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Bidirectional sun-induced chlorophyll fluorescence emission is influenced by leaf structure and light scattering properties — A bottom-up approach

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ABSTRACT

Sun-induced chlorophyll fluorescence (SIF) at leaf level is emitted in both upward and downward directions in the red and far-red part of the spectrum (650–850 nm) when a leaf is illuminated from the upper leaf surface. Hence, total SIF is represented by the sum of the upward and downward emission components. Nevertheless, the downward component of leaf SIF is often not considered despite that downward fluorescence yield (↓FY) can amount up to 40% of the total fluorescence yield (FY_{tot}). Downward SIF is mainly emitted in the far-red, since this part of fluoresced light is highly scattered within leaves, unlike red Chl fluorescence, which is mostly reabsorbed. While total FY can be quite different among distinct species, the relative partitioning between upward and downward fluorescence shows more similarities among different leaf types, especially in the far-red. It is shown that bidirectional SIF emission properties in the far-red can be attributed to the scattering properties of the leaf, whereby an equifacial leaf follows a different trend compared to bifacial leaves. This was done by comparing SIF data with simultaneously measured reflectance, transmittance and fluorescence data by means of the FluoWat leaf clip coupled with an ASD hyperspectral spectroradiometer. These results could further improve Chl fluorescence modeling at leaf level, and help to advance the interpretation of SIF at the canopy level.

1. Introduction

Sun-induced fluorescence (SIF) emitted by chlorophyll (Chl) pigments is one of the de-excitation mechanisms of plants to cope with an excess in light energy. It competes with photochemical processes, energy transfer and radiationless decay for light excited singlet Chl molecules (Krause & Weis, 1991). Although the direct link with photosynthesis may not be trivial (Meroni et al., 2009), mainly due to the interplay with the other dissipation mechanisms, SIF emitted by vegetation is seen as a meaningful signature of photosynthesis (Porcar-Castell et al., 2014). A major advantage of the SIF signal is that it is a more physiological related signal compared to the reflectance signal, and moreover uniquely originates from vegetation. Whereas the reflectance signal registered by remote sensors reveals rather definitive structural changes in leaves or vegetation canopy covers such as Chl content and leaf area index (Rautiainen et al., 2010; Verrelst, Alonso, Camps-Valls, Delegido, & Moreno, 2012), SIF represents a more fine-tuned physiological signal with a diurnal dynamic (Amoros-Lopez et al., 2008; Flexas et al., 2002).

Although the emitted SIF flux is relatively small compared to the reflected sun radiation (about 2–5% in the near infrared), SIF is a broadband spectrum ranging from 650 nm to 850 nm with two emission peaks in the red around 690 nm and in the far-red around 740 nm (Papageorgiou & Govindjee, 2004). Yet, the red and far-red fluorescence flux that is propagated throughout the leaves is also subjected to internal absorbance and scattering effects, with scattering being divided in reflectance and transmittance. From a bottom-up perspective, scattering and re-absorbance effects continue further at the canopy level, therefore they may play an increasing role when observing vegetation SIF from a remote scale. With the development of hyperspectral sensors with high spectral resolution, the retrieval of SIF has become a novel area of research (Alonso et al., 2007; Guanter et al., 2010; Meroni et al., 2009, 2010), aiming at mapping SIF from a site-specific (Damm et al., 2014; Daumard et al., 2012; Moya, Daumard, Moise, Ounis, & Goulas, 2006; Perez-Priego, Zarco-Tejada, Miller, Sepulcre-Canto, & Fereres, 2005; Zarco-Tejada, Gonzalez-Dugo, & Berni, 2012) towards a global scale (Joiner et al., 2013). Thus far these studies rather emphasized on the development of SIF retrieval methods, thereby taking atmospheric absorption and scattering effects into account (Guanter et al., 2010). A top-down approach is currently pursued by the global SIF mapping community, whereby a meaningful interpretation of the

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derived SIF retrievals is searched for, e.g., through seeking for empirical relationships with gross primary production (GPP) (Frankenberg et al., 2011; Guanter et al., 2012, 2014). However, the source of the SIF flux is typically taken for granted and the within-leaf and canopy radiative transfer fluxes have been largely left unstudied. Therefore, in order to close the scaling gap, a bottom-up approach that aims to elucidate the propagation of the SIF flux through a leaf, canopy (re-)absorption and scattering effects and eventually leaving the canopy, is urgently demanded. It is primarily through a better description of (fluoresced) light interaction at the leaf level that will lead to a better interpretation of the remotely retrieved Chl fluorescence signal (Pedrós, Goulas, Jacquemoud, Louis, & Moya, 2010).

SIF is emitted by Chl *a* throughout the different leaf layers, with emission intensity depending on incoming radiation and Chl concentration. Hence, leaves with a higher Chl concentration in the upper leaf layers (typically for bifacial leaves) will produce a higher Chl fluorescence intensity in those layers (Vogelmann & Han, 2000). Internal (re-)absorption of fluoresced light occurs intensively in the red, due to the Chl absorption peak at 681 nm, which has been often demonstrated (Buschmann, 2007; D'Ambrosio, Szabo, & Lichtenthaler, 1992). Far-red SIF (700–750 nm) on the other hand, generally emitted with the highest intensity, is located in the transition zone between highly absorbed red light and highly scattered near infra-red (NIR) light (750–1400 nm). Since within-leaf light scattering will take place in all directions, SIF will be emitted from both leaf sides (Louis, Cerovic, & Moya, 2006). Upward and downward leaf Chl fluorescence spectra have been measured (Louis et al., 2006) and modeled (with FluorMODleaf) based on excitation with laser beams of different wavelengths (Miller et al., 2005). Considering that the shape of the leaf Chl fluorescence spectrum, hence also the red/far-red ratio, depends on the excitation beam's wavelength (Agati, 1998; Louis et al., 2006), laser excited Chl fluorescence probably does not provide the exact fluorescence shape emitted under natural conditions. Sun-induced hyperspectral upward and downward Chl fluorescence, on the other hand, is able to give a more realistic Chl fluorescence shape and better reference for remote sensing purposes (Van Wittenberghe, Alonso, et al., 2014; Van Wittenberghe et al., 2013).

The remote retrieval of Chl fluorescence exploits the Fraunhofer lines, i.e. narrow dark lines of the Sun and Earth's atmospheric transmittance in which irradiance is strongly reduced (Meroni et al., 2009). The most commonly used Fraunhofer features are the O_2–A absorption region at 760.4 nm (Alonso et al., 2007; Guanter et al., 2010; Meroni et al., 2010) or the NIR solar Fraunhofer lines in the range between 740 and 755 nm (Frankenberg et al., 2011; Guanter et al., 2012). Regardless of the pursued approach, currently most of them rely on one retrieval band interval in the second emission peak. Since SIF emission is highly scattered by vegetation in the NIR, description of the signal in both upward and downward directions at leaf level is of relevant importance for the interpretation of the remotely detectable signal. Therefore, the main objective of this study is to investigate the effect of leaf structure, and hence, within-leaf scattering properties onto the bidirectional Chl fluorescence emission, meaning the upward and downward emission components. For this purpose, several species with different leaf structure were examined. Further, the relationship between upward and downward Chl fluorescence is investigated, as well as their contributions to the total leaf Chl fluorescence emission. The paper closes with a discussion on interpreting the upward canopy SIF signal as detected by airborne or spaceborne spectrometers.

2. Materials and methods

2.1. Sun-induced reflectance, transmittance and Chl fluorescence

Sun-induced reflectance (R), transmittance (T) and Chl fluorescence (F) were all measured in situ on leaves attached to their branch by means of the FluoWat leaf clip (Alonso et al., 2007; Van Wittenberghe et al., 2013) connected to a hyperspectral spectroradiometer (FieldSpec, analytical spectral devices (ASDs) Inc., Boulder, USA) on leaf samples from four tree species. The design of the FluoWat leaf clip permits the insertion of the fiber optic of the spectroradiometer in two positions, one upward and one downward with respect to the leaf position (Fig. 1A). In this way, the fiber optic perpendicularly points to the leaf surface from either one of both positions. The leaf with the attached FluoWat leaf clip is then manually positioned so that the incoming solar beam enters the open aperture which is located half-way between the plane of the leaf and the perpendicular positions of the fiber optic probe, at a relative 45° position. Reflectance and transmittance measurements are then obtained using both upward and downward fiber optic insertions of the leaf clip (Fig. 1B). For more details, see Van Wittenberghe et al. (2013). After restricting light entering the aperture with a short-pass filter that cuts off light above 650 nm, upward and downward emitted Chl fluorescence are measured at the upward and downward probe positions, respectively (Fig. 1B). Following this protocol, upward and downward Chl fluorescence are measured with the fiber optic pointing at the same leaf spot were respectively reflectance and transmittance are measured, delivering a combined R–T–F dataset.

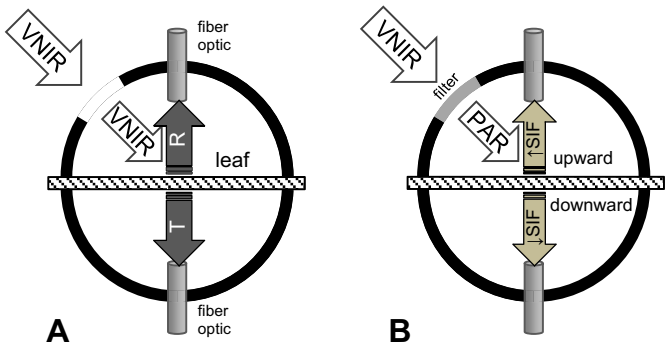

Fig. 1. Scheme of FluoWat leaf clip. Reflectance (R), transmittance (T) and sun-induced fluorescence (SIF) are measured in the visible and near-infrared (VNIR) wavelength range (400–1400 nm) by placing a fiber optic either in upward (↑) or downward (↓) position (A). After placing the short-pass to restrict incoming PAR to visible wavelengths up to 650 nm (B), upward and downward sun-induced fluorescence (↑SIF, ↓SIF) are measured (after Van Wittenberghe et al., 2013).

Leaves from four tree species, which substantially differ in leaf structure and pigment content, were collected and measured during two field campaigns in the period August 10–31 in 2011 in the city of Valencia in the framework of the BIOHYPE project (Van Wittenberghe, Alonso, et al., 2014; Van Wittenberghe et al., 2013). All sampled trees were sun-exposed and grown at different locations within the urban environment (e.g., street side, park). A first part of the dataset originated from a campaign focusing on trees across the city exposed to either a low or high traffic intensity whereby trees were sampled from the bottom canopy layer (Van Wittenberghe et al., 2013). A second part of the dataset contained leaves taken from trees, whereby three different canopy heights were sampled from three trees per species (Van Wittenberghe, Alonso, et al., 2014). Total sample size of the combined R–T–F spectral dataset of the species European nettle tree (*Celtis australis* L.) (n = 78), White mulberry (*Morus alba* L.) (n = 59), Canary Island date palm (*Phoenix canariensis* Chabaud) (n = 66) and London plane (*Platanus* × *acerifolia* (Aiton) Willd.) (n = 59) was 262 leaves. During both field campaigns, branches from each sampling site were transported to the laboratory with their stems submerged in water prior to spectral analysis. Five healthy leaves of each branch were measured outside the lab under clear sky conditions. After light adaption each leaf was inserted and the leaf clip opening was positioned towards the sun in order to receive direct sun beams on the leaf surface. Hence, the R–T–F dataset is obtained with direct sun beams under a 45° inclination with the leaf surface.

In order to compare the different samples and the different species, Chl fluorescence yield (FY, unitless) was calculated by normalizing the