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Daily and seasonal dynamics of remotely sensed photosynthetic efficiency in tree canopies

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Publication Details

Pieruschka, R., Albrecht, H., Muller, O., Berry, J. A., Klimov, D., Kolber, Z. S., Malenovsky, Z. & Rascher, U. (2014). Daily and seasonal dynamics of remotely sensed photosynthetic efficiency in tree canopies. *Tree Physiology: an international botanical journal*, 34 (7), 674-685.

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Abstract

The photosynthesis of various species or even a single plant varies dramatically in time and space, creating great spatial heterogeneity within a plant canopy. Continuous and spatially explicit monitoring is, therefore, required to assess the dynamic response of plant photosynthesis to the changing environment. This is a very challenging task when using the existing portable field instrumentation. This paper reports on the application of a technique, laser-induced fluorescence transient (LIFT), developed for ground remote measurement of photosynthetic efficiency at a distance of up to 50 m. The LIFT technique was used to monitor the seasonal dynamics of selected leaf groups within inaccessible canopies of deciduous and evergreen tree species. Electron transport rates computed from LIFT measurements varied over the growth period between the different species studied. The LIFT canopy data and light-use efficiency measured under field conditions correlated reasonably well with the single-leaf pulse amplitude-modulated measurements of broadleaf species, but differed significantly in the case of conifer tree species. The LIFT method has proven to be applicable for a remote sensing assessment of photosynthetic parameters on a diurnal and seasonal scale; further investigation is, however, needed to evaluate the influence of complex heterogeneous canopy structures on LIFT-measured chlorophyll fluorescence parameters.

Keywords

Electron transport rate, fluorescence, LIFT, PAM, photosynthesis, remote sensing

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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Daily and seasonal dynamics of remotely sensed photosynthetic efficiency in tree canopies

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Key words: fluorescence, photosynthesis, electron transport rate, remote sensing, LIFT, PAM

ABSTRACT

Photosynthesis of various species or even a single plant varies dramatically in time and space, creating a great spatial heterogeneity within a plant canopy. Continuous and spatially explicit monitoring is, therefore, required to assess the dynamic response of plant photosynthesis to the changing environment. This is a very challenging task when using the existing portable field instrumentation. This paper reports on the application of a technique, Laser Induced Fluorescence Transient (LIFT), developed for ground remote measurement of photosynthetic efficiency at a distance of up to 50 m. LIFT was used to monitor seasonal dynamics of selected leaf groups within inaccessible canopies of deciduous and evergreen tree species. Electron transport rates computed from LIFT measurements varied over the growth period between different species studied. The LIFT canopy data and light use efficiency measured under field conditions correlated reasonably well with the single-leaf Pulse Amplitude Modulated (PAM) measurements of broadleaf species, but differed significantly in case of conifer tree species. The LIFT method has proven to be applicable for a remote sensing assessment of photosynthetic parameters on a diurnal and seasonal scale, further investigation is, however, needed to evaluate influence of complex heterogeneous canopy structures on LIFT measured chlorophyll fluorescence parameters.

1 INTRODUCTION

2 Modulation of the photosynthetic processes by biological and environmental factors spans a large temporal range
3 from seconds in fluctuating light conditions to very slow seasonal changes (Rascher & Nedbal 2006; Schurr, Walter,
4 & Rascher 2006). Consideration of photosynthesis at the scale of ecosystems or the entire globe implies integration
5 of these temporal variations over many individual elements distributed over a heterogeneous spatial environment. In
6 the field the most frequently used approaches to monitor plant photosynthetic performance on the ecosystem scale
7 are based on the eddy-covariance method (Baldocchi 2003) or remote sensing observations (Hilker et al. 2008).
8 These bulk measurements of the exchange processes of the entire ecosystem are often based on many assumptions.
9 Therefore they provide little understanding of the underlining processes on the scale of single leaves or branches in
10 the plant canopy under field conditions.

11 Plant canopies are exposed to a substantial gradient in light from top of the canopy to the bottom which affects the
12 temperature and humidity and thus vapor pressure deficit within different layers of the canopy. Leaves within these
13 different layers acclimate to these conditions e.g. by different light harvesting efficiency in low or high light
14 (Niinemets 2007) or redistribution of nitrogen (Field et al. 1985). Leaves of different species and in different canopy
15 positions often respond differently to the prevailing and dynamic light conditions and environmental stress (Pearcy et
16 al. 2004). Thus, each leaf has a rather unique combination of trait values – all contributing to the canopy (Niinemets
17 2007). Integrating scales from single leaves to canopies is important e.g. to quantitatively assess and predict carbon
18 fluxes. Limited access to the leaves of many tree canopies and the limited throughput of currently available
19 approaches make detailed long-term monitoring of selected leaves and branches within the canopy difficult.

20 Non-invasive probing of chlorophyll fluorescence has become a powerful method in plant biology over the last few
21 decades (Papageorgiou & Govindjee 2005). There are several active methods to measure chlorophyll fluorescence
22 from a detailed analysis of polyphasic fluorescence rise after illumination (Strasser, Srivastava, & Govindjee 1995)
23 to the application of saturating pulses to modulate chlorophyll fluorescence and derive photosynthetic parameters
24 (Schreiber 1986). These approaches rely on an active modification of the light environment of the object of study.
25 Use of these approaches outside of the lab is limited to individual leaves in accessible parts of canopies. The passive
26 fluorescence methods monitor sun induced chlorophyll fluorescence which gives information about photosynthetic

processes over large vegetated areas (Damm et al. 2010; Malenovsky et al. 2009), but provides limited information on photosynthetic mechanisms.

The Laser Induced Fluorescence Transient (LIFT) approach was developed to bridge the gap between the laboratory and field measurements, which can be used in situ for long term and automated monitoring of selected leaves. The approach has been cross-compared with commercial Pulse Amplitude Modulated (PAM) and gas exchange measurements (Ananyev et al. 2005; Kolber et al. 2005; Pieruschka et al. 2010) and has been successfully applied in a range of studies to detect the spatial variability of light use efficiency (Nichol et al. 2012; Rascher & Pieruschka 2008) and also impact of cold stress on photosynthesis (Pieruschka et al. 2010). The widely used PAM fluorometers (Schreiber 1986) use high intensity saturating pulses, more than 2-3 folds of what leaves experience in nature, to induce a maximum level of fluorescence. This maximum fluorescence level enables, in relation to a minimum fluorescence level, the calculation of quantum yield. The LIFT technique applies a sequence of sub-saturating excitation pulses at microsecond intervals to manipulate the light environment on the leaf surface to induce a fluorescence transient. This fluorescence transient is fitted to extrapolate a maximum fluorescence level (Kolber et al. 2005), which is then interpreted in analogy to the PAM approach. In contrast to the PAM approach LIFT enables measurement from a distance up to 50 m and may provide a unique opportunity to observe remotely inaccessible vegetation canopies.

In this study, we perform a long-term experiment which is designed to monitor the daily and seasonal dynamics of photosynthesis. The aim of this study is to investigate the acclimation and dynamics of photosynthetic efficiency of tree species caused by seasonal variations in environmental conditions. In particular, we evaluate the performance of the LIFT within the tree canopies throughout a season by assessing it against the standard PAM approach. We note that this work was made possible by the seminal contributions of Charles Barry Osmond, who championed the development of the first LIFT apparatus for remote monitoring of photosynthetic parameters in land plants (Ananyev et al. 2005).

MATERIAL AND METHODS

Experimental sites and tree species

The experiments were performed with four different tree species at two experimental sites located in California, USA and Germany. At the Carnegie Institution for Science in Stanford, CA, USA (37°25' N, 122°9' W) photosynthetic efficiency of the evergreen oak *Quercus agrifolia* Née was measured between the end of February 2009 and mid-March 2009. The tree was approximately 15 m high and 30 years old. The second part of experiment was performed with three different species: *Tilia cordata* L., *Quercus petraea* L. and *Pinus sylvestris* L., at the Forschungszentrum Jülich in Germany (50°55' N, 6°21' E) between July 2009 and January 2010. All experimental trees reached a height of approximately 15-20 m and were 20-30 years old.

The field experiments

In all field experiments photosynthetic efficiency was monitored by the continuously measuring Laser Induced Fluorescence Transient (LIFT) device and with commercial PAM fluorometers, specifically designed for long-term field experiments (Monitoring PAMs, Porcar-Castell et al. 2008b). Selected leaves within tree canopies were clamped in the leaf-clips of the three available Monitoring PAMs under an angle mimicking approximately the natural position of the leaf. The Monitoring PAMs were placed at the uppermost accessible part of the canopy, ca. 15 m above ground. Every 10 minutes a saturating pulse was applied and maximal (F_m' PAM) and steady state fluorescence prior to the flash (F' PAM) were measured. Every 1-2 weeks a new leaf was camped into the leaf clip of the Monitoring PAMs to reduce any potential photoinhibition of long-term application of saturating pulses. The quantum yield of photosystem II (PSII) of light adapted leaves ($\Delta F/F_m'$ PAM) was calculated as follows: $\Delta F/F_m' \text{ PAM} = (F_m' \text{ PAM} - F_{\text{PAM}})/F_m' \text{ PAM}$. Electron transport rates (ETR) for the PAM measurements were assessed by: $\text{ETR}_{\text{PAM}} = \Delta F/F_m' \text{ PAM} * \text{PPFD}_{\text{PAM}} * 0.84 * 0.5$; with 0.84 as an estimate of absorbed photosynthetically active photon flux density (PPFD_{PAM}) measured by the sensor of the Monitoring PAMs and, 0.5 accounting for the partitioning of light absorption between photosystem I and II (Genty, Briantais, & Baker 1989). The quantum yield of PSII of dark adapted leaves ($F_v/F_{m,\text{PAM}}$) was calculated as $F_v/F_{m,\text{PAM}} = (F_{m,\text{PAM}} - F_{o,\text{PAM}})/F_{m,\text{PAM}}$, with $F_{o,\text{PAM}}$ as the minimum fluorescence and $F_{m,\text{PAM}}$ as the maximum fluorescence of dark adapted leaves (Maxwell & Johnson 2000). The $F_v/F_{m,\text{PAM}}$ was calculated as an average of values acquired 1-2 hours before sunrise.

The LIFT system was set up approximately 40-50 m away from the canopies, measuring four areas of interest (AoIs) using an excitation beam of 10 cm diameter projected at close vicinity of each of the Monitoring PAMs. LIFT measurements were performed continuously with every new data point obtained in intervals of 5-6 minutes. The LIFT approach used a laser-based excitation source (665 nm), operating in a pulse mode with variable duty cycle. At high duty cycle, 100 flashlets of 5 μ s duration and 5 μ s intervals were used to manipulate the photosynthetic activity within PSII reaction centers. At the low duty cycle, 40 flashlets lasting 5 μ s were applied with exponentially increasing intervals to observe the kinetic of photosynthetic electron transport. During the measurements 50 of so-called pulse trains were measured and averaged to increase the signal to noise ratio. The maximum (F_m' _{LIFT}) and steady state fluorescence (F_{LIFT}) of light adapted leaves were calculated by numerically fitting the fluorescence transient to a theoretical model that describes the relationship between fluorescence and photosynthetic light conversion (Kolber et al. 2005; Kolber, Prasil, & Falkowski 1998). Quantum efficiency of dark (F_v/F_m _{LIFT}) and light adapted leaves ($\Delta F/F_m'$ _{LIFT}) was calculated in analogy to the PAM approach and multiplied with an empirical factor $\phi = 1.628$. This factor accounts for the difference between the PAM and LIFT approaches derived from the differences of the linear correlation of these two approaches with gas exchange measurements (for details see Pieruschka et al. 2010). ETR_{LIFT} was calculated in analogy to the PAM approach. $PPFD_{LIFT}$ required for the calculation of the ETR_{LIFT} was taken from meteorological stations at each of the experimental sites.

Stanford site

During the experiment at the Carnegie Institution in Stanford three Monitoring PAMs were placed at the top and in the middle part of the sun exposed canopy of *Q. agrifolia* and, at a shady spot at the bottom of this tree. The LIFT was placed on a 5 m high platform at the Carnegie's building and scanned the top, middle and bottom part of the southern exposed canopy from a distance of approximately 40-50 meters. Meteorological data and short wave radiation (300-2500 nm) were measured at a meteorological station at the Carnegie Institution with a pyranometer every 30 minutes. The radiation was converted into $PPFD_{LIFT}$ by multiplying the obtained radiation ($W\ m^{-2}$) with the calibration factor equal of 4.6 (Ting & Giacomelli 1987).

Jülich site

The photosynthetic efficiency was measured within the canopies of the three tree species: *T. cordata*, *Q. petraea*, and *P. sylvestris* from mid-July 2009 to the end of September 2009. The Monitoring PAMs were located at the upper part

in the canopy of each of these trees. The LIFT was placed on the roof of a 25 m high building, monitoring the southwestern exposed side of the deciduous trees and the western exposed part of the pine tree crown from a distance of 40-50 m. As at the Stanford site, four AoIs around each of the Monitoring PAMs were assessed with LIFT. In the end of September the deciduous trees started to turn yellow and PAM fluorescence was measured only in the pine canopy until the experiment ended at the end of January. In Jülich the meteorological data were collected at the meteorological station and radiation (300-3000 nm) was measured every minute by a pyranometer (Type CM7, Kipp & Zonen, Netherlands) on the roof of the same building where the LIFT was installed. $PPFD_{LIFT}$ was calculated in analogy to the calculation at the Stanford site.

Data Analysis

The dependence of the Monitoring-PAMs fluorometer on temperature was tested with a fluorescence standard at temperatures ranging from -15°C to 25°C. A linear dependence between fluorescence measurements and temperature was found and the fluorescence values obtained during the experiments were, therefore, corrected accordingly. $PPFD_{LIFT}$ data, acquired with previously described pyranometers, were used to calculate ETR_{LIFT} when taken simultaneously with LIFT measurements. For the rest, the measured $PPFD_{LIFT}$ data were interpolated to derive irradiance data required for ETR_{LIFT} .

For the calculation of the daily PPFD ($PPFD_{day,LIFT}$) and ETR ($ETR_{day,LIFT}$) the diurnal curves were generated by linearly fitting data points between two subsequent time intervals, which were then integrated over the entire day.

We acknowledge that this approach may introduce some error in calculating ETR, especially during fluctuating light conditions. Mean daily LUE which represents the daily average operating efficiency of PS II was calculated from the relation: $LUE = ETR_{day} / PPFD_{day}$ for LIFT and PAM. Statistical analysis was performed with the Student t-test at a significance level of 0.05 and with a null hypothesis that there is no significant difference between the treatments.

Maximum electron transport rates ($ETR_{max,PAM}$) were calculated with the PAM approach by fitting the ETR vs. PPFD relation with a two parametric exponential curve (cf. Rascher, Liebig, & Lüttge 2000). F-test was used to analyze the differences between different slopes of the ETR and PPFD relations of different species, and between LIFT and PAM. The analysis was performed with the SigmaPlot software (Systat Inc., Version 11).

The relationships between ETR, PPFD and $\Delta F/F_m'$ were analyzed by using a scaling slope analysis (Renton & Poorter 2011). These values, corresponding with the multiplicative relation: $ETR = PPFD * \Delta F/F_m' * \kappa$ (where $\kappa =$

0.5*0.84 the PSII/PSI light partitioning and leaf absorbance constants), were logarithmically transformed, and $\ln(ETR)$ was plotted *versus* $\ln(PPFD)$ and $\ln(\Delta F/F_m')$. The slopes of these relations indicate relative contribution of PPFD or $\Delta F/F_m'$ to the variability ETR. To test the dependence of ETR on PPFD and temperature, the parameters were linearly fitted and the regression coefficient of determination (R^2) was computed.

RESULTS

The field experiment at the Stanford site was performed during spring with average temperatures of approximately 12°C and $PPFD_{day,LIFT}$ ranging between 15 and 25 mol m⁻² day⁻¹ (Fig. 1A). At the Jülich site the summer temperatures averaged 20°C and decreased gradually during the fall with two distinctive minima in the winter. The corresponding $PPFD_{day,LIFT}$ showed some considerable variation during the summer with values between 30-55 mol m⁻² day⁻¹ and decreased gradually in the fall to a value of approximately 5 mol m⁻² day⁻¹ (Fig. 1A). During the experiment at the Jülich site precipitation often reached more than 20 mm per week throughout the experimental period so that drought stress is very unlikely (Fig. 1B). The wind conditions during the experiment were calm with maxima of up to 10 m s⁻¹ in the fall and winter (Fig. 1B), which still caused a frequent readjustment of the monitoring PAMs within the tree canopies because leaves were ripped off the leaf clip. During the experimental period photosynthetic efficiency followed changes in $PPFD_{day,LIFT}$ with some species-specific differences, resulting in highest values for *T. cordata*, intermediate values for *Q. petraea* and lowest values for *P. sylvestris*. The $ETR_{day,LIFT}$ of *P. sylvestris* reached a minimum of approximately 0.5 mol m⁻² day⁻¹ in November and remained low until the end of the experiment (Fig 1C).

A representative day course of PPFD and fluorescence parameters for the tree species at the Jülich site is shown in Fig. 2. The $PPFD_{LIFT}$ corresponded to the irradiance outside of the canopy while the $PPFD_{PAM}$ represented incident light environment on the leaves clipped in the Monitoring PAMs (Fig. 2A-C). The resulting fluorescence parameters obtained by the LIFT indicate rather small differences between F_{LIFT} and $F_m'_{LIFT}$ in the canopy of *P. sylvestris* resulting in a not very distinct $\Delta F/F_m'^{-1}_{LIFT}$ diurnal pattern (Fig. 2D). The difference between F_{LIFT} and $F_m'_{LIFT}$ obtained for the *T. cordata* (Fig. 2E) and *Q. petraea* (Fig. 2F) resulted in a diurnal pattern of $\Delta F/F_m'^{-1}_{LIFT}$ with minima in the afternoon. NPQ_{LIFT} in particular for *T. cordata* and *Q. petraea* (Fig. 2 N&O) also showed a maximum in the afternoon. Whereas ETR_{LIFT} (Fig. 2 G-I) followed the diurnal $PPFD_{LIFT}$ course (Fig.2 A-C). The

complementary fluorescence parameters recorded by the Monitoring PAM, F_{PAM} , $F_m'_{PAM}$ and $\Delta F F_m^{-1}_{LIFT}$ (Fig. 2 G-I) as well as ETR_{PAM} (Fig. 2 J-L) and NPQ_{PAM} (Fig. 2 M-O) were largely affected by the fluctuating incident light on the measured leaves (Fig. 2 A-C).

To compare the two different approaches, ETR_{LIFT} and ETR_{PAM} were plotted vs. the respective PPFD and linearly fitted (Fig. 3). The ETR_{PAM} values of a smaller PPFD dynamic range fit within the envelope of larger ETR_{LIFT} scatterplots. The linear slopes for the PAM approach were rather similar to that of the LIFT approach for *T. cordata* (Fig. 3A), *Q. petraea* (Fig. 3B) and *Q. agrifolia* (Fig. 3C), but steeper in case of *P. sylvestris* (Fig. 3D). F-test, however, revealed no statistically significant difference between LIFT and PAM measurements of all different species (data not shown).

The quantum yield of dark adapted leaves (F_v/F_m) indicated a seasonal decrease towards the fall and winter. For both *T. cordata* and *Q. petraea* the F_v/F_m curves based on LIFT and PAM showed a good agreement (Fig. 4A&B). In mid-August the leaves on the outer part of the canopy of *Q. petraea* started to turn yellow. The decrease in leaf chlorophyll content is demonstrated as a steeper decrease in LIFT quantum yield measurements, but it was not detected by the PAM approach (Fig. 4B). LIFT F_v/F_m values of the evergreen species *Q. agrifolia* and *P. sylvestris* were mainly between 0.3 and 0.6, i.e. substantially lower than the PAM based F_v/F_m (Fig. 4C&D). In particular F_v/F_m measurements of *P. sylvestris* during the winter may indicate foliage adaptations to fall and winter conditions (Fig. 4D). To empirically investigate the effect of the leaf size and geometrical arrangement on the fluorescence signal, the ratio of F_v/F_m recorded by LIFT and PAM for the studied species and a number of species from previous studies was plotted vs. the potential maximal leaf area that can be probed by the collimated LIFT beam normalized by the size of this beam. For plant species with rather large leaves the F_v/F_m recorded by the LIFT was similar to the PAM values, but with decreasing leaf area and increasing canopy structural complexity the LIFT signal appeared to be affected by the anisotropy of the leaf clumps (Fig. 5).

Detailed information on the photosynthetic status of *Q. agrifolia* was derived by comparing different parts of the canopy of *Q. agrifolia* at the Stanford site (Tab. 1). Here the $F_v/F_{m,LIFT}$ obtained at the top and the middle of the canopy was substantially lower than at the bottom, whereas $F_v/F_{m,PAM}$ showed rather small differences between the canopy parts. LUE, derived from PAM and LIFT measurements, was lowest in the middle part of the canopy, while

the highest values were observed at the shaded bottom of the canopy. Maximum electron transport capacity (ETR_{MAX}) was largest at the top of the canopy and lowest at the bottom (Tab.1).

The seasonal dynamics of mean daily LUE_{LIFT} , ranging between 0.1 and 0.25, showed similar variation as PAM measurements, but with distinct differences between the species (Fig. 6). The heterogeneous needle-leaf canopy of *P. sylvestris* showed lower LUE_{LIFT} than broad-leaf canopy of *Q. petraea* and compact canopy of *T. cordata*, which revealed, in agreement with ETR_{LIFT} measurements (Fig. 1), the highest values. LUE_{PAM} ranged between 0.15 and 0.3, with rather small differences between the species. Another characteristic difference occurred for *P. sylvestris*, where LUE_{PAM} varied largely, mainly due to the freezing events in the winter, while LUE_{LIFT} was rather stable throughout the year, varying only slightly in August and from end of December to beginning of January (Fig. 6B&C). The scaling slope analysis was performed to analyze the impact of PPFD and light use efficiency ($\Delta F/F_m'$) on ETR. PPFD and $\Delta F/F_m'$ recorded for each day throughout the experimental period were logarithmically converted and plotted vs. the logarithmically converted ETR (Fig. 7A&B). $PPFD_{LIFT}$ with values ranging between 0.79 and 1.15 for LIFT and between 1.00 and 1.22 for $PPFD_{PAM}$, respectively (Fig. 7A), had substantially larger influence on ETR than $\Delta F/F_m'$. The scaling slope values for $\Delta F/F_m'$ vs. ETR ranged between -0.15 and 0.25 for LIFT and between -0.17 and 0.00 for PAM, respectively (Fig. 7C&D). To test the impact of the temperature on ETR, the diurnal course of temperature and ETR were plotted against each other and the resulting R^2 of this relation throughout the experimental period is shown for in Fig. 7E&F. In all cases R^2 was not larger than 0.55, which indicates a weak correlation between ETR and temperature. The analysis of the seasonal data also supported the scaling slope analysis where ETR was mostly affected by PPFD but not temperature (Tab. 2). However, during the transition from fall to winter the LIFT parameters, $F_{m,LIFT}$ and F_{LIFT} were observed to be hardly affected by the decreasing temperature (Fig. 8A&B) while the PAM based maximum fluorescence $F_{m,PAM}$ decreased substantially when the minimum temperature approached 0°C (Fig. 8D), $F_{0,PAM}$ was less affected (Fig. 8C). The light adapted fluorescence parameters, F_m' and F , showed similar dependence on temperature (data not shown).

DISCUSSION

Photosynthetic performance was monitored successfully in 4 tree species at two spatial scales over both single days (Fig. 2) and extended time periods such as seasons (Fig. 1). The LIFT approach showed photosynthetic parameters at a novel spatial scale, leaf and branches determined at 40-50m distance, which was confirmed by simultaneous

measurements of PAM measurements at leaf level. The LIFT instrument compares reasonably well with the PAM leaf measurements under field conditions in terms of ETR for *T. cordata*, *Q. petraea* and *Q. agrifolia* (Fig. 3), i.e. broadleaf canopies. Noticeable differences, however, occurred between ETR_{PAM} and ETR_{LIFT} (Fig. 3) as well as for $F_v/F_{m,LIFT}$ and $F_v/F_{m,PAM}$ for small leaves, in particular for the conifer canopy of *P. sylvestris* (Fig. 5 and 3D). As observed in previous study (Pieruschka et al. 2010), LIFT measurements conducted on smaller leaves, in particular narrow grass leaves, resulted in larger signal variability than measurements of large broad leaves. The excitation beam of the LIFT (approximately 10 cm in diameter) is in many cases larger than the measured leaves (Fig. 5) and thus the signal recorded with LIFT integrates information of several foliage layers within the canopy. Therefore, the detected fluorescence originating from the layers exposed to different light environment may differ from pulse train to pulse train. Secondly, depending on specific canopy structure and leaf biochemical/optical properties (i.e. angle distribution, clumping, chlorophyll and water content, etc.), the fluorescence signal is scattered and re-absorbed by the foliage elements as any other photon flux. As demonstrated by Knyazikhin et al. (2013), these optical processes are known to be stronger in canopies of small narrow leaves with a higher photon re-collision probability. Finally, as the foliage is moving in the wind, the fluorescence emitted from a small-leaf canopy is scattered and reabsorbed slightly differently each time being measured by the LIFT detector. Therefore, narrow leaf canopy, as for instance the pine tree, resulted in underestimation of ETR_{LIFT} vs. ETR_{PAM} or $F_v/F_{m,LIFT}$ vs. $F_v/F_{m,PAM}$, which is not obvious in case of *T. cordata*, where the relatively large leaves of the outer canopy, arranged like ‘roof tiles’, minimize the confounding canopy structural effects. Additionally, the dependence of the LIFT fluorescence signal on the leaf size and canopy structure may also be affected by a flat measurement angle of the LIFT beam relative to the canopy, which was identified in a previous study with *Beta vulgaris* (data not shown) as the cause of an underestimation of the $F_v/F_{m,LIFT}$ signals (Fig. 5).

The leaves probed with the Monitoring PAM received differed $PPFD_{PAM}$ then $PPFD_{LIFT}$ of the outer canopy layer (Fig. 3). In the case of *Q. agrifolia* the Monitoring PAM was attached to a dead branch sticking out of the canopy, which ensured that a leaf of the outer layer was clipped and hardly shaded. For the other species it appeared more difficult to access leaves of thin branches moving in the wind without self-shading, which resulted in substantially lower $PPFD_{PAM}$ (Fig. 3). The Monitoring PAMs tracked the actual incident light environment on the leaf surface, which enabled an accurate assessment of diurnal (Fig. 2) and seasonal (Fig. 1) fluorescence parameters of the selected leaves which mostly followed the variation in light. However, long-term application of saturating pulses by

the PAM may cause substantial photoinhibition (Apostol et al. 2001; Shen et al. 1996). This applies especially for needle leaves, which forced into the PAM leaf clip may be strongly affected by saturating pulses applied to the ‘side’ of the needles, while natural light environment within needle clumps is rather of a lower intensity and diffuse nature. We accounted for that by replacing and measuring new leaves every 1-2 weeks. The incident light intensity on the leaves probed with LIFT could not be measured directly and was assumed to be similar to the $PPFD_{LIFT}$ as the intensity outside the tree canopy measured with horizontally oriented sensors. The diurnal pattern of the fluorescence parameters showed a difference between the maximum $PPFD_{LIFT}$ (Fig. 2B&C) and the minimum of $F_m'_{LIFT}$ and $\Delta F F_m^{-1}_{LIFT}$ as well as the maximum for NPQ_{LIFT} for *T. cordata* (Fig. 2E, N) and *Q. petraea* (Fig. 2F, O) which occurred with some delay after the solar noon. This can be explained by physiological processes related to mid-day depression which is well known to induce stomatal closure in the afternoon and affect the fluorescence parameters (Medrano et al. 2004). Additionally, $PPFD_{LIFT}$ was recorded with a horizontal sensor whereas the leaves of the outer layer of the canopies of *T. cordata* and *Q. petraea* have an inclination resulting in a perpendicular light exposure and thus highest light exposure in the afternoon affecting fluorescence. Considering all these differences it is expected that LIFT and PAM measurements and derived photosynthetic parameters are not fully comparable, even though they compare relatively well in broadleaf cases (Fig. 3 and 4).

During the cold acclimation of *P. sylvestris* low temperatures induced a decrease and higher variability of LUE_{PAM} , whereas the LUE_{LIFT} measurements were almost unaffected. Fig. 8 shows that LIFT measured steady state (F) and maximum fluorescence (F_m) were not much affected by temperatures below 0°C during the winter period. Contrary to this, the PAM measurements, in particular $F_{m,PAM}$ decreased substantially when the minimum air temperature approached 0°C, as previously observed by Soukupová et al. (2008). The differences may be related to the above-described structural effects of narrow-leaf canopy that are alternating the leaf chlorophyll fluorescence signals. The confounding canopy effects seem to cause lower variability of LIFT fluorescence and derived LUE measurements. It is foreseen that sensitivity analyses conducted using a three dimensional canopy radiative transfer model, similar to the SCOPE model (van der Tol et al. 2009) with a capacity to simulate the forest canopy chlorophyll fluorescence, could verify and help to understand behavior of LIFT measurements illustrated in Fig. 6 and 8. Additionally, the fluorescence signal of the PAM apparatus is measured with a long-pass filter for wavelengths longer than 650 nm (Pfündel, personal communication). The LIFT apparatus uses a narrow 10 nm band-pass filter centered at 690 nm. Thus, while the PAM detects the fluorescence emitted by the PS II and I, the LIFT apparatus detects only the PS II

fluorescence. Under normal conditions the contribution of PSI to the fluorescence signal is rather low, with relatively low variability. However, it was shown that chilling stress combined with a high intensity light is able to inhibit PSI (Kudoh & Sonoike 2002; Zhang & Scheller 2004). Depending on plant species, different levels of damage of PSII, PSI or both may occur under low temperature and irradiation stress (Scheller & Haldrup 2005). While an enhanced activity of PSI induced by cycling electron transport is a well-known phenomenon (Huner, Öquist, & Sarhan 1998; Ivanov et al. 2001), PSI photoinhibition in *P. sylvestris* leaves under cold stress has never been reported. We acknowledge that attributing the differences between LIFT and PAM F_m to the PSI activity is hypothetical, and further dedicated measurements are required to verify this hypothesis.

In agreement with the study by Wulschleger (1993), which demonstrated a lower photosynthetic capacity of evergreens when compared to deciduous trees, our ETR observation are higher for the deciduous *T. cordata* and *Q. petraea* than for the evergreens *P. sylvestris* and *Q. agrifolia* (Fig. 1). To our best knowledge, the observed species were not exposed to any intensive biotic or abiotic stress (see Fig. 1A&B), but were affected only by seasonal downregulation and upregulation processes. These processes, often observed in evergreens (Porcar-Castell 2011, Porcar-Castell et al. 2008a, Ensminger, Busch, & Huner 2006; Öquist & Huner 2003; Gilmore & Ball 2000), are associated with the balance between the energy absorbed through largely temperature-insensitive photochemical processes and the energy used for temperature-sensitive biochemical processes and growth. During the spring the processes are reversed and photosynthesis is re-activated. In the fall and winter (*P. sylvestris*) and also in spring (*Q. agrifolia*), the LIFT recorded F_v/F_m was lower than in the summer (Fig. 4C&D), which may be related to the seasonal downregulation and upregulation processes. The PAM based measurements in Fig. 3D, however, indicate that the ETR_{PAM} vs. $PPFD_{PAM}$ relation for *P. sylvestris* is higher than the LIFT based values, while being comparable to the measurements obtained for *Q. petraea* (Fig. 3B). As discussed previously, we assume that structural features of tree canopies related to leaf size, angle and distribution confound the LIFT measurements and result in underestimation of the quantum yield of needle-leaves canopies.

Despite large differences in ETR_{LIFT} throughout the season (Fig. 1), the LUE ($ETR/PPFD$) showed rather small seasonal variations (Fig. 6). LUE_{LIFT} varied more in the summer than in fall and winter (Fig. 6B), whereas LUE_{PAM} varied substantially more with the onset of freezing temperatures (Fig. 6C). We assume that freezing water on the leaves at night and its melting during the day as well as some snow events caused these LUE_{PAM} fluctuations observed in December and January (Fig. 6). Using a scaling slope analysis (Renton & Poorter 2011), the relative

influence of LUE (or the analogous $\Delta F/F_m'$) and PPFD on ETR can be derived for the daily variation over the entire measurement period (Fig. 7). The PPFD had the largest influence on ETR with scaling slope values close to one (Fig. 7A&B), whereas the impact of $\Delta F/F_m'$ (actual measure of LUE) on ETR appeared to be low (Fig. 7C&D). Regression analysis over the measurement period supports the strong impact of PPFD on ETR (Tab. 2). Regression of diurnal courses of ETR vs. T (Fig. 7E&F) and the seasonal course (Tab. 2) indicate a rather small impact of temperature. This result is in agreement with previous study by Turner et al. (2003), who found that LUE was not well correlated with temperature. Although our experiments were performed with trees not exposed to severe stress impacts, some substantial variation in LUE was still observed which may be related to increasing stress levels (Fig. 6 B&C, Fig 7C&D).

LUE (and other key photosynthetic parameters) indicated differences between the sunny and shade parts of the *Q. agrifolia* canopy (Tab. 1), with higher LUE in the shaded and lower in the sunny canopy parts but higher electron transport capacity in the sunny part of the canopy. This phenomenon is usually related to light acclimation resulting in sun and shade leaves, with associated specific characteristics such as foliar pigment composition and concentration, leaf size, specific leaf area (Lichtenthaler et al. 1981, Hallik, Niinemets, & Kull 2012). Variation of PPFD over days had, however, almost no influence on LUE of the outer canopy layer ($R^2 < 0.05$, data not shown). The only exception was an increasing tendency of LUE with increasing PPFD in the conifer canopy during the fall ($R^2 = 0.15$), and LUE decrease with PPFD decrease in the evergreen *Q. agrifolia* during the spring ($R^2 = 0.19$). Acclimation of canopies to PPFD variation is very gradual and occurs rather slowly during the season, which results in almost unchanged LUE (Fig. 6). An acclimation to fast fluctuating light conditions on a daily scale would most likely require more resources than the additional gain by enhanced photosynthesis (Field 1983; Niinemets, Kull, & Tenhunen 2004). In our experiment we focused on the outer layers of the canopy and with the exception of the *Q. agrifolia* showing very typical sun and shade photosynthetic traits in different layers of the canopy (Tab. 1). The canopy of *P. sylvestris* was also measured at three different layers during the fall to winter transition. However, this free-standing tree was exposed in the fall and winter to very similar light environment within different layers. Thus, no vertical gradient in light or photosynthetic acclimation was observed within different layers but seasonal photosynthetic downregulation and upregulation processes (data not shown). Photosynthetic efficiency also depends on the fraction of absorbed PPFD (Monteith 1972). In the present study we have assumed that the leaf absorbance of the investigated species remained approximately constant. This assumption may not be true during the leaf

senescence period (Schultz 1996) and during the seasonal downregulation and upregulation processes. This potential error was, however, suggested to be rather small, in the range of about 5% (Oliveira & Penuelas 2004).

Overall, the study has demonstrated that the LIFT approach is a valuable tool to assess diurnal and seasonal dynamics of photosynthesis in inaccessible canopies as it provides a rather unique way to study spatial and temporal variation of photosynthetic parameters during seasonal plant growth and development. Still, interpretation of the LIFT narrow-leaf canopy measurements is challenging and requires more extensive field observations combined with an advanced canopy radiative transfer modelling exercise.

ACKNOWLEDGMENT

We are very grateful to Larry Giles and Todd Tobeck for valuable help in assembling a weather proof LIFT enclosure, to Dr. Axel Knaps and Dr. Birger Bohn for providing the meteorological data at FZJ. RP was supported by the Marie Curie Outgoing International Fellowships (Nr: 041060 – LIFT). We gratefully acknowledge financial support by the Transregional collaborative research centre (SFB/TR) 32 “Pattern in Soil-Vegetation-Atmosphere Systems: Monitoring, Modelling and Data Assimilation” funded by the Deutsche Forschungsgemeinschaft (DFG).

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TABLES

Tab. 1 Photosynthetic parameters of *Quercus agrifolia* measured in the early spring within different canopy layers (top, middle, bottom). The letters indicates statistically significant differences between different canopy layers.

Parameter	top	middle	bottom	n
$F_v/F_{m,LIFT}$	0.48 ± 0.08^a	0.49 ± 0.07^a	0.61 ± 0.08^b	15
$F_v/F_{m,PAM}$	0.71 ± 0.03^a	0.65 ± 0.03^b	0.68 ± 0.04^{ab}	12
LUE_{LIFT}	0.14 ± 0.04^a	0.13 ± 0.04^a	0.18 ± 0.04^b	15
LUE_{PAM}	0.17 ± 0.03^a	0.13 ± 0.03^b	0.20 ± 0.03^c	12
$ETR_{MAX,PAM}$	364 ± 4	153 ± 1	62 ± 1	1*

* $ETR_{MAX,PAM}$ was obtained by plotting the data from the entire measurement period

Tab. 2 The regression coefficient (R^2) for the seasonal correlation between ETR and PPFD or temperature obtained with the LIFT or PAM.

Plant species	LIFT/PAM	R^2	
		ETR vs. PAR	ETR vs. T
<i>T. cordata</i>	LIFT	0.7895	0.1757
	PAM	0.9347	0.1384
<i>Q. petraea</i>	LIFT	0.7430	0.2153
	PAM	0.9541	0.2080
<i>Q. agrifolia</i>	LIFT	0.9130	0.3300
	PAM	0.9872	0.0162
<i>P. sylvestris</i>	LIFT	0.8349	0.4080
	PAM	0.9096	0.0057

FIGURE LEGENDS

Figure 1 A, photosynthetic photon flux density (PPFD, solid line), temperature range (gray area) and the average temperature (dotted line) recorded during the experimental period; B, precipitation (grey bars) and wind speed (closed symbols) during the experiment; C, seasonal dynamics of electron transport rates (ETR) recorded by the LIFT for the investigated tree species. Stanford site: *Quercus agrifolia*; Jülich site: *Quercus petraea*, *Tilia cordata*, *Pinus sylvestris*.

Figure 2 A-C, diurnal course of the photosynthetic photon flux density measured outside the canopy ($PPFD_{LIFT}$) as a function of local time and $PPFD_{PAM}$ measured by the sensors of the Monitoring PAMs; (D-F) the corresponding fluorescence parameters measured by the LIFT, minimum fluorescence (F_{LIFT}), maximum fluorescence ($F'_{m,PAM}$) and the quantum efficiency ($\Delta F F_m^{-1}{}_{LIFT}$); (G-I) fluorescence parameters measured by the PAM, minimum fluorescence (F_{PAM}), maximum fluorescence ($F'_{m,PAM}$) and the quantum efficiency ($\Delta F F_m^{-1}{}_{PAM}$); (J-L) electron transport rate measured by the LIFT (ETR_{LIFT}) and PAM (ETR_{PAM}) and, (M-O) non-photochemical quenching measured by the LIFT (NPQ_{LIFT}) and PAM (NPQ_{PAM}).

Figure 3 Relation of electron transport rate recorded by the PAM (ETR_{PAM}) and LIFT (ETR_{LIFT}) and photosynthetic photon flux density (PPFD) obtained for: A, *Tilia cordata*; B, *Quercus petraea*; C, *Quercus agrifolia*; D, *Pinus sylvestris*.

Figure 4 A-D Predawn quantum yield (F_v/F_m) recorded with the LIFT (closed squares) and PAM (open symbols) for, A, *Tilia cordata*; B, *Quercus petraea*; C, *Quercus agrifolia*; D, *Pinus sylvestris*.

Figure 5 Relation of the ratio of $F_v/F_{m,LIFT}$ vs. $F_v/F_{m,PAM}$ on the leaf size described as the ratio of the potentially maximal leaf area which can be measured by a LIFT beam with a diameter of 10 cm to the size of the LTFT beam.

Data were taken from *Tilia cordata*, *Quercus petraea*, *Quercus agrifolia* and *Pinus sylvestris* (current study), *Persea americana*, *Lycopersicon esculentum*, *Capsicum annuum* (Pieruschka et al 2010), *Beta vulgaris*, *Hordeum vulgare*, *Ficus spec.* (unpublished data). The leaf size was taken from own measurements.

Figure 6 A, LUE as the average operating photochemical efficiency recorded over the experimental period by LIFT and B, PAM . Stanford site: *Quercus agrifolia*; Jülich site: *Quercus petraea*, *Tilia cordata*, *Pinus sylvestris*.

Figure 7 A and B: slope of the relation $\ln(ETR)$ vs. $\ln(PPFD)$ with A, LIFT and B, PAM based measurements. C and D slope of the relation $\ln(ETR)$ vs. $\ln(\Delta F F_m^{-1})$ with C, LIFT and D, PAM based measurements. E and F, regression coefficient for the ration of ETR vs. temperature for *Tilia cordata*, *Quercus petraea*, *Quercus agrifolia* and *Pinus sylvestris*.

Figure 8 Predawn steady state fluorescence (F) and maximum fluorescence (F_m) at the top (open symbol), middle part (gray symbol) and bottom part (black symbol) of the *Pinus sylvestris* canopy as a function of the minimum air temperature obtained during the transition from fall to winter (October - January) with the LIFT A and B, and with the PAM, C and D

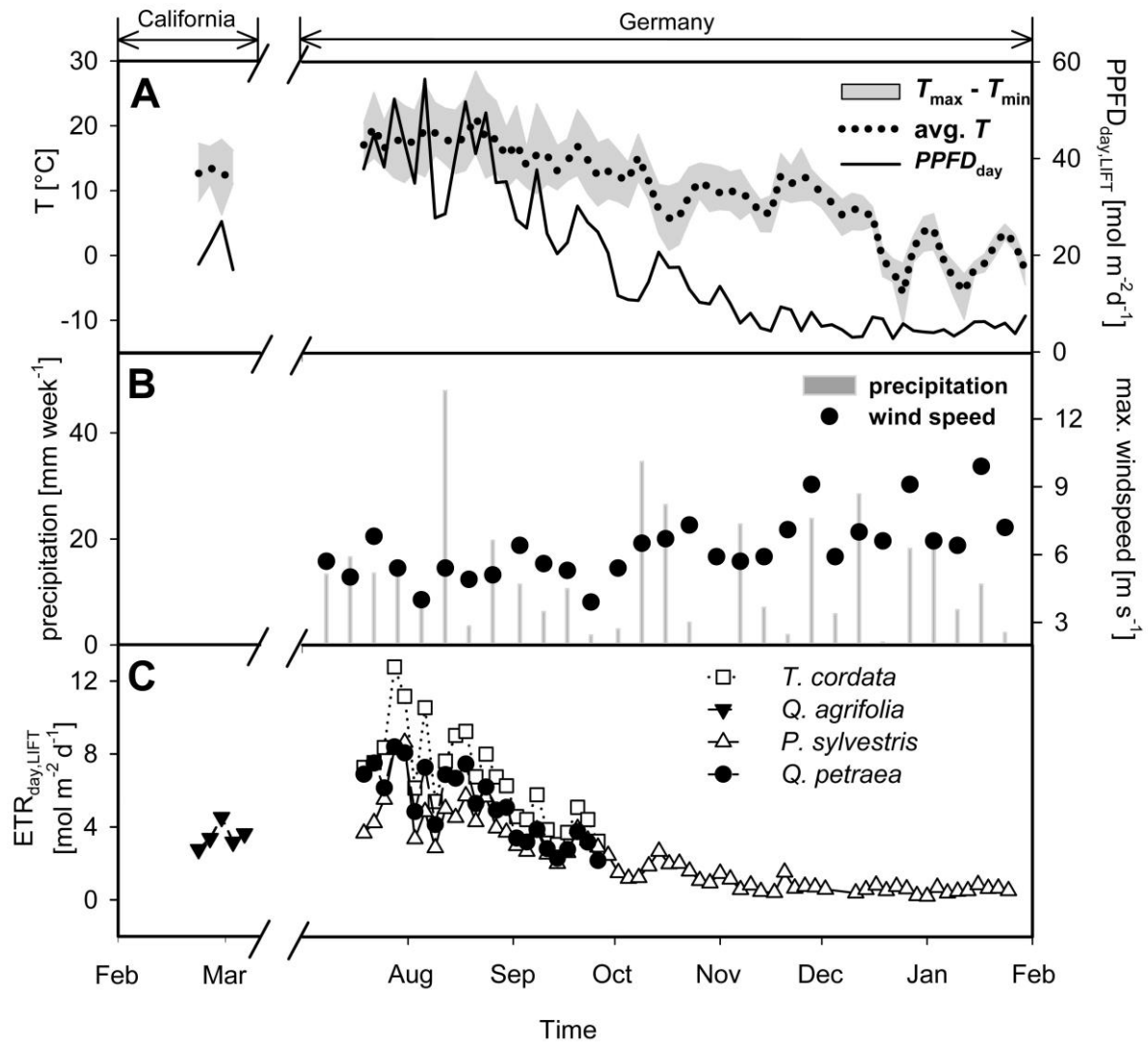


Fig. 1

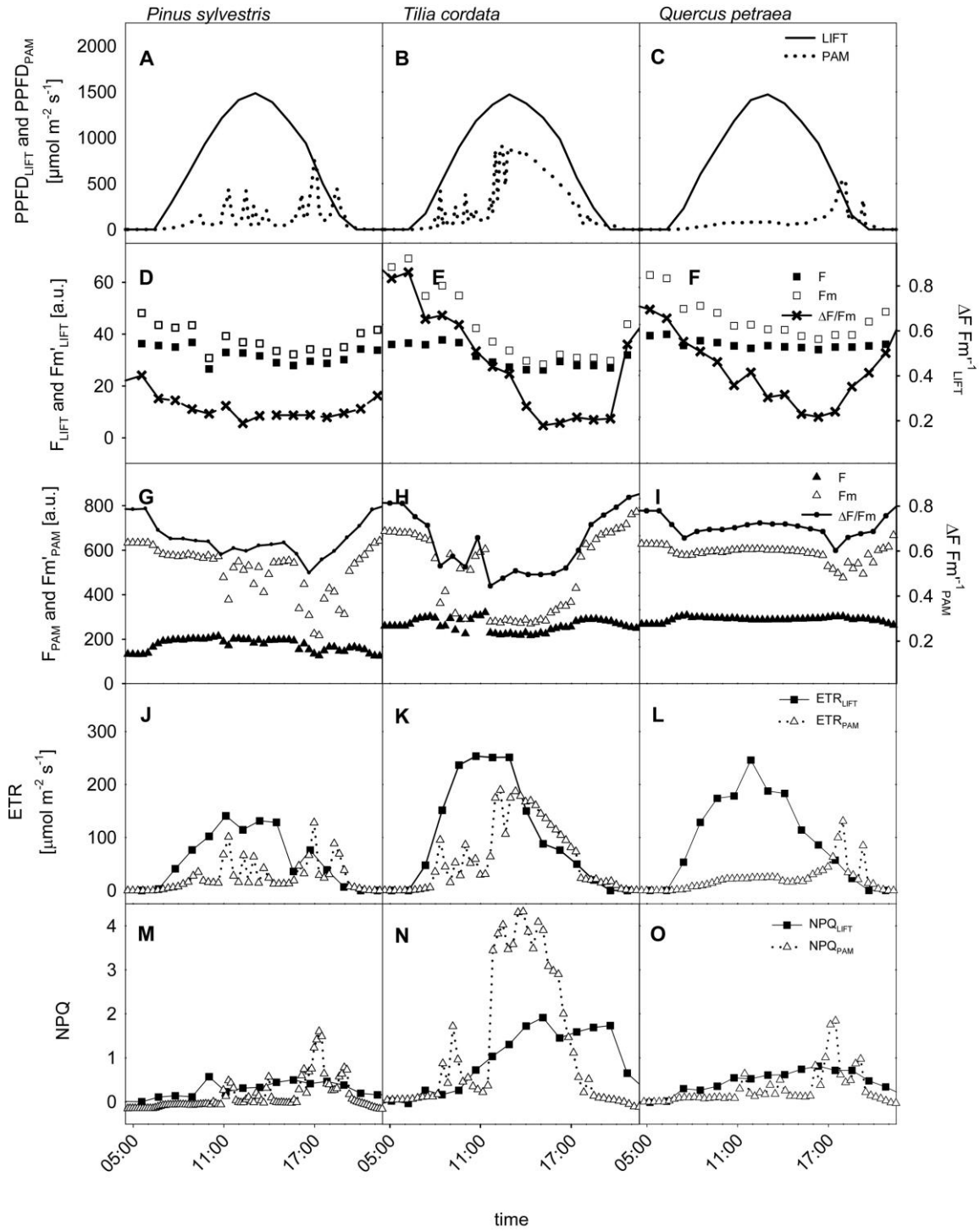
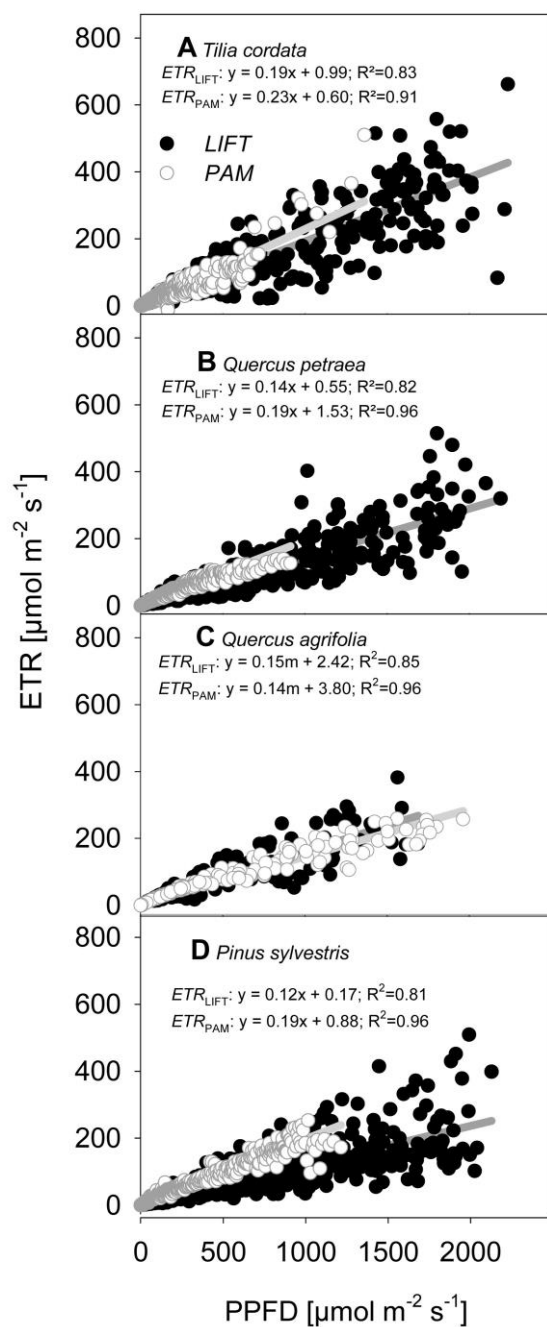
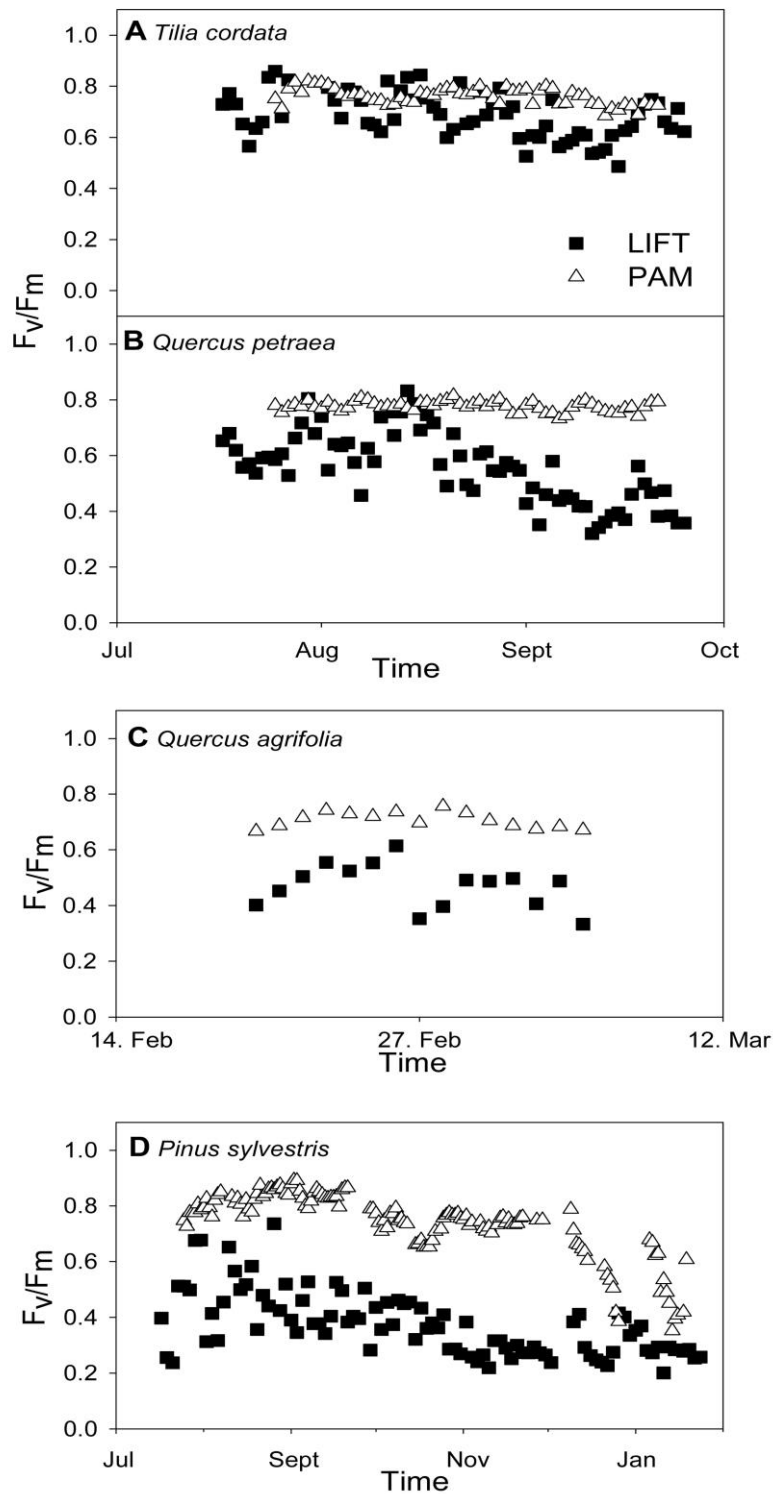


Fig. 2



1

2 Fig. 3



1

2 Fig. 4

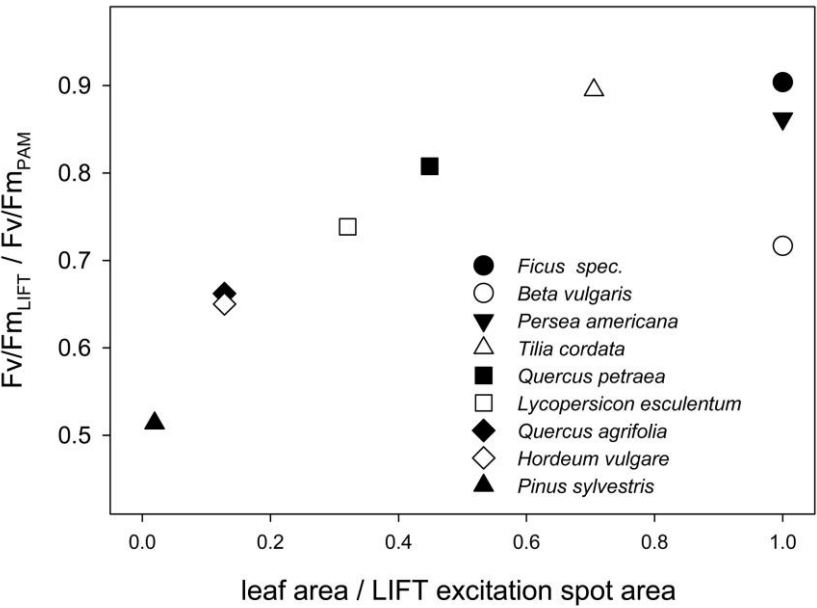


Fig. 5

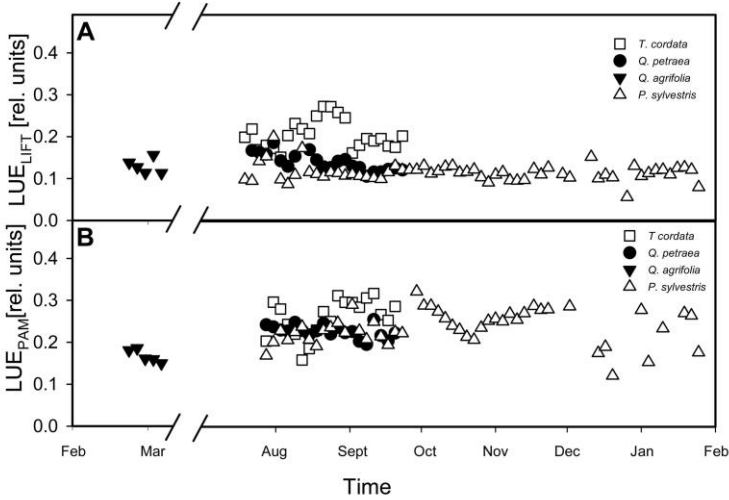


Fig. 6

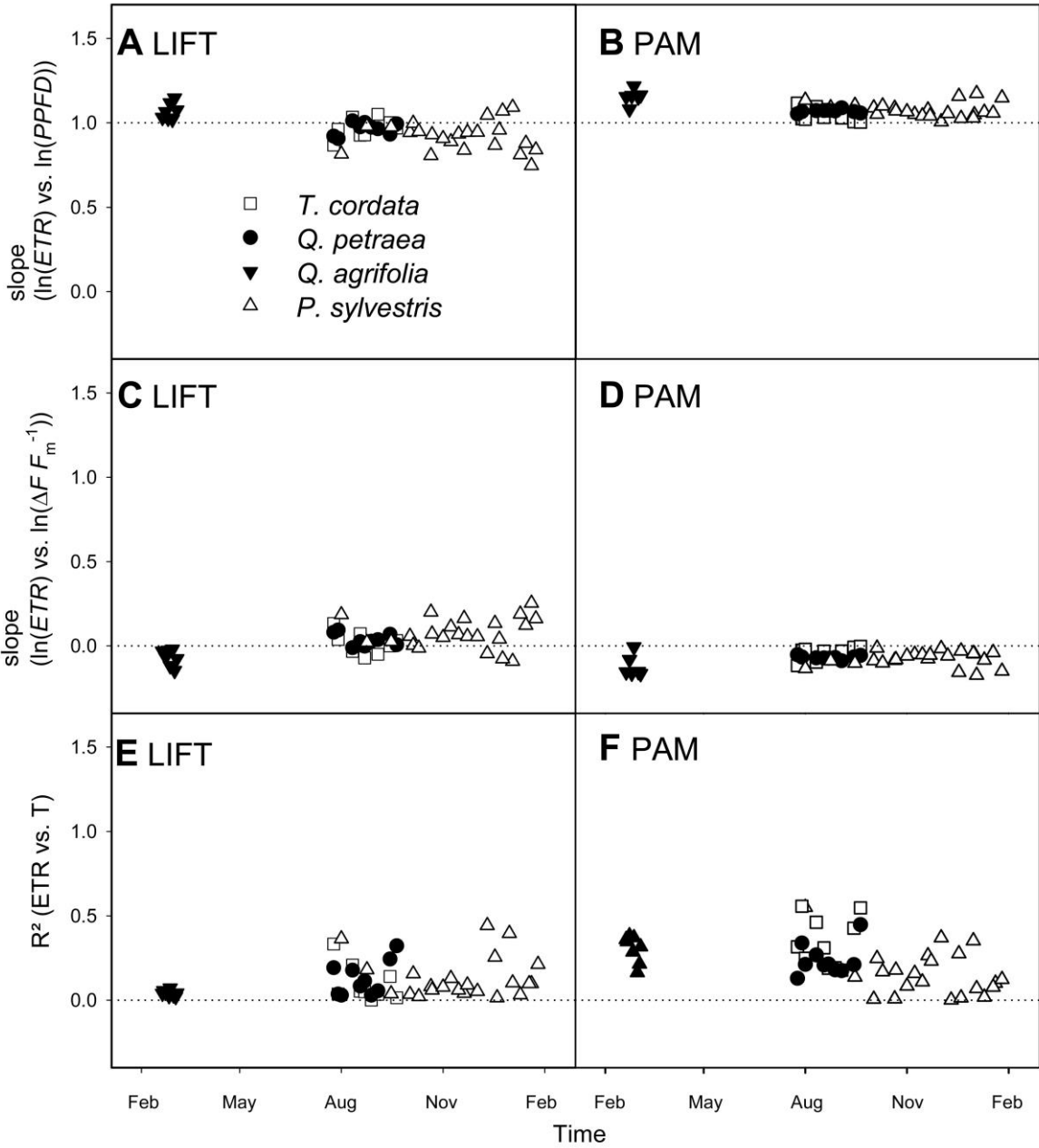
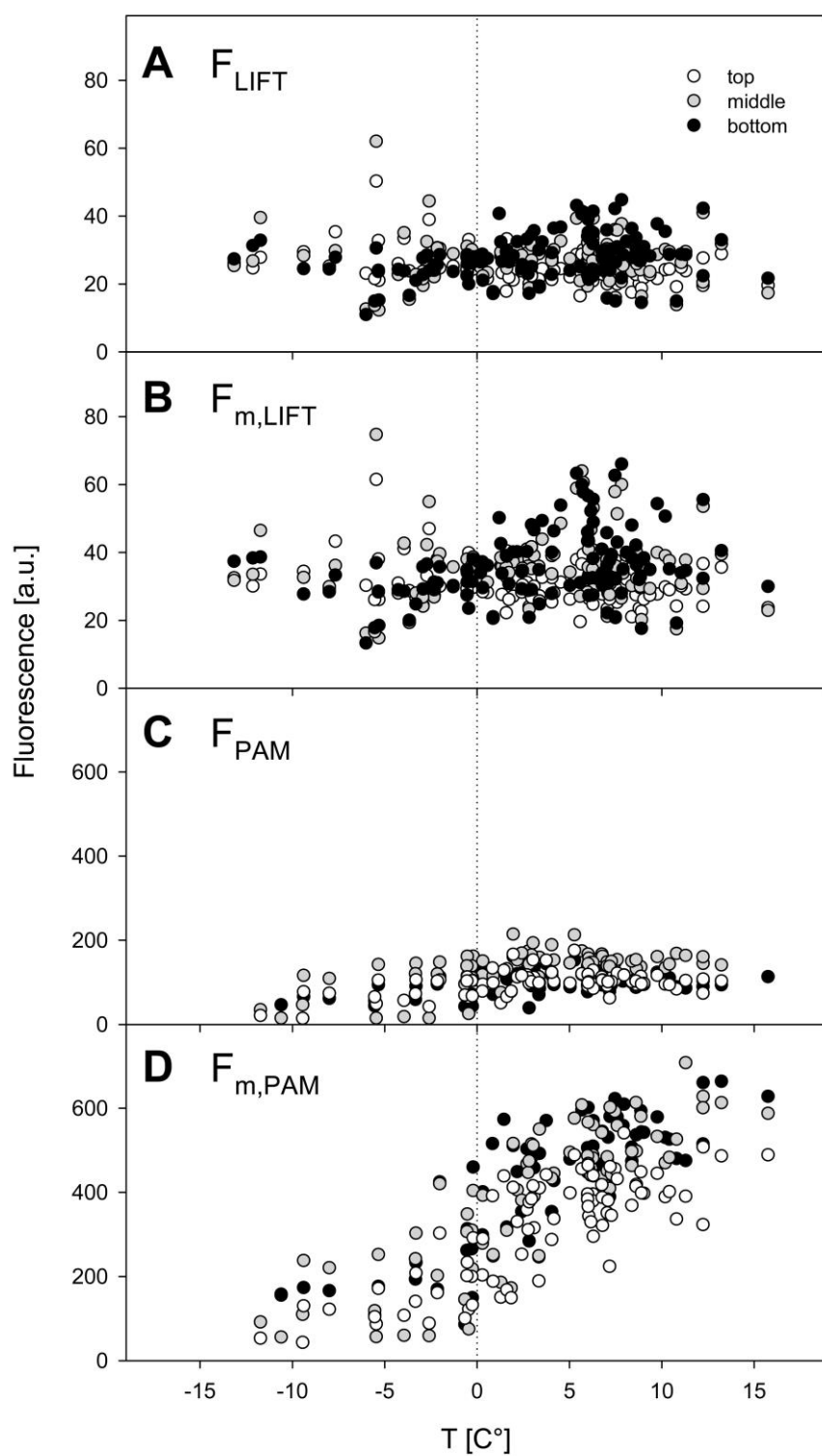


Fig. 7



1

2 Fig. 8