Assessing hyperspectral indices for tracing chlorophyll fluorescence parameters in deciduous forests

Title

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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.

Keywords: chlorophyll fluorescence; deciduous species; spectral indices; PRI

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 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 μmol 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 sec, 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 to 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.

143 2.4. Datasets

144 Two datasets (Dataset I and II) were compiled based on the series of measurements. Dataset I (control,
145 treated with deionized water only) finally contains 17 samples for F_0 and F_m and 106 leaf samples for
146 other parameters after having eliminated mismeasurements and apparent outliers. And Dataset II contains
147 13 samples including 3 samples of *Acer japonicum*, one samples of *Clethra barbinervis*, one samples of
148 *Eleutherococcus sciadophylloides*, three samples of *Lindera umbellate*, two samples of *Quercus crispula*
149 and three samples of *Viburnum furcatum* for F_0 and F_m and 46 leaf samples including 12 samples of *Acer*
150 *japonicum*, one samples of *Clethra barbinervis*, four samples of *Eleutherococcus sciadophylloides*, 12
151 samples of *Lindera umbellate*, five samples of *Quercus crispula* and 12 samples of *Viburnum furcatum* for
152 other parameters.

153

154 In order to discuss the changes in performance of the published hyperspectral indices for tracing
155 chlorophyll fluorescence parameters, we also applied an additional dataset (Dataset III) to support the
156 physiological interpretation of the identified hyperspectral indices. It was composed of a series of
157 measurements under various conditions by artificially introducing inhibitors of dibromothymoquinone
158 (DBMIB), 3 - (3, 4 - dichlorophenyl) - 1, 1 - dimethylurea (DCMU), and dithiothreitol (DTT) to alter
159 chlorophyll fluorescence and other physiological processes. All sample leaves were cut under water with
160 a sharp razor blade and the petioles were placed in a solution containing ten millimolar (mM) DTT, 0.1
161 mM DCMU, 0.05 mM DBMIB or in deionized water (controls) with dim light to ensure the solution was
162 taken up by the leaf. Steps described in the method of Gamon et al (1990) were followed. Reflectance and
163 chlorophyll fluorescence measurements were performed in the aforementioned way. This additional
164 dataset finally contained 36 samples for F_0 and F_m and 232 leaf samples for the other parameters. Among
165 them, the DBMIB treatment was supposed to increase the xanthophyll cycle pigment accumulation and
166 transcription of the β-carotene hydroxylase genes without high light irradiation (Kawabata and Takeda

2014), while DCMU is known to inhibit electron transport between QA and QB in the PS II. Further, it is assumed that PS II fluorescence is at its highest level when the plastoquinone is oxidized caused by the presence of DCMU (Delphin et al. 1996). In addition, DTT is a potent inhibitor of the xanthophyll cycle and thus provokes associated absorbance changes (Bilger et al. 1989). This additional dataset, however, was used mainly for helping to reveal the underlying physiological mechanisms, rather than directly using it for evaluation, as performance evaluation with data from inhibitor experiments could be rather complex and is not straightforward.

2.5. Published indices

In this study, eighteen hyperspectral indices raised from previous studies as listed in Supplementary Table 1 were all evaluated for their correlations with different fluorescence parameters (Table 1). Regarding PRI, its change caused by saturating light and could be applied for evaluating xanthophyll cycle activity (Wong and Gamon 2015b) and chlorophyll fluorescence (Liu et al. 2012; Wong and Gamon 2015a). Therefore, the feasibility of using ΔPRI (Gamon and Surfus 1999) has also been examined. Besides these, 12 additional indices developed by Stratoulias et al. (2015) for tracing the chlorophyll fluorescence of reeds under various conditions have also been evaluated. These 12 indices were based on simple ratios (SR, Eq. 1) or normalized differences (ND, Eq. 2) using original reflectance:

$$SR(\lambda_1,\lambda_2) = R_{\lambda_1}/R_{\lambda_2} \quad (1)$$

$$ND(\lambda_1,\lambda_2) = \left(R_{\lambda_1} - R_{\lambda_2}\right)/\left(R_{\lambda_1} + R_{\lambda_2}\right) \quad (2)$$

where R_λ is reflectance and λ_1 and λ_2 are wavelength (nm). The best combination (λ_1 and λ_2) of a given type of index was determined by linear regression to calculate the R^2 and the corresponding significance level (p-value).

Table 1. Fluorescence parameters evaluated in this study.

Fluorescence Parameters	Abbreviation	Formula
Maximum value of fluorescence in the dark-adapted state	F_m	
Minimum value of fluorescence in the dark-adapted state	F_0	
Apparent values of fluorescence in the light-adapted state	F_s	
The maximum values of fluorescence in the light-adapted state	F_m'	
Effective quantum yield of Photosystem II	Yield	$\text{Yield} = (F_m' - F_s)/F_m'$
Photochemical quenching of variable Chlorophyll fluorescence	qP	$\text{qP} = (F_m' - F_s)/(F_m' - F_o)$
Non-photochemical quenching	NPQ	$\text{NPQ} = (F_m - F_m')/F_m'$

2.6. Statistical criteria

The statistical criteria used to evaluate the performance of these indices included root mean square errors (RMSE) and the coefficient of determination (R^2), but a final selection of the best indices was based on the corrected Akaike information criterion (AICc) (Hurvich and Tsai 1989). The AIC (Akaike 1974) is a methodology for model selection when more than one model has been fitted to data during model screening.

In order to reveal in which wavelengths significant differences ($p < 0.05$) were observed between two leaf types or different treatments, the stepwise linear discriminant analysis was applied (Burns and Burns 2008;

202 Draper 1998). The stepwise linear discriminant analysis is a technique for selecting suitable predictor
203 variables (different wavelengths in this study) to be included in a multiple regression model; a combination
204 of forward and backward stepwise regression was implemented. The criterion for adding or removing
205 variables is determined by the critical significance level, which in this study was set to $p < 0.05$.
206
207 A hierarchical cluster analysis using correlation clustering (Langfelder and Horvath 2012) was conducted
208 to reveal the performance similarities of all published hyperspectral indices. This technique does not
209 require a preset number of clusters but groups the data into the optimal number automatically based on
210 the similarity between the data points. The Friedman test (Friedman 1937) was used to test for differences
211 in reflectance among the exposure times. The null hypothesis of this test is that apart from an effect of
212 blocks, the reflectance is the same in each of the groups. Furthermore, the Tukey-Kramer test (Kramer
213 1956, 1957) was applied to reveal the differences of chlorophyll fluorescence parameters among different
214 species. All analyses were conducted using R (R Core Team 2016).
215

3. Results

3.1. Spectral and fluorescence parameters in different datasets

a. Sunlit and shaded leaves (Dataset I)

The typical beech leaf spectral reflectance (sunlit or shaded) at various time points following exposure to light is illustrated in Figure 1. Generally, the reflectance of sunlit leaves was higher than that of shaded leaves. However, the reflectance of red edge and of wavelengths near 1375 nm was constant regardless of leaf type or exposure time. For shaded leaves, decreases in reflectance were observed between 750 and 1350 nm with exposure time ($p < 0.05$ based on Friedman-test). On the other hand, the reflectance at this range increased for sunlit leaves with exposure time ($p < 0.05$ based on Friedman-test), and one-quarter of the published indices used reflectance at wavelengths 750 nm or greater.

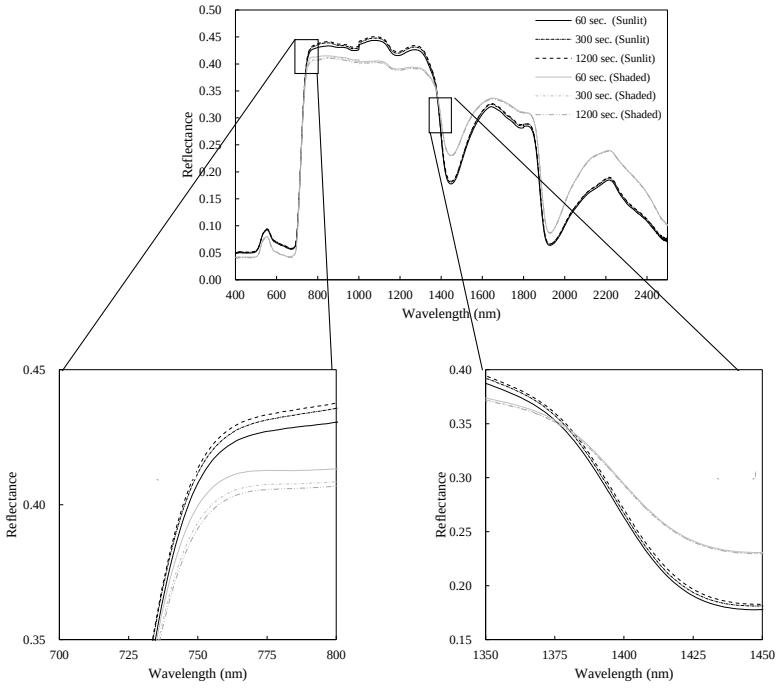

Figure 1. Example of spectral reflectance of a beech leaf at various times following sudden exposure to light (60, 300 and 600 seconds later).

Statistical results (minimum, median, mean, maximum, and standard deviation) of each fluorescence

parameters in Dataset I have been summarized in Table 2. For Dataset I, the ranges of F_m, F_0, F_s, F_m', Yield, qP and NPQ cover from 631 to 2383, 118 to 433, 121 to 1117, 170 to 1129, 0.004 to 0.503, 0.007 to 0.989, and 0.661 to 3.924 , respectively.

Table 2. Statistical descriptions of fluorescence parameters in each dataset.

		F_m	F_0	F_s	F_m'	Yield	qP	NPQ
Dataset I	Minimum	631.0	118.0	121.0	170.0	0.004	0.007	0.661
	Median	1764.0	307.0	410.0	485.5	0.098	0.441	2.472
	Mean	1609.0	284.9	409.7	493.7	0.144	0.436	2.283
	Maximum	2383.0	433.0	1117.0	1129.0	0.503	0.989	3.924
	Standard deviation	563.3	94.6	179.3	191.6	0.123	0.291	0.793
Dataset II	Minimum	1919.0	366.0	206.0	406.0	0.157	0.160	1.327
	Median	2133.0	407.0	340.0	556.0	0.343	0.351	2.763
	Mean	2138.0	409.2	379.0	590.3	0.361	0.369	2.732
	Maximum	2526.0	501.0	692.0	954.0	0.655	0.669	3.938
	Standard deviation	145.9	32.4	121.6	129.5	0.112	0.114	0.619

b. General spectral properties of other species to light stress (Dataset II)

Reflectance spectra of beech (*Fagus crenata*) and other deciduous species (*Acer japonicum*, *Clethra barbinervis*, *Eleutherococcus sciadophylloides*, *Lindera umbellate*, *Quercus crispula* and *Viburnum furcatum*) that composed Dataset II are presented in Figure 2. The reflectance patterns were very similar for 400 to 1800 nm, although three species (*Clethra barbinervis*, *Quercus crispula* and *Viburnum furcatum*) had higher reflectance while two other species (*Lindera umbellate* and *Viburnum furcatum*) had lower reflectance than that of beech. The reflectance of *Acer japonicum* was the same as the beech reflectance. Relatively low variations in reflectance values were found for both *Eleutherococcus sciadophylloides* and *Viburnum furcatum*.

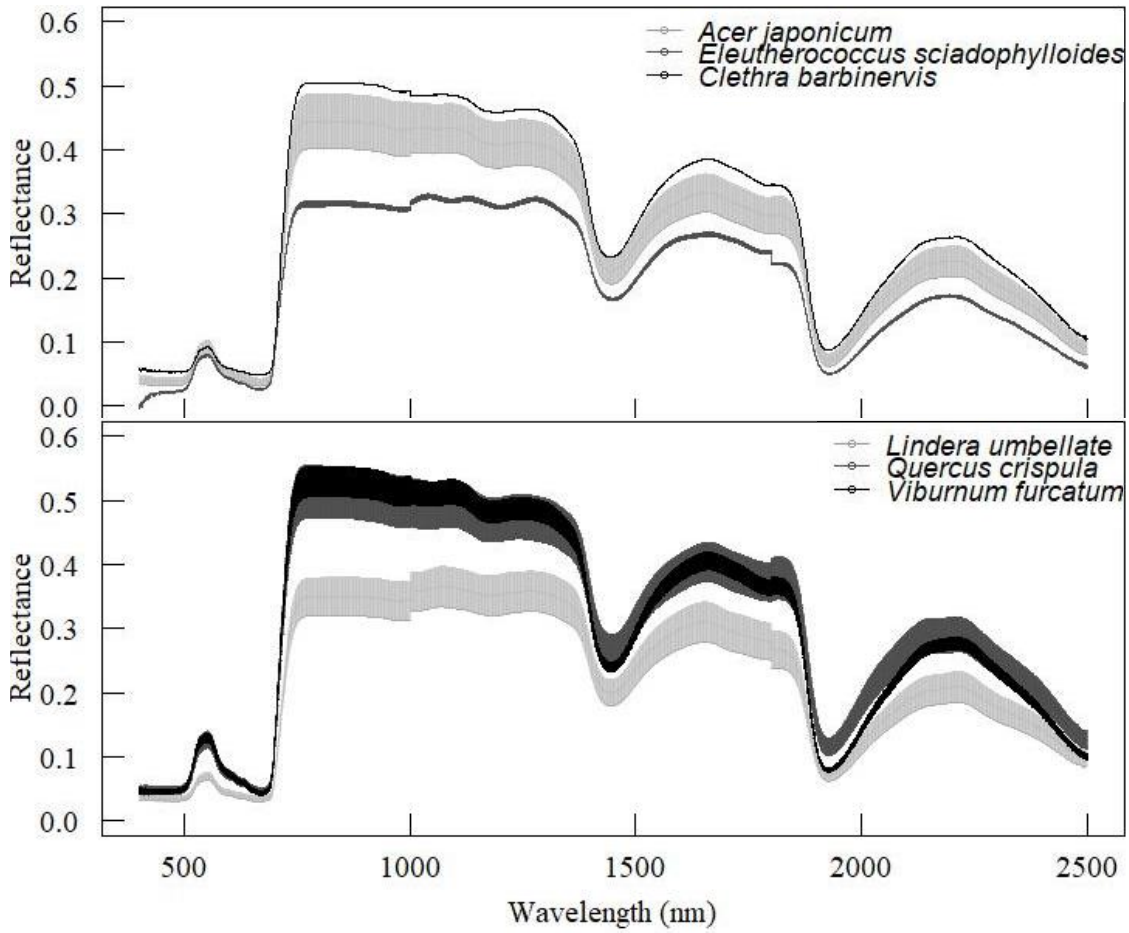

Figure 2. Mean reflectance spectra and standard deviations for six broadleaf species (*Acer japonicum*, *Clethra barbinervis*, *Eleutherococcus sciadophylloides*, *Lindera umbellate*, *Quercus crispula* and *Viburnum furcatum*).

The fluorescence parameters recorded in Dataset II range from 1919 to 2526, 366 to 501, 206 to 692, 406 to 954, 0.157 to 0.655, 0.160 to 0.669 and 1.327 to 3.938 for F_m, F_0, F_s, F_m', Yield, qP, and NPQ, respectively (Table 2).

With the exception of *Eleutherococcus sciadophylloides*, the mean values of F_m, F_0, and F_m' of these deciduous species were significantly higher than those of beech ($p<0.05$, based on Tukey-Kramer test). The four species, with the exception of *Eleutherococcus sciadophylloides* and *Lindera umbellate*, also had lower averaged F_s values than beech. The largest qP was noted for beech, followed by *Acer japonicum*,

Quercus crispula, *Viburnum furcatum*, *Clethra barbinervis*, *Lindera umbellate* and *Eleutherococcus sciadophylloides* in that order. On the other hand, the lowest Yield was also observed for beech, followed by *Eleutherococcus sciadophylloides*, *Lindera umbellate*, *Clethra barbinervis*, *Viburnum furcatum*, *Quercus crispula* and *Acer japonicum* in that order. The highest NPQ values (3.938) were confirmed for *Acer japonicum*, but the ranges of beech F_m', F_s and qP (Dataset I) covered those of the other six species (Dataset II).

3.2. Performance of published indices

The evaluation results of the 30 reported indices including the R^2, p-values, RMSE and AIC_c are presented in Supplementary Table 2. Generally, the R^2 of the regression models resulting from the published indices were high for the basic fluorescence levels (F_m, F_0, F_s and F_m') and for the derived parameters (Yield, qP and NPQ). From Supplementary table 2, PRI performed best for F_0 and F_m ', while SR (539, 560), ND (659, 687), YCAR, OCAR and PSRI performed best for F_m, F_s, Yield, qP and NPQ when based on Dataset I. Alternatively, ARI2 performed best for F_m and F_0, ND (621, 692) performed best for F_s and F_m', SR (659, 687), SR (621, 692) and EVI performed best for Yield, qP and NPQ when only using Dataset II.

In order to clarify the inconsistencies in the index performance, the performance similarities of all published indices were evaluated using correlation clustering. Figure 3 shows the clustering of the correlation relationship among all hyperspectral indices. Clearly, there were no apparent differences between SR and ND when the combination of selected wavelengths was the same, such as with RSI and NDSI. However, for the other indices, the cluster distances were not similar among the datasets; instead, there were different performance patterns for the evaluated indices for the different datasets. Although OCAR, PSRI, SR (539,560), PRI, SR (621,692), EVI and ARI2 were selected for tracing the basic fluorescence levels, the distances were different between the two datasets except for PRI and SR (539,560).

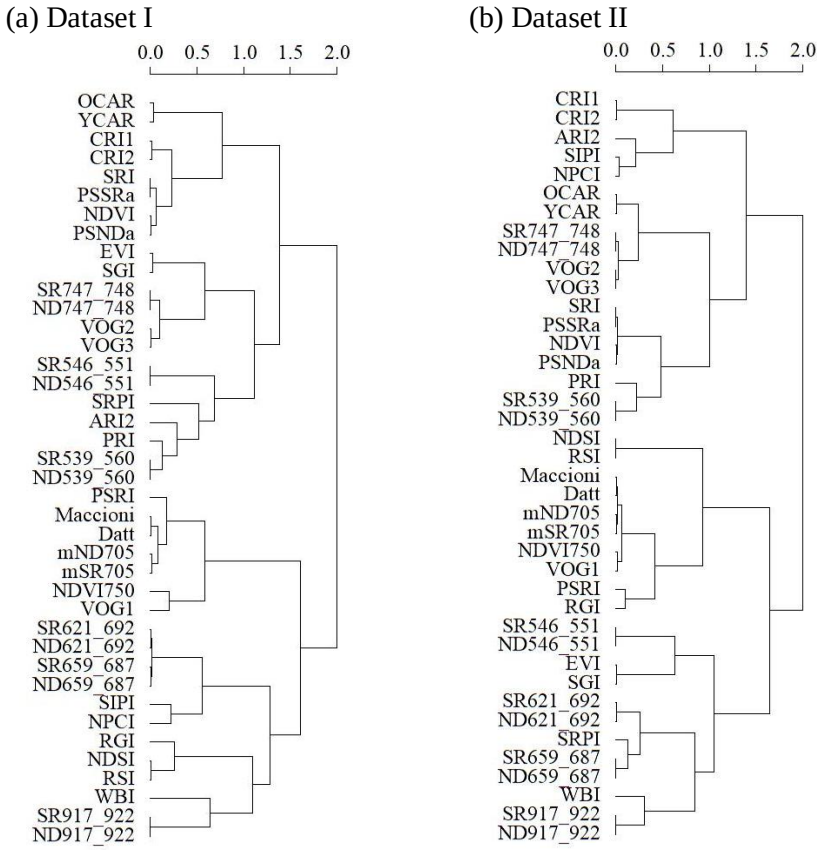

Figure 3. Clustering of correlation relationships among the hyperspectral indices.

PRI performed well for measuring F_m (R^2=0.585, AICc=255.5), F_0 (R^2=0.624, AICc=193.1), F_s (R^2=0.495, AICc=1333.7) and F_m' (R^2=0.599, AICc=1323.269) for Dataset I, while it did not perform well for Dataset II, in which different species were measured. This casts doubts on the general application of PRI when different species are investigated (AICc and R^2 were 174.0 and 0.01 (p=0.796) for F_m, 134.9 and 0.01 (p=0.775) for F_0, 577.3 and 0.01 (p=0.497) for Fs and, 582.7 and 0.02 (p=0.358) for F_m').

To determine if the inclusion of sunlit and shaded leaves caused PRI to perform poorly, evaluations

298 of different leaf groups were also carried out. For sunlit leaves of Dataset I, PRI was the best index
299 for F_m (AICc=140.4 and R^2=0.690) and F_0 (AICc=107.3 and R^2=0.719), fourth best for F_m'
300 (AICc=410.5 and R^2=0.679) and 8th best for F_s (AICc=403.6 and R^2=0.575), while insignificant
301 relationships were noted with the three derived fluorescence parameters (all $R^2 < 0.1$, p=0.889 for
302 Yield, p=0.598 for qP and p=0.250 for NPQ, respectively). Similarly, for shaded leaves PRI also
303 showed significant correlations and high R^2 for F_m (AICc=128.6 and R^2=0.408), F_0 (AICc=99.1 and
304 R^2=0.472), F_s (AICc=912.3 and R^2=0.268) and F_m' (AICc=900.3 and R^2=0.431) were confirmed.
305 Again, poor performance was observed with the three derived fluorescence parameters similar to
306 for the sunlit leaves.
307
308 Our results indicated that ΔPRI was more effective for tracing Yield and qP than PRI when based
309 on Dataset I (AICc=-158.5 and R^2=0.174 for Yield, R^2=0.264 and AICc=12.0 for qP). However,
310 ΔPRI was not applicable for tracing all of the chlorophyll fluorescence parameters when using
311 Dataset II. As for NPQ, the R^2 values were 0.005 for both the datasets only, suggesting that the
312 approach still cannot offer a universal method for tracing the parameter. Alternatively, PSRI was
313 the best index for it when based on Dataset I. However, it had worse performance for sunlit leaves
314 (AICc=56.2, R^2= 0.03, p=0.304). ,
315
316 3.3. Time courses of selected indices and chlorophyll fluorescence parameters
317 Figure 4 shows the time courses of the five fluorescence parameters, which change with exposure
318 time (i.e. F_s, F_m', Yield, qP, and NPQ), and the selected hyperspectral indices for Dataset I. The
319 patterns of time variations of fluorescence parameters were generally similar between sunlit and

shaded leaves, although the values for shaded leaves were higher than those of sunlit leaves in terms of F_s and F_m' and reversions were noted for NPQ. For F_s, it rapidly decreased in the first 100–120 seconds and slight movements were kept for both leaf types. Similar tendencies were also confirmed for F_m'. In contrast, a rapid increase in the first 120 seconds and then a gradual increase with time were observed for Yield and qP. A rapid increase for the first 80 seconds and a sudden drop after 40 seconds were observed for NPQ. Indices ND (659, 687) and PRI could trace the variation in F_s and F_m' respectively, however, for the derivative chlorophyll parameters (i.e. Yield, qP and NPQ), the selected indices were not suitable for tracing.

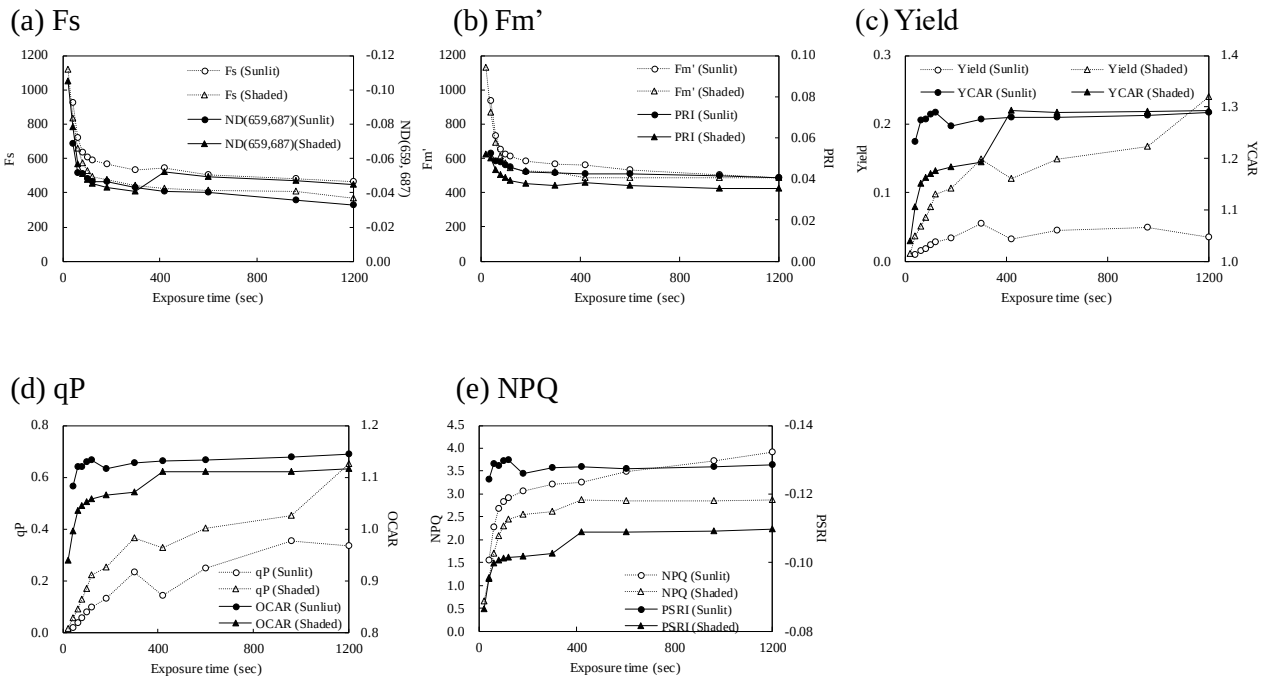

Figure 4. Time courses of fluorescence parameters and selected indices for Dataset I.

4. Discussion

Evaluation results based on Dataset I suggested that RSI and NDSI had high R^2 with F_0, which agrees with findings reported by an earlier study of (Zhang et al. 2012). However, RSI and NDSI performed poorly with Dataset II, as indicated by low R^2 values. Different ranges of fluorescence parameters in Dataset I, Dataset II, and the dataset by Zhang et al. (2012) may have explained the discrepancy, even though it may not be adequate to compare different fluorescence measurements directly. In this study, it was found that RSI and NDSI were not applicable for large F_0 or F_m cases. Thereby, RSI and NDSI both had spectral features at 935 nm, among the domain where opposite responses to the light stress of different leaf types were identified. This may partially explain the poor performance of both indices when different leaf types are not explicitly separated. Other indirect evidence for the above assertion may be strengthened by examining both indices with the additional chemically-treated dataset; they showed poor responses to other chemical treated samples for all the three inhibitors including DBMIB, DCMU, and DTT, which had effects on the various leave types as shown by the large variation in reflectance at 935 nm.

OCAR is one of the promising indices for tracing F_m as claimed by Zhang et al. (2012). The results presented here did not support this, as significant correlations were not observed for F_0 ($p=0.437$) and F_m ($p=0.499$) based on the additional chemically-treated dataset. A stepwise discriminant analysis was carried out and the results revealed that both reflectance values at 630 and 680 nm used by OCAR did not respond to inhibitor treatments. Furthermore, opposite tendencies of reflectance patterns with exposure time were noted for the two leaf types, as reflectance increased with exposure time for sunlit leaves while it decreased with exposure time for shaded leaves. These

356 results raise doubts about applying OCAR to trace the time courses of F_0 and F_m.

357

358 The different response patterns of different leave types may also explain the behavior of ARI2,
359 which was claimed to perform well by Stratoulias et al. (2015) who investigated reed reflectance
360 under various conditions. Our results indicated that ARI2 was the best index for Dataset II but not
361 for Dataset I, in which two leaf types were included. Even so, the index is attracting attention for
362 its applicability across different species of deciduous trees. In addition, further evaluation of the
363 other indices reported by Stratoulias et al. (2015) revealed that they were basically unacceptable
364 for both datasets. Interestingly, the indices based on reflectance at 659 and 687 nm developed for
365 tracing Yield by Stratoulias et al. (2015), were found to perform well for tracing F_m, F_0 and F_s for
366 Dataset I. These indices also had relatively lower AICc and higher R^2 for F_m (AICc=155.2 and
367 R^2=0.765), F_0 (AICc=117.9 and R^2=0.730) and F_s (AICc=787.6 and R^2=0.415) of Dataset II. This
368 implies that these wavelengths are effective in tracing basic fluorescence levels.

369

370 PRI has been shown to be a promising index for tracing various chlorophyll fluorescence parameters,
371 especially for NPQ, as high correlations have been reported in previous studies (Magney et al. 2014;
372 Porcar-Castell et al. 2012; Rahimzadeh-Bajgiran et al. 2012). Furthermore, changes in PRI have
373 been related to Radiation Use Efficiency (RUE) or Light Use Efficiency (LUE) (Garbulsky et al.
374 2011; Grace et al. 2007; Lees et al. 2018; Sims and Gamon 2002) as the reduction of PRI is caused
375 by a greater recourse to NPQ by an increase of the de-epoxidation level of the xanthophyll pigments
376 (Castagna et al. 2001; Elvira et al. 1998; Ranieri et al. 2001). Surprisingly, the evaluation results
377 herein presented indicate that PRI is not suitable for tracing NPQ, Yield, or qP, although PRI was

identified as the best index for tracing F_m'. This is similar to the results reported by Rapaport et al. (2017), who pointed out that it was hard to explain very large PRI variations solely by NPQ. One possible reason for this is that NPQ is calculated from dividing the difference between F_m and F_m' with F_m', while both F_m and F_m' are found linearly related with PRI and thus level out the parameter of PRI after being divided.

Another possible reason for this discrepancy can be explained by the different data ranges used for the evaluations. In earlier studies focusing on herbaceous plants, such as sunflower and eggplant or pine trees, high correlations were reported to be largely limited within an intra-daily scale. As a comparison, we focused on deciduous leaves, which were sampled from three different altitudes, from different dates, and from different species. Hence, the datasets used here for the evaluation of the indices include much more diverse cases. The poor performance of PRI for tracing the derived fluorescence parameters may therefore be explained by the different pigments contained in the evaluation datasets, as leaf spectral reflectance at 531 and 570 nm increases with decreasing chlorophyll (Sims and Gamon 2002) and PRI was found to be highly correlated with ratios of carotenoids and chlorophyll (Filella et al. 2009; Sims and Gamon 2002). This, in turn, leads to different behaviors of PRI under various environmental stress conditions, which are sometimes evaluated by the ratios of carotenoids and chlorophyll (Gamon et al., 2016).

Much of the confusion in the recent PRI literature may arise from the fact that multiple factors drive variation in PRI over different temporal scales (Barton and North 2001), and few studies have fully considered sampling effects or have attempted to distinguish the multiple causes of PRI

variation (Gamon 2015).

A recent study by Verhoef et al. (2018) reported the leaf types have great influences on the energy balance and such information is critical for modeling reflectance and fluorescence. Indeed, proportions in leaf types have been considered for estimating vegetation fluorescence emissions in previous studies (e.g. Hernández-Clemente et al. 2017). Our datasets include both sunlit and shaded leaves, which might be an important reason why PRI performed poorly. However, ignorant of leaf groups, our evaluation results suggested that PRI performed well with F_m, F_0, F_s and F_m', but poor with the three derived fluorescence parameters. In addition, much better performances were noted for ΔPRI with qP and NPQ for both leaf types. Astonishingly, our results suggested that ΔPRI was not applicable for tracing all of the chlorophyll fluorescence parameters when using Dataset II, although good performance was noted for ΔPRI with Dataset I. Overall, our results suggest that PRI is useful for tracing the basic fluorescence levels when measuring only one leaf type or when making measurements under similar conditions. The data acquired from satellites and airborne are strongly influenced by the signal from sunlit leaves and thus the abilities of PRI could be useful. As for the derived fluorescence parameters, e.g. NPQ or qP, ΔPRI is a better choice than PRI, although ΔPRI comparisons should still be limited to only one species.

As an important step towards an extensive evaluation, performance examinations for all indices were carried out on each leaf type of the sampled broadleaf trees (Supplementary Table 3). Under conditions where photosynthesis is limited by factors other than light, sunlit parts of the canopy are exposed to more excessive radiation energy than those shaded by other vegetation elements (Hilker

et al. 2010). Shaded leaves are, in general, larger and thinner than sunlit leaves. They also have half as many stomata as sunlit leaves, or even fewer, resulting in a lower respiration rate. It is well known that sunlit leaves, which develop under high irradiances, are much less susceptible to photoinhibitory damage than shaded leaves, which develop under low irradiances (Powles 1984). Evaluation results suggested that the best indices identified for both groups were different between the two leaf types. For shaded leaves, the wavelengths of the identified best indices were relatively close to that of PRI for the basic fluorescence levels (i.e. SR (539, 560) or ND (539, 560)). On the contrary, the indices using reflectance at 659 and 687 nm were selected for sunlit leaves regardless of leaf types. Indices using reflectance at 659 and 687 nm also had significant coefficients of determination for shaded leaves (AICc=127.9, 101.4, 888.3 and 922.5, R^2=0.458, 0.300, 0.477 and 0.225 for F_m, F_0, F_s and F_m', respectively). Furthermore, the reflectance at 659 and 687nm was effective for the other deciduous species (except for F_m') (AICc=168.1, 125.2 and 547.4, R^2=0.370, 0.528 and 0.482 for F_m, F_0 and F_s, respectively). Data screening of other similar wavelengths may contribute to the development of more robust indices for tracing basic fluorescence levels.

For basic fluorescence levels, PRI was still the best indicator when the leaf types were separated, and was the most reliable indicator of basic fluorescence levels overall. However, PRI was not applicable for the derived fluorescence parameters. On the other hand, ΔPRI performed better at tracing qP and NPQ, but its use is restricted to homogeneous conditions like those cases in Dataset I or II. As a result, although there are various indices for tracing chlorophyll fluorescence parameters, no index was applicable across diverse conditions and various species as evaluated in this study.

5. Conclusions

The relationships between fluorescence parameters and 30 published hyperspectral indices were evaluated based on three unique datasets including cases employing different chemical treatments, species, sites, and types of leaves. PRI was useful for tracing F_m, F_0, F_s and F_m' for beech leaves including controlled chemically treated samples, and PRI performed well when limited to a single species. YCAR was identified as the best index for tracing Yield for Dataset I, and SR (659, 687) was the best index for tracing Yield for Dataset II. For tracing qP, OCAR and SR (621, 692) were the best indices, while PSRI and EVI were the best indices for tracing NPQ for Dataset I and II, respectively. However, some of these indices were not acceptable for sunlit beech leaves. Furthermore, the well-known relationships of PRI and Yield, PRI and NPQ were not observed. In addition, poor responses of current indices under inhibitor-treated conditions suggest they did not hold strong direct engagements with chlorophyll fluorescence process. As thus, further development of relevant radiative transfer models that can offer inversion retrievals and new indices that are generally applicable, is highly needed for future work. Towards it, an extensive dataset composed of diverse cases including different leaf types, different species, and different stress conditions, as well as a clear understanding of related radiative transfer processes, is needed to make general conclusions.

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