

# Remote sensing of heterogeneity in photosynthetic efficiency, electron transport and dissipation of excess light in *Populus deltoides* stands under ambient and elevated CO<sub>2</sub> concentrations, and in a tropical forest canopy, using a new laser-induced fluorescence transient device

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## Abstract

Determining the spatial and temporal diversity of photosynthetic processes in forest canopies presents a challenge to the evaluation of biological feedbacks needed for improvement of carbon and climate models. Limited access with portable instrumentation, especially in the outer canopy, makes remote sensing of these processes a priority in experimental ecosystem and climate change research. Here, we describe the application of a new, active, chlorophyll fluorescence measurement system for remote sensing of light use efficiency, based on analysis of laser-induced fluorescence transients (LIFT). We used mature stands of *Populus* grown at ambient (380 ppm) and elevated CO<sub>2</sub> (1220 ppm) in the enclosed agriforests of the Biosphere 2 Laboratory (B2L) to compare parameters of photosynthetic efficiency, photosynthetic electron transport, and dissipation of excess light measured by LIFT and by standard on-the-leaf saturating flash methods using a commercially available pulse-modulated chlorophyll fluorescence instrument (Mini-PAM). We also used LIFT to observe the diel courses of these parameters in leaves of two tropical forest dominants, *Inga* and *Pterocarpus*, growing in the enclosed model tropical forest of B2L. Midcanopy leaves of both trees showed the expected relationships among chlorophyll fluorescence-derived photosynthetic parameters in response to sun exposure, but, unusually, both displayed an afternoon increase in nonphotochemical quenching in the shade, which was ascribed to reversible inhibition of photosynthesis at high leaf temperatures in the enclosed canopy. *Inga* generally showed higher rates of photosynthetic electron transport, but greater afternoon reduction in photosynthetic efficiency. The potential for estimation of the contribution of outer canopy photosynthesis to forest CO<sub>2</sub> assimilation, and assessment of its response to environmental stress using remote sensing devices such as LIFT, is briefly discussed.

**Keywords:** Biosphere 2 Laboratory, chlorophyll fluorescence, high-temperature stress, laser-induced fluorescence transients, nonphotochemical quenching, PSII efficiency, remote sensing

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## Introduction

Ozanne *et al.* (2003) emphasized that the forest canopy is 'the functional interface between 90% of Earth's terrestrial biomass and the atmosphere'. Spatial and temporal heterogeneity of photosynthesis in the canopy, arising from diverse contributions of plant genetic, morphological and architectural components to core ecosystem functions such as carbon and water vapor fluxes, responses to stress and nutrient cycling, is a challenging feature of biological feedback between canopy processes, solar radiation and the atmosphere. These feedbacks have been recognized as critical factors in the interactions between the terrestrial biosphere and climate on time scales of centuries. Unfortunately, limited canopy access and the small scale of observations available with portable instrumentation make it difficult to scale this heterogeneity to canopy units. Particular difficulties arise in the estimation of the light environment in canopies (Asner, 1998; Méthy, 2000) and of changes in light with time, ranging from short-term sunflecks (seconds–minutes) to slower changes in light intensity throughout the day and season.

The potential application of radiation-based remote-sensing methods to assess properties such as photosynthetic light use efficiency (LUE) in canopies has been evident for some time (Field *et al.*, 1995), and substantial progress has been made with reflectance measurements. For example, the photosynthetic reflectance index (PRI) reports the de-epoxidation state of the xanthophyll pigments in leaves (Gamon *et al.*, 1990, 1997; Stylinski *et al.*, 2002) and indicates the extent to which light, absorbed in excess of the photosynthetic capacity of leaves, is dissipated as heat through mechanisms of nonphotochemical chlorophyll fluorescence quenching (NPQ). This index is now widely used in combination with pigment analyses and chlorophyll fluorescence measurements (Tambussi *et al.*, 2002). Helicopter-borne spectral reflectance measurements of PRI, in conjunction with flux tower estimates of canopy CO<sub>2</sub> fluxes in forest stands, can be used to remotely sense LUE (Nichol *et al.*, 2002). Canopy derivative reflectance analyses (Zarko-Tejada *et al.*, 2003) have now firmly established that a double peak feature (688, 697, 710 nm) is a function of natural steady-state chlorophyll fluorescence emission, opening the possibility for tracking fluorescence parameters from reflectance data.

Portable chlorophyll fluorescence instrumentation (Schreiber *et al.*, 1986, 1994), using pulse-modulated measuring systems that probe the yield of chlorophyll fluorescence under steady-state illumination ( $F_s$ ) and during a short saturating flash delivered at close range (1–100 mm), has been widely applied to individual

leaves in accessible canopies (Watling *et al.*, 1997; Thiele *et al.*, 1998; Adams *et al.*, 1999; Rascher *et al.*, 2000). This methodology is based on the rationale that even in full sunlight, a sufficiently strong flash will close all reaction centers in a leaf, fully reduce the acceptor  $Q_A$ , giving an estimate of maximum fluorescence yield ( $F_m'$ ). If light incident on the leaf is known, the theoretical argument (Genty *et al.*, 1989) that the ratio  $(F_m' - F_s)/F_m'$  is directly proportional to the efficiency of light use by PSII reaction centers can be used to estimate photosynthetic electron transport rate (ETR). In a recent study of drought responses under controlled conditions in the B2L model tropical forest, climbers using hand-held instruments provided evidence for differences in the response of ETR of adjacent canopy dominants to water stress (Rascher *et al.*, 2004). In principle, this parameter can be scaled to leaf (and possibly canopy) carbon assimilation. Although chlorophyll fluorescence imaging has become the method of choice for evaluation of photosynthetic heterogeneity at the cell (Baker *et al.*, 2001), leaf (Daley *et al.*, 1989; Siebke & Weis 1995; Rascher *et al.*, 2001; Meng *et al.*, 2001), and plant level (Nedbal *et al.*, 2000), it is not yet applicable to canopies because it remains impracticable to deliver saturating flashes safely at a distance over areas even as small as 100 cm<sup>2</sup>–1 m<sup>2</sup>.

Direct measurement of chlorophyll fluorescence at a distance has been an objective of many remote-sensing programs (Field *et al.*, 1995) and both passive (Carter *et al.*, 1990) and laser-based active methods (Cerovic *et al.*, 1996; Rosema *et al.*, 1998; Corp *et al.*, 2003) have been developed. Early progress, especially with the ratio of fluorescence  $F_{690}/F_{730}$  (Günther *et al.*, 1994; Valentini *et al.*, 1994), and evaluation of excitation energy requirements (Rosema & Zahn, 1997), promoted further developments based on steady-state levels of fluorescence (Flexas *et al.*, 2000). Dynamic methods are needed if the full potential of chlorophyll fluorescence analysis is to be realized. The laser-induced fluorescence transient (LIFT) apparatus described here uses a fast repetition rate technique (Kolber & Falkowski, 1993; Kolber *et al.*, 1998), modified to operate with relatively low excitation power with subsaturating flashes for measurement of dynamic chlorophyll fluorescence parameters at a range of 5–30 m.

Many of the reasons for testing the prototype LIFT in the Biosphere 2 Laboratory (B2L) were outlined previously (Osmond *et al.*, 2004). One of the more important applications of the apparatus may be the assessment of CO<sub>2</sub> fixation in canopies in the face of rising atmospheric CO<sub>2</sub> concentrations, and of responses to environmental stress associated with global climate change. The large leaves of cottonwoods grown at ambient and elevated CO<sub>2</sub> in the agriforests of B2L allowed us to

monitor leaves remotely with LIFT, and simultaneously measure CO<sub>2</sub> exchange and chlorophyll fluorescence parameters on the same leaf using traditional portable instruments. We report preliminary comparisons of these techniques that encourage us to believe the LIFT apparatus may well deliver remote estimates of light use efficiency and, potentially, remote estimates of CO<sub>2</sub> fixation. Our first experiments with LIFT in the model tropical forest mesocosm of B2L showed how photosynthetic ETR of canopy dominants could be scaled against mesocosm net CO<sub>2</sub> exchange (Osmond *et al.*, 2004). Here, we describe a more extensive study of remotely sensed heterogeneity of photosynthetic parameters in these tropical tree species in B2L. The species selected differed in the activity of the lutein-epoxide cycle, possibly affecting the extent of NPQ (Rascher *et al.*, 2005). Among other things, we tested whether LIFT could detect this subtle aspect of functional photosynthetic diversity in a tropical forest canopy.

## Materials and methods

### *Plant materials and experimental conditions*

The LIFT apparatus was deployed in ambient (380 ppm CO<sub>2</sub>) and elevated (1220 ppm CO<sub>2</sub>) bays of the B2L cottonwood plantations (intensive forest mesocosm or IFM, Turnbull *et al.*, 2002) during August 2003. Individual south-facing leaves were targeted at a range of 10–25 m in the midcanopy of second year growth in a coppiced *Populus deltoides* plantation grown 4 years under these CO<sub>2</sub> concentrations at 32 °C day/27 °C night. The apparatus was set up in the SE corner of the plantation to measure sun-exposed leaf surfaces. It was also deployed on a platform in the 12-year-old model, tropical forest mesocosm (TFM; Lin *et al.*, 1998; Leigh *et al.*, 1999), approximately 8 m above the forest floor for intensive periods of measurement from September 2002 to July 2003. Thermal stratification in the upper part of the glass pyramid enclosure was alleviated by high speed fans mounted adjacent to the climate-controlled LIFT turret. Midcanopy target leaves of up to five canopy dominants could be observed under ambient CO<sub>2</sub> at a range of 12–15 m, where previous studies showed maximum summer leaf and air temperatures of 32–34 °C and 35–37 °C, respectively (J. Adams, personal communication). Here, we report data for the two most intensively studied trees; *Inga* sp. (tentatively identified by T. Pennington as *I. sapindoides* Willd.) and *Pterocarpus indicus* (Willd.). In both mesocosms, the laser duty cycle described below was applied at 15 min intervals for up to 5 consecutive days under remote control from the research network server in the B2L, and in many experiments, the apparatus in B2L was operated

remotely, in real time, over an internet connection from Rutgers University. Highly reproducible diel data were obtained from LIFT in both locations, with very stable nocturnal values, but with large signal variation in diurnal measurements attributable to the passage of shadows from clouds and from the structural elements of the glass enclosure of B2L.

### *Technical specifications and operating principles of the LIFT apparatus*

The high-frequency, high-excitation power fluorescence induction technique (fast repetition rate fluorescence or FRRF method; Kolber *et al.*, 1998) was adapted for terrestrial use by fixing a laser source coaxially on a telescope, the focal point of which was fitted with an avalanche photodiode. The laser source comprised five red laser diodes (model 500-600-2, Boston Laser, Binghamton, NY, USA) with peak emission at 660 nm (500 mW each with five × 2 lens optical systems to focus). Pulse optical power of 2.5 W at about 20% duty cycle, produced a low light intensity beam of about 30–50 W m<sup>-2</sup> measured by an optical power meter (Model 1815-C; Newport Research Corp., Irvine, CA, USA) when dispersed to provide a target beam size of 12 × 12 cm<sup>2</sup> at a range of 12–15 m. The low power used was designed to maximize human eye safety in a situation where open access to the measurement system was required and reflections from glass surfaces were of frequent occurrence. A custom-built electronic laser driver provided pulse current up to 1.2 A for each laser diode, at 100 kHz frequency. The photodetector (a Peltier-cooled 16 mm diameter avalanche photodiode module 630-70-72-641 from Advanced Photonix Inc., Camarillo, CA, USA) was protected with a longpass red glass filter (Schott RG 665, SCHOTT North America, Elmsford, NY, USA) and an interference filter (10 nm half-bandwidth at 690 nm (XB116/5OR Omega Optical, Battleboro, VT, USA)). The laser source, telescope (250 mm F6.3 Schmidt-Cassegrain telescope model 8" Meade 200 LX, Meade Corporation, Irvine, CA, USA), controllers and computers were mounted in a thermostatically controlled turret with a curved double-walled Lexan window that was purged with warm dry air from the exhaust of an externally mounted air conditioner to facilitate fog-free observation. The curved turret window allowed the automated telescope to move through a horizontal angle of 150° and a vertical arc of ± 30°. The system operated at 25 °C and was monitored with thermocouples.

The fluorescence signal was digitized at 20 MHz with 12-bit resolution using a PCI-DAS4020/12 data acquisition board (Measurement Computing Inc., Middleboro, MA, USA) under the control of LabView version 6.1

(National Instruments, Austin, TX, USA), using a driver from Universal Library for LabView (Measurement Computing Corp., USA). Pulse sequences were generated by a counter/timer board with seven 32-bit counters of National Instruments family NI PCI-6602. The basic pulse sequence used here consisted of 4  $\mu\text{s}$  light pulses followed by 6  $\mu\text{s}$  dark intervals in clusters of 40 pulses followed by a dark period of 600  $\mu\text{s}$  (Fig. 1a). The flash-induced fluorescence yield was calculated by subtracting the background signal recorded during 6  $\mu\text{s}$  interval between pulses from that measured during the flash. The fluorescence yield increased in each cluster of flashes, but declined between clusters because of  $Q_A^-$  reoxidation (broken line in Fig. 1a). Initial ( $F$ ), and the maximal ( $F_m'$ ), fluorescence yields were calculated by numerically fitting the measured fluorescence yields to a theoretical model as described previously (Kolber *et al.*, 1998).

The measured fluorescence transient,  $f(t)$ , can be formally expressed as

$$f(t) = F + (F_m' - F) \left( C(t) \frac{1 - p}{1 - C(t)p} \right), \quad (1)$$

where  $C(t)$  is the level of  $Q_A$  reduction,  $0 < C(t) < 1$ , and  $p$  is the extent of energy transfer between photosynthetic reaction centers. However, under conditions of high ambient irradiance (above 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ )  $p$  usually decreases to very low levels, so that  $p$  is fixed at zero during daylight operation (Kolber *et al.*, 1998). When  $p$  can be assumed to be zero,  $C(t)$  can simply be expressed as

$$C(t) = 1 - q_p(t), \quad (2)$$

where  $q_p$  is the photochemical quenching.  $C(t)$  is controlled by the equilibrium between the rate of charge separation,  $R_{cs}$ , and the rate of the photosynthetic electron transport (or rate of  $Q_A^-$  reoxidation),  $R_{etr}$ :

$$\frac{\partial C}{\partial t} = R_{cs} - R_{etr}. \quad (3)$$

The  $R_{cs}$  is a function of the excitation power,  $i(t)$ , and the functional absorption cross-section of photosystem II,  $\sigma_{PSII}$ :

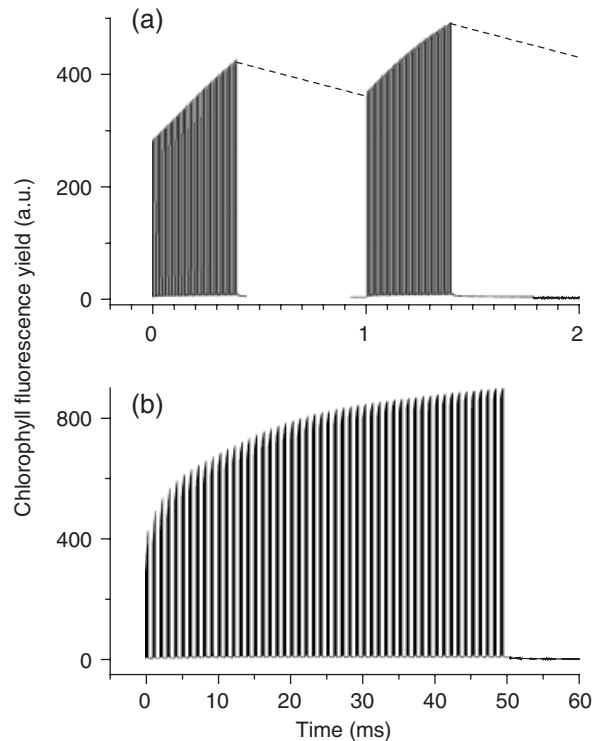
$$R_{cs}(t) = \sigma_{PSII} i(t) \frac{1 - C(t)}{1 - pC(t)}, \quad (4a)$$

$$R_{cs}(t) = \sigma_{PSII} i(t) q_p \quad \text{when } p = 0, \quad (4b)$$

while  $R_{etr}$  is controlled by the kinetics of the photosynthetic electron transport,  $k_{etr}$ :

$$R_{etr}(t) = C(t)k_{etr}(t) = C(t) \frac{1}{\tau_{Q_A}(t)}. \quad (5)$$

Photosynthetic parameters,  $F_o$ ,  $F_m$ ,  $\sigma_{PSII}$  and  $\tau_{Q_A}$  were calculated by numerically fitting the measured fluores-



**Fig. 1** Typical kinetics of chlorophyll fluorescence showing the yield measured by laser-induced fluorescence transient (LIFT) from leaves of *Populus* (grown in 800 ppm  $\text{CO}_2$ ) targeted through glass at 24 m as a function of the laser duty cycle. (a) Cumulative yield in the first two clusters of 40 individual 4  $\mu\text{s}$  laser pulses, 6  $\mu\text{s}$  interpulse dark periods. (b) Kinetic parcel of 50 clusters as in (a), used to calculate fluorescence parameters, was repeated 50 times to yield each point of the LIFT data shown in subsequent figures.

cence transients to Eqs (1)–(5) by a least-squares regression algorithm. The term ‘corrected  $\Delta F/F_m'$ , ETR or NPQ’ is used to distinguish these calculated data from the raw measurements obtained with LIFT. To improve the signal/noise ratio under full sunlight, and to minimize errors because of motion and changing orientation of leaves, 50 measurements were averaged prior to calculations of  $\Delta F/F_m'$ , ETR and NPQ. Incident photon flux density (PPFD) on the upper leaf surface of the targeted area was measured with a gallium arsenide detector (Hamamatsu, Bridgewater, NJ, USA), fitted with a diffuser in a plastic leaf clip (Hansatech Instruments, Kings Lynn, PE32 1JL, UK), calibrated against a Li-Cor quantum sensor (Model 190, Li-Cor Inc., Lincoln, NE, USA) and averaged over the fluorescence measurement cycle.

#### Comparison of remotely sensed LIFT data with leaf chamber gas exchange and Mini-PAM chlorophyll fluorescence data

We compared chlorophyll fluorescence parameters from LIFT against the commonly used ecophysiological

techniques available for leaf level measurements of photosynthesis in forest canopies by simultaneous measurements obtained with a hand-held chlorophyll fluorescence monitoring system. A Mini-PAM (H Walz, Effeltrich, Germany) was used to take three to seven measurements of  $\Delta F/F_m'$  that were averaged over the target areas of sun-exposed *P. deltoides* leaves in 380 ppm  $\text{CO}_2$  treatments before and after the 3 min LIFT measurement cycle, avoiding as far as possible variation in light intensity because of changes in leaf orientation and movement of shadows from clouds and from supporting elements of the enclosure. The Mini-PAM instrument was also hoisted into the TFM canopy and used by rope climbers to measure  $\Delta F/F_m'$  on leaves of the same species as those targeted by LIFT as described above. Routinely, 10 Mini-PAM fluorescence measurements were performed on randomly selected leaves in the target vicinity, and PFD was recorded with the sensor on the Mini-PAM. Photosynthetic ETR was calculated from incident PFD and the efficiency of PSII in the light ( $\Delta F/F_m'$ ), where  $\text{ETR} = \Delta F/F_m' \times \text{PFD} \times 0.42$ , allowing for a leaf reflectance of 16% and equal distribution of absorbed light between PSII and PSI (Genty *et al.*, 1989). Nonphotochemical chlorophyll fluorescence quenching (NPQ) was calculated as  $F_m/F_m' - 1$  (Schreiber *et al.*, 1994).

Portable gas exchange systems are also widely used to assess photosynthesis in canopies (Turnbull *et al.*, 2002). We chose to use leaves of *P. deltoides* trees grown for 4 years in elevated  $\text{CO}_2$  environment (1220 ppm) because errors associated with modeling of photosynthetic ETR from gas exchange are likely to be smaller at elevated  $\text{CO}_2$ . Calibration experiments with leaves were done by attaching the head of a gas exchange system (Model Li-Cor-6400, Li-Cor Inc.) to an area of a leaf just below the  $15 \times 10 \text{ cm}^2$  area targeted by the LIFT. The gas exchange system was programmed to measure exchange of  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , as well as the stomatal conductance repeatedly during each 3 min LIFT measurement.  $\text{CO}_2$  uptake rate ( $J_{\text{CO}_2}$ ) was measured with a portable open-flow gas exchange system (Li-Cor 6400, Li-Cor) using the standard  $2 \times 3 \text{ cm}^2$  leaf chamber with ambient light,  $\text{CO}_2$  concentration, humidity and temperature conditions. The rate of net electron transport was calculated from the rate of gross  $\text{CO}_2$  exchange (net uptake plus respiration in the light), the temperature-dependent specificity coefficient for Rubisco and the partial pressures of  $\text{O}_2$  and  $\text{CO}_2$  in the chloroplasts of the leaf, according to Eqn (A8) of von Caemmerer & Farquhar (1981).

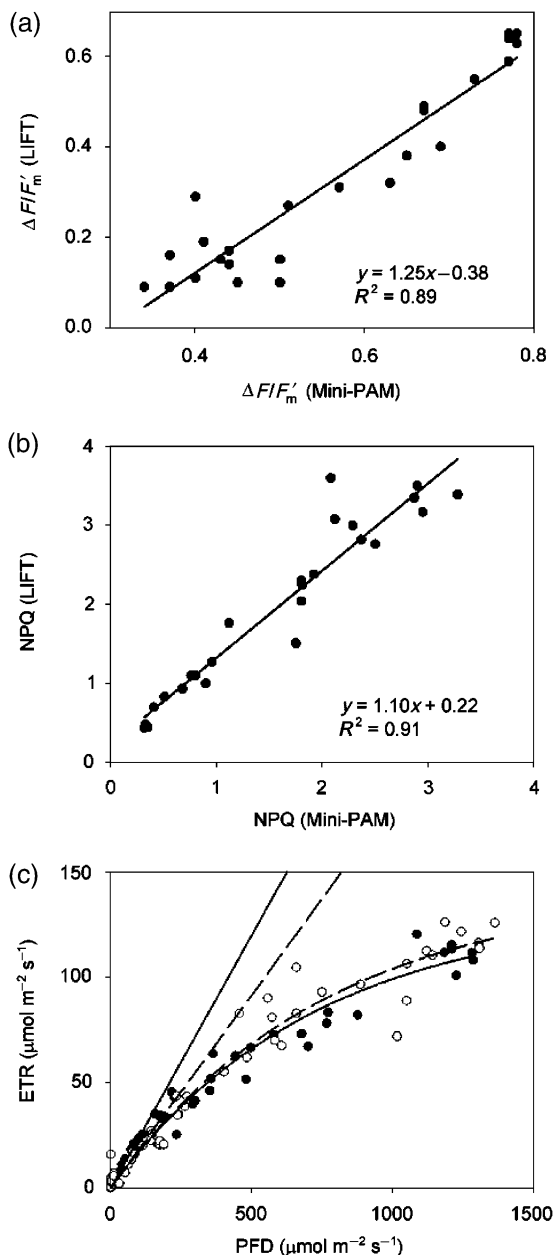
## Results

### *Comparison of LIFT-estimated photosynthetic ETR against ETR modeled from Li-Cor $\text{CO}_2$ exchange in*

### *P. deltoides at elevated $\text{CO}_2$ and ETR estimates from Mini-PAM fluorescence in the canopy of Inga sp. at ambient $\text{CO}_2$*

The correlation between  $\Delta F/F_m'$  of chlorophyll fluorescence in leaves of *Populus* measured by remote by LIFT at a range of 8 m, and the same parameters measured on the target leaf using the Mini-PAM before and after the LIFT measurements, is shown in Fig. 2a. The two very different methods of estimating  $F_m'$  produced different but well-correlated estimates of  $\Delta F/F_m'$ , with larger excursions in the values recorded by LIFT. Discrepancies between these methods are in part because of the fact that hand-held measurements could not be made at precisely the same time on the same targeted leaves. Because of this, leaf light environments were not precisely the same during the two sets of measurements, and the rapid responses of leaf fluorescence parameters to light (in 10 s or less) introduced errors in the spot measurements with hand-held instruments compared with the 3 min measurement protocol of the LIFT. Moreover, in order to operate LIFT at an eye-safe mode, it was impossible to apply saturating light pulses. Especially at high ambient light conditions, laser-induced reduction of the  $Q_A$  pool of PSII is comparably low, increasing the uncertainties in calculation of corrected values of  $F_m'$ . As expected, when the values of  $F_m'$  and  $F_m$  (recorded in dark adapted leaves) measured by the two methods were used to estimate NPQ, qualitatively similar, but slightly higher estimates of NPQ were obtained from the LIFT data (Fig. 2b). The light dependence of photosynthetic ETR in leaves of *P. deltoides* growing in 1220 ppm  $\text{CO}_2$  was calculated from  $\Delta F/F_m'$  measured by LIFT at a range of 8 m using the model of Genty *et al.* (1989). Photosynthetic ETR was also calculated from parameters of  $\text{CO}_2$  exchange measured by the Li-Cor system on an adjacent part of the same leaf, using the model of von Caemmerer & Farquhar (1981). The curves show similar saturation properties but different initial slopes (0.240 vs. 0.183), with the LIFT reporting a higher ETR at low PFD than predicted from the gas exchange data (Fig. 2c).

The diel changes in PFD,  $\Delta F/F_m'$  and NPQ measured on *Populus* leaves in the plantations under ambient (Figs 3a, b) and elevated  $\text{CO}_2$  (Figs 3d, e) imply that in spite of higher cloud cover during measurements in the elevated  $\text{CO}_2$  treatment, the diel decline and recovery of these parameters was relatively smooth. A small rise in PFD in the ambient  $\text{CO}_2$  treatment late afternoon (an artifact of the enclosure, because of additional reflected light from the north wall of the glass structure) slowed the recovery of  $\Delta F/F_m'$  and sustained a modest level of NPQ. Remotely sensed ETR calculated from these data

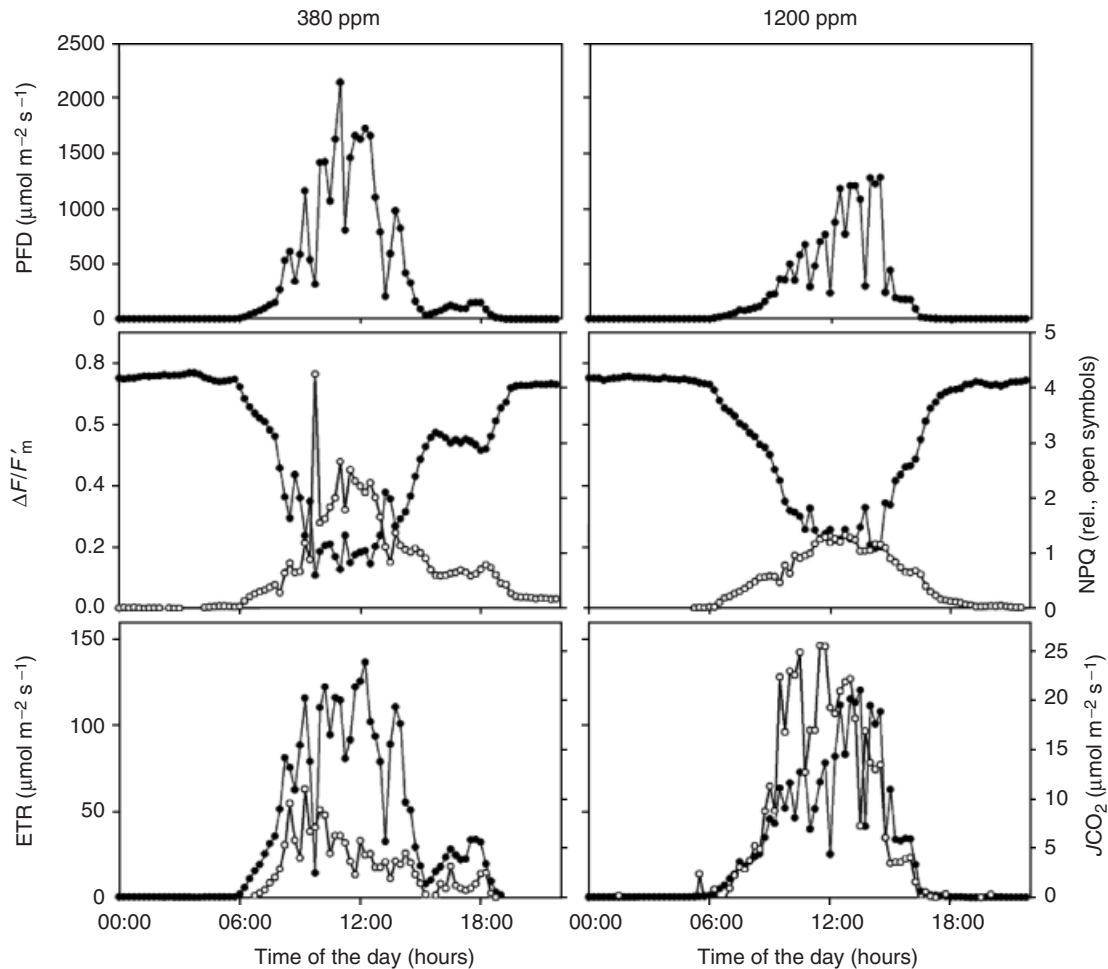


**Fig. 2** Comparisons (a) of efficiency of photosystem II ( $\Delta F/F_m'$ ) measured remotely by laser-induced fluorescence transient (LIFT) and on the same leaf with Mini-PAM, (b) of nonphotochemical chlorophyll fluorescence quenching (NPQ) calculated from data in (a) and (c) of light dependence of photosynthetic electron transport rate calculated from  $\Delta F/F_m'$  measured by LIFT and modeled from leaf gas exchange measured on the same leaf. The LIFT data are shown in solid symbols and lines; the open symbols and dashed lines show the data modeled from gas exchange measurements. Data were obtained in *Populus* stands during a 12 h light period at ambient  $\text{CO}_2$  (a, b), and at 1220 ppm  $\text{CO}_2$  (c). Data in (c) were fitted using a single exponential function ( $f(x) = a(1 - e^{-bx})$ ; Rascher *et al.* 2000). The initial slope of electron transport rate (ETR) was determined by linear regression of data points ( $n = 64$ ) measured at photon flux density (PFD)  $< 100$ .

and net  $\text{CO}_2$  exchange measured on the same leaf are plotted in the diels of Figs 3c, f. Under ambient  $\text{CO}_2$  conditions, the calculated ETR and net  $\text{CO}_2$  exchange tracked each other well at lower light intensities in the morning and afternoon, but diverged markedly at midday, when stomatal closure and decline in intercellular  $\text{CO}_2$  concentration was recorded by the gas exchange system (data not shown). Leaves in elevated  $\text{CO}_2$  (Fig. 3f) experienced lower PFD and displayed slightly lower ETR, again tracking net  $\text{CO}_2$  fixation in the morning and evening. Nevertheless, the elevated  $\text{CO}_2$  concentration drove net  $\text{CO}_2$  exchange higher at midday, despite lower PFD, slightly elevated stomatal closure, and some decline in intercellular  $\text{CO}_2$  concentration (data not shown). Plots of ETR vs.  $\text{CO}_2$  fixation in the light-limited region of the diel curves ( $< 250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) reveals the higher energy cost of carbon assimilation at ambient vs. elevated  $\text{CO}_2$ , consistent with higher photorespiratory activity in ambient  $\text{CO}_2$ .

#### *Remote sensing of photosynthetic parameters in canopy dominants in a model tropical forest using LIFT*

Direct comparisons between LIFT and Mini-PAM measurement techniques were not easily achieved in the tropical forest canopy. It was impossible to anchor the Mini-PAM to outer canopy leaves of *Inga* sp. for direct comparisons of  $\Delta F/F_m'$  on exactly the same target, and difficult to tilt LIFT deep enough into the canopy to target leaves that were accessible for handheld Mini-PAM measurements at the top of a ladder. The compromise in Fig. 4 shows measurements performed on leaves exposed to similar irradiance between 07:00 and 10:00 hours in different parts of the canopy with a fixed, programmed Mini-PAM and LIFT. Although  $\Delta F/F_m'$  measured by LIFT responded almost identically to Mini-PAM in the morning, it settled at a lower level until midday when a downward drift continued in the afternoon. In spite of differences in the light exposures in the two sets of measurements (data not shown), the greater excursion of  $\Delta F/F_m'$  in the LIFT measurements (Fig. 4a), the relative responses of NPQ in the two systems were qualitatively similar (Fig. 4b). We are aware that LIFT may underestimate  $\Delta F/F_m'$  resulting in an underestimation of ETR. This underestimation can be ascribed mainly to the nonsaturating light intensity of the laser. However, subsequent evaluations using the next generation of LIFT and a modified measuring protocol indicate that a linear correction can be developed for future ecosystem screening (Kolber *et al.*, 2005). Both measurement techniques revealed a sustained afternoon NPQ of 2–3 units, followed by a slow decline towards dusk.

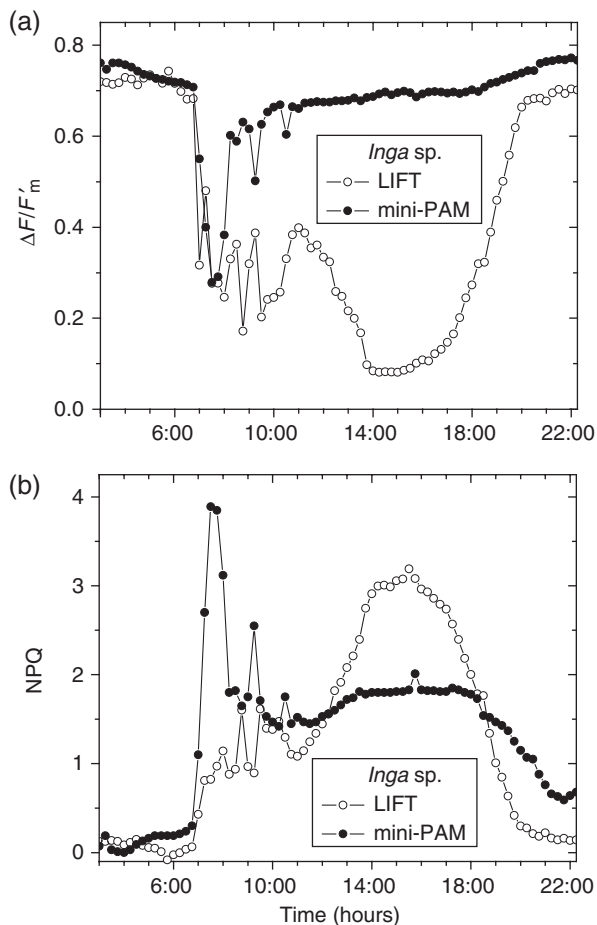


**Fig. 3** Diel patterns of chlorophyll fluorescence parameters in leaves in *Populus* stands grown at 380 ppm (left) and 1220 ppm (right) remotely sensed with laser-induced fluorescence transient, and compared with gas exchange measurements of net CO<sub>2</sub> fixation.

Midcanopy leaves of *Inga* were exposed to relatively high PFD from 06:00 to 11:00 hours (Fig. 5a) and the efficiency of PSII ( $\Delta F/F_m'$ ) detected by LIFT declined quickly after dawn. As noted above,  $\Delta F/F_m'$  declined further and steadily at low PFD in the afternoon, and recovered slowly towards dusk. As a result, ETR remained low throughout the afternoon, and NPQ showed a substantial rise above the more or less steady level attained at high PFD between 07:00 and 11:00 hours (Fig. 5b). This distinctive feature was found in all experiments done in the TFM in Summer 2003. A large mirror was used to reflect a patch of sunlight onto the surface of the canopy during midafternoon (Fig. 6a). The additional light produced no measurable change in ETR, and was presumably all dissipated by the large increase in NPQ (Fig. 6b). In other late afternoon sunfleck experiments with younger sun-exposed leaves of *Inga*, both ETR and NPQ rose rapidly in response to the increase in PFD (data not shown). We targeted more

sun-exposed and more shaded patches of the *Inga* canopy on two successive cloudless days (Fig. 7a, b). The sunnier patch showed sustained NPQ until PFD decreased to about  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  at 13:00 hours, but then increased steadily over the next 3 h. In the more shaded patch NPQ rose modestly between 07:00 and 09:00 hours in response to an hour of bright light ( $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) then declined until about 13:00 hours, when it increased with similar kinetics but lower amplitude to that observed in the sun-exposed patch of the canopy.

Although leaves of an adjacent dominant canopy, *Pterocarpus*, experienced somewhat lower PFD than those of *Inga*, they showed very similar trends in  $\Delta F/F_m'$  and NPQ, with somewhat lower estimates of ETR (cf. Figs 4–8). The afternoon drifts in photosynthetic efficiency were qualitatively similar to those observed for *Inga*, but  $\Delta F/F_m'$  usually only declined to 0.3 (cf. 0.1 in *Inga*). The mirror sunfleck treatment forced  $\Delta F/F_m'$

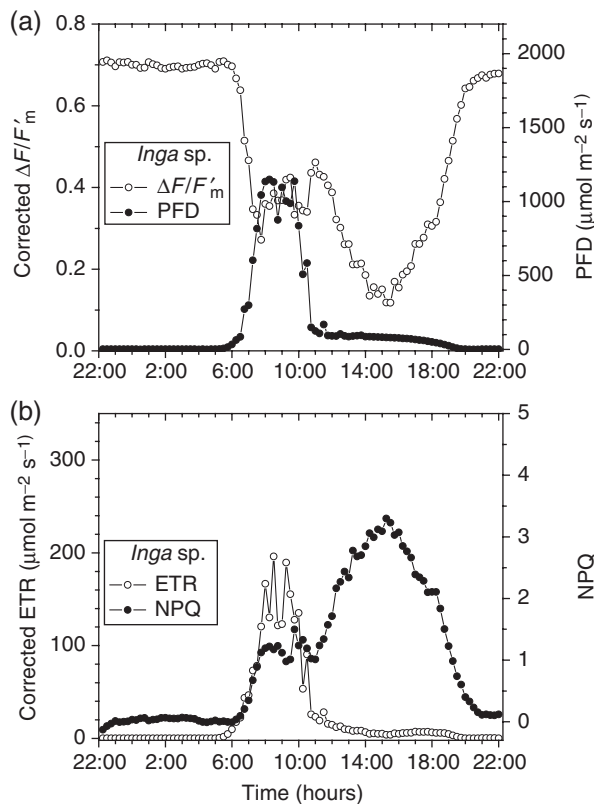


**Fig. 4** Comparison of the diurnal course of  $\Delta F/F_m'$  and NPQ measured in leaves of *Inga* in adjacent, similarly sun-exposed parts of the canopy using saturating pulse and chlorophyll fluorescence transient methods with Mini-PAM and laser-induced fluorescence transient (LIFT), respectively.

somewhat lower in *Pterocarpus* and produced a large increase in both NPQ and ETR (Fig. 9).

## Discussion

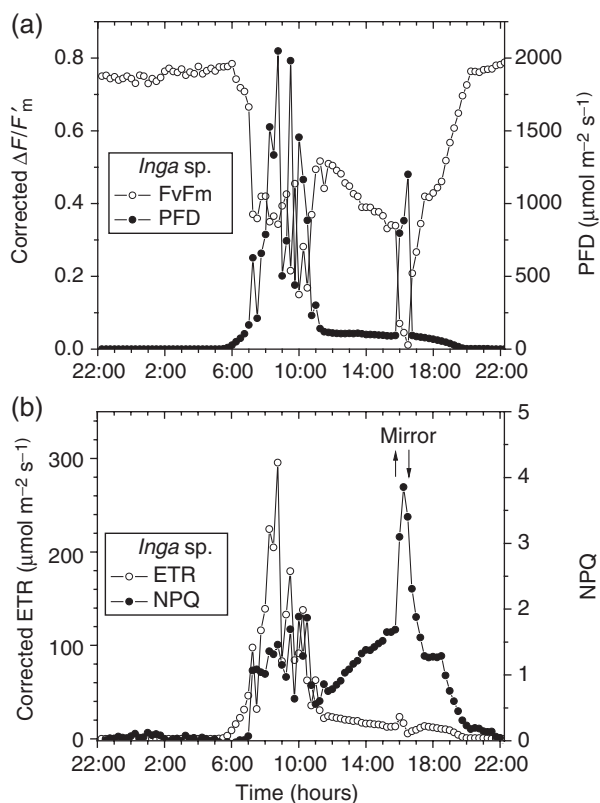
The prototype LIFT instrument described here demonstrates the potential and the limitations of remote sensing of photosynthetic efficiency and ETR using actively stimulated chlorophyll fluorescence. Our experiments show that although the remote LIFT apparatus recorded lower values of  $\Delta F/F_m'$  than obtained through on-the-leaf measurements with the saturating flash method of Mini-PAM, estimates of NPQ from the two methods were similar and well correlated in *Populus* leaves, and both methods detected the elevation of NPQ in the afternoon in leaves of *Inga* in the tropical forest. The light response profiles of photosynthetic ETR calculated from measurements by LIFT



**Fig. 5** Diel course of chlorophyll fluorescence parameters in midcanopy leaves of *Inga*, remotely sensed by laser-induced fluorescence transient at a distance of 12 m. The data are means of 3 successive cloud free days of measurement in June 2003.

closely correspond to those modeled on the basis of gas exchange measurements.

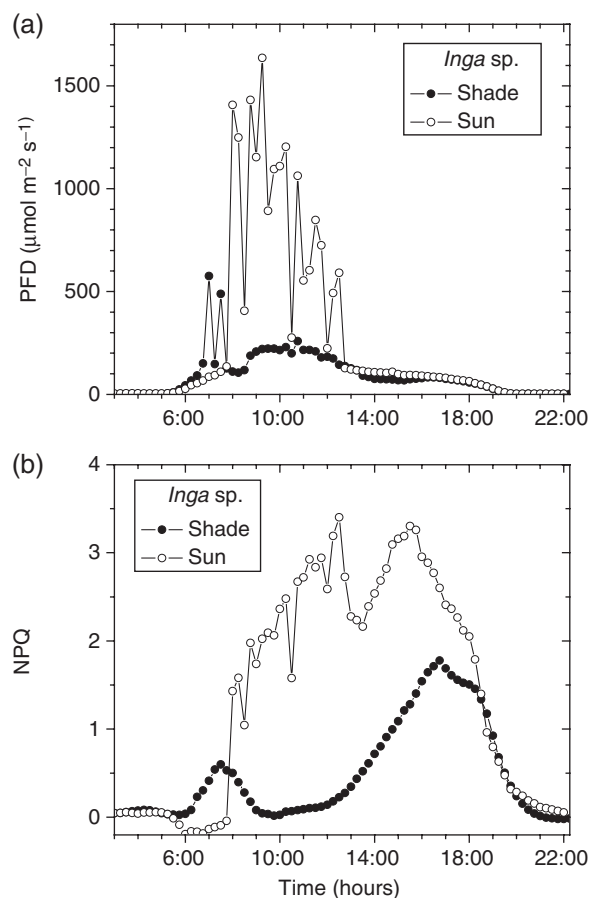
Indeed, LIFT revealed a feature of outer canopy photosynthetic efficiency that had previously eluded detection by rope climbers using the hand-held Mini-PAM. The persistent low efficiency and high NPQ reported by LIFT in low light during the afternoon in the tropical forest canopy is presumably because of high afternoon temperatures in the enclosed mesocosm. Previous measurements in the TFM revealed 7–8 °C higher leaf and air temperatures in the upper canopy compared with the forest floor. Previous studies also showed inhibition of net CO<sub>2</sub> assimilation in midcanopy leaves of other species in the TFM at high temperatures (G. Lin, unpublished data), and this would be expected to lead to sustained high NPQ. Indeed, it is well known that high temperature, above the optimum, but well below critical temperatures, causes reversible inactivation of leaf CO<sub>2</sub> fixation, and markedly increased NPQ (Weis, 1981; Weis & Berry, 1988; Law & Crafts-Brandner, 1999). In all the diel experiments monitored by LIFT,  $\Delta F/F_m'$  returned to the same predawn level, indicating no sustained



**Fig. 6** Diel course of chlorophyll fluorescence parameters in midcanopy leaves of *Inga*, remotely sensed by laser-induced fluorescence transient at a distance of 12 m. A mirror was used to apply a strong sunfleck to the targeted leaves at 15:45 hours, after they had been 4 h in the shade.

photoinhibition. Thermal stratification was much less in the *Populus* canopies (data not shown) and sustained elevated afternoon NPQ was not detected in these experiments (Fig. 4). We conclude that the apparent anomaly detected by LIFT is real and indicates an important feature of reduced light use efficiency at elevated leaf temperatures in the enclosed tropical forest canopy.

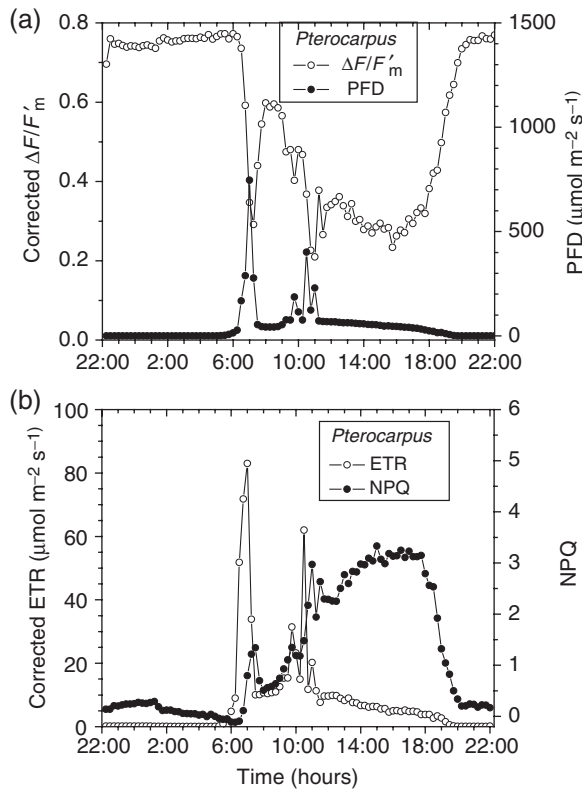
There are relatively few studies of photosynthetic properties (Zotz & Winter, 1994), light dissipation dynamics and photoprotection in the outer canopy of tropical forests presumably because access is so difficult. Most studies address more accessible understory plants of smaller stature (Valdares *et al.*, 1997; Watling *et al.*, 1997; Thiele *et al.*, 1998; Adams *et al.*, 1999). The outer canopy environment differs markedly from the understory in that it is exposed to a higher range of PFD, is less well buffered against changes in VPD and leaves of different species display very different responses to soil water stress (Rascher *et al.*, 2004). For example, spot measurements made by rope climbers using Mini-PAM showed more severe reduc-



**Fig. 7** Daily course of light environment and NPQ estimated from chlorophyll fluorescence parameters measured by laser-induced fluorescence transient in two patches of midcanopy leaves of *Inga* differing in sun exposure.

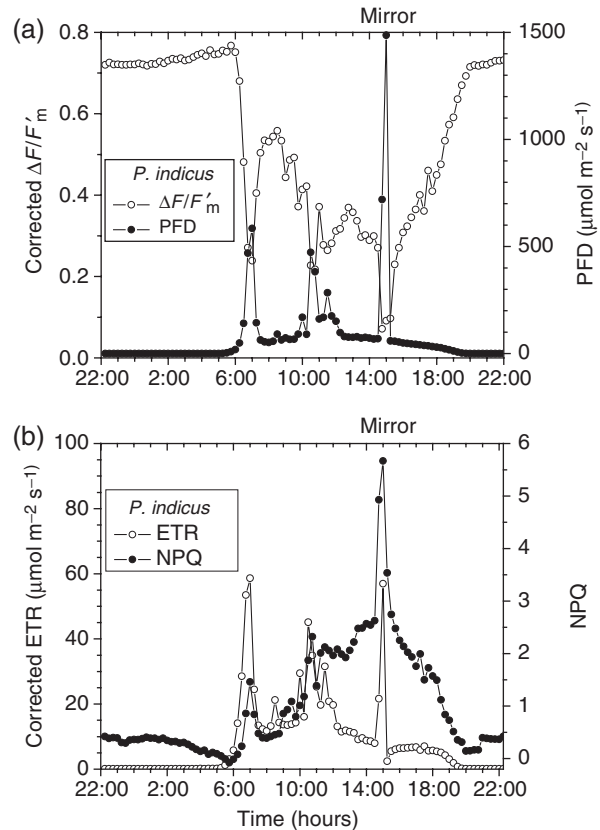
tion of ETR during drought in leaves of *Pterocarpus* compared with *Inga* (to 56% vs. 80%, respectively). Although detached, rapidly dehydrated leaves of *Inga* showed a much steeper decline in the relationship between photosynthetic reflectance index and NPQ than *Pterocarpus* (Rascher *et al.*, 2005), possibly suggesting an amplification of xanthophyll-stabilized NPQ in the lutein epoxide-rich leaves of *Inga* (Matsubara *et al.*, 2005). We were unable to detect this subtle difference between well-hydrated leaves of these species in the canopy using LIFT. The afternoon low  $\Delta F/F_m'$ , high NPQ syndrome was similar in *Inga* (high Lx species) and *Pterocarpus* (low Lx species), but remotely sensed photosynthetic ETR in *Inga* sometimes responded less to artificially imposed afternoon sunflecks than in *Pterocarpus*.

Remote-sensing devices such as LIFT will be especially useful in exploring these questions in the outer canopy. The LIFT apparatus seems to offer new opportunities for the remote sensing of photosynthetic



**Fig. 8** Diel course of chlorophyll fluorescence parameters in midcanopy leaves of *Pterocarpus* remotely sensed by laser-induced fluorescence transient at a distance of 12 m.

ETR and the evaluation of outer canopy architecture and exposure as determinants of  $\text{CO}_2$  fixation in the face of increasing atmospheric  $\text{CO}_2$  concentrations and environmental stress associated with global climate change. For example, diurnal tracking of the contributions of different parts of the *Inga* and *Pterocarpus* canopies to carbon gain in the TFM can be inferred from LIFT estimates of ETR and mapped against net system  $\text{CO}_2$  exchange (Osmond *et al.*, 2004). Further calibration of LIFT estimated ETR in different species, perhaps in conjunction with flux-tower measurements, could facilitate remote sensing of ecosystem net  $\text{CO}_2$  exchange. An improved version of LIFT (Kolber *et al.*, 2005) with greater range (to 30 m) and better time resolution (2 s) may be especially useful to unravel other potentially diverse mechanisms of light use efficiency in photosynthesis. Coupled with the accurate positioning capabilities of the telescope, it is also in principle feasible to scan large areas of a canopy with LIFT, and to map changing patterns of photosynthetic efficiency and ETR. Much depends on improving the measurement of light environment at the time and place of targeted leaves. At the very least, integration of the areas under families of diurnal curves for ETR in



**Fig. 9** Diel course of chlorophyll fluorescence parameters in midcanopy leaves of *Pterocarpus*, remotely sensed by laser-induced fluorescence transient at a distance of 12 m. A mirror was used to apply a strong sunfleck to the targeted leaves at 14:30 hours, after they had spent 2 h in the shade.

representative species and canopy environments seems more likely to provide clear insight into outer canopy contributions to system  $\text{CO}_2$  assimilation than laborious spot measurements with hand-held instruments. Although there seems little prospect 'at the moment' of repeating the above experiments in B2L, we look forward to exploring these possibilities in other controlled environments, such as those available in the tropical forest of the Eden Project ([www.edenproject.com](http://www.edenproject.com)), and eventually under field conditions.

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