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SPECIAL ISSUE: SOLAR-INDUCED FLUORESCENCE: FROM THE LEAF TO BEYOND THE CANOPY

Modification of a gas exchange system to measure active and passive chlorophyll fluorescence simultaneously under field conditions

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Abstract

Solar-induced fluorescence (SIF) is a promising tool to estimate photosynthesis across scales; however, there has been limited research done at the leaf level to investigate the relationship between SIF and photosynthesis. To help bridge this gap, a LI-COR LI-6800 gas exchange instrument was modified with a visible-near-infrared (VIS-NIR) spectrometer to measure active and passive fluorescence simultaneously. The system was adapted by drilling a hole into the bottom plate of the leaf chamber and inserting a fibre-optic to measure passive steady-state fluorescence ($F_{t,\lambda}$, analogous to SIF) from the abaxial surface of a leaf. This new modification can concurrently measure gas exchange, passive fluorescence and active fluorescence over the same leaf area and will allow researchers to measure leaf-level $F_{t,\lambda}$ in the field to validate tower-based and satellite measurements. To test the modified instrument, measurements were performed on leaves of well-watered and water-stressed walnut plants at three light levels and a constant air temperature. Measurements on these same plants were also conducted using a similarly modified Walz GFS-3000 gas exchange instrument to compare results. We found a positive linear correlation between $F_{t,\lambda}$ measurements from the modified LI-6800 and GFS-3000 instruments. We also report a positive linear relationship between $F_{t,\lambda}$ and normalized steady-state chlorophyll fluorescence (F_t/F_o) from the pulse-amplitude modulation (PAM) fluorometer of the LI-6800 system. Accordingly, this modification will inform the link between spectrally resolved $F_{t,\lambda}$ and gas exchange—leading to improved interpretation of how remotely sensed SIF tracks changes in the light reactions of photosynthesis.

Keywords: Chlorophyll fluorescence; leaf-level; LI-6800; photosynthesis; SIF.

Introduction

Light absorbed by chlorophyll pigments in plants can be utilized in three pathways. When photons excite the electrons in a plant's photosystems, the energy can drive the light reactions of photosynthesis (photochemistry), dissipate as heat (non-photochemical quenching, NPQ) or re-emit as light at longer wavelengths (650–850 nm, chlorophyll fluorescence). With three

alternative pathways, disentangling the distribution of light energy can be difficult unless one of the pathways is inhibited. Most commonly, NPQ is inhibited by pulse-amplitude modulation (PAM) fluorimetry at the leaf-level through rapid saturation of the photosystems with a pulse of light at a time scale where NPQ is negligible (Maxwell and Johnson 2000). As such, leaf-level PAM fluorimetry has become a powerful tool for assessing changes in photosynthetic machinery and plant performance (Krause and Weis 1991; Bilger *et al.* 1995; Govindjee 1995). The availability and relative ease of operation of commercial handheld fluorometers has made PAM an attractive technique to probe the first steps of photosynthesis. Pulse-amplitude modulation fluorimetry measurements are used to assess the efficiency of photosystem II (PSII) photochemistry, which has been found to correlate strongly with CO_2 fixation under normal conditions (Genty *et al.* 1989; Edwards and Baker 1993). Such comparisons have been enabled through the use of gas exchange instruments using infrared gas analysers (IRGA), which measure carbon and water fluxes at the leaf-scale (Long and Bernacchi 2003). Gas exchange measurements and PAM fluorescence can be used in a complimentary fashion to understand the relationships between net CO_2 assimilation (A_{net}), PSII yields and NPQ (e.g. Flexas *et al.* 1999; Magney *et al.* 2017).

Chlorophyll fluorescence can be classified as active (PAM) or passive, based on the source of excitation light. However, the direct link between active and passive fluorescence and its functional relationship to carbon uptake via photosynthesis remain unclear (Porcar-Castell *et al.* 2014). While PAM measurements are versatile and commonplace across plant sciences, they are limited to the individual leaf-scale and a specific point in time. In an effort to scale these measurements from the leaf to the canopy and from seconds to seasons, the solar-induced fluorescence (SIF) technique was derived using the same principle as PAM by measuring radiative loss of energy of absorbed photons by chlorophyll in the 650- to 850-nm range (Meroni *et al.* 2009). As a result, there has been recent interest in developing techniques to continuously measure chlorophyll fluorescence at increasing spatial scales with ground-based (Porcar-Castell 2011) and remote sensing platforms (Yang *et al.* 2015; Magney *et al.* 2019a). Solar-induced fluorescence measurements do not require a modulating light and are considered to be the most closely related remote sensing proxy of photosynthesis from towers and satellites (Mohammed *et al.* 2019). Several challenges exist with passive retrieval of chlorophyll fluorescence compared to the retrieval of PAM fluorescence. First, the SIF signal is relatively weak (~1–5 % of reflected light) compared to the reflectivity of leaves across the 650- to 850-nm range (Meroni *et al.* 2009). Additionally, up to 90 % of red fluorescence photons can be re-absorbed by the leaf because of the overlapping nature of the chlorophyll emission and absorption spectra (Gitelson *et al.* 1998). Lastly, the PAM technique measures a broad spectral region (>650 nm) while remote sensing measurements are made across individual wavelengths, where the spectral shape can be quite dynamic (Magney *et al.* 2019b). This makes it difficult to directly relate leaf-level PAM measurements to SIF retrieved from remote sensing systems at a range of scales. For these reasons, there has been interest in developing leaf-scale measurement techniques under controlled conditions to simultaneously measure SIF ($F_{t,\lambda}$), PAM fluorescence and gas exchange to validate fluorescence signals from remote sensing platforms.

Magney *et al.* (2017) developed a novel method of connecting passive and active fluorescence measurements at the leaf-level by modifying a Walz GFS-3000 gas exchange system (described in more detail in the Materials and Methods section). This new method was effective but limited to lab measurements due to portability constraints, temperature/humidity sensitivities of the spectrometer and imperfect transmission of incident light through the shortpass filter at the top of the leaf chamber.

Here, we outline a method to address these limitations by modifying a LI-COR LI-6800 gas exchange system in combination with a visible-near-infrared (VIS-NIR) spectrometer that: (i) is suitable for field conditions; (ii) measures gas exchange, passive fluorescence and active fluorescence concurrently; and (iii) measures the same leaf area under a wide range of controlled conditions for *in vivo* experiments. To accomplish this, a hole was drilled into the bottom chamber plate of an LI-6800 instrument to measure $F_{t,\lambda}$ from the bottom surface (abaxial) of leaves because the fluorometer on the LI-6800 does not allow spectral measurements from the top. The system was tested by measuring gas exchange and fluorescence in the field with the LI-6800 and comparing to fluorescence measurements of the same plant made by the GFS-3000.

Theory

A schematic of the propagation of emitted red and far-red Chl fluorescence molecules is provided in Fig. 1. Because PAM fluorescence is retrieved from the top of the leaf in the LI-6800, there may be some differences in active fluorescence from the adaxial side (top) of the leaf and passive fluorescence measured on the abaxial (underside) of the leaf. However, we expect that changes in fluorescence yield (normalized for light intensity) will scale similarly. To understand this in detail, it is necessary to distinguish between passive fluorescence measured via spectrometer ($F_{t,\lambda}$) and steady-state active fluorescence from PAM (F_t). $F_{t,\lambda}$ is the wavelength specific radiant energy flux of chlorophyll fluorescence that can be defined as:

$$F_{t,\lambda} = aPAR * \Phi F * \varepsilon_\lambda$$

where aPAR is the absorbed photosynthetically active radiation by chlorophyll, ΦF is the probability a photon absorbed by a leaf will be fluoresced and ε_λ is the probability of the photon escaping the leaf at wavelength λ. F_t derived from PAM fluorometry is defined as:

$$F_t = aPAR_{ML} * \Phi F * \varepsilon_p$$

where $aPAR_{ML}$ is the absorbed photosynthetically active radiation from the modulating light source and ε_p is the integral of ε_λ beyond a longpass filter (>650 nm) (Magney *et al.* 2017).

To compare passive and active fluorescence from the GFS-3000 and the LI-6800, two normalized fluorescence values were computed. F_t,λ yield measured by spectrometer was calculated by the following equation:

$$F_{t,\lambda} \text{ yield} = \frac{F_{t,740}}{R_{red\ peak}}$$

where $F_{t,740}$ represents the peak magnitude of fluorescence at 740 nm (Figs 1 and 3B) and $R_{red\ peak}$ is the maximum value of reflected red energy at 640 nm (Figs 1 and 3A). Passive fluorescence was normalized with the red peak to eliminate possible error from movement of the fibre-optic or differences in leaf thickness or incoming irradiance. Fluorescence yield measured by PAM fluorometry was calculated by the following equation:

$$\text{Fluorescence yield} = \frac{F_t}{F_o}$$

where F_o is the baseline fluorescence of the dark-adapted leaf and F_t is steady-state fluorescence in the light (Flexas *et al.* 2002).
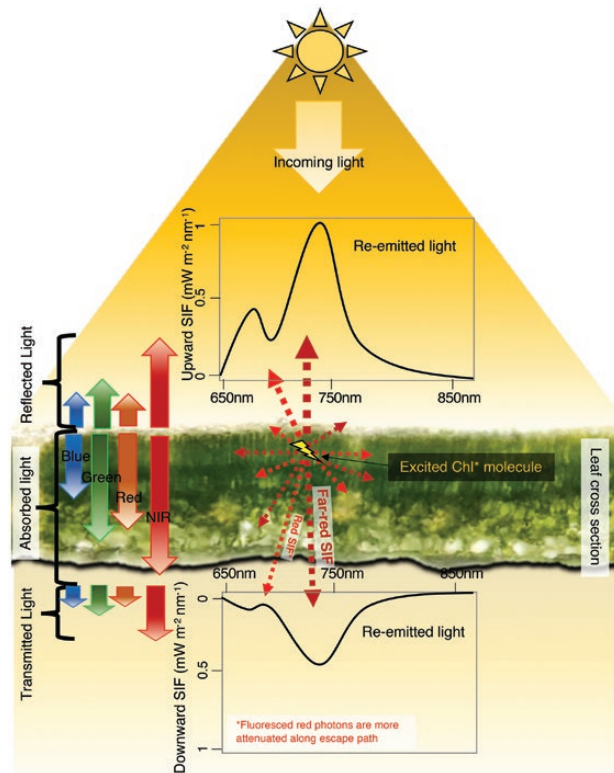
Figure 1. Conceptual figure of an excited chlorophyll molecule from the adaxial side of a *J. regia* leaf. The *J. regia* cross-section was imaged using a confocal microscope at 10× (Nikon C2+, Nikon Instruments Inc., Melville, NY, USA). When a chlorophyll molecule near the top of the leaf is excited, red and far-red photons are emitted in all directions, with red photons being re-absorbed by other Chl molecules within the leaf. Meanwhile, both red and far-red photons are attenuated as they travel through the leaf, resulting in a lower magnitude in abaxial retrieved SIF ($F_{t,\lambda}$) vs. adaxial retrieved SIF ($F_{t,\lambda}$). This is consistent with the absorption of visible and near-infrared light, resulting in reduced transmittance on the abaxial leaf surface. Measurements made from the system described in this paper are made on the abaxial side of the leaf, where we expect a greater reduction in overall $F_{t,\lambda}$ and in the red:far-red $F_{t,\lambda}$ ratio.

Materials and Methods

Modified LI-6800 with Flame spectrometer

An LI-6800 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA) was modified slightly to accommodate dual measurements of leaf gas exchange and $F_{t,\lambda}$ by drilling a hole into the bottom chamber plate (part number 9968-315) of the Multiphase Flash™ Fluorometer measuring head using a precision drill and a 1.5-mm drill bit. The spectrometer fibre-optic was fed through the hole and held in place at a set distance from the sample with two O-rings (size 003, Fig. 2) to measure passive fluorescence from the abaxial surface of leaves. To ensure that the chamber was sealed, adhesive putty was applied around the fibre-optic outside of the chamber.

PAM fluorometer and light source. The LI-COR LI-6800 Multiphase Flash™ Fluorometer with 6-cm² circular chamber was used to measure active chlorophyll fluorescence parameters. The PAM fluorometer has red actinic and saturating flash peak wavelength of 625 nm (Fig. 3A). The rectangular saturation pulse emitted 15 000 µmol m⁻² s⁻¹ of light for 0.8 s. Three saturating pulses were taken at each light level, and the steady-state fluorescence yield (F_t) was recorded before each pulse. F_t values were measured and
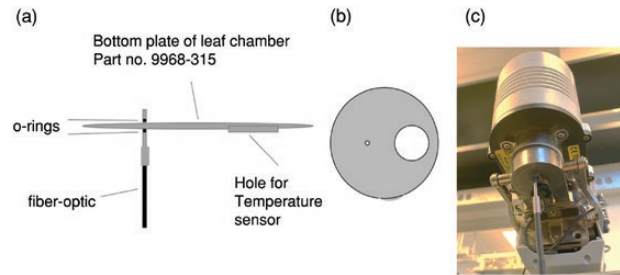
Figure 2. (A) Side-view schematic of the of the modified LI-6800 leaf chamber plate with inserted spectrometer fibre-optic. (B) Top-view schematic of the modified plate, which highlights the orientation of the 1.5-mm drilled hole in reference to the temperature sensor hole. (C) Photo of the modified system with fibre-optic inserted into the leaf chamber.

obtained immediately before saturation pulses when net CO_2 assimilation and stomatal conductance reached steady state (details below).

Gas exchange. The LI-6800 uses two IRGAs to calculate the change in water and carbon dioxide concentrations between a reference chamber and the leaf sample cell chamber. Gas exchange parameters including net CO_2 assimilation (A_{net}) and stomatal conductance (g_s) were measured at a steady state (no more than a 1 % change in parameters over 1 min) at three PAR levels under a constant temperature. At 50 µmol m⁻² s⁻¹ PAR, steady state was achieved in around 10 min. At 500 µmol m⁻² s⁻¹ and 1500 µmol m⁻² s⁻¹ PAR, steady state was achieved in around 20 min.

Spectrometer. A Flame VIS-NIR Spectrometer (Ocean Optics, Dunedin, FL, USA) was used to measure abaxial fluorescence from the bottom of the LI-6800 chamber. The Flame spectrometer was used for its portability and thermal stability (https://www.spectrecology.com/2015/04/thermal-stability-of-new-flame-bench/) making it more suitable for field measurements unlike like the QE Pro (Ocean Optics, Dunedin, FL, USA) outlined in Magney *et al.* (2017). Additionally, the Flame spectrometer is powered using a 5-V USB connection, so there is no longer a need for AC power, as is required by the QE Pro. However, the Flame spectrometer comes at a decreased signal-to-noise ratio and lower spectral resolution than the QE Pro. The Flame spectrometer covers 339–1009 nm with a 1.33-nm full-width half-maximum (FWHM), and a linear silicon CCD array with an entrance slit of 25 µm, a #1 grating and 2048 pixels. Absolute calibration was performed using an integrating sphere and a reference Analytical Spectral Devices (ASD) spectrometer at the Jet Propulsion Laboratory in Pasadena, CA, USA. The spectra were measured with an integration time of 10 ms and data was recorded every 0.2 s (co-adding 20 spectra). Integration time was chosen to avoid optical sensor saturation at the maximum light level of 1500 µmol m⁻² s⁻¹ PAR. The 10 ms integration time is the minimum for the Flame spectrometer, but with an increased integration time at lower light levels, an increased signal-to-noise ratio is expected and recommended for sampling under at light intensities <100 µmol m⁻² s⁻¹ PAR. A sample light spectrum measured with the Flame spectrometer is shown in Fig. 3, highlighting the incoming LED spectrum in Fig. 3A.

Modified Walz GFS-3000 with QE Pro spectrometer

Adaxial measurements of passive and active fluorescence were done using the instrument and spectral nomenclature described
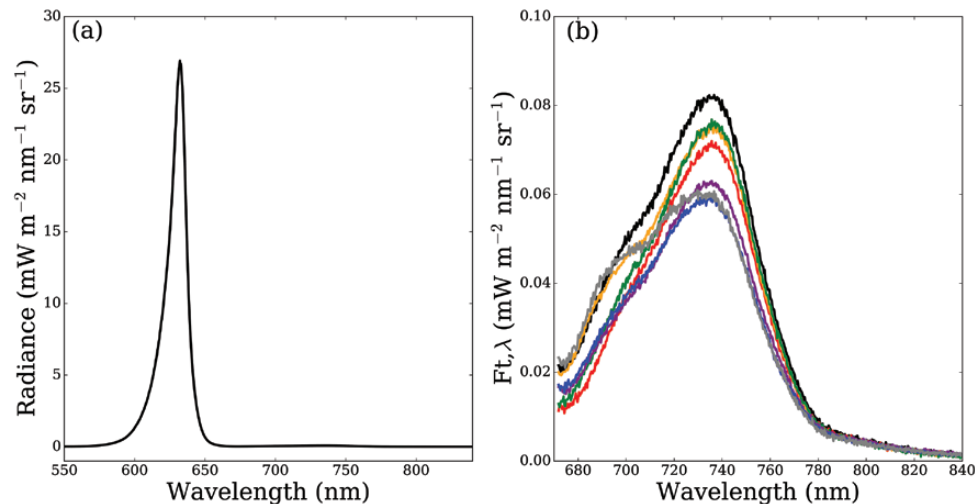
Figure 3. (A) Light spectrum of a walnut leaf at 500 µmol m⁻² s⁻¹ PAR measured with the modified LI-6800 and Flame spectrometer. (B) Enlarged spectra showing $F_{t,\lambda}$ from all *Juglans regia* samples of the experiment.

in (Magney *et al.* 2017). A portable GFS-3000 gas exchange and fluorescence system (Heinz Walz GmbH, Effeltrich, Germany) was used to validate and compare fluorescence measurements from the modified LI-6800 with Flame spectrometer set-up. The GFS-3000 was slightly modified to use a bundled fibre-optic to connect the PAM fluorescence module and QE Pro spectrometer, all details can be found in Magney *et al.* (2017).

Experimental design and analysis. Eight *Juglans regia* cv. (grafted on Vlach rootstock) saplings were planted in 5-gallon pots with UC soil mix. 44.4 mL of Osmocote® Smart-Release® Plant Food Plus fertilizer were added to each pot. The plants were grown in a UC Davis lath house. Two water treatments (well-watered and water-stressed) were implemented based on soil water mass percentage. The plants (four replicates for each treatment) were watered to maintain around 75 and 25 % of completely saturated soil by weight. Every plant was weighed individually and watered to maintain water treatment status between 4 and 6 PM every day and monitored using a Model 600 Leaf Pressure Chamber (PMS Instrument Company, Albany, OR, USA) to measure midday leaf water potentials.

The modified LI-6800 system was used for gas exchange and active and passive fluorescence measurements in the lath house. The youngest, fully expanded, intact leaf was chosen and dark-adapted for 15 min. While 15 min may not ensure complete dark adaptation, we were not conducting a full physiological evaluation of these parameters, but rather comparing two measurement techniques. Measurements were made at constant temperature (30 °C), constant relative humidity (45 %) and three different PAR levels (50, 500 and 1500 µmol m⁻² s⁻¹) that were chosen as low, medium and high light levels for a range of comparison. Carbon dioxide concentration was held constant at 420 ppm. The plants were then taken to the lab for comparative measurements with a modified Walz GFS-3000 (Magney *et al.* 2017). The same temperature, light and environmental settings used with LI-6800 measurements were used for the GFS-3000 system measurements. Measurements were taken between 9 AM and 3 PM. All code for data analysis was developed in Python v 3.7. The scipy.stats package was utilized for simple linear regression.

Results and Discussion

The adapted LI-6800 system effectively measured $F_{t,\lambda}$, PAM fluorescence and gas exchange simultaneously. We evaluate the performance of the new system by testing how effectively this array of observations responds to water-deficit treatments. The spectra in Fig. 4 show that both $F_{t,\lambda}$ magnitude and spectral shape change with water stress for both modified systems (LI-6800 & Flame spectrometer and GFS-3000 & QE Pro spectrometer). The corresponding steady-state $F_{t,\lambda}$ peaks decrease as a response to water stress, which is similar to responses of other plant species (Ač *et al.* 2015; Magney *et al.* 2019b). This result is attributed to the apparent connection between stress-induced changes in the light reactions of photosynthesis and chlorophyll fluorescence (Flexas *et al.* 2002). Water stress leads to a decline in the rate of photosynthesis due to stomatal and non-stomatal limitations, which affect chloroplast activity (Ogren and Oquist 1985; Flexas *et al.* 1999, 2002). It is important to note that changes in stomata and chlorophyll fluorescence will respond at different time scales, but generally a decline in photosynthetic capacity results in a smaller number of excited electrons in the photosystems that could be fluoresced (Gu *et al.* 2019).

The new LI-6800 and Flame spectrometer system measures emitted chlorophyll-a fluorescence from the abaxial leaf surface while the GFS-3000 & QE Pro spectrometer measures the fluorescence signal from the adaxial surface (Fig. 1). The abaxial $F_{t,\lambda}$ signal measured from the modified LI-6800 system is smaller (60.1 ± 7.8 % average decrease) than the signal gleaned from top-of-leaf $F_{t,\lambda}$ measured with the modified GFS-3000 system (Fig. 4). Additionally, the shape of the fluorescence spectra from the top and bottom of the leaf is slightly different (Fig. 4). The spectra taken from the top of the leaf (modified GFS-3000 system) contain a second smaller peak at 685 nm, typical of top-of-leaf fluorescence spectra. The absence of this second peak in the abaxial fluorescence measurements is explained by the re-absorption of red-emitted fluorescence along the optical path through the leaf (Fig. 1; Vogelmann and Evans 2002; Van Wittenberghe *et al.* 2015). While there are differences in the spectral shape from the adaxial and abaxial fluorescence measurements, the magnitude of $F_{t,\lambda}$ sensitivity to water stress is comparable. While it is important to understand

the spectral shape of abaxial fluorescence, this should not be an issue for researchers looking to compare the magnitude (not spectral shape changes) of $F_{t,\lambda}$ with PAM fluorescence and gas exchange parameters. We assume the spectral shape of far-red fluorescence is quite stable (Magney *et al.* 2019b), so research conducted using the modified LI-6800 to look at spectral variability may not yield clear results regardless of the direction of fluorescence. Additionally, because we measure from the abaxial surface of the leaf, we do not require an optical filter because much of the incoming red light is absorbed on its path through the leaf. According to the manufacturer's specifications of the LI-6800 instrument, the spectral response function of the red LED light source shows no light beyond 700 nm. Therefore, we recommend using far-red $F_{t,\lambda}$ beyond 700 nm when interpreting measurements from this modified instrument despite the seemingly nominal impact of red light at wavelengths > 670 nm.

Positive linear relationships were observed between adaxial and abaxial $F_{t,\lambda}$ at both 500 and 1500 µmol m^{-2} s^{-1} PAR (Fig. 5). $F_{t,\lambda}$ measurements at 50 µmol m^{-2} s^{-1} PAR were excluded from these results due to very small signals at the low light level (within the noise of dark measurements). Increasing the integration time may increase the signal-to-noise ratio for lower light intensities, but it was held constant during measurements to maintain consistency. Positive linear relationships between $F_{t,\lambda}$ yield from both measurement techniques suggest that the modified instrument described here is a viable method to measure $F_{t,\lambda}$ in the field. Further investigation must be done to examine differences in slope and linear fit at different light levels, but one explanation could be related to Beer's law of light attenuation, whereby an exponential decrease in the amount of light transmitted from the top to the bottom of the leaf would also impact the amount of fluoresced photons (Monsi *et al.* 2005). Additionally, the difference in slope could be attributed to changes in chloroplast positioning at different light intensities (Tholen *et al.* 2008; Van Wittenberghe *et al.* 2019), or from gasket compression effects due to taking measurements with both instruments on the same leaf area.

The relationship between SIF ($F_{t,740}$) measured with the LI-6800/Flame spectrometer and the net CO_2 assimilation is shown in Fig. 6. In general, $F_{t,\lambda}$ increases with photosynthesis. In theory and in practice, the reduction of $F_{t,\lambda}$ will be less than that of photosynthesis at the leaf scale, but previous studies have
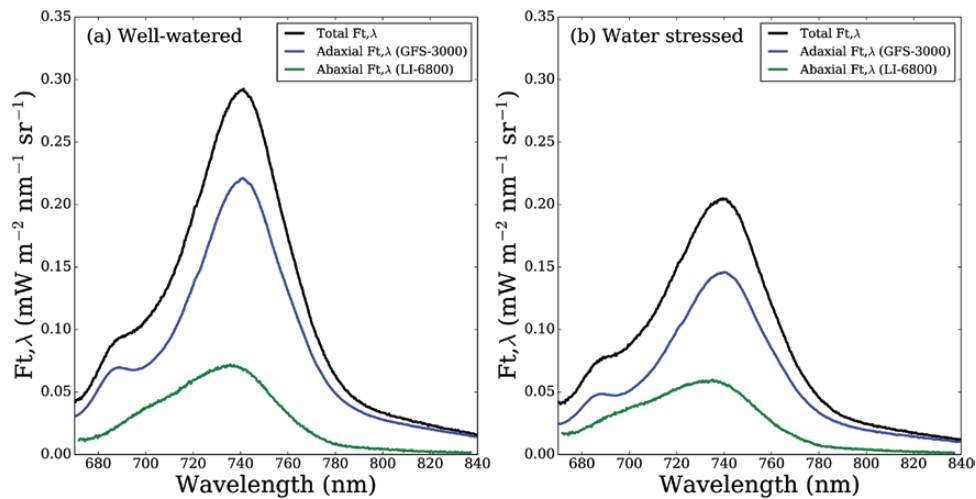

Figure 4. $F_{t,\lambda}$ spectra at 500 µmol m^{-2} s^{-1} PAR from (A) a well-watered walnut tree and (B) a water-stressed walnut tree. The plots contain fluorescence from the modified LI-6800, modified GFS-3000 and the sum of the two spectra.
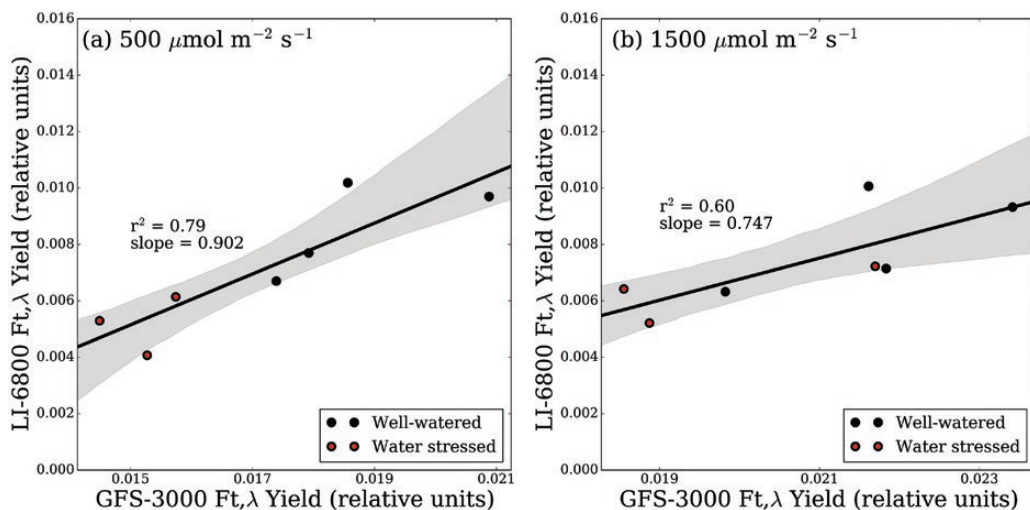

Figure 5. Relationship between $F_{t,\lambda}$ yield measured with the LI-6800 and $F_{t,\lambda}$ yield measured on the GFS-3000 at 740 nm at (A) 500 µmol m^{-2} s^{-1} PAR and (B) 1500 µmol m^{-2} s^{-1} PAR. Each point represents $F_{t,\lambda}$ yield at 740 nm measured on the same leaf area with the respective instrument.

shown that a decrease in A_{net} typically results in a decrease in SIF (Magney *et al.* 2017; Gu *et al.* 2019; Helm *et al.* 2020). Electron transport rate (ETR) measured with the LI-6800 fluorometer is plotted with $F_{t,\lambda}$ to show the modified instrument's capability to simultaneously measure passive fluorescence, active fluorescence and gas exchange.

To compare between passive and active fluorescence measurements taken with our modified LI-6800, $F_{t,\lambda}$ yield was plotted against the fluorescence yield (F_t/F_o) from the PAM fluorometer (Fig. 7). A positive linear relationship between $F_{t,\lambda}$ and PAM fluorescence was observed ($r^2 = 0.68$). Passive and active fluorescence measurements corresponding to
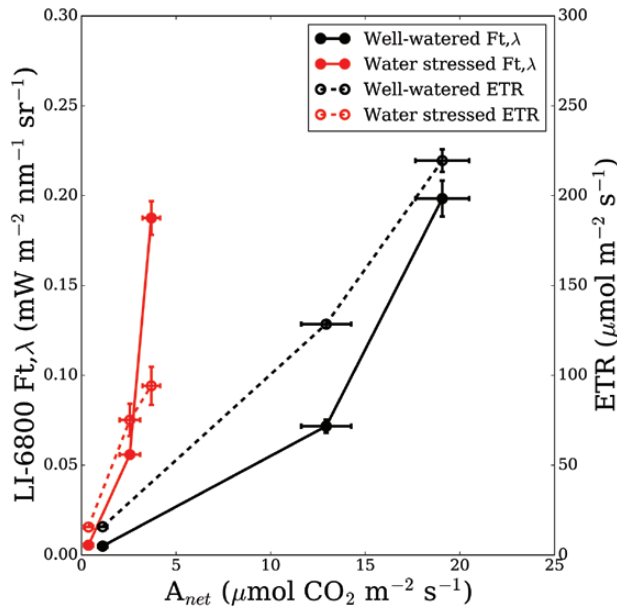

Figure 6. Relationship between $F_{t,\lambda}$ at 740 nm, ETR and net CO_2 assimilation (A_{net}). Each point corresponds to the average $F_{t,740}$, ETR and A_{net}.
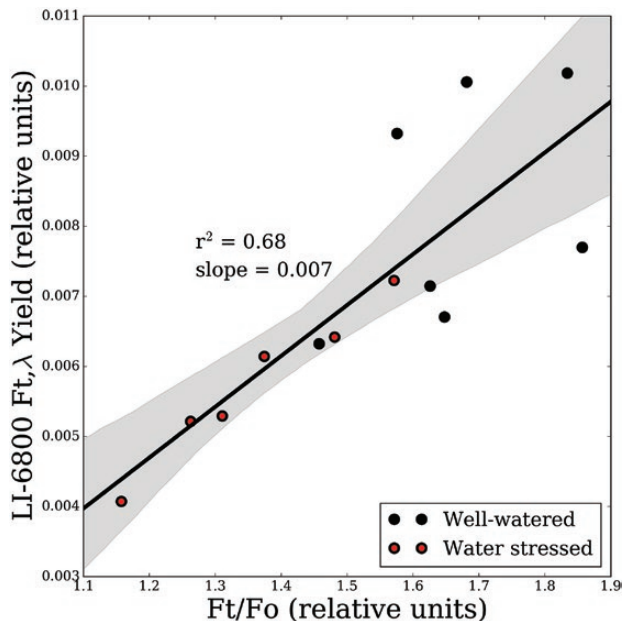

Figure 7. Relationship between LI-6800 $F_{t,\lambda}$ yield and fluorescence yield via PAM fluorometry. Points at both 500 and 1500 μmol m⁻² s⁻¹ PAR are included.

water-stressed walnut trees are lower in magnitude (and more tightly correlated) than those of the well-watered trees. The stronger relationship between passive and active fluorescence measurements of the water-stressed plants could be explained by higher water stress leading to a more homogeneous intercellular environment as the stomata close, thereby reducing the variability in photochemical quenching compared to the well-watered counterpart. M. Momayyezi *et al.* (University of California, Davis, CA, USA, unpubl. res.) found that water stress decreases leaf thickness and cell packing in *J. regia* leaves resulting in a proportional decrease of stomatal conductance. We speculate that the resulting increase in porosity creates a more homogenous intercellular environment in the water-stressed leaves. Additionally, because the exact location of the fibre-optic field of view (FOV) was slightly different for the Flame and QE Pro spectrometer methods, differences could be the result of leaf heterogeneity of chloroplast positioning, pigment concentrations and leaf thickness. F_t/F_o decreases with increasing water stress due to a series of reactions in response to drought-induced stomatal closure. Under extreme drought conditions, NPQ might become the dominant energy pathway as photochemistry and chlorophyll fluorescence become less competitive processes (Flexas *et al.* 2002). Although the source of excitation light and the interpretation of the signals of passive and active fluorescence measurements are different, both methods measure steady-state chlorophyll fluorescence, so changes in one signal should result in a similar change in the other. Unlike PAM fluorescence, the quantum yield of photochemistry cannot be directly calculated from $F_{t,\lambda}$ measurements. However, we expect that tools aiming to understand the relationship between $F_{t,\lambda}$ and PAM will help to disentangle the mechanistic relationship between remotely sensed SIF and photosynthesis (Porcar-Castell *et al.* 2014). Since SIF is primarily sensitive to the light reactions of photosynthesis, while A_{net} represents actual carbon assimilation and includes information on the carbon reactions of photosynthesis, further research using the proposed method could help to elucidate when the SIF:photosynthesis relationship converges or diverges, and what the drivers of this divergence might be.

Conclusions

The modified LI-6800 system was successful in concurrently measuring the $F_{t,\lambda}$, PAM fluorescence and gas exchange of the same leaf area. Unlike previous instruments with these measuring capabilities, this instrument is portable and can withstand changes in temperature and humidity likely to be experienced in the field. We report a $F_{t,\lambda}$ yield comparison between the abaxial $F_{t,\lambda}$ measured from the LI-6800/Flame spectrometer system and adaxial $F_{t,\lambda}$ from GFS-3000/QE Pro spectrometer system to validate the $F_{t,\lambda}$ signal and its response to changes in light and water stress. We also report the relationship between $F_{t,\lambda}$ and PAM fluorescence measured with our modified system to better understand how remotely sensed SIF may be related to photosynthesis. Due to the nature of this instrument modification (i.e. $F_{t,\lambda}$ measured abaxially while PAM fluorescence is measured adaxially), there cannot be a direct comparison between the fluorescence measurement types, but the system still facilitates a comparison to link the relationship between the two signals. This study was limited to a single species (*J. regia*) grown in a lath house. Future research with this device (or one similar) might look at multiple species aiming to advance the understanding of the biological mechanisms of $F_{t,\lambda}$. Additionally, using this device in the field with concurrent tower-based

measurements will provide a deeper look into the relationship between $F_{t',\lambda}$ and photosynthesis for remote sensing research.

Data Availability

The data displayed in Figs 5–7 of the discussion section can be found in the Supporting Information.

Contributions by the Authors

All authors contributed to the development of this manuscript. A.J.M., T.S.M. and N.B. secured the funding, all authors developed the concepts, E.W.M., T.S.M. and M.M. conducted the experiments, N.B., E.W.M. and T.S.M. designed the modifications, E.W.M., A.J.M. and T.S.M. completed the first draft of the manuscript and all authors edited the final version.

Acknowledgements

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