{s}$ signal detection under the natural environmental conditions. In this respect, the performance of VIs proposed for remote estimation of LUE (i.e. PRI) and Chl-$F_{s}$ (i.e. $R_{690}/R_{600}$ and $R_{740}/R_{600}$) is of importance. Thus, the first objective of this study was to investigate the relationships between Chl-F and reflectance signals of vegetation measured at the level of single leaves. Within the second objective we attempted the detection of a natural grassland sun-induced Chl-F signal using a commonly available imaging spectroradiometer. Finally, in daily course performance we compared selected vegetation indices proposed for the quantification of photosynthesis related processes.

Materials and methods

Study site description

The study site of the natural montane grassland is located at the permanent experimental study site Bílý Kríž (Beskydys Mts., the Czech Republic, 18.54°E, 49.49°N, 898 m a.s.l. level). The experimental plot is characterised by a cool (annual mean temperature of 5.5°C) and humid (annual mean relative air humidity of ~80%) climate, with an annual precipitation of ~1000–1400 mm. The leaf area index of the grass canopy, as measured by the plant canopy analyser (LAI-2000, Li-Cor, Lincoln, NE, USA) in 2006, was equal to 3.5 ± 0.5 m²·m⁻² (mean ± s.d.). Plant species richness of the present Nardo-Callunetea association (class Nardo-Agrostion tenetis) accounted for ~25 herbaceous species. The most abundant herbaceous species recorded at the experimental plot were: Festuca rubra agg. (L.), Hieracium sp., Plantago sp., Nardus stricta (L.) and Jacea pseudophyrygia (C.A. Meyer). Further details about dominant plant species of the experimental grassland are available in work by Urban et al. (2007).

Measurement of leaf chlorophyll fluorescence

During the field campaigns twenty intact leaves of Jacea pseudophyrygia, Festuca rubra agg., Hieracium sp., Plantago lanceolata (L.) and Alchemilla mollis (L.) in different states of seasonal ontogeny were measured by the pulse modulated fluorometer (PAM-2000, H.Walz, Effeltrich, Germany). Those leaves were marked with white flags for a later identification on hyperspectral images obtained across the experimental plot. Prior to sunrise, the maximal Chl-F yield (i.e. $F_{m}/F_{n}$) of these leaves adapted over night for dark was measured. The Chl-F signal, integrated from an area of ~80 mm² in the centre of each leaf fixed in a leaf-clip was measured at an irradiance level above 400 μmol m⁻² s⁻¹. Saturating pulses of above 1200 μmol m⁻² s⁻¹ were used to calculate the fluorescence quantum yield ($\Phi_{II}$). The maximal Chl-F yield, quantifying the highest efficiency of photon capture by open reaction centers (Kitajima and Butler
1975), was calculated as \((F_m - F_a)/F_M\) where \(F_M\) is the maximal Chl-\(F\) of a dark adapted leaf with fully closed reaction centers and \(F_a\) is the minimal Chl-\(F\) of a dark adapted leaf with fully open reaction centers. Subsequently, the actual \(\Phi_0\), denoting the actual efficiency of the PSIII photon capture at an actual irradiance level, was calculated as \((F'_m - \text{Chl-}F')/F'_M\) (Genty et al. 1989), where \(F'_m\) represents maximum Chl-\(F\) and \(\text{Chl-}F'\) is the steady-state Chl-\(F\) of a light adapted leaf. Daily courses of Chl-\(F\) parameters were measured at 0700, 1230 and 1500 hours on 4 July 2006 and at one-hourly intervals between 0800 and 1600 on 19 July 2006. The total chlorophyll content (Chl-\(a+b\)) of intact leaves was measured during both days with a chlorophyllmeter (SPAD-502, Konica Minolta Sensing, Osaka, Japan) calibrated previously in laboratory to deliver Chl-\(a+b\) per leaf area measurements.

**Ground-based imaging spectroscopy**

Daily near-nadir views of hyperspectral images, covering the experimental plot area of \(\approx 1.5 \times 4.0\) m, were acquired from a distance of \(4\) m above canopy using a visible and near-infrared Airborne Imaging Spectroradiometer for Applications (AISA-Eagle, Specim, Oulu, Finland) (Fig. S1 available as an Accessory Publication to this paper). Images of 260 spectral bands between 400–940 nm with full-width-half-maximum of 2.2 nm were recorded at a pixel-size of \(\approx 2\) mm on 4 July at 0700, 1230 and 1530 hours and on 19 July in 1-h intervals from 1000 to 1600 hours local time. The leaf Chl-\(F\) measurements were performed simultaneously with each hyperspectral image acquisition. The acquired hyperspectral images were converted into radiance values using sensor specific calibration files. Subsequently, radiance images were transformed into at-sensor reflectance data via vicarious empirical line calibration, using five near-Lambertian calibration panels of known flat reflectance signatures. An automatically supervised maximum likelihood classification (Lillesand and Kiefer 2000) was applied to distinguish the sun-lit and shaded grass pixels. Finally, an appropriate threshold filter was applied on the images of green normalised difference vegetation index (green NDVI = \((R_{654} - R_{670})/(R_{654} + R_{670})\)) (modified according to Smith et al. 1995) to separate the photosynthetically active (green) leaves from a dry litter. Only image pixels of sun-lit green vegetation were used in the subsequent analysis. The processing of hyperspectral images was conducted in a specialised environment for image processing (ENVI, Research Systems Inc., Boulder, CO, USA).

**Leaf and canopy observational levels**

The hyperspectral image processing was carried out at two observational levels: (i) leaf and (ii) canopy. In our study the ‘leaf’-level experiments were performed on a reflectance integrated from 8–20 pixels of the ground monitored leaves. VIs of these leaves computed per pixel were averaged and correlated with the PAM-2000 leaf Chl-\(F\) and SPAD-502 chlorophyll content measurements. At the ‘canopy’ level, experiments refer to an average of more than 400 000 sun-lit green vegetation pixels classified within the acquired hyperspectral images. VIs of these pixels computed per image were averaged and correlated with the averaged Chl-\(F\) of all monitored ground leaves (\(n = 20\)), located on hyperspectral images.

‘Shade-removal’ experiment

In order to create an artificially dark-adapted grass canopy, part of the experimental plot (subplot of \(0.5 \times 4\) m referred to as ‘mantled plot’ in the text) was covered by a black non-transparent blanket for 30 min before each spectral measurement. This way of darkening enabled a lateral air convection, which prevented the changes being induced by a higher than ambient air temperature. The second part of the experimental plot, i.e. the ‘control plot’, was exposed to a natural daily radiation regime. Three subsequent AISA Eagle images of both plots were always acquired at the 5th, 90th and 200th second after blanket removal. The subtraction of successive reflectance scans of the mantled plot after blanket removal was expected to detect reflectance differences of sun-lit photosynthetically active grassland pixels. The Savitzky-Golay method (Savitzky and Golay 1964) was applied to filter noise out of the reflectance difference (\(\Delta R\)) spectra.

**Optical vegetation indexes**

Based on the results of the shade-removal experiment, which revealed a dynamic response of vegetation reflectance at three wavelength regions (see Fig. 2a, b), a performance of three photosynthetic process related optical vegetation indices (VIs) was proposed to be tested. These VIs (PRI, \(R_{686}/R_{630}\) and \(R_{740}/R_{800}\)) were derived from the AISA Eagle reflectance images. No other VIs (e.g. VIs for chlorophyll content estimation) were included.

**Statistical analysis**

A curve-fitting software (TableCurve 2D, SYSTAT Inc., Evanston, IL, USA) was used to find best fitting regression relationship between variables tested. The significance of each statistical model was tested at three probability levels: *, \(P < 0.01\); **, \(P < 0.001\) and ***, \(P < 0.0001\), using the analysis of variance (ANOVA). The determination coefficient \((R^2)\) was computed to express the variation percentage of a dependent variable explained by an established regression to the independent variable. All tests were conducted in statistical software (Statistica 7.0, StatSoft Inc., Tulsa, OK, USA).

**Results**

**Chlorophyll fluorescence detection from image data**

The apparent fluorescence emission with peaks at 532, 686 and 740 nm was observed from a reflectance intensity acquired from the mantled plot, measured at the 5th and 90th s after uncovering (Fig. 1a, grey dots). In contrast to this result, the reflectance subtraction of the same image pixels acquired at the 90th and 200th s after uncovering did no longer reveal any significant reflectance changes (Fig. 1a, black dots). The daily course of \(\Delta R\) with peaks at 532 and 740 nm appeared to be less distinct than the daily course of Chl-\(F\) at 686 nm (Fig. 1b). Specifically, spectral features associated with a xantophyll pigment transformation ranged from 490 to 570 nm (Fig. 1b). Nevertheless, in the daily course that peak was less pronounced during the morning and afternoon. \(\Delta R\) for wavelengths greater than 700 nm appeared to vary more as a result of radiometric
corrections per image combined with a high vegetation absorption (Fig. 1b).

**Interpretation of hyperspectral images at leaf level**

Process-related VIs were correlated in the daily course with selected biochemical and physiological leaf parameters (Fig. 2a–c). The regression analysis showed best fits (quantified by $R^2$) for PRI and $R_{686}/R_{630}$ VIs. No significant relationships were observed in the case of $R_{740}/R_{800}$ with any of the examined parameters. The highest daily average $R^2$ values were found between ratio $R_{686}/R_{630}$ and the total chlorophyll content ($Chl_{a+b}$), and a potential quantum yield of Chl-$F$ ($\Phi_{II}$), lower for $Chl-F_s$ and lowest for the actual quantum yield of Chl-$F$ ($\Phi_{II}$) (Fig. 2a–c).

In the daily course PRI was significantly correlated with $Chl_{a+b}$ (daily average $R^2 = 0.63$; logarithmic function) except at 1000 hours (linear function) (Fig. 3a; Table 1). The exponential relationships plotted between $R_{686}/R_{630}$ and $Chl_{a+b}$ were more consistent and stable than PRI. The relation between $R_{686}/R_{630}$ and $Chl_{a+b}$ showed the lowest daily values at 1200 hours (Fig. 3b). In the case of $F_{v}/F_{m}$, both indexes showed best fits via linear regressions (Fig. 3b–d; Table 1). The relationship between Chl-$F_s$ and PRI was exponential at 1000 and 1100 hours, and logarithmic for the rest of the day (Fig. 3c; Table 1). Similar relationships were found also between Chl-$F_s$ and $R_{686}/R_{630}$, except at 1000 hours the function was linear (Fig. 3f; Table 1). Relationships with the actual quantum yield of Chl-$F$ ($\Phi_{II}$) were all found to be close to linear or logarithmic, with the lowest $\Phi_{II}$ values at 12:00 (Fig. 3g, h; Table 1).
Fig. 3. (a–h) Best fit regressions between the physiological parameters (total chlorophyll content (Chl$_{a+b}$), maximum yield of chlorophyll fluorescence ($F_v/F_m$), steady-state chlorophyll fluorescence (Chl-$F_s$) and actual quantum yield of chlorophyll fluorescence ($\Phi_s$)) and vegetation indexes (PRI and $R_{686}/R_{630}$). The numbers indicate local time of the fit during the daily course. The respective determination coefficients ($R^2$) of the regressions are summarised in Fig. 3. $n=14–18$. See Table 1 for details on best fitting equations.
**Table 1. Best fitting regressions established between vegetation indices and tested physiological parameters**

<table>
<thead>
<tr>
<th>Hours</th>
<th>Chl*</th>
<th>(x)</th>
<th>F6/Fm</th>
<th>(x)</th>
<th>Chl-</th>
<th>(x)</th>
<th>ΦII</th>
<th>(x)</th>
</tr>
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<tr>
<td>1000</td>
<td>-0.07 + 0.001x</td>
<td>-0.29 + 0.33x</td>
<td>-0.05 + 0.29x&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.047 – 0.1096 × exp(-x)</td>
<td></td>
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<tr>
<td>1100</td>
<td>-0.005 – 4.87x&lt;sup&gt;1.5&lt;/sup&gt;</td>
<td>-0.39 + 0.47x</td>
<td>-0.07 + 0.76x&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-0.01 + 0.02 × ln(x)&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>1200</td>
<td>0.02 – 6.7x&lt;sup&gt;1.5&lt;/sup&gt;</td>
<td>0.025 + 0.0019 × ln(x)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.026 – 0.009 × ln(x)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.073 + 0.101 × x&lt;sup&gt;0.5&lt;/sup&gt;</td>
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<tr>
<td>1300</td>
<td>0.01 – 5.6x&lt;sup&gt;1.5&lt;/sup&gt;</td>
<td>0.001 + 0.0016 × ln(x)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.01 + 0.02 × ln(x)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.073 + 0.101 × x&lt;sup&gt;0.5&lt;/sup&gt;</td>
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<tr>
<td>1400</td>
<td>0.07 – 0.57x&lt;sup&gt;1.5&lt;/sup&gt;</td>
<td>0.0096 + 0.0011 × ln(x)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.024 + 0.0335 × ln(x)</td>
<td>0.174 – 0.273 × exp(-x)</td>
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<tr>
<td>1500</td>
<td>0.007 – 5.0x&lt;sup&gt;1.5&lt;/sup&gt;</td>
<td>-0.42 + 0.52x</td>
<td>-0.0016 + 0.0033 × ln(x)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.174 – 0.273 × exp(-x)</td>
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**Interpretation of hyperspectral images at canopy level**

The error assessment of the maximum likelihood classification, used for the selection of vegetation exposed to the sun, revealed an accuracy between 83.1–87.8%, with the kappa coefficient between 0.78–0.83. The fraction of non-photosynthetic vegetation was ~6–7% and the fraction of sun directly illuminated vegetation varied from 5% (morning) to 32% (midday) of the total experimental plot area.

The comparison of the VI daily courses of selected regions of interest (ROI), i.e. average values of VIs of all sample leaves (total number of pixels between 150–180) and of all sun-exposed leaves in the image (total number of pixels >400 000) showed a different pattern (Fig. 4a–c). For instance, in the case of PRI the areas had their lowest values at 1100 hours, whereas the whole image showed the highest values at the same time (Fig. 4a). The absolute values of PRI derived from ROI were, in general,

**Fig. 4.** Daily courses of vegetation indexes derived from the region of interest (ROI) (●) and from sun-exposed pixels of the whole image (○) for: (a) PRI, (b) R<sub>686/R<sub>530</sub> and (c) R<sub>686-R<sub>590</sub> ROI represents the average of all measured leaves (n = 150–180) and the image represents the average of all green sun-exposed pixels (n > 400 000). R<sup>2</sup> coefficients of determination for the linear regression between the given vegetation index derived from the whole image and steady-state chlorophyll fluorescence (Chl-F<sub>II</sub>). Actual quantum yields of Chl-F<sub>II</sub> (ΦII) are indicated. (d) Daily course of Chl-F<sub>II</sub> (●) and actual quantum yield of Chl-F<sub>II</sub> (●) measured on sample leaves (ROI). Asterisks denote the significance level: ***P < 0.001.
lower than values derived from the whole image. Similarly, the ratio $R_{686}/R_{630}$ calculated from ROI was lowest at 1200 hours, whereas it was highest when calculated from the whole image. $R_{686}/R_{630}$ calculated from ROI was lower than the ratio calculated from the image except at 1500 hours (Fig. 4b). The ratio $R_{740}/R_{800}$ derived from ROI had higher absolute values than values derived from the whole image (Fig. 4c). Finally, a close linear relation was observed between Chl-$F_a$ and $\Phi_{II}(R^2 = 0.92)$ measured on sample leaves when both parameters showed a decrease around midday, from 1100 to 1400 hours (Fig. 4d).

Finally, the earlier reported reflectance change at 740 nm (far-red Chl-$F$) (Gamon and Surfus 1999; Dobrowski et al. 2005) has also been observed (Fig. 1a–c). All these outcomes support a previously established hypothesis that the Chl-$F_a$ intensity retrieved correctly from hyperspectral reflectance could be used for the estimation of vegetation carbon assimilation (Flexas et al. 2002; Dobrowski et al. 2005; Grace et al. 2007).

**Fluorescence vegetation indexes – leaf image observations**

Positive curvilinear relations fitted between PRI and Chl-$a+b$ (Figs 2a, 3b) are in line with results of other leaf- (Moran et al. 2000) and canopy-level studies (Sims et al. 2006), showing that higher leaf Chl-$a+b$ content may lead to higher LUE. However, the relationship between PRI and Chl-$a+b$ was found here to be relatively unstable in the daily course (Fig. 3a), suggesting that the PRI index might be sensitive to non-physiological factors (Barton and North 2001; Filella et al. 2004). More consistent relationships were observed between $R_{686}/R_{630}$ and Chl-$a+b$, reaching the lowest $R_{686}/R_{630}$ values at 1200 hours (Fig. 3b). This $R_{686}/R_{630}$ decrease could be related to the daily variation of Chl-$F_a$ (Fig. 4d), which is typically lower during midday (Dobrowski et al. 2005).

Compared with PRI, the reflectance ratio $R_{695}/R_{630}$ also yielded a slightly higher average $R^2$ when related to Chl-$F_a$ and $F_{v}/F_{m}$ parameters. However, correlations with $\Phi_{II}$ were of similar statistical significance (Fig. 2a, b). Although other leaf-level studies demonstrated a close correlation between $\Phi_{II}$ and PRI (Evain et al. 2004; Guo and Trotter 2006), and also a significant relationship between $\Phi_{II}$ and $R_{685}/R_{655}$ computed from reflectance airborne data (Zarco-Tejada et al. 2000b), the daily $R^2$ values for PRI-$\Phi_{II}$ and $R_{685}/R_{630}$-$\Phi_{II}$ in our study varied between 0.27–0.78 and 0.20–0.70, respectively. Such a high variation might be related to the high heterogeneity of leaf angle distribution influencing our results due to the extremely high spatial resolution of analysed pixels (Rascher et al. 2005). In general, no specific pixel size can be pronounced as ‘optimal’ for the retrievals of different geo-biophysical properties of vegetation (Rahman et al. 2003). The optimal spatial resolution undoubtedly depends on the user’s required scale of observation (biomes v. ecosystem), but also on the type of the parameter to be estimated.

The positive relationships, observed between PRI and pre-dawn $F_{v}/F_{m}$ values (Fig. 2a) in the daily course ($R^2 = 0.40–0.65$), agree with studies recently conducted in forest ecosystems (Weng et al. 2006a, 2006b). These results are suggesting the potential capability of PRI to estimate the photosynthetic leaf capacity. However, even stronger statistical daily relationships were obtained between $R_{686}/R_{630}$ and $F_{v}/F_{m}$ ($R^2 = 0.45–0.83$, Fig. 2b), which correspond to the daily relationship discovered between the measured $F_{v}/F_{m}$ and VI ratios designed around $R_{690}$ (i.e. $R_{685}/(R_{675} \times R_{690})$, $R_{685}/R_{630}$, $R_{690}/R_{630}$, $R_{680}/R_{630}$ and $R_{695}/R_{630}$) (Zarco-Tejada et al. 2000b). These findings support a conclusion that the ratio $R_{686}/R_{630}$ is less sensitive to non-physiological (e.g. structural) canopy effects than PRI.

Similar to our results (Figs 2a, 3e), a positive relation between PRI and Chl-$F_a$ has been observed in water stressed canopies of

**Discussion**

**Remote detection of chlorophyll fluorescence signals**

Chl-$F$ effects on the apparent reflectance were observed when using dark-to-light transition of grassland canopy exposed to natural light conditions (Fig. 1a–c). These results are in agreement with the previous laboratory studies of Chl-$F$ detection in single leaves (Gamon et al. 1990; Buschmann et al. 1994; Gamon and Surfus 1999; Zarco-Tejada et al. 2000a) and also at the canopy level (Zarco-Tejada et al. 2000b, 2002, 2003), which supports the applicability of remote sensing techniques for monitoring the Chl-$F$ daily cycle under natural irradiation (Fig. 1c). Observed rapid reflectance changes followed by steady state in ~90 s after uncovering the dark-adapted plot are also in agreement with the kinetic previously observed in reflectance (Zarco-Tejada et al. 2000a) and Chl-$F$ induction (Kautsky and Hirsch 1931) at the leaf level.

The reflectance change with maximum at 531 nm associated mostly with zeaxanthin formation has been reported by Gamon et al. (1992). Our results showed that the peak of this spectral feature is quite flat and broad (Fig. 1a), with a wavelength interval ranging from 490 up to 570 nm (Fig. 1b). Such a broad spectral interval suggests that many more processes, together with the dynamic zeaxanthin conversion, are mirrored in the reflectance change around 531 nm. These processes, such as energisation (Ruban et al. 1993) and acidification (e.g. Schreiber and Klughammer 2008) of the thylakoid membrane, dynamic water fluxes increasing light scattering (e.g. Heber et al. 1986), and changes in pigment-binding protein (Li et al. 2000), limit the use of PRI as a specific indicator of photosynthetic rate, not only for remote sensing applications (Grace et al. 2007). At the same time, this broadband reflectance change provides the possibility to develop an alternative approach that integrates the reflectance signal of all these wavelengths instead of the PRI index, based on only two narrow wavelengths.

The peak of reflectance change around 686 nm, as observed in our study, was previously observed in studies conducted on sunflower (Helianthus annuus L.) leaves (Gamon and Surfus 1999). Contrary to our results, two peaks, at wavelengths of 695 and at 700 nm, were reported in a study on Acer saccharum leaves and canopy (Zarco-Tejada et al. 2001). This difference can be explained by the fact that Zarco-Tejada et al. (2001) subtracted the canopy reflectance scans acquired at 0800 and 1230 hours. In such a time period, one can expect changes in atmospheric conditions, and also a different shadow fraction distribution with a different influence of vegetation structure causing likely appearance of this double peak feature.
olive (Olea europea L.; Suarez et al. 2008) and grapevines (Vitis vinifera L.; Dobrowski et al. 2005). In consistence with our results shown in Fig. 2b, Dobrowski et al. (2005) observed a stronger relationship between Chl-Fa and R740/R800 than between Chl-Fa and PRI. Nevertheless, one has to keep in mind that the experimental design by Dobrowski et al. (2005), who used heat to induce plant water stress observed by a non-imaging sensor, thereby decreasing comparability of fitted statistical models. Also, in contrast to the study by Dobrowski et al. (2005), our ratio \(R_{740}/R_{800}\) showed only a weak relation with the examined Chl-Fa parameters, reflecting the fact that the NIR wavelengths are affected by the angular reflectance anisotropy of vegetation foliage (especially at such a high spatial resolution).

Fluorescence vegetation indexes – canopy level

Compared with the studies presenting a strong relationship between \(\Phi_{II}\) or Chl-Fa and PRI retrieved from the ground (Evain et al. 2004) and aircraft (Suarez et al. 2008) canopy observations, our relation between \(\Phi_{II}\) or Chl-Fa (Fig. 4a) was rather weak. It was observed that PRI of selected leaves did not correspond well to the canopy-level PRI (Fig. 4a). Although the daily course of PRI derived from selected ROI of individual leaves supports the PRI applicability for the LUE estimation (Gammon et al. 1992; Nichol et al. 2000, 2002), the PRI derived from sun-exposed pixels of whole image suggests the opposite trend (Fig. 4a). These results revealed that a PRI response measured on single leaves could not resemble the PRI response whole canopy, most probably due to a biological and structural diversity of the natural grassland canopy. Similar behaviour was observed in case of the \(R_{690}/R_{630}\) reflectance ratio. Zarco-Tejada et al. (2000b) retrieved from a 20-m spatial resolution image data of Acer saccharum daily courses of the \(R_{690}/R_{630}\) ratio that varied by 17% while tracking the midday decrease in \(F_{a}/F_{m}\). Despite of the fact that our \(R_{690}/R_{630}\) ratio derived from ROI was following a daily trend of \(\Phi_{II}\) and Chl-Fa, reaching low values during the midday (Fig. 4d), the canopy derived \(R_{686}/R_{630}\) ratio showed the opposite daily course (Fig. 4b).

Many canopy-level studies (Grace et al. 2007; Hall et al. 2008; Hilker et al. 2008; Suarez et al. 2008) employed non-imaging sensors to integrate the sunlit and shaded canopy parts in one reflectance value. Recently, Hall et al. (2008) provided evidence that the daily PRI variation of forest canopy can be attributed solely to biological processes (changes in zeaxanthin) when accounting for the shadow fraction of vegetation estimated with airborne laser scanning. Although in our study we directly excluded shadow fraction and non-green portions of vegetation from the reflectance signal, the daily course of the whole image PRI still did not correspond to the expected daily course of LUE (i.e. lower values during midday). Potential explanation can be the extent of the scanned plot (1.5 \(\times\) 4 m) that might not be able to capture typical daily LUE course of the whole grassland ecosystem measured within a larger spatial footprint by the eddy-covariance system. Still, when considering the use of remotely sensed PRI at larger spatial scales, we strongly recommend a minimisation of angular and shading effects as well as an exclusion of non-green vegetation surfaces (Drolet et al. 2005) within the scanned canopy (Filella et al. 2004).

Conclusions

This study employed a new experimental design to provide further evidence of the ground-based remote sensing Chl-F detection. The spectrometric measurements performed with a common imaging spectroradiometer over a montane grassland in the daily course of natural light conditions revealed all the previously known vegetation fluorescence spectral features.

At the leaf level, selected VIs sensitive to Chl-F (especially PRI and \(R_{686}/R_{690}\)) were usable for tracking the spatial variation of the Chl-F parameters (i.e. \(F_{a}/F_{m}\), Chl-Fa and \(\Phi_{II}\)). In our study the ratio \(R_{686}/R_{630}\) performed slightly better than PRI in explaining the daily course variability of Chl-F and the \(R_{740}/R_{800}\) ratio was only weakly related to Chl-F parameters.

At the canopy level, a strong angular anisotropy of grassland canopy reflectance (especially at NIR wavelengths) possibly reduced the positive performance of indices found at the leaf level. Further studies investigating temporal canopy reflectance changes induced by the vegetation structural features are necessary to establish robust techniques able to retrieve accurately the vegetation Chl-F signal from a high spatial resolution imaging spectroscopy data.

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