Measuring Stress Reactions of Beech Seedlings with PRI, Fluorescence, Temperatures and Emissivity from VNIR and Thermal Field Imaging Spectroscopy

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Abstract
Photosynthesis rate was measured during the course of a day on a pot of well-watered and a pot of drought-stressed young beech trees. At the same time, hyperspectral visible/near infrared and hyperspectral thermal images of the plants were recorded with a very high spatial resolution from a 3.8 m high platform. Time series of photochemical reflectance index (PRI), sun-induced fluorescence, temperature, and emissivity of the leaves were correlated with the photosynthesis measurements. PRI (R²=0.72 and 0.78 for well-watered and drought-stressed group, respectively), fluorescence (R²=0.72 and 0.40), and temperature (R²=0.62 and 0.69) proved to be good estimators for measured photosynthesis rates, emissivity had weaker correlations (R²=0.05 and 0.18). High resolution maps of photosynthetic activity could be produced from PRI, fluorescence, and temperature.

Keywords: Dryness stress, proximal sensing, fluorescence, imaging spectroscopy, thermal infrared.

Introduction
Remote measurements of photosynthetic activity and plant stress can be an important tool for agricultural and forest management, and for modelling plant growth and the terrestrial carbon cycle. There are several remote sensing techniques to monitor photosynthetic activity: Hyperspectral measurements in the visible/near infrared (VNIR) range can be used to derive the photochemical reflectance index (PRI) or to measure the sun-induced fluorescence, measurements in the thermal infrared (TIR) range can be used to measure leaf temperature and emissivity [Zarco-Tejada et al., 2012]. Photosynthesis can be divided into a light reaction and a dark reaction. In the light reaction, water and light are used to produce NADPH from NADP+, ATP from ADP, and oxygen is exhaled, while in the dark reaction, carbon dioxide is inhaled, NADPH and ATP are reconverted to NADP+ and ADP, and carbohydrates are fixated. If the irradiation is higher than the energy demand for NADPH and ATP production by
photosynthesis, plants have the general problem of eliminating the excessive light energy. The protection against photo damage is a multilevel process [Taiz and Zeiger, 2007]. At first plants try to protect themselves by quenching the excess electron excitation as heat or fluorescence. Higher plants typically dissipate 50 to 70% of all absorbed photons to heat. The so-called non-photochemical quenching is the main way to protect plants against damages by excessive radiation [Schopfer and Brennicke, 2010]. If toxic photo oxidants are built, a variety of scavenging systems (carotenoids, superoxide dismutase, ascorbate) eliminate the reactive photo oxidants. If this detoxification is not sufficient, the photoproducts will damage photosystem II, which leads to photo inhibition [Asada, 1999].

One of the scavenging systems is the xanthophyll cycle of the three carotenoids violaxanthin, antheraxanthin and zeaxanthin. Under high light conditions, violaxanthin is converted into zeaxanthin via intermediate antheraxanthin by violaxanthin-depoxidase. At lower light conditions the process is inverted. These conformational changes lead to quenching and heat dissipation. This de-epoxidation results in a decrease in leaf reflectance at a narrow band at 531 nm wavelength [Rascher et al., 2007]. The photochemical reflectance index PRI [Gamon et al., 1992; Gamon et al., 1997] is a normalized difference reflectance index that uses the waveband at 531 nm which is affected by the de-epoxidation, and a reference band, usually at 570 nm, which is unaffected by it. At the de-epoxidated state, i.e. when the leaves are hit by more photons than they can use, the PRI values are lower than at the base state. In a recent paper by Gamon and Bond [2013], significant correlations between photosynthetic activity and PRI, both on leaf and on canopy scale, were found. The correlations were influenced by tree age, i.e. old ponderosa pine trees showed stronger midday declines in PRI than young trees, and by illumination conditions, i.e. the diurnal course of PRI was dependent on the exposition of the studied stands.

The use of sun-induced chlorophyll fluorescence for probing the status of the photosynthetic system has been examined in several studies [Meroni et al., 2009; Rascher et al., 2009; Damm et al., 2010; Zarco-Tejada et al., 2012; Liu et al., 2013; Zarco-Tejada et al., 2013]. These studies found good but complex relations between photosynthesis rate and remotely-sensed fluorescence. While at some parts of the day a near-linear relationship between the two variables exists, the relationship breaks down at other times. The measurements are further complicated by sensor noise because the fluorescence signal is quite weak and thus difficult to measure.

Hyperthermal imaging has been successfully employed for species discrimination [Ribeiro da Luz and Crowley, 2010; Ullah et al., 2012], but not yet for the characterization of biophysical or biochemical vegetation states. Broadband thermal imaging was employed by Reicosky et al. [1994], who used temperature measurements to characterize diurnal trends in photosynthesis and evaportranspiration in wheat canopies, and Zarco-Tejada et al. [2012] who used a UAV platform to detect water stress in an orchard with a micro-hyperspectral imager and a thermal camera. Reinert et al. [2012] used thermal imaging to determine leaf conductance in European beech. They found significant correlations between thermal indices and leaf conductance, relying on the position of the considered leaves in the canopy (upper, middle, or lower part).

We did an experiment on a small field scale and measured the diurnal photosynthetic activity of beech seedlings. Simultaneously, hyperspectral VNIR and TIR images of the trees were made from a 3.80 m high platform, so that the spatial distribution of reflected and emitted
Radiation could be studied in a very high temporal and spatial resolution. While the trees in one pot were well-watered, the trees in the second pot had been cut off from water supply some weeks before and were showing clear signs of drought stress. Objectives of the study were (i) to examine the photosynthesis of young beeches at light saturation and different drought stress conditions, and (ii) to measure and map the photosynthesis state by proximal sensing using a VNIR and a TIR hyperspectral camera.

**Methods**

**Drought stress experiment**

Two years before this experiment, four year old seedlings of European Beech (*Fagus sylvatica* L.) were planted into pots (30 L volume), three individuals into each pot. In 2011, a drought stress experiment was conducted on these trees. Further details on the preparation and the imaging setup are described in Buddenbaum et al. [2012] and Stern et al. [2014]. The drought stress experiment was repeated in summer 2013 with the same plants, now six years old. Half of the pots were cut off from water supply on 2nd August, while the others were continuously watered. The measurements described in this paper took place on 6th September, so the drought-stressed group had been without water supply for 5 weeks before the measurements were taken. One pot of the dry group that still had green leaves and one pot of the control group with similar tree heights as the dry group trees were selected for this experiment of diurnal variation.

**Photosynthesis measurements**

Net photosynthesis ($A_n$), transpiration rate (E) and photosynthetically active radiation (PAR) were measured on intact leaves using a LiCor LI-6400 Portable Photosynthesis System (LiCor Biosciences, Lincoln, NE, USA). Photosynthesis and transpiration rates were measured as gas exchange rates. CO$_2$ and H$_2$O were analysed with an infrared gas analyzer in the porometer head.

The measurements were conducted automatically at 30 sec intervals and measured over a time period of 10 minutes before changing the leaf. Leaves of well-watered and drought stress groups were measured alternately. Only measurements of gas exchange with PAR over 600 µmol m$^{-2}$ s$^{-1}$ (estimated light saturation of photosynthesis for beech trees) were considered for analysis (circa 80% of all measured datasets).

All measured leaves were located in the upper sunlit canopy and large enough to fill the porometer chamber with an opening of 6 cm$^2$. We started at 9:50 central European summer time when the sun first reached the pots, and finished the measurements at 15:00. The measurement day was mostly sunny, but from 11 am some cumulus clouds appeared. Maximum air temperature in the shadow at 2 m above ground was 31°C.

**Hyperspectral VNIR images**

Hyperspectral images were recorded with a HySpex VNIR-1600 camera (Norsk Elektro Optikk, Skedsmokorset, Norway) mounted on a rotation stage on a 3.8 m high platform [Buddenbaum et al., 2012]. The camera is a push-broom scanner that records lines of 1600 pixels with an across-track field of view of 17°, resulting in lines of about 1 m width. By rotating the camera images are formed. The images covered an area of about 3 m × 1 m each. The scanner was equipped with a lens for 3 m object distance with about 0.5 m depth of
focus so that most of the area was imaged in focus. A white reference target (Spectralon™, Labsphere Inc, North Sutton, USA) of known reflectance was included in the scans in order to convert the recorded radiances to reflectance values [Peddle et al., 2001; Buddenbaum and Steffens, 2011]. Black foam rubber mats were placed on the ground to reduce stray light from the background. The images were recorded under natural light conditions. Before each scan the integration time was adapted to current brightness.

The camera uses an array of 1600 × 1200 silicone detectors to record the image lines. The first dimension is the spatial across-track resolution; the second dimension is used for spectral discrimination. 320 of the 1200 lines are used, and by spectral binning of 2 neighbouring sensor elements, 160 spectral bands in the range of 414 to 994 nm are recorded. Spectral sampling distance is 3.7 nm. No information on the FWHM is available, but it is assumed to also be close to 3.7 nm.

Over the course of the day, 25 images were recorded, each accompanied by a measurement series with the gas exchange porometer.

Radiometric pre-processing of the images was done using software provided by the camera manufacturer. This software corrects dark current effects and responsivity differences of the sensor elements and converts the recorded digital numbers to radiance values.

In each image, the white reference was detected, its mean reflectance was calculated and all pixel values of the images were divided by their respective white reference values, resulting in relative reflectance values. Since the panel’s reflectivity in the spectral range of interest is close to 99% we did not multiply the values by panel reflectance to get absolute reflectance.

### PRI calculation

For PRI calculation, bands centered at 531.3 and 571.3 nm (\(\rho_{531}\) and \(\rho_{571}\)) were used, so PRI was calculated as shown in Equation [1]:

\[
PRI = \frac{\rho_{531} - \rho_{571}}{\rho_{531} + \rho_{571}} \quad [1]
\]

Figure 1 shows two sample reflectance spectra of dry and fresh leaves measured using the HySpex scanner and the position of the PRI bands.

Non-leaf elements in the images were discarded by masking out pixels with Normalized Difference Vegetation Index (NDVI) values of < 0.6, which led to a good discrimination between leaves and non-leaves by visual inspection.

In order to examine illumination effects on the relationship between photosynthesis and PRI, shaded leaves were defined as leaf pixels with less than 0.3 reflectance at 800 nm, and sunlit leaves were defined as leaf pixels with greater than 0.6 reflectance at 800 nm. The thresholds were set subjectively and led to selection of the desired pixels according to a visual inspection. Correlations between PRI and \(A_n\) were calculated separately for six classes: three leaves types (all, sunlit only, shaded only) for two different conditions (well-watered and drought-stressed).
Gamon and Bond [2013] stress the influence of irradiance on PRI values. In order to investigate whether correlations between PRI and photosynthesis are only apparent relations we derived PAR irradiance from the images by calculating the mean reflected radiance from the white reference at 414 to 700 nm wavelength. Linear regressions between PAR irradiance and PRI were calculated, separately for the six classes. Residual PRI values, i.e. differences of measured PRI values and the according PRI values estimated by the linear correlation, were derived, and correlations between the residual PRI values and $A_n$ were calculated.

**Estimation of fluorescence**

Since the amount of sun-induced fluorescence ($F_s$) is only about 1-5% of the total reflected light at a certain wavelength, quantitative measurements are quite difficult and require a sensor with a high signal to noise ratio and narrow bands [Rascher et al., 2009; Damm et al., 2010]. The HySpex sensor’s spectral resolution and spectral sampling interval should allow for fluorescence estimation, albeit with a higher root mean square error than an ideal sensor [Damm et al., 2011]. An instrument with a relatively high bandwidth like the HySpex VNIR camera includes the shoulders of the narrow absorption features used for fluorescence estimation, likely resulting in an overestimation of $F_s$ [Meroni et al., 2009]. Chlorophyll fluorescence is emitted in two broad bands with peaks at 685 and 740 nm. Remote measurements of $F_s$ usually use the O$_2$ absorption line at 760 nm (O$_2$-A), where only little sunlight reaches the earth. We derived $F_s$ according to the modified Fraunhofer line discrimination method proposed by Maier et al. [2003]. This approach assumes that $F_s$ is additive to the reflected signal and can be derived by comparing the depth of the O$_2$-A band from a non-fluorescent surface with that of the fluorescent vegetation following Equation 2:
\[ F_S = \frac{L_1 - \frac{E_1}{E_2} L_2}{1 - \frac{E_1}{E_2}} \]  \[2\]

where \( E \) is the radiance upwelling form the non-fluorescent target, \( L \) is the radiance of vegetation, and the subscripts 1 and 2 indicate the wavelengths within and outside the absorption line, respectively [Damm et al., 2010]. The band positions shift slightly across the image field of view (“smile effect”) [Segl et al., 2010]. This results in shifts in the position of the radiance minimum around the \( \text{O}_2\text{-A} \) band. Figure 2 shows radiance spectra of the white reference panel and leaves of the well-watered trees from the first measurement in the morning and the last measurement at the afternoon. The wavelength shift of the radiance minimum can be discerned. In order to mitigate the effect of the wavelength shift, we employed the mean value of the bands at 760.8 and 764.4 nm for \( L_1 \) and \( E_1 \), and the mean value of the bands at 753.5 and 771.7 nm for \( L_2 \) and \( E_2 \).

Beside photosynthetic status, fluorescence is also driven by the absolute magnitude of the incident irradiance. \( F_S \) is normalized to fluorescence quantum yield (\( F_{S\text{yield}} \)) by division through the absorbed photosynthetically active radiation (APAR) [Damm et al., 2010]:

\[ F_{S\text{yield}} = \frac{F_S}{APAR} \]  \[3\]

APAR was estimated from the images by subtracting the radiance reflected from the leaves from the radiance reflected from the white reference panel. Multiple scattering effects were ignored. All fluorescence calculations were only done on the image columns covered by the white reference panel.

Figure 2 - Radiance spectra of white reference panel (WR) and leaves from the first and the last image. In the inset the bands used for fluorescence estimation are marked.
Hyperspectral thermal images

Accompanying each VNIR hyperspectral image, a series of hyperspectral thermal images was recorded using a Hyper-Cam-LW (Telops Inc., Québec, Canada). This Fourier-transform imaging spectrometer, using a 320 × 256 pixel MCT detector, measures the spectral radiance values in the 8 to 12 µm range at a spectral resolution of up to 0.25 cm⁻¹ [Schlerf et al., 2012]. The thermal camera was mounted next to the VNIR camera and was equipped with wide-angle telescope and a 45° tilted gold-coated mirror, which allowed downward looking (nadir view), similar to the HySpex’s field of view. A spectral sampling distance of 3.3 cm⁻¹ was chosen in the spectral domain from 863 to 1297 cm⁻¹ resulting in 125 bands. A high reflectance reference target (Infragold™, Labsphere Inc, North Sutton, USA) of known reflectance was included in the scans in order to quantify downwelling radiance from the sky, reflected by the target and impeding emissivity retrieval. 22 images were recorded from 9:50 to 15:00 CEST, each consisting of 8 subsequent datacubes. The measurements were accompanied by temperature measurements (ambient, leaf of stressed and non-stressed beech seedling, wet and dry reference) for data processing and validation.

The radiometric calibration of the interferogram datacubes, including Fourier transformation, 2-point-blackbody calibration and bad pixel correction, were done using the Telops Reveal Calibrate software. The resulting radiance datacubes were then processed to spectral emissivity using a temperature and emissivity separation (TES). The TES algorithm uses an assumption that the emissivity spectra have a spectral region of maximum emissivity ranging around 97%, which is suitable for vegetation. As a result, an emissivity spectrum as well as the kinetic temperature is derived for every pixel.

On each of the pots, a square of pixels was defined that was covered nearly completely by leaves in all of the images. In order to filter out non-leaf pixels, only the central 50% of temperatures (temperatures above the 25th quantile and below the 75th quantile) were used to derive the mean kinetic temperature for the pots.

Derivation of leaf emissivities

The same areas were used to extract mean emissivities from the images. Wavenumbers above 1126 cm⁻¹ were discarded because they showed a low signal to noise ratio due to water vapour influences. The remaining values had a distinct zigzag shape of alternating high and low values. We discarded all even-numbered values and used only the remaining values at 40 wavenumbers for further analyses (Fig. 3).

While correlation analyses of the relationships between photosynthesis and PRI, fluorescence, and temperature, respectively, could be done with simple univariate methods, the hyperspectral emissivities called for a multivariate analysis. We chose partial least squares regression (PLSR) [Wold et al., 2001a; Wold et al., 2001b], a method well-suited for estimating a dependent variable even from strongly collinear and noisy independent variables [Atzberger et al., 2010; Steffens and Buddenbaum, 2013].

In a first step, PLS regressions were calculated with up to 15 latent variables with leave-one-out cross validation. The cross-validated mean squared error of estimated $A_n$ was determined to find the optimal number of latent variables (Fig. 4a). A model with 3 latent variables was chosen, because models with more variables had very similar errors, but led to noisier maps. The other parts of the figure show the PLS regression coefficients of the selected model for all wavenumbers, and estimated $A_n$ values for the training spectra.
Figure 3 - Emissivities of well-watered and drought-stressed trees; a) all values, b) only odd-numbered values up to 1126 cm\(^{-1}\) that were used for further analyses.

Figure 4 - PLS regression: a) Cross-validated mean squared error as a function of the number of PLS components (latent variables), b) PLS regression coefficients for the wavenumbers used, c) measured versus estimated photosynthesis.

Results

Photosynthesis measurements

Results of the photosynthesis measurements of the well-watered and the drought-stressed beeches are depicted in Figure 5. PAR irradiance rose from morning to noon with some dents that can be attributed to clouds or to shadows on the sensor head. Air temperature rose from 25°C to 35°C and water vapour pressure deficit at the leaf (VPDL) followed the air temperature closely. At 11:30 the leaf internal CO\(_2\) concentration of the well-watered and
the drought-stressed group converge to each other, which shows that the photosynthetic capacity of the photosynthetic apparatuses of both groups are saturated (Fig. 5b). Figure 5c shows the differences of external and internal CO₂ concentrations over time. The regression lines show that the difference gets larger for the well-watered tree while it gets smaller over time for the drought-stressed tree. This indicates that the well-watered tree has a higher photosynthetic activity than the drought-stressed tree.

The well-watered plant shows significantly higher rates of leaf conductivity, i.e. stomata openness throughout the day than the dry plant (Fig. 5d). Leaf conductivities of both plants decrease at the beginning, then stabilize at around 11:30, with some downward fluctuations in the afternoon. Transpiration rates (Fig. 5e) are more or less stable throughout the day but show the same downward fluctuations as leaf conductivity. A curve progression very similar to that of leaf conductivity can be found for $$A_n$$ (Fig. 5f). Both plants’ $$A_n$$ decrease from the beginning. The well-watered group stabilizes at about 5 µmol CO₂ m⁻² s⁻¹ at 11 am, the drought-stressed group at very low values under 1 µmol CO₂ m⁻² s⁻¹ at 11:30 am.

![Figure 5 - Gas exchange porometer measurements. (a) Photosynthetically active radiation outside the leaf (PARo), air temperature and water vapour pressure deficit at the leaf (VPDL), (b) Leaf internal (ci) and external CO₂ concentration, (c) difference of external and internal CO₂ concentration, with regression trendlines, (d) Leaf conductivity, (e) transpiration, and (f) net photosynthesis rate. Error bars show +/- one standard deviation for each measurement interval of 10 minutes.](image)
Hyperspectral VNIR images
The 25 images each show both pots, the white reference panel, and the porometer head. Figure 6 shows a true-colour depiction of the last image (15:00 hours) and a false-colour depiction of the PRI values of this image, masked as described in the PRI calculation section. Figure 1 shows typical spectra of leaves in the images.

Figure 6 - Left: True-colour HySpex image of the two beech pots. The porometer head on a tripod, the gold and white reference panels and some stones securing the black underground can be discerned. Right: Masked PRI map of the image. The dry trees are at the top of the images, the fresh trees at the bottom.

Photosynthesis-PRI relationship
The mean PRI values are depicted in Figure 7a, partitioned into all leaves, sunlit leaves, and shaded leaves of the well-watered and the drought-stressed pots. In the case of the well-watered trees, the shaded leaves have higher PRI values and the sunlit leaves have lower PRI values than all leaves. For the drought-stressed trees, this relationship is inverted for most measurements between 11 and 14 hours. The temporal course of the PRI values is similar to that of the photosynthesis rates.
Figure 7 - a) PRI values of well-watered and drought-stressed leaves over the course of the day, partitioned into all leaves, sunlit and shaded leaves; values discarded due to low irradiation are shown as outlines, b) Regressions of photosynthesis rate and shaded leaf PRI for all trees, and for well-watered and drought-stressed trees only.

Figure 8 - a) Relationship of PRI values and irradiance, separately for well-watered leaves and for all leaves, sunlit and shaded leaves, b) Regressions of photosynthesis rate and shaded leaf PRI residuals after illumination correction.

The relationship between $A_n$ and PRI is depicted in Table 1 and Figure 7b. Correlation coefficients are highest for shaded leaves and lowest for sunlit leaves, supporting the findings of illumination effects by Gamon and Bond [2013]. Figure 7b shows regressions of photosynthesis rate and PRI for all trees, well-watered trees, and drought-stressed trees separately for the shaded leaves. Since photosynthesis rates and PRI values both are significantly higher for the well-watered trees, the two groups are clearly separated in the plot.

Figure 8a shows the relationships of PRI and PAR irradiance for the six classes. It is obvious that PRI and PAR are highly correlated, especially when all leaves are considered (black symbols). Figure 8b and the last three columns of Table 1 depict the relationship between $A_n$ and the PRI residuals. In the case of shaded leaves and in the well-watered group there are still positive and slightly significant correlations, albeit smaller than between $A_n$ and non-transformed PRI.
Table 1 - Correlations between photosynthesis rate and PRI and PRI residuals.

|                | PRI                  | PRI residuals          |
|----------------|----------------------|------------------------|
|                | Both groups          | Well-watered group     | Drought-stressed group |
| All leaves     | 0.938***             | 0.879***               | 0.715*                 |
| Sunlit leaves  | 0.869***             | 0.864**                | 0.500                  |
| Shaded leaves  | 0.955***             | 0.847**                | 0.884***               |
|                |                      |                        |                        |
|                | Both groups          | Well-watered group     | Drought-stressed group |
| All leaves     | 0.090                | 0.524                  | -0.131                 |
| Sunlit leaves  | 0.124                | 0.557°                 | -0.235                 |
| Shaded leaves  | 0.172                | 0.639*                 | 0.552°                 |

Significance: p<0.1, * p<0.05, ** p<0.01, *** p<0.001

**Fluorescence**

Figure 9 displays the resulting images of $F_{Syield}$ derived from the VNIR hyperspectral data over the course of the day. Like for the PRI calculation, areas with NDVI < 0.6 have been filtered out. The images are rather noisy, but differences between the two pots and diurnal developments can be discerned. Since only the image columns with the white reference panel were used, the images show a narrower subset of the area shown in Figure 6.

![Figure 9 - Fluorescence yield images.](image)

Figure 10a shows the diurnal course of mean $F_{Syield}$ values per group. $F_{Syield}$ of both, the well-watered trees and the drought-stressed groups is steadily declining over the course of the
day. The decline of the well-watered group is steeper than that of the drought-stressed group. From about 13 hours the values are nearly the same. Figure 10b shows regressions between $F_{\text{Syield}}$ and $A_n$. Correlation for both groups is positive, i.e. more fluorescence corresponds to a higher photosynthesis rate. While under normal conditions $F_{\text{Syield}}$ and $A_n$ are negatively correlated, a positive correlation, i.e. a decline of $F_s$ when $A_n$ is low, has been observed in most studies in the presence of high light conditions and plant stress [Zarco-Tejada et al., 2003; Meroni et al., 2009].

![Figure 10 - a) Diurnal course of fluorescence yield for the well-watered and the drought-stressed plants, b) Relationship of fluorescence yield and $A_n$.](image)

**Temperature images**

Temperature images derived from the hyperthermal measurements are displayed in Figure 11. The gold and white references can be seen on the right of each image. The gold reference is always among the coldest (i.e. dark blue) objects in the image. From about the fifth image the black background mats are considerably hotter (i.e. black in the figure) than the other surfaces. The drought-stressed pot is on the right hand side, and becomes warmer than the well-watered pot in the later measurements.

![Figure 11 - Temperature images.](image)
In the beginning of the measurements, leaf temperatures of both groups were identical. During the course of the day, both groups got warmer. From 11:30 on, the drought stress trees became warmer than the well-watered group, and the difference got larger over time. From 13:30 on, the drought-stressed trees become even warmer than the air temperature (Fig. 12).

Figure 12 - a) Leaf temperatures of well-watered and drought-stressed plants, derived from Hypercam measurements, and air temperature. b) relationship of leaf temperatures to net photosynthesis.

Emissivity
Figure 13 shows maps of Photosynthesis estimated from emissivities using PLSR. As can be seen in Figure 14, they show very low coefficients of determination within the groups ($R^2 = 0.051$ and $0.179$ for well-watered and drought-stressed trees, respectively), but a high coefficient of determination for all trees ($R^2 = 0.806$). The time dependent estimated $A_n$ show that the diurnal course could not be represented, but the two groups could be discriminated using emissivities.

Figure 13 - Maps of photosynthesis estimated from the thermal emissivity images using PLSR.
**Discussion**

**Photosynthesis measurements**

The temporal trends in $A_n$ over the course of the day are clearly discernible. The drought-stressed trees are already so dry that water deficit in the plants induces low rates of photosynthesis caused by stomatal closure (Fig 2d). The cells may be so dry that the dehydratisation impacts the photosynthetic apparatus negatively. The well-watered and healthy plants show much higher photosynthesis rates that also vary more. The decline of photosynthesis after 11 am in the well-watered trees and after 10 am in the dry trees might be a case of midday depression, but the data does not allow for a clear diagnosis. More sunlight does not automatically lead to more photosynthesis in either group, because light will be at photosynthetical saturation. More light did not lead to decreasing ci-concentrations in both groups (Fig 2c) which proves the saturation of the particular photosynthesis apparatus. The drought stress group stands under high pressure of excessive light. It receives the same amount of light as the well-watered group but only the well-watered group can convert a high fraction of the available light into photosynthetic work. Therefore the heat dissipation of the drought stress group is higher than in the well-watered group, because of the excising energy which has to be quenched or dissipated before it will damage the photosynthetic apparatus by photooxidants. The relatively early onset of decreasing photosynthesis is probably species-related. Beech trees, especially seedlings, are shade-tolerant and do not usually grow in bright daylight. An additional experiment with different tree species like oaks would be an interesting field for future research.

The measurements could only be done on two pots for several reasons: First, since a single photosynthesis measurement time series takes 10 minutes and then several minutes are needed to connect another leaf to the porometer, only few trees can be measured with the desired high temporal resolution. Second, the fields of view of the two hyperspectral cameras were limited to two to three pots. It might have been possible to switch the pots under the cameras, but we decided against this in order to keep the position and orientation of the pots constant in all images.
**Photosynthesis-PRI relationship**

There are high correlations between photosynthesis rate and PRI for all considered cases. The correlations are highest for shadowed leaves. Figure 7b suggests different relationships for the drought-stressed and the well-watered plants, but the common model for all trees also works well and has the highest coefficient of determination due to the higher range of values. PRI is the only parameter with a continuous relationship to photosynthesis, i.e. the only parameter that can be used to estimate the large range of photosynthetic activities for both groups of trees with a single regression.

The normalization of PRI by PAR irradiance leads to weaker correlations to photosynthesis. A large portion of the photosynthesis-PRI correlation seems to be influenced by irradiance. The drought-stressed trees had been cut off from water supply for five weeks before the measurements and already had clearly palpably dry leaves, albeit they were still green and showed a hint of vitality. So it was expectable to see quite a different behaviour compared to the healthy trees. Future experiments should consider comparing lightly stressed plants with healthy ones.

In cases of excising light energy the xanthophyll cycle is one of the most important non-photochemical quenching systems beside higher fluorescence and higher heat dissipation to avoid damages to leaves. The xanthophyll cycle activity could be measured by the PRI index using the normalization of 531 and 570 nm reflections where the change via de-epoxization of violaxanthin will be visible. In the drought-stressed group with its closed stomata the surplus light energy is much higher than in the well-watered group. So the protection mechanisms have to quench more excess energy via normal heat dissipation, fluorescence and heat dissipation by xanthophyll cycle than the well-watered group. Increasing photosynthesis increased the PRI in the drought-stressed group because non-photochemical quenching decreases.

**Fluorescence**

Since the hyperspectral VNIR camera has wider bands than instruments specialized on fluorescence measurements, our image-based fluorescence estimations are generally noisy and show high amounts of scatter. Despite this, when averaging all pixels per group, clear diurnal trends are discernible for the pots. Separate regression models of the relationship between photosynthesis and fluorescence had to be established for well-watered and drought-stressed vegetation.

**Temperature images**

Like expected, the drought-stressed trees were not able to cool themselves by transpiration and in consequence got warmer than the air as the sun shone on them. The well-watered trees, on the other hand, stayed cooler than the air throughout the day because they were able to transpire water. For both groups, there is a clear negative correlation between temperature and photosynthesis.

**Emissivity**

Although there was a high correlation between estimated and measured photosynthesis values when both the well-watered and the drought-stressed group were taken into account, the PLS regression was unable to represent the diurnal course of photosynthesis within the groups.
But since it was possible to discriminate between the groups using emissivity, and because temperature can be used to track the diurnal course of photosynthesis, the hyperspectral thermal camera holds the potential to be used for photosynthesis measurements.

**Summary and Conclusions**

An experiment on photosynthesis of young beech trees over the main part of a day was conducted and observed with hyperspectral VNIR and TIR cameras. PRI was calculated from the hyperspectral VNIR images and correlated with photosynthesis. High correlations between photosynthesis rate and imaging spectroscopy-based PRI were found both for drought-stressed and well-watered beeches. The use of very high spatial resolution field imaging spectroscopy made it possible to separately evaluate the photosynthesis correlations of sunlit, shaded, and all leaves. Highest correlations were found for shaded leaves. The high resolution imaging spectroscopy further makes it possible to map PRI of small structures like single leaves and even intra-leaf variability and thus to act as a proximal sensing technique capable of mapping photosynthetic activity under high light conditions. PRI proved capable of mapping differences in non-photochemical quenching of excessive energy and could therefore be applied for detecting stress symptoms of oxidative stress triggered by water stress or other oxidative stress (e.g. ozone). This could be an effective tool to detect stress symptoms in vegetation.

Fluorescence has also shown to be a suitable means of characterizing plant stress. Due to its relatively wide bands the VNIR imaging spectrometer used is not perfectly suited for fluorescence measurements so that the results were noisy.

Temperature was also able to track the diurnal changes of photosynthesis activity within the groups, but unable to clearly distinguish between the groups. In the morning leaves from both trees had the same temperatures, but during the day the drought-stressed leaves were heated more intensely than the well-watered leaves.

The relationship between emissivities and photosynthesis was the other way around: Diurnal changes within the groups could not be tracked, but the groups were clearly discriminated.

In conclusion, this feasibility study could show that field-based imaging spectroscopy in the VNIR and TIR range was able to show diurnal courses of photosynthesis and differences between well-watered and drought-stressed trees. Very high spatial resolution maps of PRI, photosynthesis, temperature, and emissivity were produced that could show spatial and temporal patterns in these variables and in photosynthesis rate. Although this study has a very limited temporal and spatial frame, some conclusions can be drawn for airborne or spaceborne remote sensing applications. The very simple PRI has once more proven to be a robust measure of photosynthetic activity, while the other studied parameters are more difficult. Fluorescence holds a lot of interesting information, but it is difficult to measure and to interpret. Hyperspectral thermal remote sensing is still in an early stage of development and its use in vegetation studies has to be explored further.

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