Journal of Environmental Management 227 (2018) 172–180

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Assessing hyperspectral indices for tracing chlorophyll fluorescence parameters in deciduous forests

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ARTICLE INFO

Keywords:
Chlorophyll fluorescence
Deciduous species
Spectral indices
PRI

ABSTRACT

Chlorophyll fluorescence can be used to quantify the efficiency of photochemistry and heat dissipation. While several instruments such as Pulse-Amplitude-Modulation (PAM) fluorometers are available for taking direct measurements of parameters related to chlorophyll fluorescence, large-scale instantaneous ecosystem monitoring remains difficult. Several hyperspectral indices have been claimed to be closely related to some chlorophyll fluorescence parameters (e.g. photosystem II quantum yield (Yield), qP, NPQ), which may pave a way for efficient large-scale monitoring of fluorescence parameters. In this study, we have examined 30 published hyperspectral indices for their possible use in tracing chlorophyll fluorescence parameters. The comparison is based on a series of unique datasets with synchronous measurements of reflected hyperspectra and seven fluorescence parameters (i.e., F_m, F_0, F_s, F_m', Yield, qP and NPQ) from leaves of *Fagus crenata* and other six broadleaf species sampled in Mt. Naeba, Japan. Among them, the first dataset is composed of seasonal canopy field measurements of *Fagus crenata* leaves, while the second is composed of field measurements of other deciduous species including *Lindera umbellate*, *Clethra barbinervis*, *Viburnum furcatum*, *Eleutherococcus sciadophylloides*, *Quercus crispula* and *Acer japonicum*. Furthermore, an additional dataset composed of data resulting from various controlled experiments using inhibitors has been applied for improving physiological interpretations of indices. Results revealed that PRI had higher coefficients of determination and lower root mean square errors than other indices evaluated with a set of chlorophyll fluorescence parameters. However, this pattern was seen only for beech leaves and performed poorly across other species. As a result, no specific indices that are currently available are recommended for tracing fluorescence parameters.

1. Introduction

A delicate balance between the use of absorbed light in photosynthesis and safe dissipation of potentially harmful excess light energy is important for plants (De La Barrera and Smith, 2012). Photochemical parameters related to chlorophyll fluorescence are recognized as indicators of environmental stress. Despite several well-developed approaches to obtain these parameters, photosynthesis has to be excited actively by saturating light pulses in most cases. This greatly limits the ability to instantaneously and remotely monitor fluorescence parameters of ecosystems (Rascher et al., 2007).

The relationships between biochemical properties of vegetation and reflectance have long been investigated. As a recent development, the potential use of hyperspectral data has been evaluated and several hyperspectral vegetation indices have been reported to be good predictors of ecosystem attributes. The use of these indices may offer a good alternative for monitoring ecosystems quickly and remotely. Notably, the Photochemical Reflectance Index (PRI), calculated from reflectance at 531 and 570 nm (Gamon et al., 1992), has been claimed to successfully track changes in effective nonphotochemical quenching (NPQ), and the use of PRI for retrieving NPQ has been validated in various studies (Magney et al., 2014; Nichol et al., 2006; Porcar-Castell et al., 2012). Furthermore, several previous studies have demonstrated that PRI was useful for detecting the stress conditions induced by air pollution in its early phase by monitoring excess energy dissipation pathways such as steady-state fluorescence (F_s) and NPQ related xanthophyll-cycle (Meroni et al., 2009). In addition, changes in the PRI have been used to assess radiation use efficiency or light use efficiency as well (Garbulsky et al., 2011; Grace et al., 2007; Lees et al., 2018; Sims and Gamon, 2002). However, absolute PRI values have been found to be greatly affected by seasonal variation (Filella et al., 2009). Furthermore, Stratoulias et al. (2015) evaluated 17 hyperspectral indices (see Supplementary Table 1 and associated references for details) for tracing reed F_s, F_m', Yield, PAR, electron transport rate and leaf

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https://doi.org/10.1016/j.jenvman.2018.06.085
Received 8 September 2017; Received in revised form 25 June 2018; Accepted 27 June 2018

chlorophyll content, based on 122 samples taken from four different types of habitats (23 from terrestrial habitats, 55 from shallow water, 27 from deep water and 21 from waterfront regions). Stratoulias et al. (2015) found that all of these indices correlated with some of the chlorophyll fluorescence parameters with the exception of WBI. Zhang et al. (2012) carried out another validation study on the abilities of PSSRa and PSNDa (Blackburn, 1998a; b), $(R_{780} - R_{710})/(R_{780} + R_{680})$ (Maccioni et al., 2001), SIPI and SRPI (Penuelas et al., 1995), NPCI (Penuelas et al., 1994), $(R_{850} - R_{710})/(R_{850} + R_{680})$ (Datt, 1999), NDSI and RSI (Yang et al., 2009), OCAR and YCAR (Schlemmer et al., 2005) for tracing *Suaeda salsa* F_0, F_m, F_v/F_m, qP, Yield and NPQ parameters. This work was based on 20 samples and indicated that these indices correlated well with several chlorophyll fluorescence parameters. Among these, NDSI and RSI had higher correlation coefficients (R^2) and lower root mean square errors (RMSE) with F_0, F_m, F_v/F_m, qP and Yield, while that of Maccioni et al. (2001) was useful for tracing NPQ. Both evaluation studies were only based on one specific species.

Up to current, most validations of reported indices have been done on herbaceous species and shrubs (Naumann et al., 2008; Rascher et al., 2007; Stratoulias et al., 2015) but few validations have ever been made using deciduous leaves. Deciduous forests generally have two distinctive leaf types, namely shaded and sunlit leaves. Shaded leaves are commonly larger and thinner than sunlit leaves (Terashima et al., 2001). It is well known that sunlit leaves, which develop under high irradiances, are much less susceptible to photoinhibitory damage than shaded leaves (Powles, 1984). The difference between the two types of leaves should hence be linked also to the differences in their spectral features and therefore it is critical to validate them separately. Furthermore, accumulating evaluation studies on other hyperspectral indices besides PRI for tracing chlorophyll fluorescence parameters (Stratoulias et al., 2015; Zhang et al., 2012) are also limited to one specific species and hardly provide insights for making general conclusions.

The main target of this study is to extensively evaluate the potential of hyperspectral indices for tracing chlorophyll fluorescence parameters for deciduous forests. In total, 30 currently reported hyperspectral indices were evaluated using two unique datasets, namely: 1) sunlit and shaded beech (*Fagus creanata*) leaves; and 2) across different deciduous species. The two unique datasets contain synchronous measurements of hyperspectral reflectance and fluorescence parameters at different exposure times to light stress. An additional dataset containing the results from a series of inhibitor experiments following Gamon et al. (1990) including synchronous fluorescence and spectral information under abnormal conditions, has further been applied for providing potential physiological interpretations of hyperspectral indices.

2. Materials and methods

2.1. Study area

The samples were collected from sites in Naeba Mountain, Japan. The climate of the region is cool and temperate with an average annual temperature of 5.4–6.3 °C and annual precipitation of 2321–2391 mm. A detailed description of the sample region can be found in Wang et al. (2008). This site has also been important for SpecNet (Gamon et al., 2006) with more than 15 plots set up including four towers at 550, 900 (X1 and X5), and 1500 m (m.a.s.l.), respectively. These plots cover typical stands of the lower, middle, and upper limits of beech ecosystems. The primary understory species are *Acer japonicum*, *Clethra barbinervis*, *Eleutherococcus sciadophylloides*, *Lindera umbellate*, *Quercus crispula* and *Viburnum furcatum*. In this study, sunlit and shaded beech leaves sampled at 900 m (X1, 36°53′38″N, 138°46′01″E), at 700 m (36°55′35″N, 138°46′05″E) and at 550 m (36°55′33″N, 138°45′47″E) were used.

2.2. Sampling

Beech samples were collected using the detached leaf method (Foley et al., 2006; Richardson and Berlyn, 2002) on the 28th of July and on the 27th of August of 2012 from both, the 900 m X1 and the 700 m sites, and from the 1st of August to the 6th of August of 2010 at the 550 m site. Six other broadleaf species (*Acer japonicum*, *Clethra barbinervis*, *Eleutherococcus sciadophylloides*, *Lindera umbellate*, *Quercus crispula* and *Viburnum furcatum*) were also sampled following the same method on the 27th of August of 2013 at the 900 m X1 site. Before experiments were conducted, all sampled shoots were immediately transported to the laboratory following sampling and were kept in a dark environment inside boxes surrounded by a black douser.

2.3. Measurements

All laboratory experiments were conducted within three days after sampling. Measurements were made by abruptly exposing dark acclimated shoots to strong light from a halogen lamp with the beam adjusted to a zenith angle of 45°. This caused a sudden increase in photosynthetic photon flux density (PPFD) from less than 1 to more than 700 $\mu mol\, m^{-2} s^{-1}$. This level of light saturation has been determined in previous studies in this region (Saito and Kakubari, 1999). Following light exposure for 20 through 2400 s, the spectral reflectance was taken and leaf discs were collected for later xanthophyll pigment measurements. Spectral reflectance was measured using a FieldSpec4 (Analytical Spectral Devices Inc., Boulder, CO, USA) that was positioned at nadir, 20 cm above the samples (with a 25° FOV, resulting in a circle with 4.3 cm radius). The spectral resolutions of the three detectors were 3 nm for the region 350–1000 nm and 10 nm for the region 1000–2500 nm, which were internally resampled to sampling intervals of 1 nm in the instrument using cubic spline interpolation (Hatchell, 1999).

Chlorophyll fluorescence measurements were performed using a miniaturized pulse-amplitude modulated photosynthesis yield analyzer (Mini-PAM) (H. Walz, Effeltrich, Germany) with the leaf clip holder. Measurements of light intensity at the wavelengths between 380 and 710 nm were taken by the micro-quantum sensor of the Mini-PAM. For each sample, the minimum (F_0) and the maximum (F_m) values of fluorescence in the dark-adapted state were measured, and the apparent (F_s) and maximum (F_m') values of fluorescence in the light-adapted state were measured. Using these parameters, several calculations were made, including: the effective quantum yield of photochemistry (Yield), which is directly related to the quantum yield of CO_2 fixation in the absence of photorespiration (Baker, 2008), photochemical dissipation of absorbed energy (qP), which gives an indication of the proportion of PSII reaction centers that are open (Haboudane et al., 2004), and non-photochemical dissipation of absorbed energy (NPQ), which measures a change in the efficiency of heat dissipation.

2.4. Datasets

Two datasets (Dataset I and II) were compiled based on the series of measurements. Dataset I (control, treated with deionized water only) finally contains 17 samples for F_0 and F_m and 106 leaf samples for other parameters after having eliminated mismeasurements and apparent outliers. And Dataset II contains 13 samples including 3 samples of *Acer japonicum*, one samples of *Clethra barbinervis*, one samples of *Eleutherococcus sciadophylloides*, three samples of *Lindera umbellate*, two samples of *Quercus crispula* and three samples of *Viburnum furcatum* for F_0 and F_m and 46 leaf samples including 12 samples of *Acer japonicum*, one samples of *Clethra barbinervis*, four samples of *Eleutherococcus sciadophylloides*, 12 samples of *Lindera umbellate*, five samples of *Quercus crispula* and 12 samples of *Viburnum furcatum* for other parameters.

In order to discuss the changes in performance of the published