Linking chlorophyll a fluorescence to photosynthesis for remote sensing applications: mechanisms and challenges

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Abstract

Chlorophyll a fluorescence (ChlF) has been used for decades to study the organization, functioning, and physiology of photosynthesis at the leaf and subcellular levels. ChlF is now measurable from remote sensing platforms. This provides a new optical means to track photosynthesis and gross primary productivity of terrestrial ecosystems. Importantly, the spatiotemporal and methodological context of the new applications is dramatically different compared with most of the available ChlF literature, which raises a number of important considerations. Although we have a good mechanistic understanding of the processes that control the ChlF signal over the short term, the seasonal link between ChlF and photosynthesis remains obscure. Additionally, while the current understanding of in vivo ChlF is based on pulse amplitude-modulated (PAM) measurements, remote sensing applications are based on

Abbreviations: A0, gross photosynthetic rate (e.g. μmol CO2 fixed by the Calvin–Benson cycle m–2 s–1); APAR, the flux of photosynthetically active radiation absorbed by plants (e.g. μmol photons m–2 s–1); a, a1, relative absorption cross-section areas of PSI and PSII populations; range from zero to one; CET, cyclic electron transport; ChlF, chlorophyll a fluorescence; ETR, electron transport rate through PSII (μmol electrons m–2 s–1) equivalent to LET; F, fluorescence signal emanating from a leaf, as in Equation 11; fAPAR, fraction of incoming PAR absorbed by vegetation; range from zero to one; F670, F680 prevailing and maximal fluorescence signal at the level of PSII, respectively; F690(ΦFII), function that accounts for the shape of the fluorescence emission spectra in PSI; F690(ΦFII), function that accounts for the shape of the fluorescence emission spectra in PSI; F670, F670, fluorescence measured in the red or far-red region, respectively; F, F670, prevailing and maximal fluorescence signal as measured with PAM fluorometry (relative units, e.g. sensor mV output); F0, Fmax, minimal and maximal fluorescence signal as measured with PAM fluorometry in the dark and after a period of dark acclimation (relative units, e.g. sensor mV output); ΦF, quantum yield of fluorescence (quanta emitted/quanta absorbed); ΦP, F0, quantum yield of photochemistry in PSII (electrons transported/quanta absorbed); ΦP, F0, maximum quantum yield of photochemistry in PSI obtained after dark acclimation or during the night; GPP, gross primary productivity (e.g. total μmol CO2 assimilated by plants m–2 s–1); kq, rate constant of excitation energy transfer between neighbouring PSII units that denotes the degree of excitonic connectivity (s–1); kD, rate constant of basal or constitutive thermal energy dissipation (s–1); kE, rate constant of chlorophyll fluorescence emission (s–1); kISC, rate constant of intersystem crossing (s–1); kNPQ, rate constant of regulated thermal energy dissipation or NPQ (s–1); kq, overall rate constant of photochemistry (s–1); kq, intrinsic rate constant of photochemistry in open and fully functional reaction centres (s–1); kISC, rate constant of thermal energy dissipation by closed PSI (s–1); kT, rate constant of energy transfer between neighbouring pigments (s–1); LET, linear electron transport; LUE, light use efficiency of photosynthesis (μmol CO2 assimilated/quanta absorbed); NPQ, non-photochemical quenching of excitation energy via regulated thermal energy dissipation; PII, quantum parameter to quantify NPQ capacity, proportional to kNPQ/(kq+kD); PQ, photochemical quenching of excitation energy via electron transport; PQ, quantum parameter to quantify PQ capacity, proportional to kq/(kq+kD); PR, photosynthesis rate (μmol CO2 photosynthesized m–2 s–1); PRI, photochemical reflectance index; PSI, photosystem I; PSII, photosystem II; qf, fraction of open and functional reaction centres; range from zero to one; qL, the photochemical quenching parameter based on the model of a pseudo model assumption, an estimate of q; qP, the photochemical quenching parameter based on a model of a pseudo model assumption, an estimate of q; R, the rate of leaf-level mitochondrial day respiration (e.g. μmol CO2 respired m–2 s–1); SIF, solar-induced fluorescence (e.g. in W m–2 s–1 nm–1 s–1);
the measurement of the passive solar-induced chlorophyll fluorescence (SIF), which entails important differences and new challenges that remain to be solved. In this review we introduce and revisit the physical, physiological, and methodological factors that control the leaf-level ChlF signal in the context of the new remote sensing applications. Specifically, we present the basis of photosynthetic acclimation and its optical signals, we introduce the physical and physiological basis of ChlF from the molecular to the leaf level and beyond, and we introduce and compare PAM and SIF methodology. Finally, we evaluate and identify the challenges that still remain to be answered in order to consolidate our mechanistic understanding of the remotely sensed SIF signal.

Key words: Gross primary production, GPP, leaf level, photosystem II, photosystem I, PSII, PSI, photosynthesis dynamics, pulse amplitude modulation, PAM, PSII connectivity, remote sensing, solar-induced fluorescence, sun-induced fluorescence, SIF.

1. Introduction

Photosynthesis drives the global carbon cycle. Net photosynthesis can be quantified at the leaf level by monitoring CO₂ exchange using chamber enclosure systems combined with infrared gas analysers (Long and Bernachi, 2003), and at the ecosystem level using flux towers and eddy covariance techniques (Goulden et al., 1996; Baldocchi, 2008). At the landscape and regional levels, gross photosynthetic CO₂ assimilation, or gross primary productivity (GPP), is inferred using models and algorithms that integrate ground observations with remotely sensed data (e.g. Heinsch et al., 2006; Williams et al., 2009; Jung et al., 2011). Remotely sensed data have been extensively used to infer GPP based on the light use efficiency (LUE) model (Moneith, 1972; Kumar and Moneith, 1981; Zhao et al., 2011). In the LUE model, GPP is proportional to incoming photosynthetically active radiation (PAR), the fraction absorbed by vegetation (/APAR), and the LUE at which absorbed radiation is used by photosynthesis:

$$\text{GPP} = \frac{\text{PAR}}{\text{APAR}} \times \text{LUE} \quad (1)$$

Remote sensing has been traditionally used to estimate the first two terms of this equation (see reviews by Hilker et al., 2008; Malenovsky et al., 2009). For example, differences in surface reflectance between the red, blue, and near infrared part of the spectrum have been exploited to derive a wide range of vegetation indices to assess /APAR, green biomass, chlorophyll content, or leaf area index (e.g. Rouse et al., 1974; Huette, 1988; Qi et al., 1994; Huette et al., 1997; Daughtry et al., 2000; Haboudane et al., 2002). Typically, vegetation indices show a strong seasonal correlation with GPP in many plant communities (e.g. grasslands, croplands, and deciduous forests), but the correlation breaks down in evergreen plant communities where seasonal changes in GPP are strongly modulated by LUE as well as /APAR (Equation 1) (e.g. Sims et al., 2006; Garbulsky et al., 2008). To represent the dynamics of LUE, remote sensing data have been used to classify vegetation into plant functional types (PFTs). Subsequently, global GPP models combine spatially resolved PFT and other remote sensing products [e.g. PAR, /APAR, temperature, and vapour pressure deficit (VPD)] with functions and parameters derived from flux tower observations to estimate LUE (Heinsch et al., 2006; Williams et al., 2009; Jung et al., 2011).

Although the LUE approach provides a theoretical basis for constructing and calibrating models, there are no corresponding benchmarks to evaluate model performance at large geographical scales, and model uncertainty remains high (Beer et al., 2010). The situation could be dramatically improved if a new source of data was available that captured the dynamic behaviour of photosynthesis at the relevant scale. Fortunately, photosynthesis generates an optical signal that, in addition to PAR and /APAR, is also sensitive to LUE. This signal is chlorophyll a fluorescence (ChlF). ChlF are photons of red and far-red light that are emitted by chlorophyll a pigments nanoseconds after light absorption. Because photosynthesis and ChlF compete for the same excitation energy, ChlF carries information on LUE.

ChlF has been used for decades to elucidate the organization, function, and acclimation of the photosynthetic apparatus at the subcellular and leaf levels (see seminal reviews by Krause and Weis, 1991; Govindjee, 1995; Lázár, 1999; Maxwell and Johnson, 2000; Baker, 2008). Originally restricted to the laboratory, ChlF measurements made their move to the field with the development of the pulse amplitude-modulated (PAM) technique (an active technique that involves the use of a measuring light and a saturating light pulse), and the subsequent introduction of commercial PAM fluorometers (Schreiber et al., 1986; Bolhár-Nordenkampf et al., 1989). PAM fluorometry has facilitated the study of the acclimation of photosynthesis in situ and helped clarify the link between ChlF and photosynthetic CO₂ assimilation. Yet, despite the importance and value of PAM fluorometry, for practical reasons the technique has been restricted to the leaf level, and its applicability at the canopy and landscape levels remains unknown. To fill the gap, the field of ChlF has recently seen a new wave of developments that seek to measure ChlF from remote sensing platforms.

The remote sensing technique is based on the passive measurement of solar-induced chlorophyll fluorescence (SIF). The goal is to use the seasonal dynamics in the SIF signal measured from towers, aircrafts, and satellites as a proxy of photosynthesis (Grace et al., 2007; Hilker et al., 2008; Meroni et al., 2009; Rascher et al., 2009). During the last decade, SIF has been successfully measured from tower (Moya et al., 2004; Rossini et al., 2010; Guanter et al., 2013; Drolet et al., 2014), aircraft (Zarco-Tejada et al., 2009, 2012, 2013), and satellite platforms (Guanter et al., 2007; Joiner et al., 2011; Frankenberg et al., 2011; Guanter et al., 2012), the
The regulation of photosynthesis and its optical signals

Photosynthesis involves two main sets of reactions: the light reactions, where electromagnetic energy is absorbed by pigments and converted into chemical energy in the form of ATP and NADPH; and the carbon fixation reactions, where ATP and NADPH are used to produce sugars from atmospheric carbon dioxide. Because the light and carbon reactions exhibit different sensitivities to environmental variables such as light, temperature, or water availability, the production of ATP and NADPH by the light reactions and consumption of these metabolites by the carbon reactions do not always match (Ögren et al., 1984; Huner et al., 1996; Ensminger et al., 2006).

Energy absorbed in excess by the light reactions can damage the photosynthetic machinery (Barber and Andersson, 1992; Demmig-Adams and Adams, 2000; Tyystjärvi, 2013), for example a leaf in a sunny (high energy input) but cold (low energy consumption) environment. Accordingly, plants have evolved a number of regulatory mechanisms to adjust the energy balance between the light and carbon reactions (Walters, 2005; Demmig-Adams and Adams, 2006). The result of this continuous adjustment is that the performance of the light reactions of photosynthesis (visible to optical sensors) tends to emulate that of the carbon reactions. This establishes a link between optical data and GPP that can be implemented to the remote sensing of photosynthesis. Some processes, however, interfere with the relationship, which we discuss below (Fig. 1).

2.1 Light absorption and its regulation

Photosynthesis starts with absorption of light, mainly by chlorophyll molecules. Accordingly, an effective mechanism used by plants to regulate light absorption, or APAR, consists of adjusting the concentration of chlorophyll pigments in the leaf (Fig. 1). The relationship between chlorophyll content and light absorption is non-linear because the increment in light absorption per unit of chlorophyll decreases at high chlorophyll contents (Adams et al., 1990; Gitelson et al., 1998). Net changes in leaf-level chlorophyll are visible over time scales of days (Garcia-Plazaola and Becerril, 2001; Lu et al., 2001). In addition, certain plant species use other mechanisms to modulate photosynthetic light absorption that operate at different temporal scales: leaf movements and leaf angle adjustments (Yu and Berg, 1994; Arena et al., 2008), chloroplast movements (Brugnoli and Björkman, 1992; Sarvikas et al., 2010), changes in surface reflectance mediated by salt bladders (Mooney et al., 1977; Esteban et al., 2013), changes in leaf epicuticular wax properties (Pfündel et al., 2006; Olascoaga et al., 2014), changes in leaf
porcelain casts (Ehleringer et al., 1976; Morales et al., 2002; Galmés et al., 2007a), and changes in the concentration of non-photosynthetic pigments such as anthocyanins (Close and Beadle, 2003; Pfündel et al., 2006; Merzlyak et al., 2008) (Fig. 1). The temporal dynamics of these processes need to be considered when interpreting ChlF data because changes in light absorption have a direct impact on ChlF intensity (see Section 3). A special case is that of non-photosynthetic pigments which do not contribute to ChlF or photosynthesis, but increase leaf absorptance (Hlavinka et al., 2013).

2.2 Linear and cyclic electron transport and energy distribution between photosystems

Photosynthetic pigments are bound by proteins to form photosynthetic antenna complexes (Liu et al., 2004) that capture light energy and transfer it to a reaction centre. A reaction centre is a special pigment–protein complex that converts excitation energy to chemical energy. The combination of reaction centre and antenna is termed a photosystem. Higher plants have two types of photosystems: photosystem I (PSI) and photosystem II (PSII), which actually operate in series in the opposite order, that is with electrons being transferred from PSII to PSI (Fig. 2).

After a photon is captured by a chlorophyll molecule in PSII, the excitation energy rapidly reaches the reaction centre chlorophyll, referred to as P680 (a pigment with absorption maximum at 680 nm). Excited P680* rapidly gives an electron to the primary electron acceptor, pheophytin, which in turn reduces the quinone A (QA) electron acceptor to yield the first stable charge-separated state P680+QA–. Subsequently, QA– passes an electron to quinone B (QB) which leaves its binding site when double reduced and protonated by stromal protons.
Protonated Q \textsubscript{b} reduces plastoquinone (‘PQ’, not to be confused with photochemical quenching PQ), which is subsequently reoxidized by the cytochrome b\_f complex (Cyt b\_f), eventually transferring the protons to the thylakoid lumen (Fig. 2). At the donor side, P680\textsuperscript{+} is reduced by tyrosine Z (TyrZ); subsequently TyrZ\textsuperscript{+} takes up an electron from the oxygen-evolving complex (OEC) which is responsible for the splitting of the water molecule and the release of oxygen and protons (Antal et al., 2013). The resulting protons, together with those pumped to the lumen by the Cyt b\_f complex, accumulate in the thylakoid lumen, generating a proton concentration gradient across the thylakoid membrane (Kramer et al., 2004a). This gradient is used by ATP synthase (ATPase) to synthesize ATP. Simultaneously, energy absorbed in PSI is captured by its reaction centre chlorophyll (P700) and used to reduce the electron acceptor ferrodoxin (Fd). Oxidized P700\textsuperscript{+} is reduced back to P700 by taking an electron from plastocyanin (PC). From ferrodoxin, the electron is passed to NADP\textsuperscript{+} to produce NADPH in a reaction catalysed by ferrodoxin-NADPH reductase (FNR). This series of reactions makes up the linear electron transport (LET) (for reviews, see Ort and Yocum, 1996; Antal et al., 2013).

Efficient operation of the LET implies that the populations of PSII and PSI work in series and their reaction centres transfer electrons at approximately similar rates. Although a trivial solution would be to allocate the same relative antenna cross-section area to PSII and PSI, extra flexibility is required. For example, the absorption spectra of PSII and PSI are different due to differences in pigment composition and spectral forms (see Section 3.2), with PSI absorbing light of slightly longer wavelengths (Duyens and Sweers, 1963; Boichenko, 1998; Pfännl, 2009). In particular, PSI has a higher proportion of chlorophyll \emph{a} compared with PSII, with chlorophyll \emph{a/b} ratios of 9 for PSI compared with 2.5 for PSII, in extracted photosystem particles (Ben-Shem et al., 2003; Nield and Barber, 2006). As a result, the probabilities of light absorption by PSII and PSI will change depending on the spectral properties of incoming light, and its temporal and spatial dynamics.

Another factor that calls for extra flexibility in energy partitioning between photosystems is the operation and dynamics of cyclic electron transport (CET) (Joliot and Joliot, 2002; Rumeau et al., 2007). CET translocates electrons around PSI and pumps protons from the chloroplast stroma to the thylakoid lumen (Fig. 2). Because the electron is recycled, CET yields only ATP but no NADPH. Functionally, CET is thought to contribute to the efficient induction of the Calvin–Benson cycle upon illumination of dark-acclimated leaves (Joliot and Joliot, 2002), the regulation of the lumen pH, and thereby modulation of non-photochemical quenching in the photosystems, NPQ (see below) (Kramer et al., 2004a), or the protection of PSI against photoinhibition (Rumeau et al., 2007; Sonoike, 2011). Indeed, CET has been found to be essential for normal carbon fixation in many C\textsubscript{4} plants (Hatch, 1992). To summarize, changes in light quality and CET require flexible mechanisms capable of adjusting the energy partitioning between PSII and PSI (i.e. the relative absorption cross-sections of PSII and PSI, \emph{a}_{II} and \emph{a}_{I}, respectively) (Fig. 1).

At a time scale of minutes, the partitioning of energy between photosystems (\emph{a}_{II} and \emph{a}_{I}) is regulated through a process known as state transitions (Murata, 1969; Haldrup et al., 2001; Tikkanen et al., 2011). Under low light, if leaves are illuminated with light that favours PSII, part of the peripheral antenna complexes of PSII can migrate to serve PSI. This has the effect of balancing the energy input between the photosystems. State transitions are considered to be important only under low light conditions (Rintamäki et al., 1997; Haldrup et al., 2001; Tikkanen et al., 2011), and are therefore of little relevance for remote sensing.

At time scales of days, photosystem stoichiometry and \emph{a}_{II} and \emph{a}_{I} can adjust in response to more sustained changes in light intensity and quality (Anderson et al., 1988; Chow et al., 1990; Durnford and Falkowski, 1997; Pfannschmidt et al., 1999; Haldrup et al., 2001). Light intensity and quality co-vary within a plant canopy, with shaded parts of the canopy or understorey plants receiving light enriched in far-red due to absorption of red light by foliage above. The result is that leaves in shaded environments tend to display higher \emph{a}_{II}/\emph{a}_{I} ratios than more exposed foliage (Anderson et al., 1988; Chow et al., 1990; Rivadossi et al., 1999; Hihara and Sonoike, 2001; Eichelmann et al., 2005; Ballotari et al., 2007). In addition to their functional and energetic role, the spatiotemporal dynamics of \emph{a}_{II} and \emph{a}_{I} have important implications for the interpretation of ChlF because they affect the shape of the fluorescence spectra and the magnitude of the PSI fluorescence contribution (Palombi et al., 2011), as well as the estimation of the LET rate by means of fluorescence (see Section 4.1).

Evidence suggests that CET and energy partitioning between photosystems might be highly dynamic in response to stress and environmental conditions (Martin et al., 1978; Ivanov et al., 2001; Eichelmann et al., 2005; Rumeau et al., 2007). However, the seasonal and spatiotemporal dynamics of these traits remain poorly understood.

### 2.3 Energy partitioning at the photosystem level

Understanding the processes that control the energy partitioning in PSII is crucial to linking ChlF with photosynthetic CO\textsubscript{2} assimilation. Energy absorbed by pigments of PSI is dissipated by three main pathways: (i) it can be used by photochemistry (by LET); (ii) it can be dissipated non-radiatively as heat; or (iii) it can be re-emitted as a photon of fluorescence. A unique relationship between ChlF and photochemical efficiency cannot be established. This is because non-radiative dissipation of excitation energy is dynamic and under physiological control (see Section 3).

In low light and in the absence of stress, most of the absorbed energy is effectively used by photochemistry, and the excitation lifetime in the antenna of PSII (\emph{t}_{PSII}) is short (in the order of hundreds of picoseconds) (Dau, 1994; Lavergne and Trissl, 1995; Gilmore et al., 1995). This results in lowered fluorescence yield. This de-excitation pathway is termed photochemical quenching (PQ) (a term originally coined to denote the quenching of the fluorescence signal but herein used to address the photochemical quenching of excitation...
energy). If light intensity increases, the carbon fixation reactions and electron transport chain gradually become light saturated, causing an increase in \( \tau_{\text{PSII}} \). This results in increased fluorescence yield. The sudden increase in fluorescence yield, observed when subjecting a dark-acclimated leaf to strong illumination, and the subsequent decrease are collectively referred to as the Kautsky effect (e.g. Govindjee, 1995). These rapid fluorescence dynamics reflect the rapid reduction and re-oxidation of PSII electron acceptors and their influence on \( \tau_{\text{PSII}} \) (see Section 3.2) (Brody and Rabinovitch, 1957).

Plants are incentivized to keep \( \tau_{\text{PSII}} \) as low as possible, while still permitting photochemical trapping. This acts to minimize the formation of chlorophyll triplet states which might lead to production of singlet oxygen, a hazardous reactive oxygen species (Barber and Andersson, 1992). As a result, plants have evolved a number of regulatory mechanisms that are capable of dissipating the excess quanta as heat (see reviews by Müller et al., 2001; Demmig-Adams and Adams, 2006; Garcia-Plazaola et al., 2012). The operation of these mechanisms results in a decrease in the excitation lifetime in the antenna and subsequently a decrease in the ChIF yield. This type of de-excitation pathway is commonly referred to as non-photochemical quenching (NPQ), after being widely estimated using the fluorescence parameter NPQ (Blümel and Björkman, 1991) (see Section 4.1). However, it should be kept in mind that the regulated thermal dissipation of excitation energy (non-photochemical quenching of excitation energy) and the parameter NPQ (non-photochemical quenching of the fluorescence signal) are not always equivalent; thus, it becomes practical to separate NPQ and \( NPQ_{d} \) (Porcar-Castell et al., 2011; Garcia-Plazaola et al., 2012). For example, when state transitions reduce the PSII relative absorption cross-section \((a_{q})\), the fluorescence signal is lowered (and the NPQ parameter increases) because fewer photons are absorbed by PSII (Horton and Hague, 1988), but no change takes place in \( \tau_{\text{PSII}} \). In the following, we use NPQ and \( NPQ_{d} \) accordingly.

The regulation of NPQ in PSII involves mechanisms operating at different time scales. In the short term (seconds to hours), two \( \Delta pH \)-dependent mechanisms appear to regulate thermal energy dissipation in PSII (Müller et al., 2001; Demmig-Adams and Adams, 2006; Garcia-Plazaola et al., 2012). When the electron transport chain saturates, proton accumulation tends to decrease lumen \( pH \) (Fig. 2). Subsequently, the PsbS protein acts as a proton sensor activating and de-activating NPQ, while lumen \( pH \) regulates the activity of the enzyme violaxanthin de-epoxidase (VDE). The lowering of the \( pH \) triggers the de-epoxidation of violaxanthin to zeaxanthin, resulting in amplified NPQ (Demmig-Adams, 1990; Horton et al., 1996; Müller et al., 2001; Jahns and Holzwarth, 2012). This second mechanism operates at time scales of minutes. Together, the protonation of antenna proteins and de-epoxidation of xanthophyll cycle pigments have been traditionally addressed as energy-dependent quenching (qE) (Weis and Berry, 1987; Krause and Weis, 1991; Horton et al., 1996). Recently, another zeaxanthin-dependent (qZ) but \( \Delta pH \)-independent form of NPQ was found in *Arabidopsis* and suggested also to operate at a time scale of minutes (Nilkens et al., 2010). All these mechanisms modulate NPQ over the course of the day in response to diurnal fluctuations in light and temperature. These NPQ forms relax in the dark (e.g. overnight) and are accordingly termed flexible or reversible NPQ (Müller et al., 2001; Demmig-Adams et al., 2006; Porcar-Castell, 2011).

Over longer time scales (days to weeks) plants face more sustained changes in their environment (e.g. drought or low winter temperatures). At the seasonal scale, the light-harvesting machinery undergoes sustained changes that result in down-regulation of the photochemical quenching capacity (PQ) and up-regulation of the non-photochemical quenching capacity (NPQ) in PSII (Ottander et al., 1991, 1995; Verhoeven et al., 1996; Ensminger et al., 2004; Porcar-Castell et al., 2008a; Porcar-Castell, 2011). These adjustments are termed sustained because they do not recover overnight. The decrease in PQ is associated with the accumulation of damaged/photoinhibited PSII reaction centres. Damage and recovery of reaction centres take place simultaneously (Kok, 1956; Ohad et al., 1984; Greer et al., 1986). Thus photoinhibition becomes apparent when damage occurs faster than recovery and, since the rate constant of photoinhibition is proportional to incoming light intensity (Tyystjärvi and Aro, 1996) while the rate of recovery is temperature dependent (Greer et al., 1986), photoinhibition becomes apparent under strong light and particularly when strong light is combined with low temperatures (Strand and Lundmark, 1987; Ottander et al., 1991; Campbell and Tyystjärvi, 2012; Tyystjärvi, 2013). The term photoinhibition has also been used to denote a decrease in the maximum quantum yield of photochemistry, commonly estimated via the fluorescence parameter \( F_{v}/F_{m} \) (see Section 4.1). The seasonal decrease in \( F_{v}/F_{m} \) or ‘photoinhibition’ has been shown to be caused by reaction centre damage, the presence of sustained NPQ, or a combination of both (Porcar-Castell et al., 2008b). In this review, we use the term photoinhibition to refer to the damage of the reaction centre exclusively.

The increase in sustained NPQ has been associated with the overnight retention of a de-epoxidized xanthophyll cycle, the accumulation of the PsbS protein, the aggregation of light-harvesting complexes (LHcs) (Adams and Demmig-Adams, 1994; Ottander et al., 1995; Verhoeven et al., 1996; Ensminger et al., 2004; Zarter et al., 2006), as well as with changes in the redox properties of the electron acceptors of PSII that promote thermal dissipation in the reaction centre (Krause, 1988; Ivanov et al., 2002, 2008; Matsubara and Chow, 2004); see Verhoeven (2014) for a recent review on sustained forms of NPQ. While the correlation between NPQ (i.e. thermal energy dissipation) and the parameter \( NPQ_{d} \) (i.e. fluorescence signal quenching) is well understood over the diurnal scale, the correlation between NPQ and \( NPQ_{d} \) at the seasonal scale remains obscure (Porcar-Castell, 2011), which in turn complicates the interpretation of seasonal time series of ChIF data.

In contrast to PSII, the lifetime of excitation in PSI (\( \tau_{\text{PSI}} \)) does not seem to be affected by photochemical and non-photochemical quenching processes (at least over the short term) possibly because its reaction centre is very efficient in quenching excitation energy in the oxidized state. The result is a relatively low and constant contribution of fluorescence from...
PSI to the total signal (Genty et al., 1990b; Pfündel, 1998; Palombi et al., 2011) that can be treated as a constant signal offset (e.g. Porcar-Castell et al., 2006). However, the seasonal dynamics of PSI ChF remain unknown.

In addition to ChF, the regulation in energy partitioning at PSII generates another optical signal. The operation of the xanthophyll cycle and the fast regulation of NPQ in PSII are associated with changes in reflectance at ~531 nm (Gamon et al., 1990). This feature is exploited by the photochemical reflectance index (PRI) (Gamon et al., 1992), an index that has been shown to track changes in LUE through its correlation with NPQ (Evain et al., 2004; Nichol et al., 2006). The potential of the PRI as a remote sensing proxy of LUE has been demonstrated (Drolet et al., 2005; Garbulsky et al., 2008). However, the PRI has also been shown to be very sensitive to canopy structure, gap fraction, background, viewing angle, or leaf area index (Barton and North, 2001; Sims et al., 2006; Goerner et al., 2011), complicating the association of PRI and LUE. In addition, although the short-term variation in leaf-level PRI appears indeed to be controlled by NPQ, the seasonal variation in leaf-level PRI seems to be controlled by the slow changes in pigment pools rather than NPQ (Stylinski et al., 2002; Filella et al., 2009; Porcar-Castell et al., 2012). The mechanistic link between the PRI and LUE appears to be highly dependent on scale and remains to be fully elucidated.

2.4 Alternative electron transport sinks and metabolic pathways

ChlF can be used to estimate the rate of LET through PSII (see Section 4.1). However, a number of processes need to be taken into account if we are to infer the rate of gross photosynthetic CO₂ assimilation or the rates of ATP and NADPH production from fluorometric estimates of LET. Because CET produces ATP but no NADPH, fluorometrically estimated LET will decouple from ATP production in the presence of CET (Fig. 1). Additionally, alternative electron transport sinks such as chlororespiration (Nixon, 2000) and the Mehler reaction (Asada, 2000) reduce the overall quantum yield of NADPH, further decoupling LET from NADPH production.

Alternative electron sinks are generally assumed to be small relative to LET, although they play an important functional role and can become significant under certain conditions. For example, the rate of chlororespiration has been shown to increase under high light and high temperature (Diaz et al., 2007) and suggested to contribute to excess energy dissipation in the alpine plant Ranunculus glacialis (Laureau et al., 2013). The Mehler reaction, in turn, has been shown to be stimulated under some water stress conditions (Biehler and Fock, 1996; Flexas et al., 1999; Asada 2000). In addition, part of ATP and NADPH produced by the light reactions can be used by alternative metabolic pathways (e.g. nitrate and sulphate reduction in chloroplasts, or emission of plant volatile organic compounds) (Fig. 1). Krivosheeva et al. (1996) found that the electron transport rate and photosynthetic CO₂ uptake were decoupled in overwintering Scots pine, suggesting an increase in an alternative electron sink or metabolic pathway. Overall, the action of alternative sinks and metabolic pathways may, under certain conditions, affect the ability of ChlF to track the dynamics of photosynthetic CO₂ assimilation.

2.5 Carboxylation, oxygenation, and day respiration

The ATP and NADPH generated by the light reactions are utilized by the Calvin-Benson cycle to synthesize sugars by assimilating CO₂ (gross photosynthetic assimilation or \( A_G \)) (Fig. 1). Net photosynthetic assimilation (\( A_N \)) is the quantity that is measurable by gas exchange systems and relates to ‘true’ or gross photosynthesis (\( A_G \)) as:

\[
A_N = A_G - PR - R_d
\]

where \( R_d \) is the rate of mitochondrial day respiration and PR is the rate of photorespiration (Ogren, 1984). In photorespiration, Rubisco catalyses the oxidation (adding O₂) instead of the carboxylation (adding CO₂) of ribulose bisphosphate, with the oxidized product being partially recovered via the emission of CO₂. Because fluorometrically estimated LET (see Section 4.1) relates to \( A_G \) rather than \( A_N \), the magnitude of photorespiration and mitochondrial respiration needs to be taken into account when comparing gas exchange and ChlF measurements.

In C₄ plants (e.g. maize and sorghum), photorespiration is almost fully suppressed, and fluorometrically estimated LET correlates well with net photosynthesis (e.g. Genty et al., 1990a; Krall and Edwards, 1992). In C₃ plants, which include practically all tree species and the majority of higher plants, the energy flow going to photorespiration is considerable and variable. Flexas and Medrano (2002) showed that for a pool of different species the quantum yield of photorespiration increased in response to mild water stress, from 18% to 22%, while the quantum yield of photochemistry stayed approximately constant. Thus, processes such as photorespiration may undermine the capacity of ChlF to track plant stress under certain conditions.

The temperature sensitivity of mitochondrial respiration may vary seasonally, diurnally, or within a plant, in response to variations in maintenance respiration (Atkin et al., 2005). For example, \( R_d \) has been found to increase from 15% of \( A_N \) to as much as 50% in response to drought stress (Flexas et al., 2005; Galmés et al., 2007b), and a similar temporal pattern has been observed in boreal Scots pine foliage, with higher \( R_d \) values during spring recovery of photosynthesis compared with summer (Kolari et al., 2007). In summary, CET, alternative electron sinks, photorespiration, and mitochondrial respiration can all decouple optical data such as ChlF from net photosynthetic CO₂ assimilation.

3. Physical and physiological controls of chlorophyll a fluorescence across space and time

In this section we describe how the intensity, spectrum, and dynamics of the ChlF signal in vivo are controlled by a number of scale-dependent physical and physiological factors (including photosynthesis).
Fluorescence is radiative loss of the energy of absorbed photons. Because part of the energy of the absorbed photon is lost as heat, the energy of the emitted photon is usually lower (longer wavelength) than that of the absorbed photon, a phenomenon known as Stokes shift. The quantum yield of fluorescence emission depends on both the properties of the fluorescing chromophore and its surroundings. Chlorophylls in ether are highly fluorescent, with quantum yields of 30% and 15% for chlorophyll \(a\) and chlorophyll \(b\), respectively (Latimer et al., 1956; Barber et al., 1989). Carotenoids, in turn, yield very little fluorescence (Gillbro and Cogdell, 1989). In contrast, the quantum yield of ChlF \(in\) vivo does not exceed 10%, with typical values under steady-state illumination of 0.5–3% (Latimer et al., 1956; Brody and Rabinovitch, 1957; Krause and Weis, 1991). This drastic decrease is due to the photochemical and non-photochemical quenching of excitation energy in the photosynthetic antennae, making ChlF such a valuable tool for assessing photosynthesis.

3.1 Chlorophyll fluorescence at the molecular level

Energy levels of atomic and molecular orbitals are quantized, but molecules present wide, continuous absorption and emission (fluorescence) spectra because vibrational energies of the molecule are superimposed on each electronic energy level.

A photon can be absorbed if its energy equals the difference in the sum of electronic, vibrational, and rotational energies between an excited-state orbital and the ground-state orbital. Vibrational energy is vibration of atoms about their equilibrium positions in the molecule. Because photosynthetic pigments \(in\) vivo are tightly packed into a protein matrix, the vibration of the chemical bonds in the pigment–protein complex add additional variability to the absorption and emission spectra of pigments \(in\) vivo. This phenomenon, known as inhomogeneous broadening, explains why photosynthetic pigments display different spectral forms \(in\) vivo (Vassiliev and Bruce, 2008) but not when isolated. Rotational energy is associated with the rotation of the molecule around its axis, which is negligible in the solid state.

In Fig. 3 we portray an idealized representation of the energy levels and possible energy dissipation pathways for a chlorophyll \(a\) molecule embedded in a photosynthetic antenna. Chlorophyll \(a\) absorbs photons of blue and red light very efficiently. Absorption of a blue photon raises an electron from the ground-state orbital (\(S_0\)) to an orbital of a high excited state. Part of the energy is transformed to molecular vibrations and rapidly dissipated (Jennings et al., 2003). Subsequently, because vibrational energy levels from higher excited states overlap with those of the \(S_1\) level, electrons originally in an orbital of a higher excited state can switch to

Fig. 3. Idealized Jablonski diagram illustrating the energy partitioning of absorbed photons in a chlorophyll \(a\) molecule. Upon absorption of a photon of blue light, an electron from the ground state is raised to a higher energy state. The energy is rapidly dissipated non-radiatively as heat mainly by internal conversion, and the electron rapidly relaxes to the first excited state (\(S_1\)). In contrast, absorption of a red photon produces the \(S_0\) state directly. It is from \(S_0\) that photosynthetic energy partitioning starts. The electron can relax to the ground state via emission of a chlorophyll fluorescence photon (with an associated rate constant \(k_F\)), via non-radiative thermal dissipation, by means of either constitutive (\(k_D\)) or physiologically regulated mechanisms (\(k_{NPQ}\)), via energy transfer to another pigment or to the reaction centre chlorophyll (\(k_T\)), or via intersystem crossing to form a chlorophyll triplet state (\(k_{ISC}\)). In turn, triplet states can be deactivated via phosphorescence, reaction with oxygen to form singlet oxygen, or by transfer to a carotenoid.
the vibrational manifold of the S₁ level, and the vibrational energy is again rapidly dissipated. The phenomenon is called internal conversion and it populates the first excited state of a chlorophyll molecule in hundreds of femtoseconds to 10 picoseconds after absorption of a photon (Dau and Sauer, 1996; Jennings et al., 2003; Clegg, 2004). A photon of red light produces the S₁ state directly. It is important to note that the difference in energy between a photon of red light and a photon of shorter wavelengths (higher energy) is virtually always dissipated as heat; for this reason, it is more practical to express photosynthetic efficiencies on a quantum rather than an energy basis.

Energy partitioning takes place from S₁. Excitations in the S₁ state live a thousand times longer compared with those in the higher energy states, and therefore other processes compete with internal conversion for dissipation of excitation from S₁ to S₀ (Fig. 3). In addition to internal conversion, the excitation energy can be transferred to another pigment. Excitation energy can also be lost via emission of a fluorescence photon. Furthermore, a change in the spin state can occur and produce a chlorophyll triplet state, a process called intersystem crossing (Clegg, 2004). The triplet state is long lived and relaxes either by reacting with oxygen to produce reactive singlet oxygen (Tyystjärvi, 2004), by transfer of energy to a carotenoid or other prenyllipid, by internal conversion to the ground state, or by emission of a phosphorescence photon (Hoff, 1986). Finally, the rate constant of thermal dissipation of S₁ states appears to be under physiological control at the photosystem level, at least in PSII. This regulated component of thermal dissipation corresponds to the NPQ mechanism described in Section 2.3. There is no consensus on whether NPQ is enhancement of internal conversion (e.g. by a mechanism that brings chlorophyll a molecules close to each other), enhancement of transfer of energy from chlorophyll a to zeaxanthin (followed by internal conversion producing the ground state of the carotenoid), or both (Owens et al., 1992; Holt et al., 2005; van Grondelle and Novoderezhkin, 2006).

We choose to introduce NPQ already at the molecular level for consistency although NPQ would be meaningful at the photosystem level, at least in PSII. This regulated component of thermal dissipation rate obtained in the absence of stress and down-regulation. Any increase in thermal dissipation on top of that is attributed to NPQ.

The quantum efficiency of a process (i) that competes for excitation energy with n other processes, and where kᵢ is the first-order rate constant of the i-th process, can be expressed as (Govindjee, 2004):

$$\Phi_i = \frac{k_i}{\sum_{i=0}^{n} k_i}$$  \hspace{1cm} (3)

Accordingly, the quantum yield of fluorescence at the molecular level (Fig. 3) can be expressed as:

$$\Phi_{F_{\text{Chl}}} = \frac{k_F}{k_D + k_F + k_{\text{ISC}} + k_{PB} + k_T (1+k_{\text{NPQ}})}$$  \hspace{1cm} (4)

where k_D is assumed to be the first-order rate constant associated with the process of internal conversion, k_F is the rate constant of fluorescence emission, k_{ISC} is the rate constant of intersystem crossing leading to chlorophyll triplet states, k_{PB} the rate constant of photobleaching or destruction of the chlorophyll molecule, k_T the rate constant of energy transfer to a neighbouring pigment molecule, and k_{NPQ} the rate constant of regulated thermal energy dissipation (NPQ). All reactions are of the first order and therefore the rate constants are expressed as l/time unit (s⁻¹). The lifetime of excitation is the inverse of the sum of all rate constants ($T = 1/k_T$). The rate constant of fluorescence ($k_F$) depends on the properties of the chlorophyll molecule and is assumed to remain constant in physiological processes (Butler and Kitajima, 1975; Clegg, 2004). The rate constant k_D is invariable by definition (i.e. any variation is embedded in k_{NPQ}). The rate constants k_{ISC} and k_{PB} are orders of magnitude smaller than k_D or k_F (Clegg, 2004; Santabarbara et al., 2007) and, since they are not relevant for energy partitioning, we hereafter consider them as part of k_D. Finally, the rate constant of energy transfer between pigments k_T includes two main mechanisms: coherent energy transfer, and Förster or fluorescence resonance energy transfer (FRET) (Nedbal and Szöcs, 1986; Clegg, 2004; Engel et al., 2007; Novoderezhkin and van Grondelle, 2010). In general, energy transfer between closely coupled pigments within a single antenna protein can be described as movement of delocalized excitons (coherent energy transfer), whereas energy transfer between pigment–protein complexes requires consideration of localized excited states (Förster energy transfer) (Novoderezhkin and van Grondelle, 2010).

3.2 Chlorophyll fluorescence at the photosystem and thylakoid membrane level

Interpreting ChlF dynamics at the level of thylakoid membrane requires the use of a number of assumptions the validity of which depends on the temporal context of the application. The assumptions listed below are generally accepted when interpreting slow ChlF dynamics (seconds to minutes), but they are too simplified to interpret fast fluorescence kinetics successfully (picosecond to second range) (e.g. Lazár, 1999; Zhu et al., 2005; Stirbet, 2013). Similarly, some of the assumptions may be equally challenged at the seasonal scale, something that will be addressed in this section.

Assumption (A): single pool model. Photosynthetic antennae have evolved to collect photons and effectively deliver the energy to a reaction centre (Fig. 4). Efficient energy transfer from pigment to pigment and to the reaction centre is very rapid (time constants 10 fs to 10 ps) (Clegg, 2004; Engel et al., 2007; Novoderezhkin and van Grondelle, 2010) in comparison with the 200–500 ps required for stable charge separation (Roelofs et al., 1992; Vassiliev and Bruce, 2008). A large body of evidence supports the idea of rapid excitation equilibration in the antennae of PSII and PSI (Schatz et al., 1988; Croce et al., 1996; Dau and Sauer, 1996; Andrizhiyevskaya et al., 2004; Miloslavina et al., 2006). If we assume rapid excitation equilibration, the antenna, core, and reaction centre can be treated as a single pigment pool.
Assumption (B): no spillover. We consider that the populations of PSII and PSI are energetically isolated and that the rate of transfer of excitations from PSII to PSI (Kitajima and Butler, 1975b; Trissl and Wilhelm, 1993; Tan et al., 1998), known as spillover, can be assumed to be insignificant at ambient temperature.

Assumption (C): no thermal dissipation by closed reaction centres. We consider that thermal energy dissipation by closed reaction centres (with P680+) is only relevant when analysing ChlF data that have been excited using intense laser sources, in which the time elapsed between absorption of two consecutive photons by PSII is shorter than the lifetime of P680’ (Shinkarev and Govindjee, 1993).

Assumption (D): no quenching by oxidized plastoquinone. Excitation quenching by oxidized plastoquinone is relatively small (Vernotte et al., 1979), with a rate constant that reaches 0.15(\(k_f+ k_D\)) when at its maximum (Zhu et al., 2005) (i.e. equivalent to \(NPQ=0.15\)).

Assumption (E): perfect connectivity/lake model. The main link between ChlF dynamics and photosynthesis dynamics originates at the level of PSII, via the photochemical reaction. The photochemical reaction is defined as the stable charge separation including reduction of the QA electron acceptor and advancement of the Kok

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cycle (Britt, 1996). Photochemistry can only take place in reaction centres that are open and functional (i.e. are associated with an oxidized Qa and fully operational). We define the fraction of open and functional reaction centres \( q \), which ranges from zero (when all reaction centres are closed) to one (when reaction centres are open and functional). Accordingly, ChlF will increase with decreasing \( q \) but the relationship depends on the degree to which excitation can move from closed to open PSII centres, a phenomenon known as connectivity (Joliot and Joliot, 1964; Havaux et al., 1991; Lavergne and Trissl, 1995; Kramer et al., 2004b) and recently reviewed by Stirbet (2013).

If \( k_C \) is the rate constant of the excitation energy transfer between two connected PSII units (see Appendix 1), then three different types of connectivity models can be defined (Dau, 1994; Lavergne and Trissl, 1995; Stirbet, 2013): a separate units or puddle model, where \( k_{CI}=0 \); a lake model, where all photosystems share excitation from a single pool and \( k_{CI}=\infty \); and models with finite connectivity \((0<k_{CI}<\infty)\). Important, except for the special cases when \( q=0 \) (termed maximal fluorescence \( F_M \)), or when \( q=1 \) (termed minimal fluorescence \( F_0 \) (Kitajima and Butler, 1975a; Dau, 1994; Lavergne and Trissl, 1995), connectivity will interact with the relationship between ChlF and \( q \). Accordingly, it is important to select a model that approximates the level of connectivity in the sample. Two extreme approaches have been used to estimate \( q \): (i) the \( qP \) parameter (Schreiber et al., 1986; van Kooten and Snel, 1990) based on a separate units model; and (ii) the \( qL \) parameter (Kramer et al., 2004b; Baker, 2008; Porcar-Castell, 2011) based on a lake model, where \( qL=q \) for a system with perfect connectivity, and \( qP=q \) for a system with no connectivity at all (Appendix 1), with intermediate \( qL \) and \( qP \) performances depending on connectivity (see Fig. 5 and text below).

The bulk of experimental data favours the view that PSII units are connected to some extent (Joliot and Joliot, 1964; Dau et al., 1994; Lavergne and Trissl, 1995; Kramer et al., 2004b; Tyystjärvi et al., 2009) although some recent data favour a model of isolated centres (Oja and Laisk, 2012). Overall, the lake model has been described as a reasonable assumption to study the slow ChlF dynamics dealt with here (Kramer et al., 2004b; Baker, 2008). These conclusions need to be re-assessed at the seasonal scale for three reasons: (i) The effect of connectivity (excitation transfer from closed to open centres) could expand to photoinhibited reaction centres (excitation transfer from damaged to functional centres); (ii) connectivity might undergo changes at the seasonal scale; and (iii) connectivity effects have been analysed in the absence of or under constant and relatively low levels of NPQ, but the interplay between connectivity and NPQ remains unknown.

Lavergne and Trissl (1995) derived equations for examining the impact of different connectivity models on fluorescence yield as a function of the fraction of open reaction centres \( q \). Applying those equations (Appendix 1), we evaluated the impact of NPQ on connectivity and the interplay between connectivity, NPQ, \( qL \), and \( qP \) (Fig. 5). The analysis demonstrates that connectivity has very little effect on ChlF in the presence of NPQ (Fig. 5A–D). Therefore, the impact of connectivity on seasonal changes of ChlF obtained under natural illumination (as in the remote sensing of SIF) is probably small. In contrast, selecting a proper connectivity model remains important even in the presence of NPQ if \( q \) needs to be estimated. In a nutshell, overestimation of \( q \) by \( qP \) increases with connectivity, and underestimation of \( q \) by \( qL \) decreases with connectivity (Fig. 5E–L). If we use the lake model assumption to interpret ChlF data, a seasonal decrease in connectivity would translate into underestimation of \( q \) and consequently to underestimation of \( \Phi P \) (Equation 9). A decrease in connectivity would also decrease the overall probability of photochemical excitation trapping by reaction centres. Accordingly, the effects of connectivity on ChlF and on the ‘true’ \( \Phi P \) tend to cancel out (Weis and Berry, 1987), and therefore fluorometric estimates of \( \Phi P \) are expected to be good proxies of ‘true’ \( \Phi P \) irrespective of connectivity issues. Keeping this in mind, we use the lake model assumption and the parameter \( qL \) as a proxy of \( q \) to demonstrate the derivation of the quantum yield of photochemistry in PSII (see Section 4.1 for application to PAM fluorometry).

The effective rate constant of photochemistry \( (k_p) \) depends on the maximum intrinsic rate constant of photochemistry \( (k_{PSII}) \) proportionally to \( qL \) as:

\[
qL = qL_k_{PSII} \tag{5}
\]

Subsequently, the fluorescence flux of PSII \( (F_{II}) \) (\( \mu \)mol photons \( m^{-2} s^{-1} \)) emanating from a sample with area \( (m^{-2}) \) that contains a large population of both PSII and PSI units can be expressed as a function of the quantum yield of fluorescence emission \( (\Phi F_{II}) \):

\[
F_{II} = IAq_{II} \Phi F_{II} \tag{6}
\]

where \( I \) is the incident flux of photosynthetic active radiation (\( \mu \)mol photons \( m^{-2} s^{-1} \)), \( A \) is the absorbance coefficient, and \( q_{II} \) the relative absorption cross-section area of the PSII population in the sample. Applying Equation 3, \( \Phi F_{II} \) can be expressed as:

\[
\Phi F_{II} = \frac{k_F}{k_D + k_F + k_{NPQ} + qLk_{PSII}} \tag{7}
\]

Equations 6 and 7 introduce the link between ChlF and photochemistry at the level of PSII. It should be clarified that these equations (and the rate constants therein) are only meaningful when applied to large populations of photosystems. Because both \( k_{NPQ} \) and \( qL \) vary in response to the acclimation of photosynthesis, a direct link between ChlF and photochemistry cannot be readily established with Equation 7 alone. An additional fluorescence measurement in a different state is needed. Estimation of the quantum yield of photochemistry of PSII \( (\Phi P) \) is accomplished by measuring ChlF in the presence \((0<q<1)\) and in the absence of photochemistry \((q=0)\).

This has been traditionally accomplished using herbicides such as DCMU (e.g. Horton and Hague, 1988), liquid nitrogen temperature (Björkman and Demmig, 1987), and more...
commonly saturating light pulses (Schreiber et al., 1986; see Section 4.1). The result is an increase in $F_{II}$ to a maximal level ($F_{IIM}$). Where,

$$F_{IIM} = IAa_{II} \frac{k_F}{k_D + k_F + k_{NPQ}}$$  \hspace{1cm} (8)

Since the sum of the quantum yields of all processes competing for excitation energy equals one, the quantum yield of photochemistry in PSII ($\Phi_P$) can be expressed in fluorescence terms as:

$$\Phi_P = 1 - \frac{qLk_{PSII}}{k_D + k_F + k_{NPQ} + qLk_{PSII}} = 1 - \frac{k_F}{k_D + k_F + k_{NPQ}}$$  \hspace{1cm} (9)

$$= 1 - \frac{k_D + k_F + k_{NPQ} + qLk_{PSII}}{k_F}$$

Fig. 5. Theoretical analysis of the interaction between photosystem II connectivity, the fraction of open reaction centres $q$, NPQ, and the parameters $q_L$ and $q_P$. (A–D) The effect of increasing levels of NPQ on the relationship between $F$ and $q$ at different levels of connectivity, where $k_{CII}$ is the rate constant of energy transfer between photosystems, ranging from zero, for the case of separate units (SU), to infinity, for a lake model. (E–L) Theoretical relationship between $q$ and the fluorometric estimates of $q$ based on a separate units assumption ($q_P$) or on a lake model ($q_L$) under different levels of NPQ and under two different connectivity scenarios. Derived using parameters and equations after Lavergne and Trissl (1995); see Appendix 1 for details.
which corresponds to the parameter derived by Genty et al., (1989) at the level of PSII, and becomes $\Phi_{P_{\text{max}}}$ (the widely used parameter $F_v/F_M$; see Section 4.1) when $q_L=1$ and $k_{\text{NPQ}}=0$.

Because the rate constants $k_D$ and $k_F$ remain constant, variations in $\Phi_P$ can be expressed as a function of photochemical and non-photochemical processes, with $k_D+k_F=1$. This simplification gives rise to the photochemical (PQ) and non-photochemical quenching (NPQ) parameters, where

$$\text{PQ} = q_L \frac{k_{\text{PSII}}}{k_D+k_F} \quad \text{and} \quad \text{NPQ} = \frac{k_{\text{NPQ}}}{k_D+k_F} \quad (\text{Laisk et al., 1997; Porcar-Castell, 2011}),$$

and the quantum yield of photochemistry can be expressed as:

$$\Phi_P = \frac{\text{PQ}}{1+\text{NPQ}+\text{PQ}} \quad (10)$$

Note that the two forms of photochemical quenching parameter, PQ and $q_L$, carry exactly the same information and can be used interchangeably depending on the application. For example, PQ facilitates the comparative analysis with NPQ because it has the same relative range of variation, whereas $q_L$ gives a more visual picture of the degree of reaction centre openness.

In contrast to PSII, the fluorescence yield of PSI is generally low and remains constant under illumination. Chlorophyll cations such as P700$^+$ and P680$^+$ are known to be very efficient in dissipating excitation energy as heat. However, in contrast to P680$^+$, the lifetime of P700$^+$ is longer and P700$^+$ operates as a very effective quencher of excitation energy in PSI (Dau, 1994; Lavergne and Trissl, 1995). The fluorescence spectral properties of PSI and PSII are also different (Fig. 6). At room temperature, ChlF from plant PSI presents a shoulder at 690 nm and a peak at 730 nm (Croce et al., 1996), whereas fluorescence from PSII peaks at ~685 nm and presents vibrational satellite bands in wavelengths >700 nm (Govindjee, 1995) overlapping with PSI fluorescence (Franck et al., 2002; Fig. 6). As a result of differences in yield and spectral properties between photosystems, PSI fluorescence has been found to contribute between 0 and 50% of total fluorescence depending on the method, measuring conditions, species, and especially the spectral region, with the contribution of PSI being insignificant in the red region and maximum in the near infrared (Fig. 6) (Genty et al., 1990b; Dau, 1994; Pfündel, 1998; Agati et al., 2000; Peterson et al., 2001; Franck et al., 2002; Palombi et al., 2011; Pfündel et al., 2013).

Analogously to PSII, we express the fluorescence flux emanating from a population of PSI units as:

$$F_I = I A_{\text{PSI}} \frac{k_F}{k_D + k_F (+k_{\text{NPQ}}) + q k_{\text{PSI}} + k_{\text{RCI}}} \quad (11)$$

where $A_{\text{PSI}}$ is the relative absorption cross-section area of PSI in the sample, $q$ is the fraction of open PSI centres, $k_{\text{PSI}}$ is the intrinsic rate constant of PSI photochemistry, and $k_{\text{RCI}}$ is the rate constant of quenching by P700$^+$. Finally, $k_{\text{NPQ}}$ is the rate constant of regulated thermal energy dissipation in PSI, the significance of which remains to be elucidated (see Section 5).

3.3 Chlorophyll fluorescence at the leaf level and beyond

A new set of factors and phenomena need to be considered when linking ChlF data and photosynthetic CO2 assimilation at the leaf level and beyond (Fig. 4). The efficient absorption of blue and red light by chlorophyll within a leaf or by leaves within a plant canopy plays two important roles when scaling from the photosystem to the leaf level and beyond: (i) wavelength-dependent light penetration; and (ii) wavelength-dependent fluorescence reabsorption.
First, red light penetrates deeper into the leaf and is more scattered than blue light (Agati, 1998; Buschmann and Lichtenthaler, 1998; Vogelmann and Evans, 2002), whereas green light may have an optical path up to five times longer than that of red or blue light due to weaker absorption and higher scattering (Vogelmann, 1993; Rappaport et al., 2007). Wavelength-dependent scattering and absorption within a leaf or a plant canopy generates important gradients in light quality and intensity which translate into similar gradients in thylakoid composition, stoichiometry, and physiological state. For example, the number of PSII reaction centres is known to increase with irradiance (Anderson et al., 1988) or in response to far-red light (Chow et al., 1990), whereas the antenna sizes of the photosystem (Melis, 1991; Anderson et al., 1988; Ballottari et al., 2007), as well as the absorption cross-section of PSII relative to PSI (Eichelmann et al., 2003; Porcar-Castell et al., 2008a). In addition, chloroplasts or leaves exposed to high light conditions tend to have lower photochemical efficiencies and larger NPQ compared with more shaded chloroplasts or leaves.

Secondly, because the spectra of ChIF emission overlaps with that of chlorophyll absorption, red fluorescence photons ($F_R$) can be reabsorbed by chlorophyll itself within the leaf or inside a plant canopy (Brody and Brody, 1962; Govindjee and Yang, 1966; Gitelson et al., 1998). Reabsorption of $F_R$ within the leaf can be as high as 90% (Gitelson et al., 1998).

The overall result of the above two phenomena is that the biological footprint of a fluorescence measurement depends on the spectral properties of both the excitation light and the wavelengths across which fluorescence is retrieved (Fig. 7). The $F_R$ signal (detected in the red region) is enriched in photosystems close to the leaf surface or leaves from the top of the canopy, whereas the $F_{FR}$ signal (detected in the far-red) may have a stronger contribution from a deeper leaf or canopy layer, especially when the excitation light penetrates deep into the leaf or the canopy (Peterson et al., 2001; Rappaport et al., 2007; Pfundel, 2009). These differences are particularly relevant to the interpretation of SIF data where the signal can be obtained in different spectral regions that will consequently carry information from different layers of the leaf or the canopy (see Section 4.2).

Although Equations 6 and 7 provide the basis for linking ChIF and photochemistry, the effects of leaf and canopy structure also affect the ChIF signal and need consideration. A quantitative treatment of the impact of structure requires the use of leaf and canopy radiative transfer models. Here we use a simplified expression to introduce the main physical and physiological controls behind leaf-level ChIF (for similar formulations, see also Agati et al., 1995; Dau and Sauer, 1996; Franck et al., 2002; Pedrös et al., 2008; Palombi et al., 2011). The intensity and spectral properties of the chlorophyll fluorescence signal emanating from a leaf $F$ can be represented (in $\mu$mol photons m$^{-2}$ s$^{-1}$ nm$^{-1}$) as:

$$F[I(\lambda_{ex})A(\lambda_{ex})a_{II}(\lambda_{ex})a_I(\lambda_{ex}),F_{PSII}(\lambda_{em}),F_{PSI}(\lambda_{em}),NPQ,qL,p_I(\lambda_{em},Chl)] =$$

$$I(\lambda_{ex})A(\lambda_{ex})\Phi F_{II}K_{NPQ},qL,F_{PSII}(\lambda_{em}) +$$

$$a_I(\lambda_{ex})\Phi F_I F_{PSII}(\lambda_{em})[1-p_I(\lambda_{em},Chl)]$$

where $I(\lambda_{ex})$ and $A(\lambda_{ex})$ are the irradiance flux at the leaf surface and the leaf-level absorption as a function of the excitation wavelength; and $a_{II}(\lambda_{ex})$ and $a_I(\lambda_{ex})$ are the relative absorption cross-sections for each population of photosystems as a function of the excitation wavelength (0$\leq$'$a_{II}, a_I$$\leq$1). For example, $a_{II}$ and $a_I$ were found to remain rather constant in spinach leaves for the range 400–680 nm, but $a_I$ rapidly increases to 1 at > 675–680 nm (Kitajima and Butler, 1975b; Butler, 1978) where PSII no longer absorbs. $F_{PSII}(\lambda_{em})$ and $F_{PSI}(\lambda_{em})$ are the

Fig. 7. Wavelength dependency of light penetration and fluorescence reabsorption within a leaf and a plant canopy. Red light penetrates deeper in the leaf or within a plant canopy compared with blue light due to higher scattering. In turn, red fluorescence ($F_R$) has a larger probability of being reabsorbed by chlorophyll within the leaf and canopy compared with far-red fluorescence ($F_{FR}$), due to the characteristics of the chlorophyll absorption spectra.
wavelength-dependent functions that account for the shape of the fluorescence emission spectra for PSII and PSI, respectively (Croce et al., 1996; Franck et al., 2002; Palombi et al., 2011); and \( p_q(\lambda_{em}, \text{Chl}) \) is a function that accounts for fluorescence reabsorption and depends on fluorescence wavelength, chlorophyll content, and leaf morphology. In the following, we use Equation 12 to discuss the differences between PAM and SIF fluorescence.

### 4. Measuring chlorophyll fluorescence

Two main methods are used to measure ChlF in the field: those based on PAM systems (active methods) and those based on the retrieval of SIF (passive methods). Although active methods based on laser technology have been also developed (e.g. Kolber et al., 2005; Pieruschka et al., 2010), their applicability at the canopy and landscape level remains to be assessed. More generally, active methods are applied at the leaf level whereas passive methods are being commonly applied at the canopy level and beyond. In the following, we describe the background of these measurements, their differences, and some of the underlying challenges. For further details, we suggest reviews by Maxwell and Johnson (2000), Schreiber (2004), or Baker (2008) for PAM fluorometry, or that of Meroni et al., (2009) for SIF.

#### 4.1 Pulse amplitude-modulated fluorescence

The main feature of PAM fluorometers is that a weak and pulsed measuring light (ML) is used to excite fluorescence (Duy sens, 1979). Widths of measuring pulses are in the micro-second range and the pulse amplitude is constant. Pulse frequencies can range from 10 Hz up to 200kHz depending on fluorometer and instrument setting. At low frequencies, dark intervals between measuring pulses are relatively long so that the average photon flux density of the ML is very small (<1 μmol m\(^{-2}\) s\(^{-1}\)) and does not cause any significant change in the degree of PSII openness (\( q \)) (see Section 3.2). PAM fluorometers subtract the fluorescence signal shortly before or after a measuring pulse from the fluorescence signal during this measuring pulse. This subtraction method is highly selective and eliminates virtually all non-PAM fluorescence from the measurement, for example the fluorescence excited by sunlight in field experiments. Because PAM fluorometers register only the fluorescence excited by this constant ML, variations in PAM fluorescence reflect variations in the efficiency by which the sample transforms modulated excitation light into fluorescence.

PAM fluorometers are characterized by the wavelength of the ML as well as the wavelength range at which modulated fluorescence is being registered. Recent advances in light-emitting diode (LED) technology allow PAM excitation in the entire visible range with a full width at half maximum (FWHM) between 20 nm and 40 nm. The spectral window for fluorescence detection is typically confined to wavelengths >700 nm using a long-pass colour filter, in order to minimize reabsorption effects. Using such filters, the PAM ChlF signal is typically integrated over tens of nanometres, providing a broadband measure of ChlF. These characteristics differ from instrument to instrument.

Using Equation 12, the PAM fluorescence signal (\( F_r \)) in relative units (e.g. mV) emanating from a leaf under illumination can be expressed as:

\[
F_r = \beta (\text{ML}) (\lambda_{em}) A(\text{ML}) \left[ \frac{a_q (\lambda_{em}) k_f (\lambda_{em})}{k_f + k_q + k_{NPQ} + q_L k_{PSII}} F_{PSII} (\lambda_{retrieval}) + a_q (\lambda_{em}) \Phi F_{PSII} (\lambda_{retrieval}) \right] \left[ 1 - p_q (\lambda_{retrieval}, \text{Chl}) \right]
\]

(13)

where \( \beta \) is a parameter that accounts for the properties and sensitivity of the detector system (e.g. mV m\(^{-2}\) s\(^{-1}\) μmol photons m\(^{-2}\) s\(^{-1}\)) with wavelength (\( \lambda_{ML} \)); \( I_{ML} (\lambda_{ML}) \) is the intensity of the PAM measuring light (μmol photons m\(^{-2}\) s\(^{-1}\)) with wavelength (\( \lambda_{ML} \)); \( A(\lambda_{ML}) \), \( a_q(\lambda_{ML}) \), and \( a_q(\lambda_{ML}) \) are the absorbance and relative absorption cross-section area of PSII and PSI, respectively, at the wavelength of the measuring light (\( \lambda_{ML} \)); and \( F_{PSII}(\lambda_{retrieval}) \) and \( F_{PSII}(\lambda_{retrieval}) \) account for the shape of the fluorescence emission spectra for PSII and PSI, respectively, averaged for the spectral range of the retrieval. \( \Phi F_{PSII} \) is the fluorescence yield of PSI (see Equation 11) and \( p_q(\lambda_{retrieval}, \text{Chl}) \) the average reabsorption probability for the wavelength range at which fluorescence is being retrieved and for the prevailing leaf morphology and chlorophyll content.

Equation 13 can be greatly simplified as a result of PAM design and by using a number of assumptions. (i) Because PAM fluorometers typically (but not always) register fluorescence >700 nm, we can assume they are not affected by reabsorption, i.e. \( p_q(>700 \text{ nm, Chl})=0 \). (ii) Since the amplitude of the ML is constant, we can assume \( I_{ML}=\text{constant} \). Therefore, at constant \( A \) and \( a_q(\lambda_{ML}) \), \( F_r \) is proportional to \( \Phi F_{PSII} \). (iii) The contribution of PSI fluorescence to the total signal is usually assumed to be constant or negligible. (iv) The characteristic emission spectra for PSII and PSI are assumed to remain constant for the duration of the measurements [\( F_{PSII}(\lambda_{retrieval}) \) and \( F_{PSII}(\lambda_{retrieval}) \)]. Indeed, because PAM ChlF is acquired over a broadband, the resulting signal is less likely to be affected by seasonal shifts in the fluorescence emission spectra compared with SIF. (v) \( A(\lambda_{ML}) \) and \( a_q(\lambda_{ML}) \) are often assumed to remain constant or their variation negligible. Given these simplifications, \( F \) in Equation 13 corresponds to \( \Phi F_{PSII} \) in Equation 7.

The operating quantum yield of photochemistry in PSII (\( \Phi_P \)) can be obtained by comparing the fluorescence level in the presence (\( F_r \)) (Equation 7) and in the absence (\( F_{M} \)) of photochemistry (Equation 8). PAM fluorometers estimate maximal fluorescence (\( F_{M} \)) by providing a pulse of saturating light (SP) for several hundreds of milliseconds (typically 0.6–1 s). The SP momentarily reduces all the electron acceptors of PSII so that primary photochemistry tends to zero and \( q_L=0 \), and thus \( k_P=0 \) (Bradbury and Baker, 1981; Schreiber et al., 1986). As a result \( F_r \) increases to a maximal level \( F_{M} \). Accordingly, \( \Phi_P \) can be then estimated from PAM data following Equation 9, as:
Following from Equation 14 and provided that \( I(\lambda) \), \( A(\lambda) \), and \( a_\| (\lambda) \) are known for the range 400–700 nm (PAR), the rate of LET through PSII can be estimated as \( F_\Phi \) (Genty et al., 1989; Schreiber, 2004; Baker, 2008):

\[
\Phi P = 1 - \frac{k_D + k_F + k_{NPQ} + qL_{PSII}}{k_F} = 1 - \frac{F'}{F_M} \quad (14)
\]

Commercial fluorometers usually provide an estimate of the electron transport rate (ETR) by assuming that PAR leaf absorptance equals 0.84 (Baker, 2008) and that absorbed photons are equally distributed between the two photosystems (i.e. \( a_\| = 0.5 \)). This approximation is reasonable for comparison of ETR values between optically similar samples such as leaves of cultivars of a single plant species. When the data expand over several species or extend to a long period time, then the replication of species-specific, within-canopy gradients or seasonal variation in \( A \) and \( a_\| \) need to be taken into account when calculating ETR. The same applies to other fluorescence parameters (Baker and Oxborough, 2004; Logan et al., 2007).

Other important fluorescence parameters can be estimated by dark-acclimating the leaf for a period of time (15 min to 2 h). The purpose of dark acclimation is to re-oxidize the QA electron acceptors in every PSII (which takes only several seconds) and to relax all the reversible NPQ, which may take a few minutes or hours, depending on temperature (Bilger and Björkman, 1991; Eskling, 1997; Demmig-Adams and Adams, 2006). By definition, dark acclimation of sufficient length decreases NPQ to zero, \( k_{NPQ} = 0 \) in Equation 8. Under these conditions, minimal \( (F_\Phi) \) and maximal \( (F_M) \) fluorescence levels of the dark-acclimated leaf are obtained.

Substituting \( F' \) by \( F_\Phi \) (i.e. \( qL \) approaches unity), and \( F_M' \) by \( F_M \) (i.e. NPQ approaches zero), we can use Equation 14 to estimate the maximum quantum yield of photochemistry \( (\Phi P_{\max}) \), as (Kitajima and Butler, 1975a):

\[
\Phi P_{\max} = 1 - \frac{F_\Phi}{F_M} = \frac{F_M - F_\Phi}{F_M} = \frac{F_V}{F_M} \quad (16)
\]

The \( F_V/F_M \) parameter or \( \Phi P_{\max} \) has been found to remain rather constant in non-stressed C3 plants, with values of ~0.83 (Björkman and Demmig, 1987). Consequently, a decrease in \( F_V/F_M \) below the value found in healthy plants of the same species has been used as an indicator of decreased photochemical performance caused, for example, by photoinhibition of reaction centres or sustained forms of NPQ (Ottander, 1991; Adams and Demmig-Adams, 2004; Ensminger et al., 2004; Porcar-Castell et al. 2008a, b).

Provided that factors such as light absorptance \( (A) \) and PSII relative absorption cross-section \( (a_\|) \) remain constant, differences between \( F_V' \) and \( F_M \) can be used to estimate the regulated non-photochemical quenching or NPQ, via the parameter \( NPQ \) (Bilger and Björkman, 1991):

\[
NPQ = \frac{k_{NPQ}}{k_F + k_D} = \frac{F_M - F_M'}{F_M} \quad (17)
\]

Similarly, \( F' \), \( F_M' \), and \( F_M \) can be combined to estimate the photochemical quenching or PQ, via the parameter \( PQ \) (Porcar-Castell, 2011):

\[
PQ = qL_{PSII} \frac{k_{PSII}}{k_F + k_D} = \frac{F_M - F_M'}{F_M} \quad (18)
\]

These are just some of the many parameters that can be obtained using PAM fluorescence. Further photochemical and non-photochemical quenching parameters and process quantum yields can be found elsewhere (e.g. Roháček, 2002; Krause and Jahns, 2004; Schreiber, 2004; Baker, 2008; Porcar-Castell, 2011).

When the underlying assumptions are carefully considered (Maxwell and Johnson, 2000; Logan et al., 2007; Baker, 2008), PAM fluorometry becomes a very versatile tool that provides highly informative data to track the acclimation of the light reactions of photosynthesis. PAM fluorometry also provides the possibility of studying in detail and modelling the link between ChlF and photosynthesis in the new spatiotemporal domain (seasonal, within-canopy, multiple species). We expect that the rapid spread of gas exchange systems combined with PAM fluorometers, the recent availability of long-term PAM monitoring systems (Porcar-Castell et al., 2008c; Porcar-Castell, 2011), and the development of other active techniques such as the laser-induced fluorescence transient (LIFT) (Kolber et al., 2005; Pieruschka et al., 2010) will favour these developments.

4.2 Solar-induced fluorescence and passive remote sensing

SIF is chlorophyll fluorescence that originates from excitations caused by the absorption of sunlight. SIF can be measured using passive remote sensing at a range of scales; from leaves in the laboratory (Gamon 1990) to satellite-based retrievals across the landscape (Guanter et al., 2007; Frankenberg et al., 2011; Joiner et al., 2011). As discussed in the Section 4.1, PAM fluorometers measure fluorescence induced by a (modulating) ML source which makes the observations independent from any other irradiance source (such as the sun). In contrast, careful characterization of the incident irradiance field is required if SIF-based ChlF observations are to be meaningfully compared with PAM fluorescence measurements.

The principal passive remote sensing technique used to estimate SIF is the Fraunhofer line depth/discriminator (FLD) method (Plaszyk, 1975). Fraunhofer lines are dark, narrow regions of the solar spectrum that are caused by gaseous absorption in the Sun’s photosphere. There are also several dark regions in the spectrum caused by gaseous absorption in the Earth’s atmosphere, so-called telluric lines. Several Fraunhofer and telluric lines are found at wavelengths coincident with fluorescence emission and are literally filled in by fluorescence (examples are shown as grey vertical lines
on Fig. 8A). In reality, several spectrally adjacent lines may contribute to a specific Fraunhofer ‘feature’. It is across these features that the FLD algorithm is applied [in a slight abuse of terminology, we use ‘line(s)’ and ‘feature’ interchangeably in this section].

Changes in the fractional depths of Fraunhofer lines are used as the foundation of the FLD retrieval. In essence, the relatively weak fluorescence signal is amplified in these features of the spectrum. The FLD method can be applied to many different lines including the Fraunhofer hydrogen-α band (656 nm), potassium D1 (769 nm), the telluric oxygen-B (690 nm), and oxygen-A (760 nm) bands. To compute SIF using the FLD method it is not necessary to measure whole radiance or irradiance spectra; rather, four measurements are needed in total. One measurement of solar irradiance and target radiance is taken ‘inside’ the line, and one measurement of solar irradiance and spectral radiance is taken directly next to the line. The following expression is used to retrieve fluorescence across the line (SIF

\[
SIF_{\text{retrieval}} = \frac{L_{\text{inside}}E_{\text{outside}} - L_{\text{outside}}E_{\text{inside}}}{L_{\text{inside}} - E_{\text{outside}}}
\]  

(19)

L and E refer to radiance and irradiance measurements taken at a wavelength within (inside) and outside particular line feature. The key assumption is that the bands are sufficiently close so that both inherent reflectance and fluorescence are constant across the lines. In practice, these assumptions are often violated, which has motivated the development of several improved FLD-based retrievals [see the description of the spectral fitting method below, and Meroni (2009) for an in-depth review of improved methods].

The physiological interpretation of the resulting SIF signal depends on the spectral line chosen for the retrieval, SIF\text{retrieval}. Consistent with PAM fluorescence (Equation 13), the SIF signal can be expressed using Equation 11 for a specific retrieval wavelength, as:

\[
SIF_{\text{retrieval}} = \int_{\lambda_{\text{uns}}}^{\lambda_{\text{sun}}} L_{\text{sun}}(\lambda)I(\lambda, \text{sun})A(\lambda, \text{sun})[a_t(\lambda, \text{sun}) \Phi F_{\text{PSI}}(\lambda, \text{retrieval})] + a_t(\lambda, \text{sun}) \Phi F_{\text{PSI}}(\lambda, \text{retrieval})[1 - p_t(\lambda, \text{retrieval}, \text{Chl})] \, d\lambda_{\text{sun}}
\]  

(20)

From Equation 20 it is evident that the interpretation of the resulting SIF signal depends on the spectral window chosen to retrieve SIF. The SIF signal measured at the O2B line, SIF\text{O2B}, is very close to the chlorophyll absorption peak and is therefore very sensitive to reabsorption of fluorescence by chlorophyll. This is not the case for SIF\text{760} which is measured in the O2A line. Therefore, the SIF signal carries different physical–physiological information depending on the region used in the retrieval (Fig. 7). In addition, the contribution from PSI fluorescence will also vary depending on the retrieval line wavelength. This is due to differences in wavelength dependencies of \(F_{\text{PSI}}(\lambda, \text{retrieval})\) and \(F_{\text{PSI}}(\lambda, \text{retrieval})\) in Equation 20 which cause a higher relative contribution of PSI fluorescence at longer wavelengths since \(F_{\text{PSI}}(690) >> F_{\text{PSI}}(760)\) (Fig. 6). A further challenge faced when retrieving SIF from space is accurately correcting for atmospheric scattering and absorption effects. These may act to modify the observed SIF signal. In particular, when using telluric lines to retrieve SIF, the same chemical species that absorbs solar irradiance in the Earth’s atmosphere (causing the feature) also absorbs the emitted SIF signal. This means that precise atmospheric correction is essential if accurate canopy-leaving estimates of SIF are to be retrieved from at-sensor observations.

A promising development of the FLD method is the spectral fitting method (SFM) (Meroni et al., 2009). The SFM solves the inverse problem of estimating fluorescence across a Fraunhofer feature by using a least-squares approach. In the SFM, FLD observations are fitted with synthetic spectra modelled using simple polynomial functions. The SFM was designed to reduce noise and to be more robust to contamination by atmospheric effects than the FLD method and was demonstrated to increase the accuracy of SIF retrievals in comparison with the basic FLD algorithm (Meroni et al., 2009).
In addition to FLD-based methods, reflectance spectroscopy can be used to gain insight into the relationship between ChlF and photosynthesis. This is because the fluorescence emission spectrum is superimposed on the leaf (or canopy) reflectance spectrum. The reflectance spectrum is measured, typically in the laboratory or the field, as the ratio of reflected radiance to incident irradiance using hyperspectral spectroradiometers (Fig. 8B). A number of reflectance band ratios (algebraic combinations of ≥2 narrow spectral reflectance ‘bands’) have been developed to quantify the effect of fluorescence emission on the reflectance spectrum (Meroni et al., 2009). These methods are particularly popular at the leaf scale where they are often compared and contrasted to reflectance indices that measure related physiological processes such as the photochemical reflectance index (PRI) (Gamon et al., 1992) or chlorophyll-based indices (Richardson and Berlyn, 2002). Reflectance difference spectra are useful as measures of the dynamic fluorescence contribution to reflectance. Difference spectra can be calculated from sets of reflectance spectra both as a function of time (Gamon et al., 1990) and using a filter-based approach (Zarco-Tejada et al., 2000; Campbell et al., 2008; Meroni et al., 2009). Filter-based approaches are used to extricate the chlorophyll fluorescence contribution from the measured apparent reflectance spectrum.

5. Remaining challenges: from diurnal to seasonal, from the leaf to the landscape, from active to passive fluorescence.

Time-resolved fluorescence spectra and lifetime analysis have proved essential for elucidating structural and functional features of photosynthesis (Roelofs et al., 1992; Dau, 1994; Govindjee, 1995; Croce et al., 1996; Lázár, 1999; Jennings et al., 2003; Vassiliev and Bruce, 2008). Likewise, PAM fluorescence measurements have been valuable in studying the ecophysiology of photosynthesis at the leaf level and in situ (Bradbury and Baker, 1991; Ottander et al., 1991; Adams and Demmig-Adams, 1994; Verhoeven et al., 1996; Ogaya and Peñuelas, 2003; Ensminger et al., 2004; Porcar-Castell et al., 2008a, b, c; Porcar-Castell, 2011).

The ChlF signal is now being applied to the study of photosynthesis from remote sensing platforms. Before we can mechanistically link SIF and GPP, we need to expand the knowledge we have gained from short-term measurements using mainly PAM techniques at leaf level, to the seasonal and canopy level using SIF instead. This involves a number of challenges that are introduced in this section and can be summarized as follows. (i) Temporal up-scaling. Factors that control the seasonal variation of ChlF properties remain unclear. (ii) Spatial up-scaling. In addition to characterizing and modelling the radiative transfer of the signal, up-scaling from the leaf to the landscape also requires understanding of the vertical and species-specific variation in physiological traits, an area with very limited information. (iii) Mechanistic up-scaling from SIF to gross photosynthesis. In contrast to PAM fluorescence, the quantum yield of photochemistry cannot be directly resolved from SIF. This problem can only be bypassed by developing robust models to link SIF to GPP, an area where a quantum leap forward is urgently needed.

5.1 Temporal up-scaling: from the diurnal to the seasonal scale

Over the short term (seconds to days) it is reasonable to assume that most of the factors that control the ChlF signal at the leaf level (Equation 12) remain constant, so that variations in fluorescence can be largely attributed to photochemical or non-photochemical processes in PSII (i.e. qL and NPQ) (Maxwell and Johnson, 2000; Logan et al., 2007; Baker, 2008), as well as to changes in illumination $I(\lambda_{in})$ in the case of SIF. However, it remains largely unknown whether these simplifying assumptions hold at the seasonal scale (days to months).

Changes in chlorophyll contents, leaf absorptance, and fluorescence reabsorption

Leaf chlorophyll concentrations vary at the seasonal time scale (García-Plazaola and Becerril, 2001; Lu et al., 2001). This is particularly evident during leaf development and senescence, but adjustments in pigment concentrations occur also in evergreen foliage during the course of the seasons. For example, boreal Scots pine trees decrease their needle chlorophyll concentration by up to ~40% in winter compared with summer (Ensminger et al., 2004; Porcar-Castell et al., 2012). An increase in leaf-level chlorophyll content may have mixed effects on ChlF.

Chlorophyll concentration modulates light absorptance $A(\lambda)$ and, by extension, ChlF (Equation 12). The relationship between chlorophyll content and light absorptance is positive and non-linear, saturating at high chlorophyll contents (Björkman and Demmig, 1987; Adams et al., 1990; Gitelson et al., 1998). In agreement with this, the relationship between leaf-level chlorophyll concentration and the fluorescence signal also saturates at high chlorophyll contents (Adams et al., 1990).

Similarly, chlorophyll concentration also modulates fluorescence reabsorption (Equation 12) and, by extension, ChlF. An increase in absorption $A(\lambda)$ will cause an increase in fluorescence at all wavelengths, whereas an increase in reabsorption will decrease red but not far-red ChlF (Lichtenthaler and Rinderle, 1988; Gitelson et al., 1998). This phenomenon is built in the ratio of red to far-red fluorescence ($F_R/F_{FR}$) to estimate chlorophyll concentrations. Interestingly, the processes controlling $F_R/F_{FR}$ depend on scale. At the diurnal scale, $F_R/F_{FR}$ varies in response to the action of NPQ (Agati et al., 1995), whereas at the seasonal scale or when comparing leaves with different chlorophyll contents, $F_R/F_{FR}$ reflects changes in chlorophyll concentration (Lichtenthaler and Rinderle, 1988; Gitelson et al., 1998). This scale-dependent relationship also affects the correlation between $F_R/F_{FR}$ and LUE. The ratio $F_R/F_{FR}$ is directly proportional to LUE over the diurnal scale (Agati et al., 1995), but becomes inversely proportional to LUE at the seasonal scale (Freedman, 2008c).
Leaf chlorophyll concentration is therefore expected to have a mixed effect on the ChlF signal, with absorption effects dominating in leaves with low chlorophyll concentrations (i.e. more chlorophyll more fluorescence), and reabsorption effects dominating in leaves with high chlorophyll contents (i.e. more chlorophyll less fluorescence) (unless PAM fluorometer design discriminates all red fluorescence). This is consistent with results obtained in *Hedera canariensis* and *Platamus occidentalis* where the shift from absorption- to reabsorption-dominated effects took place at chlorophyll concentrations around 250 mg Chl m⁻² (Björkman and Demmig, 1987; Adams et al., 1990). Further characterization of the interplay between chlorophyll contents, leaf absorption, and wavelength-dependent fluorescence properties across leaves with different morphologies is warranted to model these effects.

**Changes in relative absorption cross-sections of PSII (a₂) and PSI (a₁)**

PSII and PSI have different fluorescent properties. The quantum yield of PSI (Φ₁) is typically much smaller than Φ₂, and PSI fluorescence peaks at longer wavelengths than PSII fluorescence (Fig. 6). Consequently, changes in the relative absorption cross-sections of PSII (a₂) and PSI (a₁) affect the fluorescence yield and spectra of the leaf. Over the short term, state transitions are known to reduce a₂ and increase a₁, and, since Φ₂<Φ₁, the intensity of the fluorescence signal decreases. This quenching has been described in PAM fluorescence terms as δT (Krause and Weis, 1991), although δT does not contribute to NPQ as such (non-radiative quenching of excitation energy in PSI) but simply quenches F' by decreasing a₂, which results in a decrease in F₄₂ (Equation 8) and a subsequent increase in the parameter NPQ (Equation 17). In addition, state transitions also produce a change in the spectral properties of the F signal, with F₄ being decreased to a larger proportion than F₂₄ due to a larger contribution of PSII fluorescence in the red relative to far-red. This phenomenon is particularly visible at liquid nitrogen temperature (77 °K) when the PSII and PSI fluorescence peaks can be separated, with F₄ (originating mainly in PSI) and F₂₄ (originating mainly in PSII) (Butler, 1978; Govindjee, 1995). Changes in the 77 °K fluorescence ratio F₄/F₂₄ have been used to study state transitions (e.g. Tan et al., 1998).

The question is, how stable/dynamic are the relative absorption cross-sections at the seasonal scale? If a₂ and a₁ would change over the season, we could expect a similar effect to that of state transitions, and possibly modifications in the relationship between ChlF, NPQ, and photochemistry. Direct measurement of the relative absorption cross-sections of PSII and PSI remains a challenge (Laisk et al., 2002; Eichelmann et al., 2005). Studies that directly measure a₂ and a₁ in conjunction with fluorescence spectral properties at the seasonal scale will help elucidate the dynamics and impact of a₂ and a₁ on ChlF.

**Down-regulation of photosynthesis**

Leaf-level fluorescence spectra might also change in response to the structural re-organization of the thylakoid observed, for example, in response to low temperature. The aggregation of LHCs in PSII has been suggested to be part of the mechanism of sustained stress-induced down-regulation of PSII (Ottander et al., 1995; Ensminger et al., 2004; Verhoeven, 2014). Winter down-regulation of the photosystem is known to be accompanied by up-regulation in SpS proteins and xanthophyll cycle pigments (Ensminger et al., 2004; Zarter et al., 2006). These sustained quenching phenomena enhance NPQ and decrease the quantum yield of fluorescence and photochemistry, but also induce changes in fluorescence spectra. Gilmore and Ball (2000) identified what they called the cold-hard band (CHB) in overwintering snow gum leaves, where they showed that the leaf fluorescence spectrum was blue-shifted (i.e. moved towards shorter wavelengths) in response to cold acclimation. Interestingly, the CHB was shown to be fully reversible under constant chlorophyll content during recovery, suggesting that NPQ and perhaps also fluorescence spectra of the photosystems, F₄₄₁(λ_em) and F₄₄₃(λ_em), modulate the fluorescence spectral properties during down-regulation episodes. Measuring the fluorescence spectral properties of leaves and isolated PSI units under different levels and forms of NPQ (i.e. reversible and sustained) could serve to study how fluorescence spectra change in response to down-regulation of photosynthesis and its impact on both PAM and SIF fluorescence.

**Effect of temperature**

Long-term field observations (such as those in remote sensing) are often conducted under a wide range of temperatures that can easily exceed 60 °C. The yield and shape of the fluorescence spectrum are known to change in a thermodynamic process that takes place independently of plant physiological status (Croce et al., 1996; Gobets and van Grondelle, 2001). When temperature is decreased from ambient to liquid nitrogen temperature (77 °K) the fluorescence yield of PSI Φ₂ decreases because excitations are trapped in the so-called red chlorophylls (Croce et al., 1996; Gobets and van Grondelle, 2001; Jennings et al., 2003).

Red chlorophylls absorb at longer wavelengths than the rest of the antenna, but, at physiological temperatures, uphill energy transfer from the red chlorophylls to the bulk antenna is possible through thermal activation using vibrational energy from the phonon bath (i.e. energy contained in the vibrations of the pigment–protein matrix) (Jennings et al., 2003; van Grondelle and Novoderezhkin, 2006). At low temperature (e.g. 77 °K), the phonon bath does not provide sufficient energy for the uphill energy transfer (Jennings et al., 2003), and excitations get trapped in these chlorophylls, giving rise to an increase in fluorescence yield as well as a slight red shift in peak position (Croce et al., 1996). Low temperature also limits energy transfer between PSI proteins, and two spectral peaks or shoulders (685 nm and 695 nm) appear at 77 °K instead of one single peak observed at room temperature (Govindjee, 1995; Keränen et al., 1999; Maxwell and Johnson, 2000).
Because these thermodynamic effects take place gradually between ambient and liquid nitrogen temperature (Brody and Brody, 1962; Gylle et al., 2012), they may need consideration when analysing ChlF data sets collected under a wide range of temperatures. For example, the ChlF of a *Chlorella* sp. suspension increased by 30% when lowering temperature from 25 °C to −30 °C at 720 nm (Brody and Brody, 1962). In addition, the differential dependence of both $F_0$ and $F_M$ on temperature at a physiologically relevant range has also been observed in leaves (Weis and Berry, 1988; Kuropawat et al., 1992; Pospíšil et al., 1998). Similar increases in ChlF in both the red and near infrared with decreasing temperature have been observed in intact leaves (e.g. Agati, 1998; Agati et al., 2000). Physiological and thermodynamic effects cannot always be decoupled empirically, although physiological factors determine the differential effect on $F_0$ and $F_M$ (Pospíšil et al., 1998) or the dependence of the temperature effect on the needle age found in spruce (Ilík et al., 1998). The impact and seasonal dynamics of the thermodynamic phenomena deserve to be quantified and characterized with dedicated experiments using various species and leaves presenting different physiological states, so that the effect can be modelled and corrected if needed.

Seasonal changes in connectivity between PSII units

Connectivity is the phenomenon by which excitation energy moves between neighbouring photosystems. This affects the relationship between fluorescence and the fraction of open/functional reaction centres ($q$) (Fig. 5) (Joliot and Joliot, 1964; Havaux et al., 1991; Lavergne and Trissl, 1995; Kramer et al., 2004b). In the absence of connectivity (separate units model), fluorescence yield is linearly related to $q$ (Fig. 5A). In contrast, a system with connected PSII units will result in lower fluorescence yield for the same levels of reaction centre closure ($q$), because excitation energy in closed reaction centres can be quenched by neighbouring open reaction centres. Therefore, changes in connectivity per se can generate a change in ΦF at constant $q$ and NPQ, complicating the interpretation of ChlF data. Connectivity between PSII units has been shown to change in response to protein phosphorylation (Kyle et al., 1982), salt stress (Mehta et al., 2010), and thylakoid grana stacking (Chow et al., 2005). Because the impact of connectivity on ChlF decreases with NPQ (Fig. 5A–D), and because SIF is by default measured under illumination in the presence of NPQ, it is likely that connectivity and its potential dynamics do not exert a significant control on SIF. However, because seasonal changes in connectivity are likely to occur, the lake model assumption requires validation at the seasonal scale. The potential impact of seasonal changes in connectivity needs to be considered when attributing changes in photochemical quenching parameters such as $q_P$ or $q_L$ to processes such as photoinhibition of reaction centres, or when a connectivity assumption is used to derive fluorescence parameters as in the LIFT approach (Kolber et al., 2005).

The contribution of PSI fluorescence to total fluorescence

The dynamics of the ChlF signal are most often associated with PSII and used to derive information on the acclimation of PSII. PSI has been typically ignored or corrected for using a constant offset value. Using different techniques and species, the contribution of PSI fluorescence has been found to range from close to zero in the red portion of the fluorescence spectra and up to 50% (Genty et al., 1990b; Dau, 1994; Pfündel, 1998; Agati et al., 2000; Peterson et al., 2001; Franck et al., 2002; Palombi et al., 2011; Pfündel et al., 2013). Franck et al. (2002) presented a method to resolve the spectral fluorescence contribution at the $F_0$ and $F_M$ states (Fig. 6) that illustrates the wavelength dependency of the PSI fluorescence contribution. The contribution of PSI to total fluorescence decreases with decreasing photochemical quenching in PSI, becoming relatively insignificant at the $F_M$ level ($q=0$). Under natural illumination $F_0$ and SIF levels are typically much closer to $F_0$ than to $F_M$, and the contribution that should be taken as reference of PSI fluorescence ‘offset’ is that obtained at the $F_0$ level and not the $F_M$. Clearly, PSI contribution to total fluorescence is important and cannot be ignored. However, can we expect this ChlF ‘offset’ to remain constant over time?

There are three different mechanisms by which PSI fluorescence contribution can vary. (i) Changes in chlorophyll content affect reabsorption of $F_R$ (Lichtenthaler and Rinderle, 1988; Gitelson et al., 1998). Consequently, because the red region is enriched in PSI fluorescence (Fig. 6), increasing chlorophyll contents will tend to increase the overall PSI fluorescence contribution. (ii) PSI contribution might dramatically increase in response to sustained NPQ and down-regulation of PSI. Although $F_0$ represents a theoretical minimal fluorescence, fluorescence is known to decrease below summer/non-stressed $F_0$ levels when the photosystems are deeply down-regulated (Porcar-Castell et al., 2008a; Soukupová et al., 2008; Porcar-Castell, 2011). Under these conditions, and unless sustained forms of NPQ operate in a similar fashion in both photosystems, the contribution of PSI fluorescence to the total signal could dramatically increase relative to the contribution estimated at the summer $F_0$. Finally, (iii) seasonal changes in the relative absorption cross-sections of PSI and PSII would equally affect PSI contribution. The seasonal dynamics in the PSI contribution to the total ChlF signal await further study so that we can relate the seasonal changes in the ChlF signal to processes taking place only in PSI, or in both photosystems.

In particular, the significance of sustained forms of NPQ in PSI and its putative impact on $\Phi F_1$ remain controversial. On one hand, there is no evidence of a significant effect of NPQ on PSI fluorescence over the short term (Genty et al., 1990b; Krause and Jahns, 2004; Pfündel et al., 2013), although it is known that 30–50% of the xanthophyll cycle pigments are associated with PSI, and de-epoxidation reactions also take place in PSI (Thayer and Björkman, 1992; Lee and Thornber, 1995; Färber, 1997). In contrast, NPQ has been found to have no effect on the redox state of the electron transport chain (Tikkanen et al., 2011), which suggests that NPQ might operate in an equal fashion in both photosystems, unless CET is highly dynamic. The hypothesis of a constant $\Phi F_1$ awaits validation at the seasonal scale.
5.2 Spatial up-scaling

With the development of ChlF measurement techniques that can monitor targets of different size (leaf, canopy, and landscape), the question of scaling of process knowledge becomes increasingly important (Fig. 4). One of the main challenges in up-scaling is how to represent the spatial variability in physical and physiological factors within a leaf or a plant canopy (Malenovský et al., 2009). When measuring SIF emitted by a leaf, the resulting signal can be expected to emanate predominantly from the top layers which are exposed to higher irradiance levels, as discussed in Section 3.3. Similarly, when measuring SIF of a plant canopy, the SIF signal will mainly consist of fluorescence originating from the top of the canopy, which is exposed to higher irradiance. For canopy-level observations, an additional problem is that the signal depends on canopy structure, sun elevation, and view angle, with different fractions of sunlit and shaded leaves visible from the perspective of the observer when looking at the same canopy from different angles. SIF thus depends on the position of the observer, whereas the emission at the photosystem level does not (van der Tol et al., 2009).

Similarly, as discussed in Section 3.3, the fact that the resulting fluorescence signal needs to travel through a variable depth of leaf or canopy before it reaches the sensor will change the spectral properties of the signal due to variable and selective reabsorption of red fluorescence (Gitelson et al., 1998; Franck et al., 2002). The signal will be enriched in top canopy leaves for SIF (red), for example SIF660, with a larger contribution from leaves deeper in the canopy for SIF (far-red), for example SIF690. Daumard et al. (2012) analysed measurements of top-of-canopy SIF in a crop in the red and far-red during an entire season, and concluded that the far-red contains clear information about the development and photosynthetic capacity of the crop, whereas the F660/F690 ratio was sensitive to chlorophyll concentrations and the structure of the vegetation.

Radiative transfer models have been developed to simulate the effects of leaf and canopy structure and geometry on the wavelength-dependent radiative fluxes, ranging from simple turbid medium approaches (Allen, 1964) to ray tracing in realistic vegetation models (Gastellu-Etchegorry et al., 2004). Of intermediate complexity (and realism) are models that treat the canopy as a stochastically distributed arrangement of leaves (Verhoef, 1984). Some of these models also include the radiative transfer of ChlF emission in leaves (Pedrós et al., 2010) and canopies (Rosema et al., 1998; Miller et al., 2005; van der Tol et al., 2009; Zarco-Tejada et al., 2013). These models are designed to reproduce the fate of the emitted ChlF spectra through the leaf and canopy. It is not yet known how accurately these models represent the structural effects of vegetation on observations of SIF, or how sensitive they are to the underlying assumptions of leaf and canopy structure.

In addition to understanding radiative transfer as a physical process (physical modelling), we also need to solve the question of how physiological traits and photosynthesis dynamics (Equation 12) control the resulting fluorescence signal (physiological modelling). Understanding this aspect requires coupling radiative transfer with physiology. Models that couple radiative transfer and photosynthesis have been used for decades (Sellers, 1985; Goudriaan, 1988), but SIF has been only recently included (Miller et al., 2005; van der Tol et al., 2009). Even though these models have already been implemented for certain real-world scenarios, it remains unknown how well they represent the interactions between illumination and physiology (Malenovský et al., 2009), and whether they are meaningful to spatiotemporal scales of remote sensing (i.e. seasonal scale, and canopy/landscape level with multiple species). In particular, the temporal variation of leaf-level traits discussed above (Section 5.1), the vertical variation in leaf-level traits such as leaf absorbance (Ellsworth and Reich, 1993), PSI:PSI stoichiometry, and relative absorption cross-sections (Chow et al., 1999; Rivodossi et al., 1999) observed within a plant canopy, and the variation of leaf-level traits across species, need to be characterized and implemented within these models.

5.3 Mechanistic up-scaling: from SIF to GPP

Despite the complexity of the relationship between SIF and GPP, and despite the limited spatial and temporal resolution of the currently available remotely sensed SIF data (10 km, monthly) the resemblance of global maps of satellite-retrieved SIF and modelled GPP (Frankenberg et al., 2011; Joiner et al., 2011; Guanter et al., 2012) shows that the relationship between satellite-derived SIF and GPP is significant, albeit probably biome dependent. The question remains of whether we have enough process understanding to explain the correlation between SIF and GPP mechanistically. How can we assimilate SIF into models to improve/benchmark spatially distributed GPP estimates? The current practice of estimating GPP from remote sensing is to parameterize LUE (Equation 1) by means of either statistical (Xiao et al., 2004; Jung et al., 2011) or physically based models (e.g. Ryu et al., 2011). In both approaches, a combination of flux tower data and remote sensing products are used.

An important question when considering Equation 1 is whether SIF can be used to infer LUE, APAR (where PAR=PAR×APAR), or both. Remote sensing of green fAPAR based on reflectance measurements remains a challenge due to signal saturation, bidirectional reflectance distribution function (BRDF) effects (Morton et al., 2014), and because materials such as soil, wood, and dead biomass also absorb PAR but do not contribute to photosynthesis (Huete, 1988; Qi et al., 1994; Daugtry et al., 2000; Haboudane et al., 2002). In contrast, because ChlF originates only from chlorophyll, SIF could improve the estimation of fAPAR in comparison with current reflectance-based methods (Frankenberg et al., 2011).

The question of whether SIF is a measure for LUE is difficult to answer. In contrast to PAM measurements, it is not obvious how SIF (Equation 20) can be related to photochemistry. The information in a measurement of SIF is simply insufficient to calculate the quantum yield of photochemistry as derived from Equation 9 (unknown $F_{m}/F_{o}$ when measuring SIF). The accumulated knowledge provided by PAM fluorescence measurements may provide a path to understanding and modelling the relationship between SIF, electron transport, and GPP.
Fluorescence ($\Phi_F$) and photochemical ($\Phi_P$) yields (Equations 7 and 9, respectively) are affected by photochemical (PQ) and non-photochemical quenching (NPQ) (see Equation 10 and Fig. 9). In the field, the quantum yield of fluorescence is highly dynamic, both during the course of a day (Porcar-Castell et al., 2008b) and throughout seasons (Soukupová et al., 2008; Porcar-Castell, 2011), where $\Phi_F$ decreases during stress episodes and increases upon recovery in a process that appears to be largely controlled by the presence of sustained NPQ forms (Ensminger et al., 2004; Porcar-Castell et al., 2008a; Porcar-Castell, 2011). Therefore, $\Phi_F$ is related to $\Phi_P$ and hence to LUE. In conclusion, SIF contains information relating to LUE as well as APAR. Yet, disentangling these two contributions remains a challenge from a remotely sensed large-scale observation platform.

Over the course of a day, the relationship between $\Phi_F$ and $\Phi_P$ falls apart into two distinct phases: under low light (first morning hours and towards sunset) the changes in the quantum yield of photochemistry are controlled by PQ, with NPQ remaining approximately constant and low (Fig. 9). In contrast, under high light (noon hours) the changes in $\Phi_P$ are dominated by NPQ, with PQ remaining rather constant. Since decreasing PQ and increasing NPQ have opposite effects on $\Phi_F$ (see Equation 7), this non-complementary behaviour generates a two-phased inverted ‘V’ relationship between $\Phi_P$ and $\Phi_F$ (Fig. 9), where $\Phi_P$ and $\Phi_F$ are inversely proportional under low light ‘PQ-Phase’, and proportional under high light ‘NPQ-Phase’. The inverted ‘V’ relationship can be reproduced using current process-based understanding of the relationship between ChlF and photosynthesis (see Fig. 2 in van der Tol et al., 2009).

Remotely sensed SIF will always be obtained under high-light conditions (‘NPQ-Phase’); therefore, it could be expected that SIF and $\Phi_P$ vary concomitantly in response to stress, with SIF~$\Phi_F$ APAR. Indeed, a good correlation between $\Phi_F$ and photosynthesis has been observed in response to water stress episodes (Flexas et al., 2000) when measured under high light in the ‘NPQ-Phase’. Similarly, both $\Phi_F$ and $\Phi_P$ tend to decrease simultaneously in response...
to low temperatures due to the action of sustained NPQ (Soukupová et al., 2008; Porcar-Castell, 2011). As a caveat, it is likely that the sensitivity of the relationship changes during the season in response to sustained NPQ or photoinhibition of reaction centres, something that remains to be clarified and awaits validation. For example, the hysteresis observed in Fig. 9 between morning and afternoon observations may indicate that these processes already play a role at the diurnal scale. Overall, the general relationship between \( \Phi P \) and \( \Phi F \) presented in Fig. 9 demonstrates the strong control of the fluorescence signal by the dynamics of PQ and NPQ. This link has been described with empirical relationships that have been parameterized in radiative transfer models (Lee et al., 2013).

To understand further how \( \Phi F \) and \( \Phi P \), as well as \( \Phi F \) and SIF, are related at the seasonal scale, the following points need consideration. (i) Over the short term, NPQ is known to decrease both SIF and \( F' \), although for SIF the effect is known to be stronger when estimated in the red region (e.g. SIF\(_{690}\)) compared with the far-red (e.g. SIF\(_{760}\)) (Lichtenthaler and Rinderle, 1988; Agati et al., 1995), most probably due to the NPQ-insensitive contribution of PSI far-red fluorescence. At the seasonal scale, the PAM term \( F' \) may decrease in response to stress due to accumulation of sustained forms of NPQ, yet, because stress is often accompanied by a decrease in chlorophyll content (i.e. decreased fluorescence reabsorption), SIF\(_{690}\) (retrieved in the red region) may actually increase instead of decreasing like \( F' \). (ii) Similarly, seasonal changes in the contribution of PSI may equally interact with the expected relationship between \( \Phi F \) and \( \Phi P \) when using SIF\(_{760}\) (retrieved in the far-red) instead. Further long-term experimental work is needed to characterize the processes that control the relationship between SIF\(_{\text{retrieval}}\), \( F' \), \( \Phi F \), and \( \Phi P \) at the seasonal scale. Last, but not least, the seasonal dynamics of alternative electron sinks, alternative metabolic pathways, and photorespiration (Fig. 1) will need characterization before we can mechanistically link SIF and GPP.

### 6. Concluding remarks

The availability, quality, and spatiotemporal coverage of SIF data are expected to increase drastically over the next few years. At the canopy scale, field spectroradiometer prototype systems are being rapidly developed and deployed in increasing numbers (Rossini et al., 2010; Balzarolo et al., 2011; Drolet et al., 2014). These systems will promote the expansion of SIF measurements at the ecosystem scale. The increased knowledge will hopefully lead to a better understanding of the SIF signal and its relationship to GPP. In addition, airborne instruments specifically designed to map vegetation fluorescence have recently been developed (Rascher et al., 2009; Zarco-Tejada et al., 2009, 2012). This opens the way for extensive airborne campaigns to better understand the significance of spatial variability in the SIF signal and facilitate the up-scaling from the canopy to the landscape levels. Finally, and most significantly, imminent space missions will herald a new range of possibilities for retrieving SIF at the global scale. This could result in improved estimates of the global carbon budget and our capacity to track the health of terrestrial ecosystems. In particular, the ESA’s Earth Explorer Fluorescence Explorer (FLEX) (Moreno et al., 2006; Kraft et al., 2013) is expected to provide an unprecedented source of spatially continuous SIF data as well as other critical variables to facilitate the signal interpretation (e.g. photochemical reflectance index, canopy temperature, fAPAR). Likewise, satellite missions such as the NASA OCO-2 (Frankenberg et al., 2014) or the ESA Sentinels 4–5, sensors conceived to measure atmospheric properties, will also offer exciting possibilities to retrieve SIF. Despite this promising scenario and despite the empirical evidence that confirms that SIF carries novel information on the dynamics of photosynthesis compared with previous remote sensing data (Frankenberg et al., 2011; Joiner et al., 2011; Guanter et al., 2012), the mechanistic link between SIF and GPP still remains unclear.

In this review, we identified a number of areas where further research is deemed necessary to understand fully the mechanisms that control the seasonality of the SIF signal at the leaf level. Three prospective research areas for future work can be summarized.

(i) Characterization of processes that uncouple ChlF and GPP at the seasonal scale. The seasonal variation of processes such as photosynthetic light absorption, CET, adjustments in relative absorption cross-sections of PSII and PSI, alternative sinks, PSI fluorescence contribution, photorespiration, photosystem connectivity, and the thermodynamic effect of temperature need to be characterized in order to understand and mechanistically model the seasonal link between ChlF and photosynthesis.

(ii) Linking PAM and SIF fluorescence at the seasonal scale. Combined measurements of PAM and spectrally resolved ChlF are needed at the seasonal and leaf scales to elucidate how the methodology affects the information content of the signal.

(iii) Characterizing the vertical and genetic variation in physiological traits. The variation in factors listed in (i) and (ii) within a plant canopy and across species still needs to be characterized fully in order to identify possible generic functions that could be implemented when upsampling from the leaf to the canopy and landscape levels.

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Appendix 1. Interaction between PSII connectivity, fraction of open reaction centres $q$, and parameters $qL$ and $qP$ in the presence of NPQ. An analysis based on equations derived by Lavergne and Trissl, (1995)

Adaptation of the fluorescence yield equation as a function of $q$ for a connected units model

We define a system with two populations of PSII units, open (PSII$_{open}$) and closed (PSII$_{close}$). We assume rapid energy equilibration between peripheral antenna, core, and reaction centre (single pool model). We define ‘$q$’ as the fraction of PSII units with an open reaction centre. $k_{LII}$ is the sum of rate constants of all non-photochemical quenching processes operating in the photosynthetic antenna, namely constitutive thermal dissipation, fluorescence, and regulated thermal energy dissipation (NPQ), $k_{LII}=k_{rad}+k_{P}+k_{NPQ}$. We assume that thermal dissipation by open reaction centres, intersystem crossing, photobleaching, and spillover are insignificant and are embedded in $k_{D}$. Finally, $k_{PSII}$ is the intrinsic rate constant of photochemistry in open reaction centres, and $k_{CII}$ is the rate constant of energy transfer between photosynthetic units (Fig. A1).

According to the above system, three different connectivity models can be defined (Lavergne and Trissl, 1995): (i) a lake model, when $k_{CII}$ tends to infinity; (ii) a separate units model, when $k_{CII}$ tends to zero; and (iii) a connected units model when $0<k_{CII}<\infty$. We then redefine the parameters $J$, $B$, and $C$ derived for a connected units model by Lavergne and Trissl (1995) using our nomenclature, where $k_{PSII}\equiv \alpha_{P}$ (therein), $k_{LII}\equiv k_{l}$ (therein), $k_{CII}\equiv k_{l}$ (therein), and $k_{rad}\equiv k_{rad}$ (therein). Similarly, the rate constants $k_{20}$ and $k_{30}$ in Lavergne and Trissl (1995) are reformulated as (note that $\alpha_{t}$, the rate constant of thermal dissipation by open centres, is assumed to be zero):

$$k_{20} = k_{PSII} + k_{L}$$  \hspace{1cm} (A1)

$$k_{30} = k_{CII} + k_{L}$$  \hspace{1cm} (A2)

and the factors $J$, $B$, and $C$ in Lavergne and Trissl (1995) become:

$$J = \frac{k_{CII}(k_{20}-k_{30})}{k_{30}(k_{CII} + k_{L})} = \frac{k_{CII}(k_{PSII} - k_{CII})}{(k_{CII} + k_{L})(k_{PSII} + k_{L})}$$  \hspace{1cm} (A3)

Equation A8 was used to estimate $F$ and $F_{M}$ in Fig. 5, using parameter values reported in Lavergne and Trissl (1995) (Table 1A), and adjusting $k_{CII}$ and $k_{LII}$ to represent different levels of connectivity and NPQ, respectively. For example, $k_{LII}=0.3 \text{ ns}^{-1}$ (NPQ=0), but becomes 0.6 \text{ ns}^{-1} or 0.9 \text{ ns}^{-1} if NPQ=1 or 2, respectively, as NPQ=$k_{NPQ}/(k_{f}+k_{D})$.

Table 1A. Parameter values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correspondence in Lavergne and Trissl</th>
<th>Value (ns$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{PSII}$</td>
<td>$\alpha_{P}$</td>
<td>2.664</td>
</tr>
<tr>
<td>$k_{rad}$</td>
<td>$k_{rad}$</td>
<td>0.066</td>
</tr>
<tr>
<td>$k_{LII}$</td>
<td>$k_{l}$</td>
<td>0.300</td>
</tr>
</tbody>
</table>
Demonstration of qL and qP

When $k_{CII}$ approaches infinity (i.e. a lake model), Equation A8 becomes:

$$\Phi F (q)_{Lake} = \frac{k_F}{k_{LII} + qk_{PSII}} = \frac{k_F}{k_{LII} + k_P + k_{NPQ} + qk_{PSII}} \quad (A9)$$

which is equivalent to Equation 7, demonstrating that qL is equal to q when connectivity approaches infinity. Conversely, if $k_{CII}$ approaches zero (separate units model), and given that the sum of all quantum yields is by definition equal to unity, then

$$1 - \frac{k_{PSII}}{k_{PSII} + k_{LII}} = \frac{k_{LII}}{k_{PSII} + k_{LII}}, \quad (A10)$$

and we can express Equation A8 as:

$$\Phi F (q)_{SeparateUnits} = \frac{k_F}{k_{LII}} \left(1 - \frac{k_{PSII}}{k_{PSII} + k_{LII}} q \right) = \frac{k_F}{k_{LII}} \left(1 - \frac{k_{PSII}}{k_{PSII} + k_{LII}} \right)$$

$$= (1 - q) \frac{k_F}{k_{LII}} + q \left(\frac{k_F - k_{PSII}}{k_{LII}} \frac{k_{LII}(k_{PSII} + k_{LII})}{k_{LII}} \right)$$

$$= (1 - q) \frac{k_F}{k_{LII}} + q \left(\frac{k_F - k_{PSII}}{k_{LII}} \frac{k_{LII}(k_{PSII} + k_{LII})}{k_{LII}} \right)$$

$$= (1 - q) \frac{k_F}{k_{LII}} + q \left(\frac{k_F - k_{PSII}}{k_{LII}} \frac{k_{LII}(k_{PSII} + k_{LII})}{k_{LII}} \right)$$

$$= (1 - q) \frac{k_F}{k_{LII}} + q \left(\frac{k_F - k_{PSII}}{k_{LII}} \frac{k_{LII}(k_{PSII} + k_{LII})}{k_{LII}} \right)$$

$$= (1 - q) \frac{k_F}{k_{LII}} + q \left(\frac{k_F - k_{PSII}}{k_{LII}} \frac{k_{LII}(k_{PSII} + k_{LII})}{k_{LII}} \right)$$

which is equivalent to the separate units formulation of q, that corresponds to the parameter qP (Genty et al. 1989). The performance of qL and qP under intermediate connectivity scenarios and in the presence of NPQ is assessed in Fig. 5.

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