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#### **Key Points:**

- Leaf-level chlorophyll fluorescence does not exhibit a significant relationship with photosynthesis after inducing stomatal closure
- Remote fluorescence data provide insight into the light reactions of photosynthesis, but do not directly track carbon assimilation
- The link between fluorescence and primary productivity may result from shared drivers, such as chlorophyll content or energy partitioning

#### **Supporting Information:**

- Supporting Information S1
- Figure S1
- Figure S2
- Figure S3
- Figure S4
- Figure S5

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# Solar-Induced Fluorescence Does Not Track Photosynthetic Carbon Assimilation Following Induced Stomatal Closure

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**Abstract** Since 2006, six satellites measuring solar-induced chlorophyll fluorescence (SIF) have been launched to better constrain terrestrial gross primary productivity (GPP). The promise of the SIF signal as a proxy for photosynthesis with a strong relationship to GPP has been widely cited in carbon cycling studies. However, chlorophyll fluorescence originates from dynamic energy partitioning at the leaf level and does not exhibit a uniformly linear relationship with photosynthesis at finer scales. We induced stomatal closure in deciduous woody tree branches and measured SIF at a proximal scale, alongside leaf-level gas exchange, pulse amplitude modulated (PAM) fluorescence, and leaf pigment content. We found no change in SIF or steady-state PAM fluorescence, despite clear reductions in stomatal conductance, carbon assimilation, and light-use efficiency in treated leaves. These findings suggest that equating SIF and photosynthesis is an oversimplification that may undermine the utility of SIF as a biophysical parameter in GPP models.

**Plain Language Summary** Earth's vegetation plays a key role in storing carbon that would otherwise reside in the atmosphere. Recently, there has been increasing interest in measuring fluorescent light emitted by the chlorophyll in plant cells in order to track carbon uptake. Satellite fluorescence measurements show a strong, direct relationship with primary productivity. However, leaf-level chlorophyll fluorescence studies have yielded insights into the origin of this signal as one of several pathways by which plants consume excess absorbed light. At finer scales, fluorescence emission may become inversely related to photosynthetic rate, due to the additional role of heat dissipation as an alternative pathway for plants to partition energy. To investigate the contradiction between measurements across scales, we experimentally manipulated tree branches, inhibiting photosynthesis by closing the stomata through which plants exchange water and carbon dioxide gases. We observed significant reductions in leaf-level gas exchange in treated branches but found no similar change in fluorescence measured at the leaf level or from a proximal tower. While fluorescence offers physiological insights, we suggest that the close relationship with primary productivity at the satellite scale could result from a shared driver, such as chlorophyll content and that fluorescence data should be interpreted with care.

# 1. Introduction

The terrestrial biosphere is a crucial sink for anthropogenic emissions of carbon to the atmosphere (Pan et al., 2011) and a focal area of Earth systems research (Le Quéré et al., 2018). Numerous satellite programs monitor global vegetation, but there is insufficient on-the-ground data to fully constrain fluxes and feed-backs in modeled global gross primary productivity (GPP) (Anav et al., 2015; Huntzinger et al., 2017). Recent GPP modeling efforts have incorporated remotely measured solar-induced fluorescence (SIF) emitted by vegetation (Porcar-Castell et al., 2014), with satellite SIF retrievals reported to show a positive, linear relationship with upscaled GPP model outputs (Sun et al., 2018) and flux tower estimates across biomes (Verma et al., 2017; Xiao et al., 2019). However, the SIF signal originates from a pathway competing with photochemistry for absorbed light energy, suggesting that photosynthetic rate could be inversely related to fluorescence flux. We hypothesize that strong SIF-GPP correlations arise from a shared driver,



Figure 1. Potential fates for a photon incident on a leaf. Pie chart represents approximate partitioning of absorbed energy among these fates.

such as chlorophyll content or strong seasonal changes in alternative sinks for absorbed energy (Magney et al., 2019), rather than a universal ability of SIF to report real-time insights into the biophysics of photosynthesis.

SIF contains valuable physiological information. Chlorophyll fluorescence emission has been the subject of several decades' work revealing energy partitioning at the leaf level, typically measured using pulse-amplitude-modulated (PAM) fluorometers (Maxwell & Johnson, 2000). Such instruments characterize the competing pathways by which leaves maximize the use of energy from absorbed light to power photochemistry, while balancing the need to dissipate excess energy to prevent singlet oxygen ( $^{1}O_{2}$ )-mediated cellular damage (Baker, 2008). Figure 1 depicts the potential fates for a photon with notation, where appropriate, to indicate the associated PAM fluorescence parameters that provide information on each energy partitioning pathway. Data collected using PAM fluorometers can be used to quantify  $\Phi$ PSII, the light-use efficiency of linear electron transport (a chain of energy-generating reactions originating at Photosystem II) in the light reactions of photosynthesis ( $P_{\rm ET}$ ). Crucially, the relationship between this parameter and carbon assimilation in the dark reactions of photosynthesis ( $P_{\rm CF}$ , also referred to as  $A_{\rm net}$  when measured in the context of leaf-level gas exchange) is nonlinear, due to photorespiration ( $P_{\rm RS}$ ), particularly at low internal concentrations of CO<sub>2</sub> (CO<sub>2i</sub>).

A share of absorbed light is used to support photochemistry in unstressed, healthy leaves (Demmig-Adams, 1998), but leaves routinely absorb more photons than can be used on an instantaneous basis. While some energy is lost as heat through constitutive, nonradiative decay (D), leaves may also engage a regulated photoprotective mechanism known as energy dissipation (ED) involving carotenoids of the xanthophyll cycle (Demmig-Adams & Adams, 2006). Energy dissipation can be tracked using PAM fluorescence as decreases in Photosystem II quantum yield measured during illumination  $(F_{\nu'}/F_{m'})$  (Demmig-Adams & Adams, 2006; Logan et al., 2007). Levels of ED vary in response to excess light absorption and generally increase during exposure to environmental stress, at times rising dramatically to consume the greatest share of absorbed light (Logan et al., 1998; Verhoeven et al., 1999). Fluorescence emission accounts for the remaining small fraction of absorbed light, typically 1 % to 5 % of incoming radiance (Meroni

et al., 2009). PAM fluorometers measure  $F_s$ , a broadband, leaf-level quantification of steady-state fluorescence emission from illuminated leaves.  $F_s$  is an actively induced signal generated by the measuring beam of a PAM fluorometer, responding to many physiological drivers and providing little insight into photosynthetic activity without consideration of other physiological parameters (Logan et al., 2007). In contrast, SIF is measured as a faint, but highly temporally dynamic enhancement in the apparent reflectance inside spectrally narrow solar Fraunhofer lines or atmospheric absorption features (Meroni et al., 2009).

Since photochemistry and fluorescence represent competing fates for absorbed photons, both of which also compete with ED, we may expect them to exhibit a complex, perhaps even an inverse relationship, as is observed under conditions (e.g., low light) where ED remains low (Porcar-Castell et al., 2008, 2014). Numerous studies report a positive relationship between SIF and GPP (Sun et al., 2018; Verma et al., 2017; Xiao et al., 2019), suggesting SIF is a proxy for photosynthesis (X. Yang et al., 2015). Though the term "proxy" indicates an indirect representation used at a scale where direct measurement is impossible, its usage in highly cited SIF studies and conference sessions (e.g., Zhang et al., 2018) has come to imply a more universal substitution in the literature on global GPP modeling. Some researchers have suggested that the strong, direct SIF-GPP relationships observed by satellites at the landscape scale may be an artifact of averaging over space, time, or view angle (Gu et al., 2019; Y. Zhang et al., 2018; Z. Zhang et al., 2018), which serves to linearize the canopy-scale relationship more than would be observed at a smaller spatial or temporal resolution. Even at the leaf-scale, some have found a nonlinear relationship between SIF and GPP, particularly in high-light conditions where photosynthesis saturates while fluorescence emission continues to increase (Gu et al., 2019; Magney et al., 2017). Similarly, the magnitude of the change in SIF may not track changes in photosynthesis in response to stresses over shorter durations (Helm et al., 2020). Thus, while landscape-scale correlation between GPP and SIF is informative, the link between SIF and leaf or whole-plant physiology on the ground and on shorter time scales remains a crucial area of uncertainty.

Previous work on this question has involved intermediate-scale measurements from towers or airborne platforms, and some authors report sufficient agreement across scales to allow for the creation of methods for downscaling satellite data using transformed tower-based measurements (Duveiller & Cescatti, 2016). However, spatial or temporal aggregation is often employed: Though some authors have measured changes in SIF over a period of hours following herbicide application (Pinto et al., 2020), correlations in daily mean tower-based SIF retrievals and flux tower-based GPP are more commonly reported (H. Yang et al., 2017; Wohlfahrt et al., 2018). While this aggregation may be justified in the context of other studies reporting linear scaling from instantaneous to monthly SIF-GPP relationships (albeit at large spatial scales) (Wood et al., 2017), averaging across large diel variations limits the ability to track rapid changes in physiological activity.

# 2. Materials and Methods

# 2.1. Experimental Design

To evaluate whether remotely sensed SIF tracks changes in photosynthesis at short temporal scales, we experimentally manipulated instantaneous leaf photosynthetic carbon fixation ( $P_{CF}$ ) by simulating water stress in deciduous woody trees (Figure S1 in the supporting information). Experimental trees were selected for well-illuminated, healthy branches extending out from the site treeline, and included three individuals of *Liriodendron tulipifera*, and three oaks, two individuals of *Quercus palustris* and one of *Quercus alba*. Water stress conditions were simulated using foliar application of the plant hormone abscisic acid (ABA) or a pressure cuff to induce emboli in individual branches. Both treatments were chosen with the goal of inducing stomatal closure, which limits leaf-level exchange of water vapor for carbon dioxide and thereby reduces carbon assimilation in treated leaves without influencing leaf pigment content.

On the morning of 10 July 2019, pre-treatment measurements of SIF, leaf-level gas exchange ( $A_{net}$  and  $g_{sw}$ ), PAM fluorescence ( $F_s$ ,  $\Phi$ PSII,  $F_{v'}/F_{m'}$ ), and leaf pigments (chlorophyll pool, chlorophyll a/b ratio, and xanthophyll cycle pool) were made under clear sky conditions. Following pre-treatment measurements, experimental manipulations were performed on all branches. Heavy clouds and light afternoon rains on 11 July 2019 delayed repeat measurements until the following morning. On the morning of 12 July 2019, post-treatment measurements were performed on all branches at the same time of day to within 2 min, under nearly identical sky conditions.



#### 2.2. Site Description and FOREST Project

Data were collected at the Forested Optical Reference for Evaluating Sensor Technology (FOREST) site on the campus of the National Institute of Standards and Technology (NIST) in Gaithersburg, MD. The FOREST site is a remnant stand of large-stature forest (~165 MgC ha<sup>-1</sup>) dominated by oaks and tulip poplars and was established as an urban testbed for carbon monitoring and instrument development in 2017. Complementary biometric and environmental parameters, including soil temperature, soil moisture, air temperature, relative humidity, stem respiration, soil respiration, sap flux, and atmospheric  $CO_2$  concentration were measured at frequent intervals. Further information on data collection methodology and spatial trends can be found in Smith et al. (2019).

#### 2.3. SIF Instrumentation Deployment

Spectra used in SIF retrievals were collected using two QE Pro\* (Ocean Insight, Largo, FL, USA) grating spectrometers with a 0.25 nm spectral resolution and a 160 nm spectral range centered at ~730 nm. Spectrometers were housed inside a temperature-controlled building (24 °C  $\pm$  2 °C on average) facing the FOREST treeline, with fiber optic cables running out through wall ports.

Measurements of radiance reflected and SIF emitted from the treeline were collected using a 127 mm 1,250 mm FL (F/10) reflector telescope coupled to a fiber optic cable and situated on an external balcony (Figure S2c). When viewing over the approximately 100 m distance between balcony and treeline, the telescope field of view allowed for measurement of an approximately 0.75 m diameter area of interest. The telescope sight was aligned in the field, allowing for targeting of experimental branches (Figure S2c) with  $\pm 10$  cm accuracy. More details on alignment methodology may be found in the supporting information.

Vegetation was viewed at a nearly perpendicular angle into the side of the treeline, rather than from above the canopy (Figure S2a). Multiple scattering within leaves may result in proportionally higher near-infrared than red chlorophyll fluorescence emission from the undersides of leaves (Van Wittenberghe et al., 2015), which may be more visible at our site than from a typical tower. However, since we performed SIF retrievals using the Fraunhofer Line Depth (FLD) (Plascyk & Gabriel, 1975) method in the near-infrared  $O_2$ -A feature, this was not anticipated to pose any problems to measurement. Downwelling irradiance was measured using a fiber optic cable affixed along the external wall of the building, with an attached Ocean Insight CC-3 opal glass cosine corrector extending above the roof. The limitations of this cosine corrector at extreme Sun angles were characterized using laboratory tests with the fiber and cosine corrector mounted on a rotating stage opposite a known light source. Downwelling spectra were corrected for underestimation of incident irradiance based on solar zenith angle at the time and date of measurement.

Prior to deployment, spectrometers were radiometrically calibrated by transferring the scale from a calibrated spectroradiometer using an integrating sphere at NIST. These radiometric responsivities were determined with the telescope and cosine corrector fore-optics attached, in order to account for spectral and radiometric effects on measured signal. Linear functions determined from laboratory measurements of electronic dark current were used to correct individual field measurements based on spectrometer integration time. Spectra were corrected as in Sabater et al. (2018) to account for atmospheric O<sub>2</sub> absorption along the branch-telescope path length, as determined using a TruPulse 200 hypsometer (Laser Technology, Inc., Centennial, CO, USA). Further details on all corrections and calibrations can be found in Marrs et al. (2019).

Spectra were recorded consecutively from the area of interest targeted with the telescope (n = 20 spectra per branch per day) before and after applying experimental treatments, at the same time of day. Spectra were collected with the highest frequency allowed by spectrometer integration time, adjusted to achieve 80 % to 90 % of detector dynamic range (5.5 s to 12 s, depending on branch illumination levels). Spectra were acquired using OceanView software and were processed in RStudio (RStudio Team, 2015) with custom R (R Core Team, 2018) code. The 3FLD method (Maier et al., 2003) was evaluated alongside the standard FLD approach. We observed a linear relationship between retrievals obtained using the two methods, with lower absolute magnitudes of SIF from the 3FLD retrievals (Figure S3).

#### 2.4. Kautsky Curve

To confirm our capacity to detect a physiologically meaningful chlorophyll fluorescence signal, we measured the Kautsky effect (Kautsky & Hirsch, 1931), in which dark-acclimated photosystems suddenly exposed to



light are temporarily unable to perform electron transfer, resulting in a large spike in fluorescence intensity as a compensatory mechanism, followed by a gradual decline upon the induction of photochemical and nonphotochemical mechanisms of chlorophyll de-excitation. The resulting Kautsky curve (Figure S4) shows strong agreement in timing and relative signal magnitude in PAM fluorescence and SIF measurements, indicating that our remote spectrometer deployment captured the same fluorescence dynamics observed at the leaf level.

#### 2.5. Gas Exchange and PAM Fluorescence

Leaf-level photosynthetic  $CO_2$  assimilation and PAM chlorophyll fluorescence emission were measured using a Licor 6800 photosynthesis analyzer with the 6800-01A fluorometer head (LI-COR Biosciences, Lincoln, NE, USA) (Figure S2b). Measurements were made on full-Sun acclimated leaves in the area of interest of the SIF measurements at 1,600 µmol photons m<sup>-2</sup> s<sup>-1</sup>, a reference concentration of 400 parts per million  $CO_2$ , a temperature-control block temperature of 30 °C and a vapor pressure deficit of 2.0, which were intended to simulate ambient conditions for parameters other than temperature, which was set slightly above ambient to reduce the risk of condensation within the instrument. A timeseries of local meteorological data can be found in Figure S5. Branch values represent the mean of triplicate measurements on separate leaves.

#### 2.6. ABA

To induce stomatal closure, ABA was applied as a foliar spray with 0.25 g ABA (plantmedia.com, Dublin, OH, USA) dissolved in 25 mL of ethanol. The ABA solution was diluted to 0.5 mM ABA in 0.1 % Triton X-100 (approximately 2 % ethanol in the final spray) and was liberally applied to both sides of all leaves on ABA-treated branches twice in the afternoon of the pre-treatment day, following intial measurements (Figure S2f). Leaves were treated a second time as above with freshly prepared ABA solution just after dawn on 12 July 2019.

#### 2.7. Pressure Cuff

We introduced emboli into the xylem of branches to simulate water stress, prompt stomatal closure, and lower leaf photosynthetic rates. In preliminary testing, it took several hours for pressurizations to affect leaf gas exchange. Experimental branches were pressurized in the late afternoon of 10 July 2019. To introduce emboli, we used a split pressure chamber (Hubbard et al., 2001) sealed around branches to inject pressurized nitrogen gas into the xylem. Machined blocks of aluminum were bolted together and sealed around an attached branch (Figure S2d). This chamber enclosed a 10 cm long length of a branch ranging from 1 to 2 cm in diameter. The chamber was sealed around the projecting branches using conical rubber gaskets fitted around the stems that expanded radially when compressed by tightening mated aluminum end caps into matching keyways on the chamber. Once sealed, the chamber was slowly pressurized to 6.9 MPa using compressed nitrogen gas. The chamber was held at that pressure for 10 min and was then vented slowly and allowed to return to atmospheric pressure.

In preliminary testing of species with diffuse-porous wood, the leaves on pressure-treated branches wilted significantly following pressurization, while leaves from ring-porous species did not. To avoid changes in leaf geometry that might alter remotely measured SIF in ways that were unrelated to the impacts of the stem pressurization on leaf photosynthesis, we did not pressure treat branches of *L. tulipifera* as it has diffuse-porous wood and instead focused the branch pressurizations on ring-porous *Quercus* sp. in this study. No wilting was observed in pressurized *Quercus* branches.

#### 2.8. Leaf Chlorophylls and Carotenoids-Extraction and Quantification

Leaf samples for the analysis of chlorophylls and carotenoids were collected from designated pigment-monitoring leaves on each branch both before and after experimental manipulations were performed. These collections were coordinated temporally with measurements of all other parameters made on adjacent leaves of the same focal branches in the same light environment. At the point of collection, leaf samples were immediately frozen in liquid nitrogen and were stored at -80 °C until processed. Leaf pigments were extracted from these stored samples in acetone according to Adams and Demmig-Adams (1992) modified as described in de Viller et al. (2017). Following extraction, leaf pigments were quantified by high-performance liquid chromatography (HPLC) as described in de Viller et al. (2017).



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**Figure 2.** Trends in SIF, gas exchange, and PAM fluorescence parameters. Error bars represent 95 % confidence intervals determined from LME models. Points are means from pre-treament ("Pre") and post-treatment ("Post") days. Parameters include net carbon assimilation ( $A_{net}$ ), stomatal conductance to water ( $g_{sw}$ ), SIF, steady-state PAM fluorescence ( $F_s$ ), light-use efficiency of Photosystem II ( $\Phi$ PSII), and photosystem II quantum yield under illumination ( $F_v'/F_m'$ ).

#### 2.9. Statistical Analyses

For each tree, one adjacent healthy branch was designated as a control for comparison to treated branches on the same tree. To account for environmental or other external changes across field measurement days, control branch data were used as a baseline for comparison with treated branches on the same tree, with the assumption that all neighboring branches on a tree shared similar physiological conditions before the experiment began. However, in order to compare across sets of branches, it was necessary to control for between-tree variability. Such variability was especially evident in SIF retrievals (see Figure 3), but analyses were performed identically for all parameters. Data were analyzed using linear mixed-effects (LME) models of the interaction between time (pre-treatment vs. post-treatment) and treatment type, with tree identity held as a random effect. Statistical analyses were performed with R in Rstudio. LME models were constructed and run using the lme4 package (Bates et al., 2015), and 95 % confidence intervals around the coefficient for the interaction term were calculated using the effects package (Fox & Weisber, 2019).

#### 3. Results

Following experimental manipulations, both  $A_{net}$  and stomatal conductance to water ( $g_{sw}$ ) decreased significantly, falling nearly to zero (Figure 2). These results indicate that both types of manipulations were effective in reducing stomatal conductance to water and CO<sub>2</sub>, resulting in a nearly complete shutdown in  $P_{CF}$ . In order to relate these changes back to SIF, they must be assessed in the context of overall energy partitioning in these treated leaves. PAM fluorescence measurements of  $\Phi$ PSII show a significant reduction following treatment, albeit of a smaller magnitude than observed in  $g_{sw}$  and  $A_{net}$ . This indicates decreased light-use efficiency of PSII and suggests, in the context of the lower carbon fixation observed in leaf-level gas exchange measurements, that a greater fraction of the energy from  $P_{ET}$  is being used for photorespiration after stomatal closure. With observed reductions to energy use in  $P_{CF}$ , we expected to see increases elsewhere. Values of  $F_v'/F_m'$  decreased by 20 ± 7 % after treatment, indicating increased levels of ED as a compensating





**Figure 3.** Variability in fluorescence measurements. Individual measurements of steady-state PAM fluorescence ( $F_s$ ) and SIF. Boxes show 25th percentile, median, and 75th percentile with lines extending to 1.5 times the interquartile range. Oaks 1 and 3 are *Quercus palustris*; oak 2 is *Quercus alba*.

mechanism. These observations show that our experimental treatments altered leaf-level energy partitioning, with gas exchange and  $P_{CF}$  dramatically reduced, and complementary increases in  $P_{RS}$  and ED. Critically, we observe no consistent trends in SIF or  $F_s$  that would be indicative of these dramatic changes observed in other energy partitioning parameters.

Individual SIF retrievals from trees targeted in this experiment are variable, with large branch-to-branch differences in magnitude (Figure 3). While this variability is common and the motivation for spatial and temporal aggregation performed in many studies, we present all data points here for transparency and ease of comparison with  $F_s$  values measured from the same branches. SIF varies strongly with instrument field of view, incident light variations, leaf density and angle, and chlorophyll content (Verrelst et al., 2015). Some SIF retrievals on branches with a smaller proportion of illuminated vegetation in the instrument field of view result in small negative values. Negative SIF is a physical impossibility and an artifact of the calculations underlying SIF retrievals, but it also highlights the importance of external drivers on the final magnitude

Leaf Chlorophyll and Xanthophyll Concentrations						
	Control			Treated		
Parameter	Species	Pre-treatment	Post-treatment	Treatment	Pre-treatment	Post-treatment
Chlorophyll Pool	Quercus species	457 ± 109.9	491 ± 110.3	ABA	435 ± 109.9	427 ± 109.9
(Chl a + b)				Pressurized	433 <u>+</u> 116.2	$431 \pm 116.2$
$(\mu mol m^{-2})$	Tulip Poplar	549 ± 69.4	533 ± 76.4	ABA	520 ± 69.4	537 ± 69.4
Chl a:b Ratio	Quercus species	$3.4 \pm 0.19$	$3.4 \pm 0.19$	ABA	$3.3 \pm 0.19$	$3.4 \pm 0.19$
				Pressurized	$3.3 \pm 0.15$	$3.3 \pm 0.15$
	Tulip Poplar	$2.7 \pm 0.06$	$2.7 \pm 0.07$	ABA	$2.7 \pm 0.06$	$2.7 \pm 0.06$
Xanthophyll Pool	Quercus species	34.3 ± 6.25	34.9 ± 6.43	ABA	34.1 ± 6.25	$42.3 \pm 6.25$
(V + A + Z)				Pressurized	29.2 ± 5.19	$50.0 \pm 5.19$
$(\mu mol m^{-2})$	Tulip Poplar	31.7 ± 12.19	$39.7 \pm 13.02$	ABA	$32.5 \pm 12.19$	$45.1 \pm 12.19$

*Note.* Error ranges represent 95 % confidence intervals determined from linear mixed effects models. Xanthophyll pigments include V = violaxanthin, A = antheraxanthin, and Z = zeaxanthin.

Table 1

of a recorded SIF value. Similarly,  $F_s$  values are variable, with no consistent direction of change between pre-treatment and post-treatment values from treated branches. We observe no changes in leaf chlorophyll pools and a significant change in xanthophyll cycle pool size in only pressurized *Quercus* branches post-treatment (Table 1), and thus can rule out the scenario in which such changes might obscure an otherwise significant trend in SIF or  $F_s$ . Neither fluorescence parameter, if used as a stand-alone monitoring method, would have provided insight into the nearly complete elimination of photosynthetic carbon assimilation, nor given clear insight into resulting adjustments in leaf-level energy partitioning. Note that the data presented here were collected following experimental manipulations to three tree species at one study site, this does not necessarily provide universal insight into the range of physiological conditions under which SIF may be measured.

# 4. Conclusions

How do we reconcile the ambiguity in our observations with the strong SIF-GPP relationships found at larger scales? Traditional models of GPP define it as the product of the light-use efficiency (LUE) of vegetation and absorbed PAR (APAR, the product of PAR intensity and the fraction absorbed by photosynthetic elements on the ground) (Monteith, 1972). SIF, as a phenomenon intrinsically connected to the use or dissipation of incoming light, should contain information about both LUE and APAR (Porcar-Castell et al., 2014), meaning that it can be related back to modeled GPP. A typical formulation of this relationship sets SIF =  $\Phi F \cdot APAR \cdot \Omega_c$ , where  $\Phi F$  is fluorescence yield (Berry et al., 2013) and  $\Omega_c$  represents the escape probability of fluorescence (Yoshida et al., 2015). The latter is a crucial factor when modeling the proportion of fluorescence emitted at the leaf level that will reach the outer canopy without being scattered or reabsorbed (Dechant et al., 2020; Verrelst et al., 2015), but one that we can assume to remain constant over a daily course of measurements. Most work on SIF assumes constant  $\Phi F$ : LUE in order to formulate the linear relationship between SIF and GPP that matches observations at large scales. However, recent work questions these assumptions, suggesting SIF tracks rates of linear electron transport, thus only providing insights into the light reactions of photosynthesis (Gu et al., 2019).

If SIF is directly affected by the light reactions, we should not expect it to mirror changes in gas exchange and carbon assimilation, consistent with our experiments. Instead, we propose the hypothesis that the SIF-GPP relationship at large spatial and temporal scales is the result of a shared driver. Chlorophyll content in the vegetated area of interest is a fundamental property relating these two processes; areas with denser vegetation or higher chlorophyll concentrations may fix more carbon and emit more chlorophyll fluorescence. The link between chlorophyll content and absorbed PAR has motivated the use of traditional greenness indices as GPP model inputs that may perform nearly as well as SIF when used in combination with other environmental parameters (Sims et al., 2008) and has been demonstrated to drive fluorescence dynamics within canopies (Maguire et al., 2020). Nonetheless, the influence of xanthophyll pigments and ED must also be considered in order to explain seasonal variability in SIF from evergreen forests (Magney et al., 2019).

SIF has often been described as a proxy for photosynthesis. However, findings from the PAM fluorescence literature show that this is an oversimplification at small spatial and temporal scales, one that may undermine the utility of the SIF signal as part of a complex, but fundamental biophysical process. Previous studies using herbicides that fully inhibit  $P_{\rm ET}$  show increased SIF after treatment (Pinto et al., 2020; Rossini et al., 2015), indicating a clear linkage to leaf-level energy partitioning, though with the opposite trend as satellite-based SIF-GPP relationships. In order to take full advantage of information provided by remotely measured chlorophyll fluorescence, a fuller picture of energy partitioning on the ground is needed. In particular, this includes a need to better characterize energy dissipation dynamics. This may be accomplished in a number of ways; for example, the photochemical reflectance index (PRI) (Gamon et al., 1992) provides insight into the xanthophyll cycle by which energy dissipation is regulated and can be measured at a variety of scales to match tower, airborne, or satellite SIF measurements. The ability to relate leaf-level phenomena to global processes requires data collection and synthesis across multiple scales. SIF offers tremendous promise for improving the characterization of terrestrial carbon exchange, and a fuller understanding of the boundaries on its utility and interpretation will help to create more reliable models of global productivity.



# Data Availability Statement

Data supporting the conclusions of this manuscript may be accessed through the Harvard Dataverse at this site (https://doi.org/10.7910/DVN/1GKVM4).

#### Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

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